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The Mating Behavior of Diamondback Moth (Lepidoptera: Plutellidae) 【Research report】

小菜蛾之交尾習性【研究報告】

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Received: Accepted: Available online: 1995/03/01

Abstract

Studies were conducted to better understand mating and other reproductive activities of diamondback moth, *Plutella xylostella* (L.), in order to improve the use of synthetic sex pheromone in managing this crucifer pest. Adult emergence occurred during photophase. The majority of males mated several times during one night. Duration of mating ranged from 50 to 109 minutes. When one male was confined with one female, 93.7% of males mated once, 5.6% twice, and less than 1% more frequently during 24 hours. When three females were confined with one male, 57.1% males mated once, 27.1% twice, 12.9% thrice, and 1.4% four or five times. Four-to seven-day-old males were more aggressive than younger or older ones in seeking and mating virgin females. Mating did not affect the response of male adults to synthetic sex pheromone. When only males were present in an area, more than two-thirds of them were trapped in a sex pheromone-baited trap. However, when females were introduced to provide a sex ratio of 1 : 2 female : male, the number of males captured was reduced by 50%. The active space for the diamondback moth sex pheromone was 4 m.

摘要

小菜蛾是十字花科蔬菜作物主要害蟲之一，為了有效的應用合成性費洛蒙於蟲害管理上，在亞洲蔬菜研究發展中心進行小菜蛾交尾繁殖行為之研究。此蟲大多在白天羽化，9:00AM前羽化雄蛾當夜即可交尾，大部份雄蛾一夜可多次交尾，一次交尾時間可長達50-109分鐘。當一雄一雌配對時，93.7%雄蛾一夜只交尾一次，僅5.6%交尾兩次，而少於1%的雄蛾交尾超過兩次。然而當一隻雄蛾與三隻雌蛾配對時，雄蛾一夜間只交尾一次者降至57.1%，交尾二次者提高至27.1%，交尾三次者有12.9%，更有11.4%雄蛾交尾超過三次。4-7日齡雄蛾具最強的找尋及與處女雌蛾交尾之能力。已交尾雄蛾仍對合成性費洛蒙具有反應。在此雄蛾存在之空間內有2/3以上的雄蛾可被性費洛蒙捕捉器捕獲，但在雌雄性比為1:2的空間內被捕獲雄蛾數可減少50%。本實驗所用合成性費洛蒙之有效範圍可達4公尺。

Key words: *Plutella xylostella*, mating, mating periodicity, mating duration, sex pheromone.

關鍵詞: 小菜蛾、交尾、性費洛蒙、交尾日週期、交尾持續時間。

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The Mating Behavior of Diamondback Moth (Lepidoptera: Plutellidae)

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ABSTRACT

Studies were conducted to better understand mating and other reproductive activities of diamondback moth, *Plutella xylostella* (L.), in order to improve the use of synthetic sex pheromone in managing this crucifer pest. Adult emergence occurred during photophase. The majority of males mated several times during one night. Duration of mating ranged from 50 to 109 minutes. When one male was confined with one female, 93.7% of males mated once, 5.6% twice, and less than 1% more frequently during 24 hours. When three females were confined with one male, 57.1% males mated once, 27.1% twice, 12.9% thrice, and 1.4% four or five times. Four- to seven-day-old males were more aggressive than younger or older ones in seeking and mating virgin females. Mating did not affect the response of male adults to synthetic sex pheromone. When only males were present in an area, more than two-thirds of them were trapped in a sex pheromone-baited trap. However, when females were introduced to provide a sex ratio of 1:2 female:male, the number of males captured was reduced by 50%. The active space for the diamondback moth sex pheromone was 4 m.

Key words: *Plutella xylostella*, mating, mating periodicity, mating duration, sex pheromone

Introduction

Diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), is a destructive pest of all economically important crucifers throughout tropical regions of the world, especially in Southeast Asia (Talekar and Shelton, 1993). The

lack of effective species of natural enemies and the elimination of existing and adapted ones by frequent use of insecticides has resulted in diamondback moth becoming a serious pest in the tropics. Intensive use of insecticides has also culminated in this insect becoming resistant to most chemicals (Miyata *et al.*,

1986; Sun, 1992). Because of this, considerable research is being done on alternative control measures (Talekar and Griggs, 1986; Talekar, 1992). The use of synthetic sex pheromone to alter mating behavior is one such measure. Sex pheromones of approximately 1611 insect species, including 1335 of Lepidoptera, have been identified and synthesized (Mayer and McLaughlin, 1991). A three component sex pheromone of diamondback moth: cis-11-hexadecenal, cis-11-hexadecenyl acetate and cis-11-hexadecenol has been identified (Chow *et al.*, 1977; Ando *et al.*, 1979; Chisholm *et al.*, 1979), synthesized and is commercially available. However, its use has been limited due to low persistence, short effective distance, and high cost. To improve the utilization of the pheromone, we conducted some basic studies on the reproductive behavior of diamondback moth.

Materials and Methods

Mating periodicity and frequency

In the first test we placed one 18-26 hour-old male and one same age female adults in a plastic cup (4 cm × 2.7 cm × 4 cm) used routinely for rearing diamondback moth in our laboratory. A total of 242 pairs of insects were used. Starting at 1000 hours, at 30-minute intervals, we counted the number of pairs in copula for 24 hours. In the second test, we placed one 18-26 hour-old male and three same age females in the insect rearing cup and recorded the number of pairs in copula every 30 minutes for 24 hours. A total of 114 males and 342 females were used. Timings of sunset and sunrise were recorded.

In the third test we confined one two-day-old virgin female and one unmated male adults that emerged from pupae at the following 3- or 4- hour intervals of a day: 0800 to 1100, 1101 to 1500, 1501 to 1900, 1901 to 2300, 2301 to 0200 or 0201 to 0500 hours. A total of 154 pairs of insects

were used. We recorded the time when they started mating and then calculated the pre-mating period.

Mating duration study

One two-day-old unmated male and three same age virgin females were placed in an insect-rearing cup and frequency and duration of mating was observed once every 10 minutes from 1800 hours to 0700 hours of the next day. A total of 63 males and 189 females were used. The mated males were classified as once-, twice- or thrice-mated.

Effect of age on mating ability of males

Eleven males ranging from 1 to 11 days old were confined in a glass jar (30 cm height, 14 cm diameter). They were marked on the thorax with specific colors to indicate their age. Starting at 1830 hours, we introduced the first of the six two-day-old virgin female in the jar for mating. Copulating pair was immediately removed, the age of the male noted, and was given a score of 6. A second 2-day-old virgin female was then introduced. The copulating pair was immediately removed, the age of the male was noted, and was given a score of 5. A third 2-day-old virgin female was then introduced. The copulating pair was immediately removed, the age of the male was noted, and was given a score of 4. This procedure was repeated until all six females were exposed. The males that failed to mate with any of the six females were given a score of 0. This experiment was done 15 times. The total scores of 15 tests were used in judging the effect of age on mating ability of diamondback moth males.

Periodicity of response of diamondback moth males to virgin females and synthetic sex pheromone

This experiment was carried out in a field planted to Chinese cabbage (*Brassi-*

ca campestris ssp *pekinensis*). Ten two-day-old virgin females were confined in a 4 cm diameter 6 cm long fine mesh stainless steel wire container and the container was placed inside a sticky paper trap. The traps baited with females were placed 50 cm above soil surface in four 25 × 24 m Chinese cabbage fields. In the same fields, other traps baited with 100 µg synthetic sex pheromone (cis-11-hexadecenal, cis-11-hexadecenyl acetate, cis-11-hexadecenol; 5:5:0.1) were also placed 15 m away from the virgin female-baited traps. The number of males caught in each trap was recorded every hour for 24 hours starting at 1000 hours.

Effect of mating on the response of males to synthetic sex pheromone

Every day for seven consecutive days, five 0–24 hour-old males and five similar age females were confined together in each of the five glass tubes (2.8 cm diameter, 12.8 cm length) and allowed them to mate. Each day's male insects were marked on their thoraxes with a specific color to indicate their age. Simultaneously, five males only were also placed daily in each of the five glass tubes. All insects were maintained at 25 ± 2°C and given dilute honey for food. On the seventh day we installed one sticky paper trap, baited with 3 µg sex pheromone, in a 270-cm-long × 170-cm-wide × 180-cm-high nylon net cage containing 30 potted common cabbage (*Brassica oleracea* var *capitata*) plants. The marked males of each age group were released in the cage. The next morning the number of marked males caught in the trap was recorded. This experiment was done three times.

Influence of sex ratio on the effectiveness of sex pheromone

Two-day-old unmated adults were separated into the following four groups: (a) 30 males only, (b) 15 females and 30 males, (c) 30 females and 30 males, and (d) 60 females and 30 males. The insects were

released in the late afternoon into four separate nylon-net cages, each cage containing two sticky paper traps baited with 3 µg of sex pheromone. The number of adults caught in the traps were recorded the next morning. This experiment was repeated for three consecutive days using different two-day-old adults and fresh sex pheromone.

Pheromone effectiveness distance study

A large number of males obtained from laboratory culture were separated in 10 groups. Each group of insects was marked with distinguishing paint on their thoraxes. A trap baited with about 3 µg sex pheromone was placed in an empty field at 1900 hours and marked males were released at 0.5, 1, 2, 3, 4, 5, 6, 7, 8 or 10 m from the trap. At 2000 hours we recorded the number of marked males caught in the trap. This experiment was repeated 10 times during January–February 1986. The mean minimum and maximum temperatures during the period were 9°C and 22.5°C, respectively; RH, 67–94% and wind velocity ranged from 0.03 to 2.02 m/second. From the number of insects trapped from each distance we calculated the active space of diamondback moth sex pheromone activity by method developed by Hartstack *et al.* (1971).

Results and Discussion

Mating periodicity and frequency

Mating began at the onset of dark and peaked within one hour. This mating peak lasted for 3 hours. Another peak of mating activity occurred about 5 hours after the onset of darkness. In the second experiment two additional peaks were observed. These peaks could be due to the differences in sex ratios maintained in this experiment. Irrespective of sex ratios, most of the mating occurred in the first 3 hours after initiation of dark. Our results obtained in Taiwan are similar to the

findings of Sakanoshita and Yanagita (1972) in Japan and Pivnick *et al.* (1990) in Canada. This information was used by Talekar *et al.* (1986) who proposed controlling diamondback moth by applying sprinkler irrigation to cabbage at dusk which will disrupt the insect mating and reproduction.

Emergence of adults from pupae occurred during photophase. Adults which emerged before 1100 hours copulated the same night (Fig. 1); of those that emerged between 1100 and 1900 hours, a few males copulated the same night and those that emerged after 1900 hours copulated only the next evening. Adult emergence timings were considered as critical in predicting the occurrence of mating during the night.

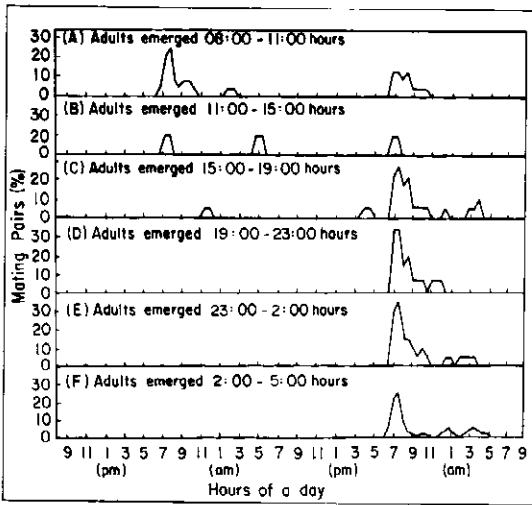


Fig. 1. The relationship between the time of adult emergence and occurrence of first mating by single paired diamondback moth. (Initiation of light, 0500 hours; dark, 1850 hours).

When one male and one female were placed together, 93.7% of the males mated once; 5.6% mated twice and less than 1% thrice (Table 1). Ohira (1979) observed several matings, with a maximum of four in a single day, in about 25% of the pairs

of adults. In our study when three females were confined with a single male, mating frequency increased: only 57.1% males mated once, 27.1% twice, 12.9% thrice, and 1.4% four or five times. Yamada (1979) reports even higher frequency of mating, up to nine when five females were confined with one male. Preponderance of females in diamondback moth population thus increases the frequency of mating. Such multiple mating is likely to increase fecundity and pest population build-up.

Table 1. Mating frequency of male diamondback moth

Frequency of matings per day	% of mated males when paired with	
	1 female	3 females
1	93.7	57.1
2	5.6	27.1
3	0.8	12.9
4	0.0	1.4
5	0.0	1.4

Mating duration study

The majority of males mated several times in one night. Single matings lasted 109 minutes each whereas multiple matings lasted 50 to 100 minutes each when more than one took place in one night (Fig. 2). The second mating occurred within 10 minutes after completion of the first. To our knowledge this is the first report of duration of mating in diamondback moth.

Effect of age on mating ability of males

Although males ranging in age from 1 to 11 days were able to mate with receptive females, it is the four- to seven-day-old males that were more aggressive in mating over both the younger and older males (Fig. 3). Although, as we observed earlier in this study, males can mate within 24 hours of emergence from

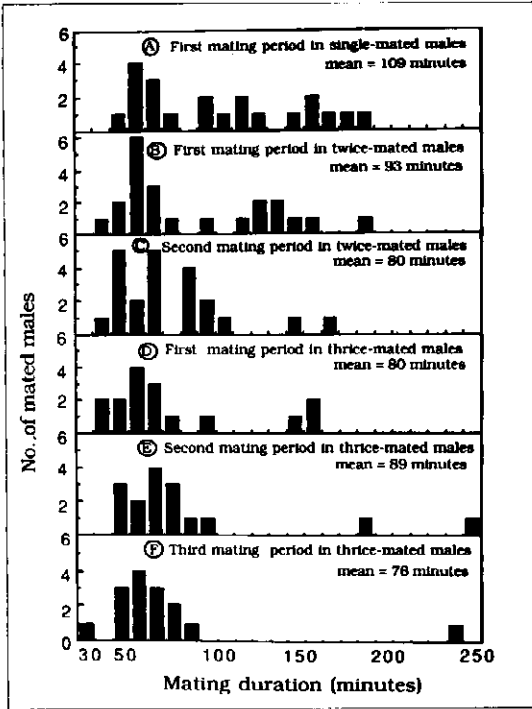


Fig. 2. Duration of mating of diamondback moth males in relation to mating frequency.

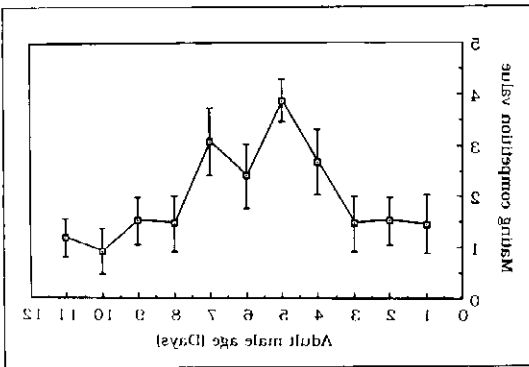


Fig. 3. Influence of age on mating ability of diamondback moth male adults.

pupae, they do not seem to reach peak sexual maturity until they are 3-4 days old. Male moths that are 8-days and older are too old and probably passed active reproductive period, hence there was decline in the frequency of mating as the

insects became older than 8 days.

Periodicity of response of males to virgin females and synthetic sex pheromone

The periodicity of capture of males in traps baited with virgin females and synthetic sex pheromone was practically identical (Fig. 4). Traps baited with 10 virgin females attracted substantially more males than the traps baited with 100 μg of the synthetic sex pheromone. In virgin female-baited traps, two population maximum peaks were observed, one within two hours from onset of scotophase and one 4 hours later. In the synthetic sex pheromone traps, only the first population peak was observed. It is possible that the concentration of synthetic pheromone we used was lower than the amount emitted by 10 females. It could also be due to the differences in the proportion of various chemical components that make up the natural pheromone emitted by the virgin females and the proportion of these components in the synthetic sex pheromone blend we used. Weather conditions and differences in the strains of the insect affect the proportion of various pheromone components produced by the virgin female (Maa, 1986). The proportions of various components we used in the synthetic pheromone blend, therefore, may not be ideal and identical to the one emitted by the strain of diamondback moth we used in our study. The possibility that virgin females emitted pheromone twice within an interval of 4 hours which resulted in the appearance of two population maxima could not be ruled out.

Effect of mating on the response of males to synthetic sex pheromone

There was no significant difference in the recapture of the males in the synthetic sex pheromone trap whether the males were mated or unmated (Table 2). This applied to all males ranging from one to

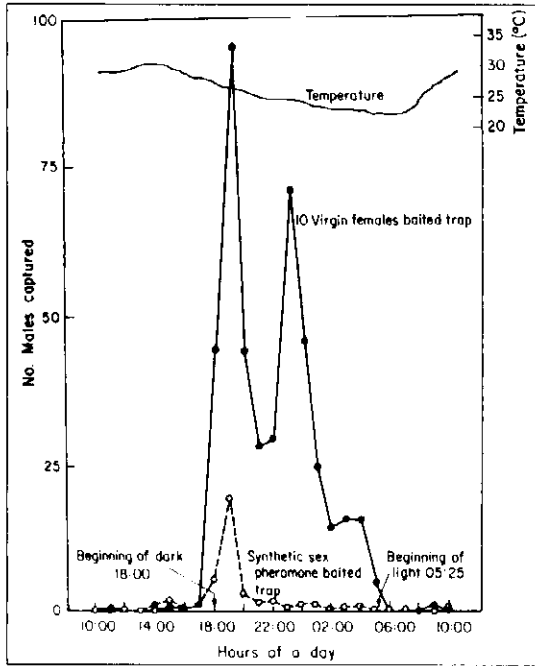


Fig. 4. The daily periodic response of males of diamondback moth to 10 virgin females and to synthetic sex pheromone baited traps placed in a Chinese cabbage field.

seven days old. Under practical field condition, capture of unmated males undoubtedly would help in reducing chances of mating which in turn will reduce insect population. However, since males can mate several times, especially when females outnumber the males, trapping of even mated males in pheromone trap is of economic significance because it will stop them from future mating and subsequent reproduction.

Influence of sex ratio on the effectiveness of sex pheromone

When only males were present in an insect population, more than two-thirds were captured in the pheromone trap. However, introduction of virgin females to adjust to the sex ratio of 1 female to 2 males, reduced the male insect capture in the synthetic pheromone traps by 50% (Table 3). Increasing sex ratio to 1:1 did

Table 2. Effect of mating on efficiency of synthetic sex pheromone in attracting various aged males of diamondback moth

Male age (days)	Recapture(%)	
	mated	Unmated
1	9.8c	8.6c
2	40.3abc	42.0abc
3	51.6ab	50.0ab
4	34.3abc	32.9abc
5	31.9abc	37.3abc
6	37.9abc	58.7ab
7	66.0a	65.5ab

The means in the vertical column followed by the same letter are not significantly different at 5% level by Duncan's (1955) Multiple Range Test. Data are means of three replicates.

not affect the male insect capture over the ratio of 1:2, but doubling of female population further to 2 females to 1 male further reduced the capture of diamondback moth males in the pheromone baited traps. It is possible that the more the females are present in an insect population the more males will be attracted to the sex pheromone produced by the females and proportionately less will be attracted to sex pheromone-baited traps. This fact should be factored in while extending results of laboratory tests with isolated males in deciding upon the concentration of sex pheromone for field use where mixed sex population exists.

Pheromone effectiveness distance study

The number of males captured in synthetic sex pheromone-baited trap from various distances at which the insects were placed immediately before the introduction of sex pheromone, are summarized in Fig. 5. As the distance between the trap and male insect release site increased, the number of insects trapped decreased. The environmental factors such as humidity, wind velocity, and temperature can affect the movement

Table 3. Influence of adult sex ratio on the efficiency of synthetic sex pheromone in trapping males of diamondback moth

Sex ratio male:female	% males captured at various sex ratio (male:female)			
	Test 1	Test 2	Test 3	Mean ¹
30:0	87.9	77.8	63.3	76.3a
30:15	33.3	36.7	45.0	38.3b
30:30	40.0	26.7	50.0	38.9b
30:60	23.3	30.0	26.7	26.7b

¹Means in the vertical columns followed by the same letter are not significantly different at 5% level by Duncan's (1955) Multiple Range Test.

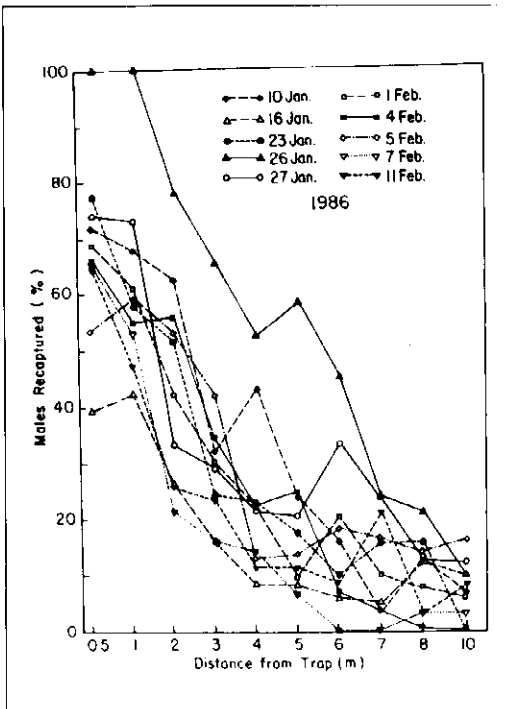


Fig. 5. Recapture rate of males of diamondback moth one hour after release of unmated males at various distances from the sex pheromone trap.

of insects and their attraction to sex pheromone source. These factors could have influenced outcome of our test. This relationship in our test is explained by an equation: $R = 61.909 + 57.90W - 36.177W^2 - 15.486D + 0.927D^2$ where R = recapture rate, W = wind velocity, and D = distance. The active space of the pheromone in our

study was 4 m which is higher than that observed by Ishii *et al.* (1981) and Chow *et al.* (1984). We are unable to explain the reasons for greater response of males to the sex pheromone in this study.

Acknowledgments

This is Asian Vegetable Research and Development Center Journal Paper No. JP 145.

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Received for publication September 29, 1994;
Revised manuscript accepted April 13, 1995.

小菜蛾之交尾習性

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摘 要

小菜蛾是十字花科蔬菜作物主要害蟲之一，為了有效的應用合成性費洛蒙於蟲害管理上，在亞洲蔬菜研究發展中心進行小菜蛾交尾繁殖行為之研究。此蟲大多在白天羽化，9:00AM前羽化雄蛾當夜即可交尾，大部份雄蛾一夜可多次交尾，一次交尾時間可長達50-109分鐘。當一雄一雌配對時，93.7%雄蛾一夜只交尾一次，僅5.6%交尾兩次，而少於1%的雄蛾交尾超過兩次。然而當一隻雄蛾與三隻雌蛾配對時，雄蛾一夜間只交尾一次者降至57.1%，交尾二次者提高至27.1%，交尾三次者有12.9%，更有11.4%雄蛾交尾超過三次。4-7日齡雄蛾具最強的找尋及與處女雌蛾交尾之能力。已交尾雄蛾仍對合成性費洛蒙具有反應。在此雄蛾存在之空間內有2/3以上的雄蛾可被性費洛蒙捕捉器捕獲，但在雌雄性比為1:2的空間內被捕獲雄蛾數可減少50%。本實驗所用合成性費洛蒙之有效範圍可達4公尺。

關鍵詞：小菜蛾、交尾、性費洛蒙、交尾日週期、交尾持續時間。