

Effect of male and female multiple mating on the fecundity, fertility, and longevity of diamondback moth, *Plutella xylostella* (L.)

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Abstract: The effect of diamondback moth (DBM), *Plutella xylostella* (Lep., Plutellidae) male and female multiple mating on fecundity, fertility, and longevity was studied. Males could mate for five times with virgin females during scotophase. The successful copulation rates, fecundity of female, and longevity of both females and males decreased when male mating times increased, whereas copulation duration increased. Correlation coefficient between copulation duration and male mating times was significant ($r = 0.7358$, $P = 0.0001$, spearman rank-order correlation). There were linear relationships between mating history of males and longevities of males and females, and regression relationships between them were significant. Mated females had similar daily reproductive pattern, which laid the most eggs on the first day after mating in spite of their mates' mating history. Virgin females laid some infertile eggs before they died. Most of the females mated once during their lifespan but 19.9% of females mated twice when one female kept with one male during scotophase. There were no significant differences in the fecundity, fertility and longevity between the single- and twice-mated females. Correlation coefficient between copulation duration and female mating times was not significant ($r = 0.0860$, $P = 0.8575$). Results suggested that DBM females may be monandrous. Multiple mating did not increase male or female mating fitness.

Key words: *Plutella xylostella*, fecundity, fertility, longevity, multiple mating

1 Introduction

Multiple mating is a widespread phenomenon among various insect species, especially in Lepidoptera, where both males and females are capable of mating more than once (DRUMMOND, 1984). Nearly all polyandrous insect species benefit from multiple mating (RIDLEY, 1988). Some reasons have been proposed for multiple mating of male and female. For example, they can generally increase their fitness, increase reproductive success, increase genetic diversity of offspring, and so on when mate multiply (ARNQVIST and NILSSON, 2000; TREGENZA and WEDELL, 2002). Female fecundity, fertility and longevity can be affected by multiple mating of themselves and their mate. For female with nuptial feeding or not, multiple mating increases their average egg and offspring production and fertility, while weakly affecting longevity. For those without nuptial feeding, multiple mating in general reduces longevity (WARD and LANDOLT, 1995; LAMUNYON, 1997; KAWAGOE et al., 2001; SADEK, 2001). Normally monandrous females may mate again if kept with males, but in this case re-mating is not able to increase female fecundity (RIDLEY, 1988; FOSTER and AYEARS, 1996; MCNEIL et al., 1997).

Diamondback moth (DBM), *Plutella xylostella* (L.) is the most destructive insect of cruciferous plants throughout the world, and it is estimated that the annual cost for managing is US\$1 billion (TALEKAR and SHELTON, 1993). There were several researches on mating behaviour and factors affecting fecundity, fertility and longevity of DBM females (HARCOURT, 1957; YAMADA and UMEYA, 1972; KE and FANG, 1979; YAMADA, 1979; YAMADA and KAWASAKI, 1983; UEMATSU et al., 1989; LEE et al., 1995). However, very few researches studied the effect of multiple mating on the fecundity, fertility and longevity for both males and females. Through this study we can learn the mating fitness of DBM female and male. At the same time multiple mating may affect control effect on pest with sex pheromone. We can predict the affect of mating behaviour on the control effect of DBM with female sex pheromone.

2 Materials and Methods

DBM larvae were collected from the wild field in Hubei province (108°08'-116°07', 29°05'-33°21') and bred in laboratory

for nearly 20 generations. DBM were bred at $25^{\circ}\text{C} \pm 1^{\circ}\text{C}$, 75% RH under 16 : 8 (L : D) photoperiodic regime. Larvae were fed on fresh cabbage leaves every day. Pupae were kept separately in glass tube (5 cm \times 3 cm ID) with pored cover for aeration. Newly emerged adults were sexed, and fed on a 10% honey solution soaked in cotton. Leaves of cabbage (*Brassica deracea* var. *capitata* Linn) were placed in the tube as an oviposition substrate mating and changed every 2 days during the experiment.

1. Male multiple mating experiment: one group of males was kept as virgins until they died. Another group of males was provided with new virgin females at the beginning of each scotophase for 5 days, if they had mated in the previous one.

2. Female multiple mating: one group of females was kept as virgins until they died. Another group of virgin females was paired with a virgin male in the first scotophase and again with new virgin males in the second scotophase if they mated.

Copulation was observed during the scotophase. Males and females were separately kept in different tubes after copulation. Females were removed to new tubes and the eggs laid were counted everyday. Eggs were kept till the black-head stage when the percentage of fertile eggs was determined. Successful copulation rate and copulation duration were recorded. Successful copulation was determined by viability of eggs the female laid. Daily reproductive pattern, fecundity, fertility of female, and longevity of females or males were recorded. If the males or females died during the course of the experiments, they were discarded. Each treatment was repeated for five times.

2.1 Data analysis

Analysis of data was performed using ANOVA followed by SNK test or Student's *t*-test for comparing mean values (SAS version 8.0). Percentage data were arcsine-transformed before analysis. Analysis of correlation between copulation duration and mating times was performed with Spearman rank-order correlation.

3 Results

3.1 Multiple mating of male

The successful copulation rates of males were influenced by male mating frequency. The successful copulation rate was 78.5% in once-mated males, but it decreased with the increase of mating frequency. Significant differences were found in rates of successful

copulation between males mated for first to third time and fifth time (table 1).

The mating history of males had a significant influence on fecundity, and had little influence on fertility. Females mated with virgin males had the largest fecundity, significantly greater than those mated with once to fifth mate males. Female egg fertility was >94% in spite of their mate mating history (table 1).

Longevities of females and males were affected by the mating history of males significantly ($P < 0.05$). Virgin females or virgin males had the longest life, and the lifespan of both sexes became shorter with the increase of male mating times (table 1).

Copulation durations were increased as male mating frequency increased. Correlation coefficient of copulation duration and numbers of mating was 0.7358 ($P < 0.0001$).

Mating history of males and longevities of males and females can build linear regression equation. The regression relationships between them were significant (table 2).

Irrespective of male mating history, females had similar daily reproductive pattern, the number of eggs laid was the greatest in the first day after mating, and decreased thereafter in the following days. Most of the virgin females began to lay eggs on the fourth or fifth day after emergence, indicating that mating stimulated female oviposition (fig. 1).

3.2 Multiple mating of female

When females paired with virgin males, 78.5% of females mated once and only 19.3% females mated twice. The rates of mating females reduced significantly after their first mating.

There were no significant differences in copulation duration between once- and second-mated females. Correlation coefficient of numbers of mating and copulation duration was 0.0860 ($P = 0.8575$).

Although virgin females could lay eggs, their numbers were much less than those of mated ones and the eggs were infertile. Virgin females had the longest life of 9.2 days, significantly longer than once- and twice-mated females. Fecundity, fertility and longevity of once-mated females were not significantly different from those of twice-mated females (table 3).

Table 1. Effect of males mating history on longevity of males and fecundity, fertility, and longevity of females

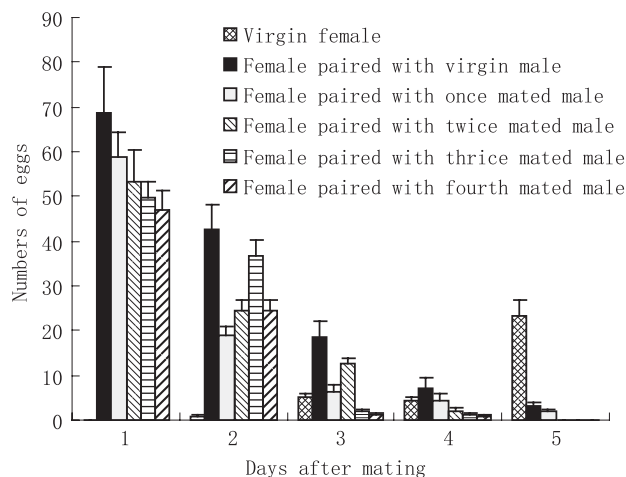
Male mating (times)	No. female observed	Successful copulation rate (%)	Copulation duration (min)	Fecundity (no.)	Fertility (%)	Female longevity (day)	Male longevity (day)
0	53	–	–	–	–	9.24 \pm 1.24 a	12.25 \pm 2.13 a
1	42	78.50 \pm 13.91 a	55.98 \pm 2.69 a	127.87 \pm 21.98 a	98.67 \pm 0.58 a	6.86 \pm 0.60 b	9.15 \pm 2.00 b
2	35	65.16 \pm 6.16 a	59.51 \pm 4.68 a	76.75 \pm 16.30 b	98.00 \pm 1.00 a	6.83 \pm 0.82 b	8.31 \pm 1.73 bc
3	33	62.71 \pm 7.50 a	61.77 \pm 9.15 a	89.05 \pm 3.05 b	94.67 \pm 4.04 a	5.81 \pm 0.73 b	7.89 \pm 1.19 c
4	27	50.96 \pm 5.94 ab	66.35 \pm 1.96 a	85.82 \pm 9.06 b	97.00 \pm 1.00 a	5.91 \pm 0.55 b	7.68 \pm 1.70 c
5	13	24.84 \pm 10.9 b	65.68 \pm 2.55 a	71.27 \pm 4.68 b	94.00 \pm 1.00 a	4.81 \pm 0.57 c	6.26 \pm 1.09 d

Values are mean \pm SEm. Different letters in the same range are significant ($P < 0.05$) by SNK test. –, indicates no research.

Table 2. Relationship between the mating history of males and longevity of males and females

Treatment	Equation	F-value	Correlation coefficient
Mating history of male and longevity of male	$y = 9.7810 - 0.6410x^\dagger$	35.11	-0.9598**
Mating history of male and longevity of female	$y = 11.0743 - 0.9937x^\ddagger$	21.24	-0.9174**
Mating history of male and longevity of male	$y = 7.5500 - 0.5020x^\dagger$	21.05	-0.9356*
Mating history of male and longevity of female	$y = 8.4352 - 0.7434x^\ddagger$	22.50	-0.9215**

Significantly different: * $P < 0.05$, ** $P < 0.01$.
 † Virgin male or female excluded.
 ‡ Virgin male or female included.

**Fig. 1.** Daily reproductive pattern of females with different mating history male partners

4 Discussion

The successful copulation rates, fecundity, longevities of females and males decrease with increased male mating times. DBM copulation duration increases with increased male mating times. The phenomenon also happens in other insect species. For example, in *Jalmenus evagoras* copulation duration increased from 2.4 h at first mating to over 4 h at the fourth mating (HAUGHES et al., 2000). Longer copulation duration may be a disadvantage to insects. For example, longer copulation duration may increase the risk to be eaten by predators, physical injury, etc. Lifespan of DBM females and males was significantly reduced as the number of males mating increased. A similar relationship between female longevity and male mating history has been found in other lepidopteran insects (RUTOWSKI, 1982; SAVALLI and FOX, 1999; HAUGHES et al., 2000). The mechanism by which female lifespan is affected by the male mating history is unclear but could be related to male-derived nutrients or other physiological events. This study indicates that males may engage in multiple

mating, but additional mating may lead to diminishing male reproductive returns. Male mating fitness may reduce when it mates many times.

Daily reproductive patterns of females were similar regardless of whether females paired with virgin males or mated males. Females laid most of the eggs on the first day after copulation. The duration of egg production was longest in females mated with virgin males. Virgin females did not lay eggs until 4–5 days after emergence. For DBM females mating could stimulate egg laying, similar to other species (DANTHANARAYANA and GU, 1991; UNNITHAN and PAYE, 1991).

The successful copulation rates differ significantly for female for the first and second mating, whereas fecundity, fertility and longevity did not change much. It can predict that most of the females choose to mate for only once during the lifespan. From the slight increase in fecundity, longevity we can see that females may accept nuptial feeding during mating, but DBM females do not benefit much from the second mating. For females a single mating is enough, multiple mating did not increase mating fitness much. As DBM females retain the amount of sperm far exceeding the number needed to fertilize all the eggs, and form a spermatophore after mating (YANG and CHOW, 1978), a single mating is sufficient to enable DBM females to fertilize all eggs. DBM had a similar response as the above-mentioned insect species. JUSTUS and MITCHELL (1999) reported DBM female could form a mating plug in the copulatory duct deterring additional mating (DICKINSON and RUTOWSKI, 1989). The above facts suggest that the females of DBM should be monandrous.

The ratio of males and females is about 1 : 1 either in the field or laboratory (HARCOURT, 1957; KE and FANG, 1979). In the field if mass trapping using sex pheromone lures is performed, then the sex ratio could be biased to females. This may result in a reduction in female mating chance, and surviving males may have to mate with females for many times. In this case, the mating quality of males could be largely decreased, causing less fecundity of females. Thus, mass trapping

Table 3. Mating history of female on the fecundity, fertility and longevity of themselves

Female mating (times)	No. female observed	Copulation duration (min)	Successful copulation rate (%)	Fecundity (no.)	Fertility (%)	Longevity (days)
0	40	–	–	29.24 ± 3.25 b	0 b	9.24 ± 1.24 a
1	32	55.98 ± 2.70 a	78.50 ± 13.91 a	127.87 ± 21.98 a	98.67 ± 0.58 a	6.86 ± 0.60 b
2	8	59.13 ± 8.02 a	19.89 ± 7.09 b	143.18 ± 29.43 a	98.91 ± 0.74 a	7.17 ± 0.64 b

Values are mean ± SEM. Different letters in the same range are significant ($P < 0.05$) by SNK test. –, indicates no research.

of males may result in a population reduction of the next generation. From this point, mass trapping with sex pheromone lures has a great potential for controlling DBM. In addition, due to imbalanced sex ratios with mass trapping, mating could be delayed or inhibited. In many other insects delayed mating can reduce the fecundity and fertility of females (KRANN and STRATEN, 1988; JONES and AIHARA-SASAKI, 2001); this could also be a potential mechanism for controlling DBM with sex pheromone. This also can apply to other insects with a similar phenomenon.

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