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ORIGINAL ARTICLE

Effects of delayed mating on the fecundity, fertility and longevity of females of diamondback moth, *Plutella xylostella*

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Abstract The effects of delayed mating on the copulation duration, female fertility, fecundity, egg fertility, longevity and the number days alive after mating of females of diamondback moth (DBM), *Plutella xylostella*, were studied. When male mating was delayed, the female fertility, fecundity, egg fertility, longevity and number days alive after mating of DBM decreased, and there was a negative correlation between the age of the moth with those variables except copulation duration. When female mating was delayed, the female fertility, fecundity, percent egg fertility and number days alive after mating of DBM also decreased, but the longevity increased, which also showed a negative relationship between the age of the moth with the variables except copulation duration and longevity. When both males and females delayed mating, the female fertility and fecundity decreased; egg fertility was affected marginally, and the longevity of females increased. The moth age was negatively correlated with those variables.

Key words delayed mating, egg fertility, fecundity, female fertility, longevity, *Plutella xylostella*

Introduction

Delayed mating is common in nature. Mating may be delayed by many factors, including temperature, rain, wind or control methods such as mating disruption and mass trapping (Cardé & Minks, 1995). The effects of delayed mating on the reproductive output of certain insect orders, such as the Lepidopterans, Homopterans, Dipterans and Coleopterans were reviewed by Wenninger and Averill (2006). There is clear experimental evidence that in many Lepidopteron species the aging of virgin females has a detrimental effect on reproduction, either by decreasing female readiness to mate or by reducing fecundity or fertility (Ellis & Steele, 1982; Henneberry & Clayton, 1985; Hiroki & Obara, 1997; Fadamiro & Baker, 1999; Fraser & Trimble, 2001; Fitzpatrick, 2006; Jones *et al*., 2008; Stelinski & Gut, 2009).

Diamondback moth (DBM), *Plutella xylostella* (L.), is the most destructive insect of cruciferous plants throughout the world (Talekar & Shelton, 1993). It has been found that one effective form of control and management of pest insects can be achieved through control or manipulation of mating events (Cardé & Minks, 1995). Some research has been done on mating behavior and other factors that affect the fecundity, fertility and longevity of DBM females (Yamada & Umeya, 1972; Ke & Fang, 1979; Uematsu *et al*., 1989). However, little research exists about the specific effects of delayed mating of DBM on their fecundity, fertility and longevity. This study was undertaken to fill the gap, and to provide insight into potential pest bio-control methods.

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Materials and methods

Insect rearing and test conditions

Diamondback moth larvae were collected from the field in Hubei Province, China (108°08′–116°07′E, 29°05′–33°21′N) and reared in a laboratory for nearly 20 generations. DBM were reared at 25 ± 1°C, 75% RH and 16 : 8 (L : D) photoperiod. Larvae were fed with fresh cabbage leaves daily. Pupae were kept separately in glass tubes (5 cm × 3 cm internal diameter) with perforated lids. Newly emerged adults were sexed, and fed on a 10% honey solution.

Mating experiments

Three types of mating experiments were performed:

(i) Male-delayed mating experiment: single virgin males of different ages (1–7 days since adult emergence) were paired with single 1-day-old virgin females in glass tubes (20 cm × 3 cm ID). Adults were 1 day old when they were less than 24 h old since adult emergence; adults were 2 days old when they were between 24 h and 48 h old, and so forth.
(ii) Female-delayed mating experiment: single virgin females of different ages (1–7 days) were paired with single 1-day-old virgin males in glass tubes.
(iii) Both female- and male-delayed mating experiment: single virgin females of different ages (1–7 days) were paired with single virgin males of the same age in glass tubes.

Copulation was observed during scotophase. Copulation duration, female fertility (percent of females laying fertile eggs), fecundity (total number of eggs laid) and percent egg fertility (percent developing to blackhead stage) were recorded. After copulation, females were transferred to new tubes with some cabbage leaf tissue as a site for oviposition. Each treatment was repeated five times, using 10 pairs of females and males. If the males or females died during the course of the experiments, they were discarded. The five replicated data were used for analysis.

Data analysis

Analysis of data was performed using analysis of variance (ANOVA) followed by the q test (SAS version 8.0., SAS Institute, Cary, NC, US). Percentages of female fertility and egg fertility were arcsine square root transformed. Analysis of correlations between age of moth and female fertility, fecundity, egg fertility, longevity and the number days alive after mating was performed using the Spearman rank order correlation test; the age of the moths was transformed by denary logarithm.

Results

Effect of male-delayed mating

The female fertility, fecundity, egg fertility and longevity of females decreased significantly with the age of the male mating partner (Table 1). Copulation duration was shortest when the female was 1 day old (Table 1). Correlation coefficients between the age of male and female fertility, fecundity, egg fertility, longevity and the number days alive after mating of females were significant ($P < 0.05$) (Table 1).

Effect of female-delayed mating

The female fertility, fecundity and egg fertility decreased significantly with the age of females (Table 2). The copulation duration was shorter when the female was 1 day old or 7 days old (Table 2). The longevity of females that mated when they were 3 days old was shortest, and then they increased. Correlation coefficients between the age of DBM females and female fertility, fecundity, egg fertility and the number days alive after mating were significant ($P < 0.05$) (Table 2).

Effect of both female- and male-delayed mating

When the ages of both males and females increased for mating, female fertility and fecundity decreased significantly with increasing age. Fertility was high (> 95%) irrespective of the age of the mating partner. Although there was a tendency for females to live longer when the longer mating was postponed, this was not statistically significant. The age of the mating partners had some effect on mating duration. Correlation coefficients between the age of DBM and female fertility, fecundity, egg fertility and the number days alive after mating were significant (Table 3).

Discussion

The results showed that female fertility, fecundity and egg fertility decreased with age at copulation for both males and females. This indicates that delayed mating is disadvantageous for reproduction for both sexes. This

Table 1 Effect of *Plutella xylostella* (DBM) male-delayed mating on female fertility, fecundity, egg fertility, female longevity and number of days alive after mating.

Age of male (days)	No. females	Copulation duration (min) (mean† ± SD)‡	Female fertility (%)	Fecundity (egg) (mean† ± SD)‡	Egg fertility (%)	Longevity of female (days) (mean† ± SD)‡	No. days alive after mating (mean† ± SD)‡
1	50	81.3 ± 11.7 b	87.24	109.2 ± 23.9 ab	98.50	7.9 ± 0.9 a	6.9 ± 0.9 a
2	50	112.8 ± 17.5 a	75.17	121.6 ± 18.1 a	97.64	7.3 ± 0.7 ab	6.3 ± 0.7 b
3	50	107.7 ± 11.7 ab	62.36	97.6 ± 10.2 bc	90.83	6.7 ± 1.4 ab	5.7 ± 1.4 c
4	43	95.6 ± 20.2 ab	43.79	90.2 ± 13.0 bc	84.75	6.7 ± 1.2 ab	5.7 ± 1.2 c
5	38	92.1 ± 27.2 ab	35.43	88.8 ± 13.8 bc	64.76	6.3 ± 1.6 abc	5.3 ± 1.6 c
6	35	98.0 ± 5.7 ab	37.18	95.2 ± 12.0 bc	65.45	5.7 ± 1.4 bc	4.7 ± 1.4 c
7	27	87.0 ± 12.4 ab	33.24	81.2 ± 11.0 c	66.43	5.1 ± 1.2 c	4.1 ± 1.2 c
Correlation coefficient		–§	−0.9488*	−0.8231*	−0.9389*	−0.9494*	−0.9494*

†Average of five replicates.
‡Values followed by the same letters are not significantly different ($P > 0.05$) by Student-Newman-Keuls test.
§Indicates the correlation coefficient between DBM age and the copulation duration was not significant ($P > 0.05$).
*Indicates the correlation coefficients between DBM age and female fertility, fecundity, egg fertility, longevity and the number of days alive after mating of females were significant at 0.05.

phenomenon was congruent with other research (Ellis & Steele, 1982; Kiritani & Kanoh, 1984; Henneberry & Clayton, 1985; Hiroki & Obara, 1997; Fadamiro & Baker, 1999; Fraser & Trimble, 2001; Torres *et al*., 2002; Fitzpatrick, 2006; Jones *et al*., 2008; Stelinski & Gut, 2009). Hence interventions in order to delay mating could be used in pest control.

The results further demonstrated that the copulation duration was affected by the age of the mating partners, and the moths completed the copulation with shortest

Table 2 Effect of *Plutella xylostella* (DBM) female-delayed mating on female fertility, fecundity, egg fertility, female longevity and number of days alive after mating.

Age of female (days)	No. females	Copulation duration (min) (mean† ± SD)‡	Female fertility (%)	Fecundity (egg) (mean† ± SD)‡	Egg fertility (%)	Longevity of female (days) (mean† ± SD)‡	No. days alive after mating (mean† ± S.D)‡
1	50	79.7 ± 19.9 bc	86.34	133.0 ± 38.8 a	99.40	7.7 ± 1.4 b	6.7 ± 1.4 a
2	50	84.4 ± 25.5 bc	63.13	153.0 ± 10.9 a	98.50	6.5 ± 1.1 bc	4.5 ± 1.1 b
3	50	87.7 ± 10.1 bc	64.76	129.5 ± 11.3 b	91.80	6.0 ± 1.6 c	3.0 ± 1.6 c
4	45	109.5 ± 11.1 ab	45.91	72.8 ± 5.2 c	85.72	6.5 ± 1.4 bc	2.5 ± 1.4 c
5	38	108.5 ± 11.4 abc	34.33	48.4 ± 11.1 d	68.96	6.8 ± 1.2 bc	1.8 ± 1.2 d
6	32	120.2 ± 25.6 a	36.24	44.8 ± 13.8 d	66.49	7.9 ± 0.7 b	2.9 ± 0.7 c
7	27	78.5 ± 15.6 c	32.34	40.3 ± 0.9 d	67.87	9.7 ± 1.9 a	2.7 ± 1.9 c
Correlation coefficient		–§	−0.9400*	−0.9223*	−0.9540*	–§	−0.7663*

†Average of five replicates.
‡Values followed by the same letters are not significantly different ($P > 0.05$) by Student-Newman-Keuls test.
§Indicates the correlation coefficients between DBM age and copulation duration, longevity of females were not significant ($P > 0.05$).
*Indicates the correlation coefficients between DBM age and female fertility, fecundity, egg fertility and the number days alive after mating of females were significant at 0.05.

Table 3 Effect of both *Plutella xylostella* (DBM) male- and female-delayed mating on female fertility, fecundity, egg fertility, female longevity and number of days alive after mating.

Age of male and female (days)	No. females	Copulation duration (min) (mean† ± SD)‡	Female fertility (%)	Fecundity (egg) (mean† ± SD)‡	Egg fertility (%)	Longevity of female (days) (mean† ± SD)‡	No. days alive after mating (mean† ± SD)‡
1	50	88.4 ± 22.4 ab	82.56	159.8 ± 28.4 a	99.46	8.9 ± 2.2 b	7.9 ± 2.2 a
2	50	76.7 ± 23.1 b	65.51	117.5 ± 24.7 b	98.54	8.9 ± 2.2 b	6.9 ± 2.2 b
3	50	97.5 ± 27.1 ab	74.45	115.6 ± 30.3 b	95.81	9.9 ± 1.9 b	6.9 ± 1.9 b
4	35	91.3 ± 26.0 ab	54.05	90.2 ± 28.0b c	95.72	9.4 ± 2.4 b	5.4 ± 2.4 c
5	31	106.2 ± 29.3 a	51.67	81.8 ± 11.7 c	98.96	10.3 ± 2.8 ab	5.3 ± 2.8 c
6	20	107.0 ± 22.7 a	50.65	82.0 ± 22.8 c	97.43	10.7 ± 2.2 ab	4.7 ± 2.2 c
7	21	89.3 ± 6.7 ab	42.57	25.7 ± 5.9 d	96.67	12.2 ± 2.3 a	5.2 ± 2.3 c
Correlation coefficient		_§	−0.9241*	−0.9447*	−0.9147*	_§	−0.9195*

†Average of five replicates.

‡Values followed by the same letters are not significantly different ($P > 0.05$) by Student-Newman-Keuls test.

§Indicates the correlation coefficients between DBM age and copulation duration and longevity of females were not significant ($P > 0.05$).

*Indicates the correlation coefficients between DBM age and female fertility, fecundity, egg fertility and the number days alive after mating of females were significant at 0.05.

time at 1 day or 2 days old. This finding is the same as results found for DBM that mated more than once, in which males and females can complete copulation rapidly as they mate without delay (Wang & Zhang, 2005). It may be advantageous for DBM to finish their copulation in the shortest time to reduce the risk of being preyed upon or injured, or subjected to other dangers.

When males and females of the same age mated, an increase of age was associated with a rapid decrease in fecundity, but the fertility was maintained at more than 95%. This shows that although delayed mating is disadvantageous for fecundity, when males and females were the same age, the male can still fully inseminate the female even at a low level of fecundity. In general, both male- and female-delayed mating was unfavorable for the DBM population.

Age at copulation of both the male and female also affected female longevity. When young females were mated with older males, their longevity decreased. When older females were mated with young males or same-age males, they had a longer life. The results were the same as those seen in *Homona magnanima* Diaknoff, and in *Cydia pomonella* (L) (Kiritani & Kanoh, 1984; Vickers, 1997). However, this differs from *Plodia interpunctella* (Hübner), where female longevity and female delay or both male and female delay had a positive linear regression relationship (Huang & Subramanyam, 2003). For different insect pests, the response to delayed mating may be different. Usually virgin females live the longest (Varshney *et al*., 1971; Vickers, 1997; Wang & Zhang, 2005). The results showed that copulation needed energy and physiological factors. When female mating is delayed, this may lifespan may be prolonged in order to provide more chances of mating, but then when older females mated, female fertility, fecundity and egg fertility decreased.

Mating disruption or mass trapping is a useful method for controlling Lepidopteron pests. Mating disruption or mass trapping does not completely inhibit mating: inevitably some mating does take place and offspring are produced by the females that do mate (Cardé & Minks, 1995; Knight, 1997; Unnithan & Paye, 1991). It is possible that for some the pheromone-permeated air may have delayed mating. Delayed mating could reduce the number of the next generation and possibly even future generations (Jones & Aihara-Sasaki, 2001). Research shows that DBM can continue to fertilize their eggs when mated just once during their life (Wang & Zhang, 2005). DBM can form a mating plug in the copulatory duct that deters additional mating, so even though DBM can mate more, that may not help to increase their fertility (Justus & Mitchell, 1999; Dickinson & Rutowski, 1989). From our experiments we can learn that delayed mating was not beneficial for DBM reproduction. If control methods such as mass trapping or mating disruption could delay DBM mating for more than 3 days, it may be an effective technique for reducing the number of DBM in the field, which still requires further research.

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