Analysis of the Mating and Reproductive Traits of *Plutella xylostella* (Lepidoptera: Plutellidae)

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**ABSTRACT.** The reproductive traits of the diamondback moth, *Plutella xylostella* (L) (Lepidoptera: Plutellidae) were investigated and analyzed by different analytical methods. Simple statistical analysis showed relatively higher mating rates maintained from 21:00 to 2:00, thereafter dropping to a minimum at about 18:00. Mating rates were affected by female and male age. Mating was most likely to take place between females and males that were 1 d old. Correlation and factor analysis indicated that mating delayed females have a relatively lower and unsuccessful mating rate and relatively shorter copulation duration, with lower egg hatchability and fecundity; in addition, the mating delayed male would reduce female's fertility. Delay of mating prolonged life of both males and females. A higher and successful mating rate would cause a higher egg hatchability and fecundity. Canonical correlation analysis showed that mating age and successful copulation of female play a decisive role for her fecundity and longevity, and mating age and mating rates of male play a decisive role for his longevity.

**Key Words:** fecundity, longevity, mating delay, multifactorial analysis, mating rate

Diamondback moth *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) is one of the most serious pests of cruciferous crops throughout the world, particularly in tropical and subtropical countries (CABI Compendium 2004). Crop damage is caused by its larval feeding and excretion (Vanichpakorn et al. 2010). This pest is hard to control due to its high reproductive rate and the level of resistance it has developed to various insecticides (Shelton et al. 1993, Mohan and Gujar 2003, Sayyed and Wright 2005, Zhao et al. 2006). Simultaneously, there is increased interest in ecofriendly methods to control diamondback moth, such as mating disruption, which can only be successful following studies of reproductive behavior and chemical ecology of diamondback moth (Schroeder et al. 2000, Maxwell et al. 2006, Guo and Qin 2010).

During a mating disruption program, many females should miss their optimum age of mating, resulting in decreased fecundity (Huang and Subramanyam 2003, Fitzpatrick 2006, Jones et al. 2008). Wang et al. (2011) investigated the effects of delayed mating on the fecundity, longevity, and fertility of diamondback moth females. Besides mating age, many mating traits including mating rate, time of mating, copulative rate, copulative duration, etc. had some relationship with diamondback moth reproduction (Uematsu et al. 1989, Pivnick et al. 1990, Talekar and Shelton 1993). Disruption of any of these traits will affect the total reproductive output of diamondback moth. However, little data exist about all of these mating traits of diamondback moth on their reproduction. This is caused by the need for a systematic experimental set-up and practical statistical analysis methods.

There are complex relations among mating and reproductive variables. Many times, it is difficult to find a regular pattern among these variables by a simple analysis. Multiple statistical examines the relationships among multiple variables. Among the methods of multiple statistical analyses, principal components analysis (PCA), and factor analysis are frequently used in the researches of animal ecology and behavior. They aim to reduce numerous measures to a small set of the most important summary scores (Tabachnick and Fidell 1996, Budaev 2010). Researchers often try to interpret principal components and significant clusters to obtain a better understanding of the patterns of correlations between the original variables. Canonical correlation analysis is also a multivariate statistical model that facilitates the study of interrelationships among sets of multiple dependent variables and multiple independent variables (Green and Carroll 1978).

Up to now, some correlations between the traits of diamondback moth are not clear, e.g., Tan et al. (2011) indicated a positive correlation between the moth age and copulation duration, but Wang et al. (2011) did not show a significant correlation between them. In practice, a better understanding of the traits and their correlations are useful to guide the biocontrol of diamondback moth. For example, diamondback moth reproduction was reduced by disturbing its mating behaviors through interference with sex pheromone transmission; in the future, the insect pest can be controlled by breaking the links among the traits that determine reproductive success. In this study, 11 mating and reproductive traits of diamondback moth were investigated and analyzed by several analytical methods. Our hypothesis is that the reproduction of diamondback moth is under the control of a set of mating and reproductive traits. Elucidation of the mating and reproductive traits of diamondback moth will provide some insight into potential pest biocontrol methods.

**Materials and Methods**

**Insect Rearing.** Larvae of diamondback moth, *P. xylostella* (L.) (Lepidoptera: Plutellidae) were collected from cabbage plants (*Brassica oleracea* (L.) in Beijing (39.5° N, 116.2° E), China, from July to August 2011, and reared in an insectarium under the conditions of 26 ± 2°C, 14:10 (L:D) h photoperiod, and 60–70% relative humidity (RH), until pupation. Two-wk-old radish plants (*Raphanus sativus* (L)) with two fully expanded true leaves were used as larval food. After eclosion, the moths were fed continuously with 10% sucrose solution.

Male and female were segregated by their form and structure at their pupal stage, and then all virgin moths used in the bioassays were kept under the same environmental conditions as that described earlier. An adult was considered 1-d old on the day of its eclosion.

**Data Collection.** Pupae were placed singly into glass tubes (5 cm tall by 3 cm in internal diameter) with moist cotton in the bottom and covered with a small mass of cotton. The date of each adult emergence was recorded on the tube. All moths were sorted into groups based
on age in days from 1 to 6 d old. Overall, there were 14 age combinations of female and male ages. Each age combination contained an equal number (N = 40) of female and male individuals (Table 1).

Moth traits were divided into 11 variables according to the characters of mating and reproduction of P. xylostella (Table 1). For the canonical correlation analysis, two groups of 11 variables were separated as follows: X1–X6 as independent variables, and X7–X11 as dependent variables. These 11 variables were defined as follows:

X1: Mating age of female (days): the days a female adult from emergence to its mating. X2: Mating age of male (days): the days a male adult from emergence to its death. X3: Mating rate (%): (Reproduced copulations/Total copulations) × 100%. X4: Mating times: the number of mating of diamondback moth), were put into a big beaker (Ø = 10 cm), then one male (1-, 2-, or 3-d old) was put into the beaker, which was covered with a piece of gauze. Copulation was observed under red light at room temperature from 20:00 to 21:00. In total, nine combinations of the choice experiment were examined: one male of 1 d old with six females of the 6 ages (3 repeats), one male of 2 d old with six females of the 6 ages (3 repeats), and one male of 3-d old with six females of the 6 ages (3 repeats).

Data Analysis. Correlations between the 11 variables were tested first. Following this, PCA was conducted to create uncorrelated principal components from the original variables. Factor analysis was used to find the variables which were most useful for discriminating between 11 variables of reproduction. Finally, for giving a synthetical correlation analysis, canonical correlation coefficients were calculated from the two data sets. These 11 variables were divided into two groups. The first one was an independent group related to mating traits (X1–X6) and the second one was dependent group related to reproduction traits (X7–X11).

Table 1. Data of reproductive traits of diamondback moth, P. xylostella (L.)

| Mating age of females (days) | Mating age of males (days) | Mating rates (%) | Mating times | Successful copulation rate (%) | Copulative duration (min) | Female fertility (%) | Egg hatchability (%) | Fecundity (egg) | Longevity of females (days) | Longevity of males (days) |
|----------------------------|---------------------------|------------------|--------------|-------------------------------|--------------------------|---------------------|-------------------|-----------------|--------------------------|--------------------------|
| X1                         | X2                         | X3               | X4            | X5                            | X6                       | X7                  | X8                | X9              | X10                      | X11                      |
| 1                          | 1                          | 100              | 2.75 ± 0.44   | 84.17 ± 5.10                  | 59.09 ± 3.22             | 85.00               | 88.93 ± 1.71      | 95.06 ± 5.07     | 5.50 ± 0.14      | 5.00 ± 0.13             |
| 2                          | 2                          | 100              | 2.33 ± 0.76   | 89.36 ± 6.18                  | 53.18 ± 4.23             | 90.00               | 80.71 ± 8.02      | 79.13 ± 12.67     | 5.70 ± 0.21      | 5.10 ± 0.10             |
| 3                          | 3                          | 90               | 2.11 ± 0.39   | 70.37 ± 15.16                 | 41.58 ± 5.53             | 70.00               | 67.69 ± 4.33      | 71.57 ± 12.00     | 7.40 ± 0.27      | 6.20 ± 0.13             |
| 4                          | 4                          | 55               | 1.83 ± 0.37   | 73.05 ± 10.40                 | 44.09 ± 5.87             | 72.22               | 57.20 ± 6.52      | 63.67 ± 7.11      | 7.72 ± 0.11      | 6.39 ± 0.12             |
| 5                          | 5                          | 80               | 1.79 ± 0.31   | 75.00 ± 12.50                 | 53.33 ± 5.27             | 70.00               | 63.35 ± 6.27      | 43.93 ± 5.75      | 8.70 ± 0.19      | 7.50 ± 0.15             |
| 6                          | 6                          | 50               | 1.50 ± 0.34   | 85.71 ± 8.17                  | 49.00 ± 5.93             | 47.37               | 37.63 ± 8.87      | 43.09 ± 7.97      | 7.94 ± 0.27      | 9.05 ± 0.21             |
| 7                          | 7                          | 40               | 2.07 ± 0.30   | 92.92 ± 5.24                  | 60.00 ± 3.92             | 80.00               | 74.11 ± 4.80      | 70.56 ± 13.39     | 7.45 ± 0.25      | 7.53 ± 0.20             |
| 8                          | 8                          | 60               | 2.58 ± 0.38   | 68.75 ± 13.35                 | 50.00 ± 6.83             | 90.00               | 67.95 ± 10.01     | 56.67 ± 15.56     | 7.33 ± 0.37      | 5.56 ± 0.24             |
| 9                          | 9                          | 54               | 2.28 ± 0.42   | 68.21 ± 10.76                 | 53.21 ± 5.73             | 78.95               | 56.48 ± 7.63      | 56.50 ± 8.12      | 8.90 ± 0.18      | 5.40 ± 0.27             |
| 10                         | 10                         | 50               | 2.88 ± 0.85   | 64.81 ± 12.56                 | 40.50 ± 6.22             | 88.89               | 43.56 ± 10.52     | 56.38 ± 12.96     | 9.89 ± 0.31      | 6.22 ± 0.28             |
| 11                         | 11                         | 45               | 2.07 ± 0.30   | 83.34 ± 7.76                  | 58.28 ± 5.30             | 73.68               | 77.66 ± 3.57      | 82.00 ± 5.79      | 6.13 ± 0.26      | 5.67 ± 0.16             |
| 12                         | 12                         | 40               | 2.58 ± 0.38   | 89.42 ± 4.81                  | 59.39 ± 3.20             | 72.22               | 70.18 ± 4.31      | 83.32 ± 6.19      | 6.30 ± 0.15      | 6.70 ± 0.15             |
| 13                         | 13                         | 60               | 2.28 ± 0.42   | 82.55 ± 6.35                  | 52.65 ± 3.31             | 76.47               | 76.76 ± 3.49      | 72.65 ± 10.61     | 6.11 ± 0.15      | 7.63 ± 0.16             |
| 14                         | 14                         | 70               | 2.88 ± 0.85   | 95.20 ± 5.62                  | 69.13 ± 6.97             | 62.50               | 80.17 ± 6.80      | 70.88 ± 10.96     | 6.11 ± 0.20      | 8.11 ± 0.20             |

There were totally 14 age combinations of female and male, each age combination contained a number 40 of female and male (N = 40). The number after “±” is standard errors. "VAR" variables: X1–X6; "WITH" variables: X7–X11.
positive correlations appeared respectively between mating rate (X3), egg hatchability (X8), and fecundity (X9); successful copulation rate (X5) and copulative duration (X6); egg hatchability (X8) and fecundity (X9). All of these indicate that the higher the mating rate and successful copulation rate the higher the egg hatchability and copulative duration, and the fecundity and hatchability are positively correlated.

**Factor Analysis and Clustering.** PCA of the reproductive trait analysis extracted two factors with eigenvalues of more than one. Cumulatively, these factors explained 79.68% of the total variability in the data. The first factor (F1) explained 51.16% of the total variability, whereas the second factor (F2) explained 28.52% of the total variability. A classification was done by FA (Kaiser-Meyer-Olkin = 0.552, Bartlett Approx. Chi-Square = 155.128, df = 55, Significance = 0.000) that sorted out four significant clusters from 11 variables, in which, X1–X10 characterized female mating age and her longevity; X2–X11 characterized male mating age and his longevity; X4–X7 characterized mating times and female fertility, and X3, X5, X6, X8, and X9 characterized the multimating property and fecundity. Each of these clusters from a specific or a synthetical angle showed the closer relationships between the relevant variables.

**Canonical Correlation.** Tests of dimensionality for the canonical correlation analysis indicated that two of the five canonical dimensions are statistically significant at the 0.05 level. The first canonical correlation coefficient had a canonical correlation of 70%, and second was 26%. The absolute correlation information from the two canonical correlation coefficients was over 96% of the total.

**Discussion**

The results from this study showed that relatively higher mating rates were observed from 21:00 to 2:00, decreasing after this until about 18:00. This contrasts with a study showed three mating peaks during a day, namely 12:30, 0:30, and 6:30 (Tan et al. 2011). By a comparison, it found some difference of the insect rearing conditions between them and ours (Tan et al.: 25 ± 0.5°C, 12:12 (L:D) h photoperiod, and 80% RH; this study: 26 ± 2°C, 14:10 (L:D) h photoperiod, and 60–70% RH). The discrepancies of these results maybe mainly come from the difference between light and dark conditions. And considered with the present results, it is possible that diamondback moth can mate at different times within a day and night. During daytime, a relatively higher mating rate appeared when 1-d-old male mates with a female over that age (mating delayed female); conversely, relatively lower mating rates were observed when 1-d-old female mates with a male over that age.

By raising its copulative frequencies, female may make up for the loss of deterioration in viability of ova with time (Wenninger and Averill 2006). Male age does not appear to be a major factor determining mating success in diamondback moth, as long as the female is young. From this study, mating is most likely to take place between 1-d-old females and males during night time. That is consistent with the results from Pivnick et al. (1990) and Ohira (1979) which showed that the mating occurs during the first 4–15 h following emergence with a peak between 1 and 2 h of scotophase.

Any delay in mating age will affect reproduction. In this study older females had lower mating rate, successful copulation, copulative duration, egg hatchability, and fecundity; and the mating delayed male would reduce the female fertilization. In this study, the females laid their eggs in the presence of their male partner. The results could not

**Table 2. Correlation matrix between 11 variables of diamondback moth, *P. xylostella* (L.)**

|                    | X1  | X2  | X3  | X4  | X5  | X6  | X7  | X8  | X9  | X10 | X11 |
|--------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Mating age of females (days) | 1   |     |     |     |     |     |     |     |     |     |     |
| Mating age of males (days)   |     | 1   |     |     |     |     |     |     |     |     |     |
| Mating rates (%)             |     |     | X2  |     |     |     |     |     |     |     |     |
| Mating times                |     |     |     | X3  |     |     |     |     |     |     |     |
| Successful copulation rate (%)|     |     |     |     | X4  |     |     |     |     |     |     |
| Copulative duration (min)    |     |     |     |     |     | X5  |     |     |     |     |     |
| Female fertility (%)         |     |     |     |     |     |     | X6  |     |     |     |     |
| Egg hatchability (%)        |     |     |     |     |     |     |     | X7  |     |     |     |
| Fecundity (egg)             |     |     |     |     |     |     |     |     | X8  |     |     |
| Longevity of females (days) |     |     |     |     |     |     |     |     |     | X9  |     |
| Longevity of males (days)   |     |     |     |     |     |     |     |     |     |     | X10 |

*r*~significant at the level $\alpha = 0.05$, and $r^{**}$~extremely significant at the level $\alpha = 0.01$; df = 12.
Alternation of diamondback moth (Lepidoptera: Plutellidae) to volatile
(Plodia interpunctella) Plutella xylostella Drosophila elegans

Fraser and Trimble 2001

L. (Lepidoptera: Plutellidae). Proc. Assoc. Plant
Torres-
Vickers
2002

Cluster of the reproductive traits by factor analysis. Extraction
methods: Principal Component Analysis. Rotation method: Varimax
with Kaiser Normalization. Rotation converged in 3 iterations.

Table 3. Standardized canonical coefficients for the independent (I)
and dependent (D) variables

\[
I_1 = -0.8967x_1 + 0.1527x_2 - 0.0006x_3 + 0.1667x_4 + 0.2743x_5 - 0.2658x_6
\]

\[
D_1 = 0.1295x_7 - 0.0373x_8 + 0.5819x_9 - 0.5961x_{10} + 0.5266x_{11}
\]

\[
I_2 = -0.1068x_1 - 0.9076x_2 + 0.2553x_3 - 0.0154x_4 - 0.2157x_5 + 0.9076x_2
\]

\[
D_2 = 0.2733x_7 + 0.4719x_8 + 0.3506x_9 + 0.7767x_{10} - 0.6223x_{11}
\]

tell that without the presence of the male if females were left to lay their
eggs, and whether a single copulation was enough to have a decent brood.

This study indicated a negative correlation between the moth age
and copulation duration, which are different to the studies of Wang
et al. (2011) and Tan et al. (2011). The former did not show a significant
 correlation between them but the latter indicated a positive correlation.
A positive correlation between copulation duration and male mating
times was reported by Wang et al. (2005), but this correlation was not
existent from this study. Partial reason of these differences maybe come
from the insect-rearing conditions among the studies (Tan et al.: 25 ± 0.5°C,
12:12 (L:D) h photoperiod, and 80% RH; Wang et al.: 25 ± 1.0°C, 16:8 (L:D)
h photoperiod, and 75% RH; this study: 26 ± 2°C, 14:10 (L:D) h photoperiod,
and 60–70% RH). All of these aspects need additional researches.

Some researchers explained that females by extending mating
time can get a little more sperm and nutrients transferred from male
semen (Hirai and Kimura 1999). Males by extending mating times
not only transfer more sperm and nutrition to females, but also as a
way to defend against other males and avoid sperm competition
(Alcock 1995, Simmonds 2001). Mating delay phenomenon is similar
as those seen in other insects (Ummithan and Paye 1991, Vickers
1997, Fadamiro and Baker 1999, Fraser and Trimble 2001, Torres-
vila et al. 2002, Stelinski and Gut 2009). It may be attributed to the
energy conservation. If the moths cannot be mated quickly after their
emergence, both males and females have to spend part of their energy
surviving in order to have a chance to mate. The longer they wait for
mating the less of their energy preserved, as soon as they are mated,
the energy for further copulation is less than before, fertility and
fecundity are decreased afterwards. This implies that energy con-
sumption for reproduction is critical and correlated to the energy
resources (food availability). The results of this study emphasize that
1-d-old females are at peak reproductive capacity, any delay in mat-
ing beyond this age may result in reduced reproduction. In practice,
these results are useful for understand the control method of mating
disruption of diamondback moth, in which a lot of males can be
trapped and killed, such that female moths cannot find a male to mate
with immediately so that their longevity will be prolonged and their
fecundity will be decreased.

In this study, besides the close correlations between diamondback
moth mating age and reproduction traits, many significant correlations
arose from a specific or a synthesis angle of the multiple statistical
analysis. From the simple correlations, the closer relationships arose
between the relevant variables, such as mating rate and female longevity,
egg hatchability and fecundity, successful copulation rate and copu-
lative duration etc. From a specific or a synthetic angle factor analysis
and canonical correlation analysis showed the closer relationships
between the relevant variables.

In summary, any disadvantageous effect on diamondback moth mat-
ing will cause a reproduction defect. We think, these results should be
helpful for the plant protection workers to work out new diamondback
moth controlling plans.

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Conflict of Interest

There is no conflict of interest between authors.

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