Factors influencing mating incidence and reproduction in codling moth *Cydia pomonella* L.
(Lepidoptera: Tortricidae)

H. Makee*1, I. Idris, K. Hussian
Atomic Energy Commission of Syria (AECS), Syrian Arab Republic, P.O. Box 6091, Damascus, Syria.

Key words: *Cydia pomonella*, fecundity, fertility, mating ability, mating frequency.

Abstract: The codling moth *Cydia pomonella* L., is a primary pest of apple and various studies have been performed to assess the possibility of applying sterile insect technique as a control method against this pest. In support of this technique, the present work aims to examine the effects of adult age and weight on mating ability, number of matings, fecundity and fertility in *C. pomonella*. The relationship between number of matings, fecundity, and fertility of females was also studied. Female and male weights were found to have an effect on the number of times individuals mate, but male weight only influenced mating success. Unlike male weight, female weight affected fecundity and fertility. Negative correlations were found between mating success, fecundity and fertility and adult age. Multiply-mated females and those which did not accept a second mating showed higher fecundity and fertility than their counterparts that were not given the opportunity to remate. Our results provide essential information necessary to increase the effectiveness of sterile insect technique as a control method against *C. pomonella*.

1. Introduction

Apple is a very important fruit tree in Syria with total apple acreage of about 41 000 ha. Codling moth *Cydia pomonella* L. (Lepidoptera: Tortricidae) is considered the most important insect pest of apple in Syria and insecticides are widely used to control it. Such control methods are costly, nonselective, environmentally unsafe and effective for only a short period in the treated area. Moreover, *C. pomonella* has already developed resistance to various insecticides (Varela et al., 1993). Therefore, a more reliable and environmentally safe control method is required.

Knipling (1970) and Myers et al. (1998) reported that the sterile insect technique (SIT) can be considered an important component of an area-wide approach to insect control programs. The possibility of applying SIT as an alternative control method to suppress *C. pomonella* populations has been determined by many researchers (Bloem et al., 1999, 2001). This approach relies on mass-rearing and release of both sexes of irradiated moths into wild pest populations.

The success of this technique against codling moth depends largely on the release of sexually competitive insects capable of locating and carrying out mating with several feral individuals (Knipling, 1981). It is widely agreed that insects with a long lifespan, good dispersal pattern, high incidence of mating, ability to transfer sperm successfully, and a noticeable fecundity would exhibit an acceptable level of mating competitiveness (Carpentar et al., 1989).

Mating in Lepidoptera involves the following sequence of events: copulation, spermatophore transfer, insemination and egg fertilization. Bues et al. (1992) observed that most lepidopteran females tend to mate within 24 h of emergence. The effect of adult age on mating varies considerably between species as well as sex. Previous studies have stated that younger females of *Pectinophora gossypiella* Saunders (Lingren et al., 1988), *Eoreuma loftini* Dyar (Spurgeon et al., 1995), *Lymantria dispar* L. (Proshold, 1996), and *Phthorimaea operculella* Zeller (Makee and Saour, 2001) had a higher occurrence of successful matings compared to older individuals. On the contrary, young females of *Ephestia kuehniella* Zeller showed a low mating incidence. However, the newly emerged males of *E. kuehniella* were more likely to transfer spermatophores than older males (Calvert and Corbet, 1973). The effect of adult age on reproductive capacity is well known in several lepidopteran species (Ellis and Steele, 1982). It has been shown that the reproduction of *Plutella xylostella* (L.) females was significantly reduced by age (Nemoto et al., 1992).

Insect weight plays an important role in mating and reproduction. Large males of *Ephestia elutella* Hübner were more likely to mate than smaller ones (Phelan and Barker 1986). Similarly, a significant relationship between body size and successful mating has also been reported in the dipteran *Drosophila melanogaster* Meigen (Partridge and Farquhor, 1983). Studies have shown that in hemipteran *Podisus macu-
liventris and P. connexivus the heavier females presented a better reproductive rate (Evans, 1982; Zanuncio et al., 1992).

To maximize the mating competitiveness of released insects, the influence of adult age and weight on mating incidence and reproduction capacity of codling moth were examined. In addition, the effect of the number of matings on fecundity and fertility of this species was tested.

2. Materials and Methods

A C. pomonella colony has been maintained in our laboratory for several years and was used for the present study. Newly emerged adults were crossed in Petri dishes (12 cm dim), five pairs in each. A wet cotton wool was placed in each Petri dish as drinking source. After 3-4 days, the dishes which had eggs were collected and soaked with 2% Sodium hypochlorite solution for 2 min for egg sterilization. Then the dishes were washed with tap water and left to dry. Eggs were checked daily for hatching. Newly hatched larvae were placed on artificial media consisted of the following ingredients: agar-agar, maize, wheat germ, casein, yeast, Wesson salts, benzoic acid, fumidil, ascorbic acid, vitamins and nipagine (Anisimov, personal communication). All insect stages were kept under constant temperature at 25±1°C with 70±5% RH, and a photoperiod of 16:8 h (L:D).

In all the experiments, pupae were sexed and individually placed in small plastic tubes until eclosion. For oviposition, newly emerged females and males were paired in Petri dishes (12 cm dim) having a feeding source (a wet cotton wool). “Mating incidence” in this study is used to indicate successful spermatophore transfer and/or spermatophore presence in the bursa copulatrix of the female. The number of matings is reflected by the number of spermatophores in the bursa copulatrix.

The effect of adult age

Ten different groups of virgin females (n= 20 in each group) aged 1-10 d were employed. Females of each group were individually paired with newly emerged males (<18 h). After 24 h, males were removed and females were kept for oviposition until death. All eggs were collected, counted and allowed to hatch. After death, the females were dissected and examined for the presence of spermatophores in the bursa copulatrix.

Ten different groups of virgin males (n=20 in each group) aged 1-10 d were examined to determine the effect of male age on successful spermatophore transfer. Males of each group were individually paired with newly emerged virgin females (<18 h). After 24 h, males were removed and females were kept for oviposition until death. All eggs were collected, counted and allowed to hatch. After death, the females were dissected and examined for the presence of spermatophores.

The effect of adult weight

To determine the influence of male and female weight on incidence of mating, number of matings, fecundity and fertility pupae were weighed, sexed, and divided into three groups based on weight: light, medium and heavy. Female pupal weights were, respectively, 34-37, 39-42 and 44-47 mg, while male pupal weights were, respectively, 25-31, 36-45 and 47-49 mg. Emerged females and males in each group were individually paired with newly emerged adults of the opposite sex. In male and female groups, males were paired with females weighing 36.2±0.5 mg, while females were paired with males weighing 30.6±1.3 mg.

In each group, the females and males were kept together until death. All eggs were collected, counted and left to hatch. The females were dissected and the number of spermatophores in the bursa copulatrix were counted. To determine the relationship between adult weight and fecundity and fertility, only mated females were considered.

Effect of sex ratio on mating ability

Two experiments were carried out. In the first experiment, 1-d-old males (n = 20) were individually confined with three newly emerged females (1 male: 3 females). Males and females were kept together for 24 h, after which time the females were dissected and the number of spermatophores was determined.

In the second experiment, 1-d-old females (n = 20) were singly confined with three newly emerged males (1 female: 3 males). After 24 h, females were removed, dissected and the number of spermatophores was determined. A ratio of 1 female: 1 male was used as a control group for the two experiments.

Effect of female multiple mating on fecundity and fertility

Two groups of virgin females were used. Females of the first group (n= 20) were individually paired with newly emerged males. After 24 h, males were removed and females were kept for oviposition until death. In the second group, females (n= 45) were individually paired with 1-d-old males. Males were removed after 24 h and replaced with new 1-d-old males. The same procedure was followed for seven successive days. In both groups, all eggs were collected, counted and allowed to hatch. After death, the females were dissected to determine the presence and number of spermatophores.

Statistical analysis was carried out using the STATISTIC program version 6 (Statsoft, Inc. 2003) at 5% level (P= 0.05). A simple linear regression analysis was done to study the relationship between adult age and incidence of mating and fertility. Data were subjected to analysis of variance for determination of differences between means, which were tested for significance using Tukey HSD test. The percentages were analyzed by applying normal approximation test (analysis of proportion).

3. Results

Effect of adult age

Figure 1 illustrates the effect of male and female mating ability of C. pomonella with regard to age. A regres-
A linear regression line was fitted to present the relationship between incidence of mating and adult age. The percentage of mating ability was significantly correlated with adult age ($y = -7.2303x + 95.667$, $R^2 = 0.77$, $P<0.05$; $y = -7.8788x + 90.133$, $R^2 = 0.86$, $P<0.05$ for females and males, respectively). A significant increase in mating ability was recorded when males and females became 2 d old. After that, a significant reduction in the mating ability was noticed 4 d and 5 d after male and female emergence (Fig. 1). The mating ability of 1-d-old males and females was similar, and afterwards the mating ability of females was higher than that of males, regardless of adult age.

To determine the effect of adult age on fecundity and fertility only mated females were used in the analysis. Figure 2 reveals that the number of eggs increased when the adults became 2-d-old; the number of eggs then significantly declined ($F=13.19$; d.f $=9,190$; $P<0.05$ for female and $F=12.9$; d.f $=9,190$; $P<0.05$ for male). Significant differences were noticed between males and females at each tested age, except when both sexes were 1-d-old (Fig. 2).

Effect of body weight

The percentage of mating ability of the females was not affected by their weight. No differences in mating ability were found among the three tested female weight groups (Fig. 4). In contrast, the number of female matings was influenced by their weight. The mean number of matings of group 3 females (the heaviest) was significantly higher than that of groups 1 and 2 ($F=8.6$; d.f $=2,74$; $P<0.05$). However, the mean number of matings of group 2 females did not significantly differ from that of group 1 females (Fig. 5).
A significant difference in the percentage of mating incidence was observed among male weight groups 1 and 3 (Fig. 4). All males of group 3 (the heaviest) were able to transfer spermatophores, whereas only half of group 1 males (the lightest) was able to do so. The mating incidence of group 2 males did not significantly differ from that of group 3 (Fig. 4). The mean number of matings of group 1 males was significantly lower than that of groups 2 and 3 ($F=5.19$; d.f.=2,72; $P<0.05$). No differences in the number of matings were detected between males in groups 2 and 3 (Fig. 5).

Results from the study show that female body weight significantly affects fecundity ($F=6.24$; d.f.=2,74; $P<0.05$) and fertility ($F=7.09$; d.f.=2,74; $P<0.05$) of the codling moth. The mean number of eggs laid and mean number of eggs hatch increased significantly when female weight increased (Table 1).

<table>
<thead>
<tr>
<th>Female weight (mg)</th>
<th>Mean fecundity</th>
<th>Mean fertility</th>
</tr>
</thead>
<tbody>
<tr>
<td>34-37</td>
<td>66.2±11.35 b</td>
<td>50.6±9.37 b</td>
</tr>
<tr>
<td>39-42</td>
<td>99.8±12.70 ab</td>
<td>68.9±11.74 b</td>
</tr>
<tr>
<td>44-47</td>
<td>134.1±16.03 a</td>
<td>112.5±14.14 a</td>
</tr>
</tbody>
</table>

Means followed by different letters (columns) are significantly different at $P<0.05$ (Tukey HSD test).

Unlike female weight, male weight did not affect fecundity and fertility. There were no significant differences in fecundity and fertility between heavy and light males (Table 2).

<table>
<thead>
<tr>
<th>Male weight (mg)</th>
<th>Mean fecundity</th>
<th>fertility</th>
</tr>
</thead>
<tbody>
<tr>
<td>25-31</td>
<td>63.1±12.85 a</td>
<td>52.3±10.8 a</td>
</tr>
<tr>
<td>36-45</td>
<td>85.0±9.88 a</td>
<td>57.8±8.6 a</td>
</tr>
<tr>
<td>47-49</td>
<td>95.3±12.2 a</td>
<td>67.0±9.8 a</td>
</tr>
</tbody>
</table>

Means followed by different letters (columns) are significantly different at $P<0.05$ (Tukey HSD test).

The mean number of eggs and fertility of group 1 females, in which the females were not given an opportunity to remate, were significantly lower than those of group 2 females, in which the females were given a chance to remate ($F=6.7$; d.f.=6,59; $P<0.05$ for fecundity, $F=6.7$; d.f.=6,59; $P<0.05$, respectively). Regardless of the number of matings of group 2 females, the mean number of eggs and fertility did not differ significantly.

### Table 3 - Effect of number of females and males on number of mating of C. pomonella during a 24-h period

<table>
<thead>
<tr>
<th>Sex</th>
<th>Sex Ratio</th>
<th>% mated adult per no. of mating</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Female</td>
<td>1:1</td>
<td>B35 ab</td>
</tr>
<tr>
<td></td>
<td>1:3</td>
<td>B40 a</td>
</tr>
<tr>
<td>Male</td>
<td>1:1</td>
<td>B30 ab</td>
</tr>
<tr>
<td></td>
<td>3:1</td>
<td>B20 b</td>
</tr>
</tbody>
</table>

Percentages preceded by different capital letters (rows) and followed by different small letters (columns) are significantly different at $P<0.05$ (normal approximation test).

### Table 4 - Effects of repeated mating on the mean number of eggs and egg hatch percentage of C. pomonella females

<table>
<thead>
<tr>
<th>Female group</th>
<th>No. of matings</th>
<th>Females (%)</th>
<th>Mean no. of eggs/female±se</th>
<th>Mean fertility/female± se</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>60</td>
<td>93.6±19.9 b</td>
<td>93.6±19.9 b</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>7</td>
<td>134.7±25.6 b</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>18</td>
<td>237.1±32.1 a</td>
<td>237.1±32.1 a</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>35</td>
<td>225.8±20.5 a</td>
<td>225.8±20.5 a</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>22</td>
<td>260.9±27.9 a</td>
<td>260.9±27.9 a</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>11</td>
<td>238.6±8.5 a</td>
<td>238.6±8.5 a</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>270.0±25.8 a</td>
<td>270.0±25.8 a</td>
<td></td>
</tr>
</tbody>
</table>

Means and percentages followed by different letters (columns) are significantly different at $P<0.05$ (Tukey HSD test).

### 4. Discussion and Conclusions

The tendency and number of matings of C. pomonella may be affected by various factors. Our results indicate that old males and females of this species were less likely to mate than young individuals. However, differences appeared when the patterns of mating ability of females and males were compared. The female mating ability was greater than that of males. However, the mating ability of females declined less rapidly than that of males with age (Fig. 1). In Lepidoptera, the ability to release adequate sex pheromone and/or to respond to the sex pheromone of the
opposite sex generally reduces with age (Spurgeon et al., 1995; Proshold, 1996; Makee and Saour, 2001). Our result confirms that senescence might influence male moths to a greater degree than females.

In the current study, male age greatly influenced female fecundity and fertility of *C. pomonella* (Figs. 1 and 2). Conflicting results have been reported on the impact of male age on female fecundity and fertility of *C. pomonella*. Vickers (1997) reported that male age had no effect on female fecundity and fertility, whereas Knight (2007) showed that female fecundity after mating with 1-d-old males was significantly lower than after mating with 3-d-old males. Similarly, female fecundity and fertility of *Platella xylostella* L. decreased when the females mated with old males (Nemoto et al., 1992; Wang et al., 2011). Female discrimination against older males has been demonstrated in several species (Ritchie et al., 1995; Jones and Elgar, 2004). Thus, *C. pomonella* females preferred mating with younger males since mating with older males diminishes female fecundity and fertility. Such reduction in reproductively could be due to an age-correlated reduction in sperm quality (Crow, 1997; Hansen and Price, 1999).

The results from the present work clearly indicate both fecundity and fertility were affected by female age at mating, both of which decreased with an increase in age (Figs. 1 and 2). Similar effects have been observed previously in several species such as *Spodoptera exigua* (Hübner), (Rogers and Marti, 1996), *Lobesia botrana* (Dennis & Schiffermüller) (Torres-Vila et al., 2002) and *P. xylostella* (Wang et al., 2011). The reduction of egg production and viability when mating of *C. pomonella* was delayed after emergence could be related to utilization of the fat body, which is essential source of vitellogenins and lipids for oocyte maturation, for non reproduction metabolism by older females (Barrer, 1976).

Unlike male weight, female weight did not play a role in mating ability in *C. pomonella* (Fig. 4); both light and heavy females had similar mating tendencies. However, female weight did affect the number of matings (Fig. 5). Similar results were reported in *P. operculella* (Makee and Saour, 2001). Male weight had important impact on mating ability and number of matings of *C. pomonella* (Figs. 4 and 5). There may be two main reasons for the relationship between male weight, ability to produce and transfer spermatophore, and number of matings: (1) heavy males may be able to produce sufficient quantity of sex pheromone to attract females (Thornhill and Alcock, 1983; Phelan and Barker, 1986); (2) *C. pomonella* females tend to mate with heavier males, as confirmed by our data (i.e. they mated with 100 and 50% of heavy and light males, respectively) (Fig. 4).

Our results show that reproductively of heavy females was greater than for light females (Table 1). Strong correlation between adult weight and fecundity has been noted in various insect species (Evans, 1982). Honek (1993) reported that genetic and environmental factors could influence insect weight. There are several environmental factors including food type and temperature (Mohaghegh et al., 1999). Generally, female weight partly reflects the size of fat-body. This organ is essential for oocyte maturation since it is a site of lipid and yolk protein synthesis (Chapman, 1982). Therefore, heavy females are able to produce more eggs since they have a larger fat-body. In contrast to female weight, male weight did not influence the fecundity and fertility of *C. pomonella* (Tables 1 and 2).

Like most lepidopteran, when a *C. pomonella* male was paired with one female for 24 h, it was able to produce and transfer only one spermatophore (Makee and Saour, 2001). Nevertheless, if several virgin females were available, *C. pomonella* males were able to transfer more than one spermatophore during one scotophase (Table 3). A comparable result has been noted in males of *Grapholita molesta* Busck, *Spodoptera frugiperda* (J.E. Smith) and *P. operculella* (George and Howard, 1968; Simmons and Marti, 1992; Makee and Saour, 2001).

Nevertheless, *C. pomonella* females mated once in 24 h even when they were confined with three newly emerged males (Table 3). Thus, females needed a lapse of time to remate, regardless of the number of males available. A similar result was reported in *P. operculella* (Makee and Saour, 2001). After mating, females released special volatile materials that reduced their receptivity (Tompkins and Hall, 1981).

When *C. pomonella* females were exposed to newly emerged males for seven successive days, only 18% of them mated once, 35% twice and 40% several times (Table 4). *C. pomonella* females were capable of mating more than five times when they were paired with virgin males for seven successive days. Conversely, *P. operculella* females were unable to mate more than three times when they were paired with virgin males for seven successive days (Makee and Saour, 2001). Whatever the number of matings, the mean number of eggs and fertility of females were similar in both species. This may imply that once-mated females would not seek additional matings since they received sufficient effective sperm during their first mating. Several studies reported that *P. operculella*, *Heliothis virescens* F. and *L. dispar* females that did not receive an adequate quantity and quality of sperm during the first mating needed to remate (Lingren et al., 1988; Proshold, 1995; Makee and Saour, 2001). On the contrary, Knight (2007) stated that *C. pomonella* females that had mated three times had a significantly higher fecundity than singly-mated moths.

Fecundity and fertility of females with an opportunity to remate were higher than those of once-mated females that were not allowed to remate (Table 4). The relationship between the number of matings and the female’s reproductivity could be attributed to: (1) sperm replenishment which is required for egg fertilization; (2) nutrients derived from spermatophores that are utilized by the female in egg production (Greenfield, 1983).

The present study provides useful information for situations where sterile insect technique could be considered against *C. pomonella*: (1) repeated releases of young sterile insects should be executed rather than one major release; (2) production of heavy insects is preferable in mass-rearing procedures; (3) the effectiveness of sterile in-
sect technique against *C. pomonella* would not restrain by the release of both sterile males and females, since during one scotophase *C. pomonella* males could remate whereas females were unable to do that.

References


PROSHOLD F.I., 1995 - Remating by gypsy moths (Lepidoptera: Lymantriidae) mated with *F1*-sterile males as a function of sperm within the spermatheca. - J. Econ. Entomol., 88: 644-648.


