Impact of Mating Frequency on Fecundity, Fertility and Longevity of Red Palm Weevil, *Rhynchophorus ferrugineus* (Olivier) (Coleoptera: Curculionidae)

Mahmoud Mohamed Abdel-Azim, Polana Sri Panduranga Vithal Vidyasagar, Saleh Abdullah Aldosari and Rashid Mumtaz

Chair of Date Palm Research, College of Food and Agriculture Sciences, King Saud University, Riyadh 11451, Saudi Arabia

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**Abstract:** The reproductive behavior of red palm weevil, *Rhynchophorus ferrugineus* Olivier was studied in laboratory with several sets of unmated females paired with unmated males for different periods. The treatments in these controlled mating experiments included single mating, multiple mating for 24 h, multiple mating weekly, multiple mating on alternate days and multiple mating uninterrupted with a control group. During the study, observations were made on the number of eggs laid, incubation period, hatching percentage, ovipositional periods and adult longevity. Though unmated females laid a large number of eggs, these were infertile. This study clearly showed significant differences in the egg-laying capacity of females exposed to different frequencies of mating. The average number of eggs laid varied from 98.9 ± 9.7 eggs in a single-time mated female to 195.8 ± 24.3 eggs in females allowed to mate on alternate days throughout their life. As far as the percentage of egg-hatching is concerned, there were significant differences in single and multiple mated females with 22.4 ± 3.3% hatching in one time mated females as compared to 85.2 ± 1.9% in alternate day mated females. This study also showed direct impact of female matings on the number of eggs laid and their hatching percentage. Moreover, the unmated and mated female groups showed no significant differences in oviposition and post-ovipositional period and also there was very little difference in male and female adult longevity.

**Key words:** Mating frequency, fecundity, hatchability, adult longevity, red palm weevil, *Rhynchophorus ferrugineus*.

1. **Introduction**

The red palm weevil, *Rhynchophorus ferrugineus* Olivier (Coleoptera: Curculionidae), is one of the most economically important destructive stem tissue-boring pest of date palm *Phoenix dactylifera* L. in many parts of the world. The insect was first described in India as a serious pest of coconut palm [1-3] and later on of date palm [4]. This weevil has been spreading westwards very rapidly from mid 1980’s [5, 6]. Ever since its accidental introduction in 1987, the pest has caused severe damage to date palms in Saudi Arabia, UAE, and Oman [7, 8], Egypt [9] and later in Canary island palms in Southern Spain [10]. *R. ferrugineus* is quite difficult to detect in early stages of attack in palms because they hide inside host plant [2, 11].

Biology and life cycle studies of laboratory-reared insects were reported by several researchers working with sugarcane [12-15] and date palm [16, 8]. Some studies were conducted on the sterilization of male by irradiation to explore the feasibility of using sterile insect technique (SIT) in India [17, 18]. However, it was found that a female with single mating is capable of producing viable eggs throughout its life.

Multiple mating with the same male or multiple males (polyandry) is a widespread behavior, but the causes and consequences of this behavior are often very complex and little understood [19, 20]. Benefits
to females from multiple matings can be the availability of extra sperm or male-derived substances [21] and genetic benefits of mating with higher-quality males [21, 22]. In red palm weevil very little information is available on the number of copulations and their effect on the progeny.

Thus, we have conducted the present study to find out the effect of frequency of mating on the fecundity, hatchability, longevity of adults and other biological parameters. These studies will provide detailed information on reproductive behavior of *R. ferrugineus* and help in formulating future research on insect populations.

2. Materials and Methods

After collection from infested palms in Al-Kharj region of Saudi Arabia, a population of *R. ferrugineus* has been reared in laboratory. The culture of the red palm weevil was maintained on sugarcane stems in the rearing room at 23 ± 2 °C temperature, 30 ± 4% RH and 14:10 h (D:L) photoperiod. Two weeks after pupation, cocoons were harvested from the sugarcane stems and incubated individually in round plastic jars measuring 70 mm × 90 mm (diameter × height) with perforated screw cap and were checked daily for adult emergence. The emerged adults were removed, sexed, separated and placed individually in round plastic jars measuring 90 mm × 110 mm (diameter × height) and covered with perforated caps prior to the beginning of the experiments.

Fresh sugarcane stems of about 3 cm diameters were cleaned and cut into 9 cm long bits and then these were longitudinally split. Adults were introduced into adult rearing jar with two split sugarcane pieces for feeding and egg laying. Following are the treatments.

2.1 No Mating (NM)

A set of 10 each newly emerged adult females and males were individually placed in separate jars without mating (virgins). Females remained unmated and were never exposed to males during their entire life span.

2.2 Single Mating (SM)

Ten newly emerged females and males were paired in 10 separate jars for one mating only. For each pair the pre-copulation period was about 15-20 min and the copulation took place for 3-4 min and the whole process was finished in less than 30 min. After visual observation of single copulation, the males were removed to separate jars to record their longevity and females were allowed to feed and lay eggs for the rest of their life.

2.3 Multiple Matings for 24 h (MMD)

Newly emerged females and males were paired in the rearing jars for multiple matings for 24 h and then males were removed to their respective jars. Females were then allowed to feed and lay eggs till their death.

2.4 Multiple Matings for 24 h per Week (MMW)

Pairing was done with newly emerged females and males and each was left in rearing jar for 24 h, then males were removed to numbered jars with food and mated females continued in the respective rearing jar for feeding and laying eggs. The same males were reintroduced into the respective female jars after 6 days for another 24 h. This introduction and removal of males at weekly interval and other observations were continued till the death of all individual insects in this treatment.

2.5 Multiple Matings on Alternate Days (MMAD)

The newly emerged females and males were paired and allowed to have multiple matings for 24 h, and then males were removed. Again after a gap of 24 h the males were reintroduced in to the female jars for multiple matings for another 24 h. This process of alternate day mating was repeated till one of the insects in each pair died, but other observations were continued till the death of all insects.

2.6 Multiple Matings Unrestricted (MMUR)

In this experiment, newly emerged ten females and
males were paired and left in the rearing jar for unrestricted and continuous multiple matings without separation.

All jars in all treatments were inspected daily for the collection of eggs. The eggs collected from each female jar were routinely examined, counted and transferred to pieces of sugarcane stems in separate boxes. The total number of eggs laid by each female and the number of eggs hatched were recorded. Sugarcane stem pieces were replaced with fresh ones as and when required.

Apart from observing the total egg laying behavior of females, the specific effect of treatments on the monthly egg laying pattern was also studied. We also recorded incubation period, hatching percentage, oviposition period and the longevity of adults.

2.7 Statistical Analysis

Results were given as mean ± SE. Data were subjected to statistical analysis using SPSS one–way ANOVA (SPSS Release 15.00). The means were separated using Scheffe multiple test and the least significant difference (LSD) test at $P = 0.05$. The confidence limit was 95%. The mean differences were considered significant at values $P \leq 0.05$ level.

3. Results

The results on the effect of mating frequency on various biological parameters including egg laying capacity, percentage of hatching, oviposition period, as well as adult longevity in unmated, single and multiple mated females and males of *R. ferrugineus* are presented here.

3.1 Fecundity

Significant differences were found in the number of eggs laid by the females of six groups (Table 1). Average number of eggs deposited per female was 78.8 ± 10.5 (range 46-162) in no mating females (NM) as compared to 195.8 ± 24.3 (range 98-304) eggs by females of multiple matings on alternate days (MMAD) group and 98.9 ± 9.7 (range 59-169) days in females with single mating group (SM). These results showed highly significant differences in the egg production in unmated and frequently mated female groups ($P < 0.00001$) (Table 1).

The influence of multiple matings and their frequency on the total number of eggs laid by females in various groups between 0-30, 30-60, 60-90 and 90-120 day intervals is presented in Fig. 1. The results indicate that the number of eggs laid by MMW, MMAD and MMUR females between 30-60 days was significantly higher than in NM, SM and MMD females. Moreover, the females in groups MMAD and MMUR laid significantly higher number of eggs than in the other groups between 60-90 days.

3.2 Incubation Period

In comparison made between the incubation period of eggs laid by female groups exposed to different

<table>
<thead>
<tr>
<th>Female mating treatments</th>
<th>Number of eggs laid</th>
<th>Incubation period (days)</th>
<th>Egg hatching %</th>
</tr>
</thead>
<tbody>
<tr>
<td>NM</td>
<td>78.8 ± 10.5 a</td>
<td>0.0 ± 0.0 b</td>
<td>0.0 ± 0.0 a</td>
</tr>
<tr>
<td>SM</td>
<td>98.9 ± 9.7 ab</td>
<td>4.0 ± 0.3 a</td>
<td>22.4 ± 3.3 b</td>
</tr>
<tr>
<td>MMD</td>
<td>112.4 ± 11.5 ab</td>
<td>4.6 ±0.5 a</td>
<td>31.5 ± 3.7 c</td>
</tr>
<tr>
<td>MMW</td>
<td>158.4 ± 15.0 bc</td>
<td>4.4 ± 0.6 a</td>
<td>73.8 ± 1.8 d</td>
</tr>
<tr>
<td>MMAD</td>
<td>195.8 ± 24.3 c</td>
<td>4.3 ± 0.4 a</td>
<td>85.2 ± 1.9 e</td>
</tr>
<tr>
<td>MMUR</td>
<td>175.9 ± 18.6 bc</td>
<td>4.6 ± 0.6 a</td>
<td>85.9 ± 1.9 e</td>
</tr>
<tr>
<td>$F, P$</td>
<td>8.721, &lt; 0.0001</td>
<td>4.314, &lt; 0.002</td>
<td>228,854, &lt; 0.0001</td>
</tr>
</tbody>
</table>

Mean (± SE) in a column of the same variable not followed by the same letter are significantly different at the $P = 0.05$ level (df = 5, 54; Scheffe; and LSD test). NM-No Mating (Virgins), SM-Single mating, MMD-Multiple matings for 24 h, MMW-Multiple matings for 24 h per week, MMAD-Multiple matings on alternate days and MMUR-Multiple matings unrestricted.
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Fig. 1  Egg hatching percentage at 30, 60, 90 and 120 day intervals in female *R. ferrugineus* exposed to various mating treatments. Data for egg hatching in each treatment are presented as mean for 30 days. NM-No Mating (Virgins), SM-Single mating, MMD-Multiple matings for 24 h, MMW-Multiple matings for 24 h per week, MMAD-Multiple matings on alternate days and MMUR-Multiple matings unrestricted.

frequencies of mating, no significant differences were observed (F = 4.314 and P = 0.002) (Table 1). The data on incubation period of eggs showed that, the incubation period was 4.6 ± 0.6 and 4.6 ± 0.5 (range 3-9 and 2-7) days in MMUR and MMD groups, respectively.

3.3 Egg Hatching (Fertility)

Most interesting results were obtained in the percentage of egg hatching. In multiple mated females of longer durations, the percentage of egg hatching increased significantly due to increased periods of inseminations (Table 1). The per cent hatchability averaged 22.4 ± 3.3 (range 8%-38%) in SM group and increased to a maximum of 85.9 ± 1.9 (range 75%-94%) in the MMUR females which was highly significant (F = 228.854, P = 0.0001) (Table 1).

When the age and frequency of mating effect on the hatching was plotted, it was found that the groups MMAD and MMUR showed no significant differences in percentage of egg hatching throughout their life (Fig. 1). However, the females belonging to SM and MMD groups exhibited significant decline in hatchability after 30 days and continued till the end of ovipositional period (Fig. 1). The eggs laid by NM females were infertile and there was no hatching.

3.4 Pre-Oviposition, Oviposition and Post-Oviposition Period

The pre-oviposition period for female *R. ferrugineus* reared on sugarcane stem varied from 3.2 ± 0.5 d in group with just one copulation (SM) to 3.9 ± 0.6 d (range 1-8 d) in females of MMAD and was not significant (Table 2).

Maximum oviposition period of females was 84.9 ± 6.861 d (range 45-121 d) in NM females while, the minimum period of 78.8 ± 9.935 d (range 41-130) was recorded in the case of MMAD group and again was not found significant (Table 2).

As far as the post-oviposition period was concerned, it was 27.7 ± 2.5 d (range 16-42 d) in SM females while the minimum period of 19.7 ± 2.4 d (range 10-32 d) was observed in females belonging to MMAD group (Table 2).
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Table 2 Pre-oviposition, oviposition and post-oviposition period (Mean ± SE) in days of female *R. ferrugineus* exposed to different mating frequencies.

<table>
<thead>
<tr>
<th>Female mating treatments</th>
<th>Pre-oviposition period (ns)</th>
<th>Oviposition period (ns)</th>
<th>Post-oviposition period (ns)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NM</td>
<td>3.6 ± 0.7</td>
<td>84.9 ± 6.9</td>
<td>26.3 ± 2.4</td>
</tr>
<tr>
<td>SM</td>
<td>3.2 ± 0.5</td>
<td>82.8 ± 9.2</td>
<td>27.7 ± 2.5</td>
</tr>
<tr>
<td>MMD</td>
<td>3.6 ± 0.5</td>
<td>80.5 ± 4.9</td>
<td>25.8 ± 2.3</td>
</tr>
<tr>
<td>MMW</td>
<td>3.2 ± 0.5</td>
<td>84.7 ± 5.7</td>
<td>21.2 ± 2.3</td>
</tr>
<tr>
<td>MMAD</td>
<td>3.9 ± 0.6</td>
<td>78.8 ± 10.0</td>
<td>19.7 ± 2.4</td>
</tr>
<tr>
<td>MMUR</td>
<td>3.6 ± 0.5</td>
<td>83.4 ± 6.6</td>
<td>20.5 ± 2.4</td>
</tr>
<tr>
<td><em>F, P</em></td>
<td>0.232, &lt; 0.947</td>
<td>0.106, &lt; 0.990</td>
<td>2.100, &lt; 0.079</td>
</tr>
</tbody>
</table>

“ns” indicates no significant differences at the *P* = 0.05 level (df = 5, 54; Scheffe; and LSD test). NM-No Mating (Virgins), SM-Single mating, MMD-Multiple matings for 24 h, MMW-Multiple matings for 24 h per week, MMAD-Multiple matings on alternate days and MMUR-Multiple matings unrestricted.

3.5 Adult Longevity

The results on the female and male longevity are presented in Table 3. No significant differences were noticed in the life span of females which were either with a male periodically or without a male as per the treatments. The unmated females lived for 114.8 ± 6.7 days as compared to females kept with males which lived for an average of 107.5 ± 6.5 days which was not significant. In general, males outlived the females in all kinds of treatments with the lowest and highest longevity of 112.3 ± 8.2 and 133.3 ± 8.2 days having been recorded in male groups of MMUR and NM respectively.

4. Discussion

There is a relationship between the number of matings and the egg laying and viability in *R. ferrugineus*. Our studies revealed that the average number and range of eggs laid by various groups of multiple mated females are comparable to the previous records of 127-276 [23], 162-350 [24], 204 [25] and 77-283 eggs [8]. However, the number/range of eggs reported by these researchers was lower than the other studies that reported 531 [26], 355-760 [2], 200-500 [27]. When comparisons are made on the fecundity of *R. ferrugineus* there has been wide range of variation reported perhaps due to the food or diet provided and temperature at which insects were reared and method of rearing. In the present study, egg production was not directly dependent on multiple matings or mating periods.

Virgin females laid on an average 78.8 ± 10.5 eggs which is not significantly different from single mating (SM) and multiple matings daily (MMD) groups but is significantly different from multiple matings weekly (MMW), multiple matings alternate days (MMAD) and multiple matings unrestricted (MMUR) females. This study clearly shows that mating is not necessary for laying eggs and once a female is physiologically mature, it will lay eggs irrespective of mating. However, the percentage of hatching in the eggs laid by unmated females is nil, whereas females with unrestricted mating laid significantly higher number of fertile eggs.
When comparison is made between females with single complete copulation and females with multiple matings for 24 h, it was found that the number of eggs laid decreased as the age progressed. One interesting observation is that in SM group, the egg hatching was almost equal to other treatments in the first 30 days of egg laying. But, the hatching percentage declined drastically in the later period. This is presumably due to non-availability of enough sperm to fertilize the eggs. Although the hatching rate in females that were exposed to males for multiple matings for 24 h every week (MMW) is significantly lower than in MMAD or MMUR (Table 1) groups, there appears to be adequate quantity of sperm and gonadotropic factors for giving 73.8 ± 1.8% hatching.

It was reported that multiple mated females usually lay more eggs than single mated females [28-31]. According to another paper, R. ferrugineus females laid fewer eggs when confined with males than when they were isolated after mating [32]. But from our studies, there appears to be no increase in egg production in females exposed to males continuously for multiple matings. Therefore, the question arises as to what are the advantages of multiple matings in R. ferrugineus.

There was report that the mean total number of eggs laid by females decreased significantly with increasing weevil age and ranged from 65.5 eggs from 1-d-old female to 43.5 eggs from 45-d-old female [33]. Even the rate of egg hatching dropped significantly with increasing weevil age and ranged from 75.6% in 1-d-old to 47.4% in 45-d-old weevils [33]. It was also stated that females separated from males for period as long as 30 days still produced fertile eggs and indicated that sperm from one mating can produce fertile eggs for the reproductive life of the female [32]. Our observations showed that females with single mating produced viable eggs to a certain period and the hatching rate decreased subsequently, thereby demonstrating the absence of sufficient sperms. Hence the female has to mate regularly to be able to produce viable progeny. Perhaps that is one of the reasons why the females multiply mating.

Studies in other coleopterans suggested that multiple matings help females to accumulate direct material benefits such as an adequate supply of sperm [30], defensive chemicals [34], nutrients and/or oviposition stimulants [22]. Additionally, multiple matings is also associated with a number of costs, like loss of time and energy, risk of predation and infection by sexually transmitted diseases [35-38].

When live females captured through the pheromone-baited traps were reared singly and paired with males laid on an average of 112.00 and 159.00 eggs respectively [39]. However, the percentage of hatching in the former group of females was only 55.05, whereas, in the latter group it was 78.61. This observation suggests that field collected females under isolation laid much lower number of eggs with reduced hatching rate as compared to females paired with a male.

The pre-oviposition in R. ferrugineus was previously reported as 3-4 d [23, 13], 3-5 d [25], 3 d [27], and 4.5 d [8] and our present results are in conformity with them. The average post-oviposition period in field collected red palms weevils was earlier reported as 9.93 and 4.28 days in two separate batches of insects [39]. But in our studies the highest post-oviposition period was 27.7 d in single mated females (SM) followed by 26.3 d in unmated females (NM) and the shortest duration of 19.7 d was recorded in multiple matings on alternate days (MMAD). Food may influence the post-oviposition period as in the case of females fed on 6% sugarcane solution, where the post-oviposition period was reported to be 4 days only [40].

The presence of males in small jars was suggested to have interfered with oviposition or increased damage to eggs and larvae in R. cruentatus [41]. In our studies, exposing females continuously to males in multiple matings unrestricted group (MMUR) has not interfered in egg production and viability as revealed
by the data that compares well with treatments where males were removed from the jars periodically. Therefore, we are of the view that exposure of females to males may not have affected egg laying and hatching as well in R. ferrugineus.

The longevity of adults reported here is more or less identical to the previous reports of 50-90 d [23], 83.6 d for males and 60 d for females [24], and 60-90 d [2]. Considerably higher adult longevity was reported by Aldhafer et al. [13], where the adult longevity period was 161 d (range: 67-257 d) for males and 113 d (range: 70-150 d) for females. The influence of food quality and rearing temperature may also play a role in the longevity of adults. In our studies the lowest longevity was observed in MMUR females with 107.5 ± 6.5 d and the highest in NM males (133.3 ± 8.2 d). But in R. ferrugineus, there was no significant difference in the longevity of unmated and multiple mated females, suggesting that there was no nutrient benefit for the females due to mating.

It was reported in ladybirds, a slight but statistically significant increase in female longevity with increase in number of matings indicating that the male ejaculate might provide a supply of nutrients. The results also reveal that there exist an optimal number of matings for both sexes and that for females it was higher than for males [42].

5. Conclusions

Following conclusions are drawn from our investigations on the frequency of mating in R. ferrugineus. The unmated females laid a large number of infertile eggs and in single mated females the viable eggs are deposited for the first 30 days only. According to the present study for laying optimum number of eggs and hatchability, exposure of females to multiple matings at weekly intervals or alternate days or continuously with males is a prerequisite. Here we have to answer the question that why red palm weevils mate frequently in short intervals? There is another facet related to multiple matings when fitness is a matter of concern. If it benefits males, how does it help females? Our investigations on consequences of mating frequency in R. ferrugineus will further future research on population modeling and population dispersal in new regions and continents in a short period of time due to its advantageous reproductive behavior.

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