

# Reproductive behaviour in different aged adults of fall armyworm, *Spodoptera frugiperda* (J. E. Smith)

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**In insects, mating and oviposition are important reproductive behaviours that occur for a limited period in their lifecycle. The physiological age of an insect is crucial for reproductive maturity, mate selection and fitness. To study the effect of age on reproduction, an experiment was conducted to analyse the reproductive behaviour in different aged adults of *Spodoptera frugiperda* (J. E. Smith). The copulation duration varied significantly in different aged adults, with the highest duration of copulation ( $161.42 \pm 16.53$  min) in 1-day male (DM)  $\times$  3-day female (DF) mating combination and the lowest ( $85.00 \pm 14.31$  min) in 4 DM  $\times$  4 DF combination. A significant difference in fecundity, hatching percentage (fertility) and oviposition period was observed among the different mating treatments. Fecundity showed a decreasing trend along with the increasing age of the adults, with the highest fecundity ( $1769.60 \pm 181.24$  eggs) from 2 DM  $\times$  1 DF combination and the lowest ( $682.86 \pm 82.04$  eggs) from 4 DM  $\times$  4 DF mating combination. Overall, irrespective of male and/or female, an increase in age negatively impacted reproductive fitness. Therefore, any technique that delays the *S. frugiperda* breeding, such as pheromone-mediated mating disruption, might successfully control the pest. Practically our research information will be useful for the effective behavioural management of *S. frugiperda* by developing techniques like mating disruption strategies, thereby affecting its reproductive succession.**

**Keywords:** Copulation, fall armyworm, fecundity, mating frequency, scotophase.

REPRODUCTION is an important mechanism that determines the population growth of any organism. In insects, calling, mating and oviposition are important reproductive behaviours which occur for a limited period in their life cycle. The physiological age of insects matters for reproductive maturity, mate selection and reproductive capacity of insects<sup>1-4</sup>. Over time these reproductive statuses will change; therefore, mating between young adults will increase their reproductive potential<sup>1</sup>. However, biological (reproductive

maturity), environmental (unsuitable temperature, rainfall and wind) and anthropogenic factors (mating disruption, trapping) cause delayed mating<sup>5,6</sup>. Delayed mating is defined as a condition in which female and/or male mating happens at later stages of life. In most of the lepidopterans, the experimental evidence demonstrated that delayed mating is disadvantageous for females<sup>4,7-9</sup>; however, it has shown both positive and negative effects on the reproduction of different species of insects<sup>10,11</sup>.

Delayed mating has been observed to significantly affect both males and females with respect to attractiveness towards the other sex, mating frequency, fecundity and fertility in different species of Lepidoptera<sup>1,12,13</sup>. Some experimental evidence have shown that male age and mating history have a negative impact on female reproductive potential by reducing female fecundity<sup>1,7,14</sup>. This is because the quality of sperm and nitrogen-rich liquid accessory substances transferred from male to female is dependent on the male age<sup>9,14,15</sup>. When both sexes are delayed, the reproductive potential of females is drastically decreased<sup>1</sup>. Though there is experimental evidence that the age of both sexes affects the reproductive potential of females, the interactive effect of delayed mating of males and females in *S. frugiperda* has received little attention.

The fall armyworm (FAW), *S. frugiperda*, is a pest with a significant economic impact and a propensity for migration. This polyphagous pest, with its native to the Western Hemisphere, is capable of feeding over 353 plant species, including staple food crops like maize, sorghum, rice and sugarcane<sup>16-18</sup>. Through its invasive alien habit, it established itself throughout Africa and Asia within 4 years since its first report outside America. It was first reported from India in 2018 on maize<sup>19</sup>, and it spread throughout the country<sup>20</sup>. The activity of the pest was noticed both in *kharif* and *rabi* seasons<sup>21</sup>. Synthetic insecticides are frequently used to manage FAW and the crop protection cost to produce 100 kg of maize in fields increased 11.20 times between 2017 and 2020 (refs 22, 23). Integrated pest management has been practised with the use of the sex pheromone of FAW. Female sex pheromones have been identified and used for monitoring and mass trapping of FAW in India. Efforts are being made to utilize the same for mating

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disruption<sup>20,24</sup>, which not only prevents mating but also delays it, thereby reducing the number and viability of eggs produced<sup>25</sup>.

Our interests in investigating the reproductive behaviour in different aged adults of *S. frugiperda* are based on the applicability of pheromone-mediated mating disruption for this important socio-economic pest. This management tactic is currently used for FAW management in Western countries<sup>26,27</sup>, and at research trials in India<sup>20</sup>. Though this is used in integrated pest control, little is known regarding its effect on delaying mating or on the effect of delayed mating on reproductive fitness. In the present study, we address the effect of the age of adults on copulation, mating frequency, oviposition period, fecundity, fertility, and adult longevity of *S. frugiperda*.

## Materials and methods

### *Test insect*

The adults of *S. frugiperda* were obtained from the stock culture of the insect reared on a meridic diet<sup>28</sup> and maintained in a controlled condition of photoperiod of 12:12 h (L:D) at temperature  $27^{\circ} \pm 1^{\circ}\text{C}$ ,  $65 \pm 5\%$  relative humidity (RH) in Insect Physiology and Molecular Biology Laboratory, Division of Entomology, ICAR-Indian Agricultural Research Institute, New Delhi. The adults were allowed to mate, and sufficient eggs were collected for the experiment planned. The hatched larvae were cultured on a meridic diet<sup>28</sup> till pupation. Then the pupae were sexed<sup>29</sup> and placed in separate vials till emergence. The emerged adults were provided with a 10% honey solution dispensed on cotton wad till they were used for the experiment. The whole experimental setup was maintained in controlled room conditions, as mentioned above. The day of the adult's emergence was considered as day 1. The scotophase started at 18:00 h and ended at 06:00 h.

### *Experimental set-up*

In the present study, adults emerged on four continuous days and were used in different mating combinations. The adults were designated as 1D on the day of emergence (1 DM (1-day male), 1 DF (1-day female), 2D when they were delayed for one day (on the 2nd day of emergence) (2 DM, 2 DF) and so on till the 4th day. These adults were singly paired in all possible age combinations. In total, there were sixteen mating treatments namely, 1 DM  $\times$  1 DF, 1 DM  $\times$  2 DF, 1 DM  $\times$  3 DF, 1 DM  $\times$  4 DF; 2 DM  $\times$  1 DF, 2 DM  $\times$  2 DF, 2 DM  $\times$  3 DF, 2 DM  $\times$  4 DF; 3 DM  $\times$  1 DF, 3 DM  $\times$  2 DF, 3 DM  $\times$  3 DF, 3 DM  $\times$  4 DF; 4 DM  $\times$  1 DF, 4 DM  $\times$  2 DF, 4 DM  $\times$  3 DF and 4 DM  $\times$  4 DF with nine replications for each treatment combination. Single pairs (male and female @ 1 : 1) were kept in plastic containers (17.5 cm in height by 10.5 cm in diameter) with their

mouth covered with a muslin cloth. The moths were fed with 10% honey solution. The complete experimental set-up was maintained in the above-mentioned controlled conditions for the complete longevity of moths. Copulation duration and copulation percentage were recorded on the day of experimental setup throughout the complete scotophase. Paper strips were provided as oviposition substrate for females. To access the reproductive behaviour in different aged adults, nine important reproductive parameters were recorded, which include copulation duration, copulation percentage (recorded only on the experimental scotophase), fecundity, fertility, mating frequency, mating success, oviposition period, male longevity and female longevity.

### *Reproductive parameters*

The moths were observed for every 30 min interval on the first scotophase of pairing in the experimental setup. Copulation duration was recorded from the start of mating until the male and female got separated by themselves. Copulation percentage includes the number of successful mating from all nine replications for each treatment. Total fecundity is the summation of daily fecundity, and the fertility percentage was also calculated. Mating frequency was calculated by counting the number of spermatophores in the female bursa copulatrix after death. Mating success is the presence or absence of a spermatophore in the bursa copulatrix. The oviposition period is the number of days the female lays eggs during its longevity. Male and female longevity is the period from the date of emergence to death.

### *Statistical analysis*

In a completely randomized set up, the data were subjected to one-way ANOVA to test mating behaviour and reproductive attributes. The significant difference between the treatments was determined using the *F*-test, and the treatment means were separated using Tukey's Post Hoc test at  $P = 0.05$  and were represented as mean  $\pm$  SE. To determine the relationship between the longevity of adults and the number of spermatophores, simple linear regression was performed. Kaplan–Meier survival analysis was done to predict the adult survival using log-rank (Mantel–Cox) and the Gehan–Breslow–Wilcoxon test.

## Results

### *Effect of adult age on mating behaviour*

The present study shows that the mating behaviour of both male and female was significantly affected by age. The copulation duration varied significantly in different aged adults ( $F = 3.064$ ;  $df = 15$ ,  $P < 0.001$ ) (Table 1). The longer duration of copulation was observed when young aged

**Table 1.** Effect of adult age on reproductive attributes of fall armyworm, *Spodoptera frugiperda*

Mating system (male × female)	Copulation duration (min)	Copulation (%)	Mating success (%)	Mating frequency	Fecundity	Egg hatching (%)	Oviposition period (days)	Female longevity (days)	Male longevity (days)
1 DM × 1 DF	125.00 ± 9.22 <sup>abcd</sup>	44.44 ± 17.56 <sup>b</sup> (40.11 ± 15.46)	66.65 ± 16.66 (59.67 ± 14.67)	1.67 ± 0.21 <sup>abcde</sup>	1552.57 ± 282.34 <sup>ab</sup>	88.60 ± 1.17 <sup>b</sup> (70.36 ± 1.05)	1.56 ± 0.62 <sup>bcd</sup>	10.56 ± 0.29	9.78 ± 0.22
1 DM × 2 DF	150.00 ± 11.34 <sup>abc</sup>	22.23 ± 14.69 <sup>c</sup> (20.55 ± 12.94)	66.65 ± 16.66 (59.67 ± 14.67)	2.33 ± 0.21 <sup>a</sup>	1460.00 ± 196.62 <sup>abc</sup>	92.83 ± 1.01 <sup>a</sup> (74.66 ± 1.12)	2.00 ± 0.52 <sup>abc</sup>	10.78 ± 0.32	10.00 ± 0.33
1 DM × 3 DF	161.42 ± 16.53 <sup>a</sup>	55.55 ± 17.56 <sup>a</sup> (49.88 ± 15.46)	66.65 ± 16.66 (59.67 ± 14.67)	1.50 ± 0.22 <sup>abcde</sup>	1294.33 ± 206.10 <sup>abcd</sup>	84.67 ± 1.67 <sup>bcd</sup> (67.10 ± 1.33)	1.56 ± 0.52 <sup>bcd</sup>	10.89 ± 0.45	10.00 ± 0.50
1 DM × 4 DF	155.00 ± 9.21 <sup>ab</sup>	44.44 ± 17.56 <sup>b</sup> (40.11 ± 15.46)	77.76 ± 14.69 (49.89 ± 15.46)	1.14 ± 0.14 <sup>d</sup>	762.78 ± 79.80 <sup>de</sup>	80.71 ± 1.38 <sup>cde</sup> (64.03 ± 0.99)	0.33 ± 0.16 <sup>d</sup>	10.78 ± 0.36	9.56 ± 0.37
2 DM × 1 DF	90.00 ± 13.09 <sup>d</sup>	44.44 ± 17.56 <sup>b</sup> (40.11 ± 15.46)	66.65 ± 16.66 (59.67 ± 14.67)	1.50 ± 0.22 <sup>abcde</sup>	1769.60 ± 181.24 <sup>a</sup>	86.83 ± 1.51 <sup>b</sup> (68.86 ± 1.23)	2.33 ± 0.47 <sup>ab</sup>	11.56 ± 0.29	10.44 ± 0.37
2 DM × 2 DF	124.28 ± 7.82 <sup>abcd</sup>	44.44 ± 17.56 <sup>b</sup> (40.11 ± 15.46)	66.65 ± 16.66 (59.67 ± 14.67)	2.00 ± 0.36 <sup>abc</sup>	1552.85 ± 242.99 <sup>ab</sup>	92.83 ± 1.28 <sup>a</sup> (74.86 ± 1.62)	1.67 ± 0.47 <sup>abcd</sup>	10.89 ± 0.26	9.89 ± 0.38
2 DM × 3 DF	120.00 ± 11.18 <sup>abcd</sup>	99.97 ± 0 <sup>a</sup> (89.00 ± 0.00)	88.86 ± 11.10 (69.45 ± 12.94)	1.75 ± 0.35 <sup>abcde</sup>	1322.00 ± 150.73 <sup>abcd</sup>	88.63 ± 1.29 <sup>b</sup> (70.52 ± 1.24)	2.89 ± 0.48 <sup>a</sup>	10.89 ± 0.51	9.78 ± 0.57
2 DM × 4 DF	107.14 ± 8.92 <sup>d</sup>	44.44 ± 17.56 <sup>b</sup> (40.10 ± 15.46)	66.65 ± 16.66 (59.67 ± 14.67)	1.5 ± 0.22 <sup>abcde</sup>	1346.67 ± 183.55 <sup>abcd</sup>	79.17 ± 2.15 <sup>de</sup> (62.99 ± 1.52)	0.67 ± 0.33 <sup>d</sup>	11.11 ± 0.51	10.22 ± 0.52
3 DM × 1 DF	125.00 ± 9.21 <sup>abcd</sup>	22.23 ± 14.69 <sup>c</sup> (20.55 ± 12.94)	66.65 ± 16.66 (59.67 ± 14.67)	1.67 ± 0.21 <sup>abcde</sup>	1394.28 ± 191.12 <sup>abc</sup>	85.83 ± 1.38 <sup>bc</sup> (68.01 ± 1.13)	1.22 ± 0.36 <sup>bcd</sup>	11.22 ± 0.40	10.22 ± 0.43
3 DM × 2 DF	110.00 ± 27.56 <sup>cd</sup>	66.65 ± 16.66 <sup>a</sup> (59.66 ± 14.66)	77.76 ± 14.69 (79.23 ± 9.78)	1.86 ± 0.34 <sup>abcd</sup>	1338.50 ± 265.47 <sup>abcd</sup>	87.57 ± 1.56 <sup>b</sup> (69.57 ± 1.34)	2.33 ± 0.52 <sup>ab</sup>	11.33 ± 0.47	9.67 ± 0.62
3 DM × 3 DF	120.00 ± 12.67 <sup>abcd</sup>	88.86 ± 11.1 <sup>a</sup> (79.22 ± 9.78)	77.76 ± 14.69 (69.45 ± 12.94)	2.14 ± 0.36 <sup>abc</sup>	1135.00 ± 167.00 <sup>bcd</sup>	86.14 ± 2.03 <sup>bc</sup> (68.44 ± 1.66)	1.00 ± 0.33 <sup>cd</sup>	11.00 ± 0.40	9.78 ± 0.57
3 DM × 4 DF	115.00 ± 14.31 <sup>bcd</sup>	55.55 ± 17.56 <sup>a</sup> (49.88 ± 15.46)	55.55 ± 17.56 (49.89 ± 15.46)	1.17 ± 0.30 <sup>cd</sup>	945.00 ± 122.12 <sup>cde</sup>	80.00 ± 1.70 <sup>de</sup> (63.51 ± 1.23)	0.44 ± 0.24 <sup>d</sup>	11.00 ± 0.52	9.78 ± 0.46
4 DM × 1 DF	124.29 ± 10.20 <sup>abcd</sup>	33.34 ± 16.66 <sup>c</sup> (30.33 ± 14.67)	55.55 ± 17.56 (69.45 ± 12.94)	1.2 ± 0.20 <sup>cde</sup>	912.15 ± 95.018 <sup>cde</sup>	83.20 ± 2.52 <sup>bcd</sup> (66.02 ± 1.89)	0.89 ± 0.26 <sup>cd</sup>	10.89 ± 0.26	10.00 ± 0.40
4 DM × 2 DF	102.86 ± 6.06 <sup>d</sup>	22.23 ± 14.69 <sup>c</sup> (20.55 ± 12.94)	66.65 ± 16.66 (59.67 ± 14.67)	1.33 ± 0.21 <sup>bcd</sup>	928.57 ± 81.78 <sup>cde</sup>	79.67 ± 1.43 <sup>de</sup> (63.27 ± 1.03)	0.67 ± 0.23 <sup>d</sup>	11.89 ± 0.42	10.78 ± 0.43
4 DM × 3 DF	90.00 ± 7.74 <sup>d</sup>	55.55 ± 17.56 <sup>a</sup> (49.88 ± 15.46)	55.55 ± 17.56 (49.89 ± 15.46)	1.2 ± 0.20 <sup>cde</sup>	705.00 ± 106.86 <sup>c</sup>	76.40 ± 3.08 <sup>c</sup> (61.09 ± 2.01)	0.78 ± 0.27 <sup>cd</sup>	11.00 ± 0.52	9.89 ± 0.45
4 DM × 4 DF	85.00 ± 14.31 <sup>d</sup>	44.44 ± 17.56 <sup>b</sup> (40.11 ± 15.46)	44.44 ± 17.56 (40.11 ± 15.46)	1 ± 0.00 <sup>e</sup>	682.86 ± 82.04 <sup>c</sup>	68.75 ± 2.69 <sup>f</sup> (56.07 ± 1.66)	0.44 ± 0.17 <sup>d</sup>	11.33 ± 0.44	9.89 ± 0.38
<i>P</i> value	<0.0001	0.033	0.966 (NS)	0.008	<0.0001	<0.0001	<0.0001	0.838 (NS)	0.953 (NS)
<i>df</i>	15	15	15	15	15	15	15	15	15
<i>F</i> value	3.064	1.859	0.436	2.344	3.365	11.059	4.558	0.639	0.468

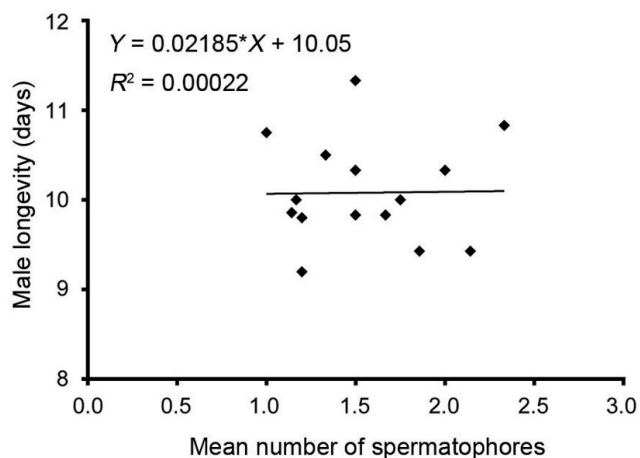
Values are represented as mean ± SE. Different lowercase letters represent significant differences across treatments, figures in the parentheses are arc sine transformed values.

males paired with delayed females (1 DM paired with different aged females), with the highest duration of copulation being  $161.42 \pm 16.53$  min in 1 DM  $\times$  3 DF mating combination; and the lowest copulation duration was observed in delayed males paired with different aged females (4 DM paired with different aged females) with  $85.00 \pm 14.31$  min in 4 DM  $\times$  4 DF combination (Table 1). Percentage copulation also significantly differed among different mating treatments ( $F = 1.859$ ;  $df = 15$ ,  $P < 0.033$ ). The highest percentage of copulation was observed in 2 DM  $\times$  3 DF combination with  $99.97 \pm 0.00$ , and the lowest was  $22.23 \pm 14.69$  in 1 DM  $\times$  2 DF, 3 DM  $\times$  1 DF as well as 4 DM  $\times$  2DF (Table 1). Though there is no significant difference ( $F = 0.436$ ;  $df = 15$ ,  $P < 0.966$ ) in the percentage mating success of different treatments, it, however, varied from  $88.86 \pm 11.10$  in 2 DM  $\times$  3 DF to  $44.44 \pm 17.56$  in 4 DM  $\times$  4 DF (Table 1). Mating frequency (successful mating) is measured by the number of spermatophores recovered from the bursa copulatrix of the female. It varied

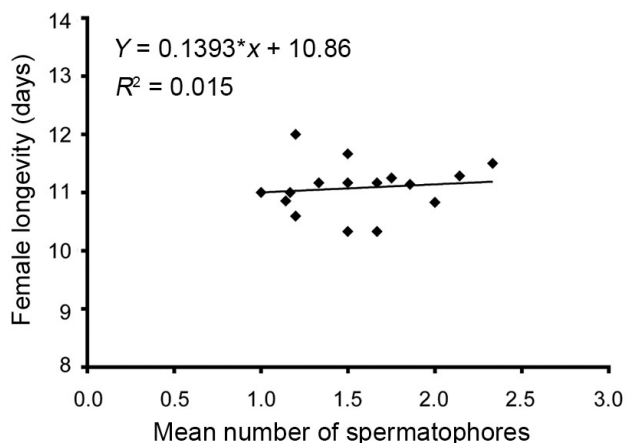
significantly among the different mating treatments ( $F = 2.344$ ;  $df = 15$ ,  $P < 0.008$ ). It was highest in 1 DM  $\times$  2 DF with a mean of  $2.33 \pm 0.21$ , and the lowest was  $1 \pm 0.00$  in the 4 DM  $\times$  4 DF combination. The 2 DF paired with different-aged males showed the highest mating frequency, averaging of 2.34 spermatophores per female (Table 1). However, there was no significant correlation between the longevity of both male ( $R^2 = 0.00022$ ;  $df = 14$ ;  $P = 0.956$ ) and female ( $R^2 = 0.014$ ;  $df = 14$ ;  $P = 0.653$ ), with the mean number of spermatophores (Figures 1 and 2).

*Effect of adult age on reproductive attributes*

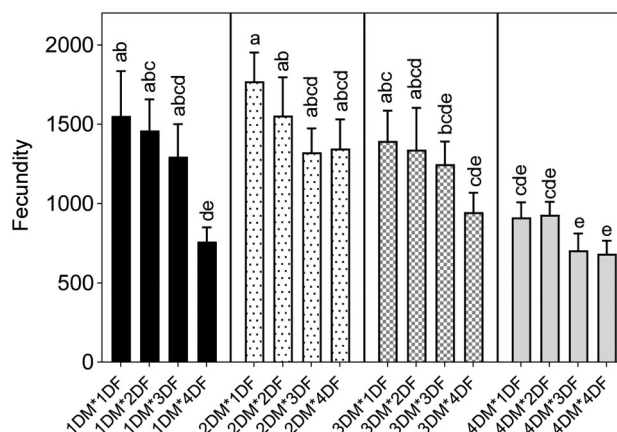
We observed a significant difference in reproductive attributes like fecundity, hatching percentage (fertility) and oviposition period among the different mating treatments. Fecundity showed a decreasing trend along with the increasing age of the adults, with the highest fecundity of  $1769.60 \pm 181.24$  eggs from the 2 DM  $\times$  1 DF combination, and the lowest was of  $682.86 \pm 82.04$  eggs from 4 DM  $\times$  4 DF combination (Table 1). Irrespective of male age, the fecundity of aged females decreased, and the 4-day male showed a significant negative effect on fecundity irrespective of female age (Figure 3). There was a significant association observed between the number of spermatophores and fecundity ( $R^2 = 0.70$ ;  $df = 142$ ;  $P < 0.0001$ ) (Figure 4). The egg-hatching percentage also showed decreasing trend with an increase in the age of adults. The highest was  $92.83 \pm 1.28$  from 2-day adults (2 DM  $\times$  2 DF combinations), and the lowest was  $68.75 \pm 2.69$  from 4-day adults (4 DM  $\times$  4 DF combinations) (Table 1 and Figure 5). The hatching percentage obtained from the crosses between 4D females across all the ages of males was significantly on par with each other, being lower than the rest of the mating treatments (Table 1 and Figure 5). Further, irrespective of the age of the males, the egg hatching was significantly higher in the cases where 2D females were used in the mating as



**Figure 1.** Relationship between number of spermatophores and male longevity of *S. frugiperda*.

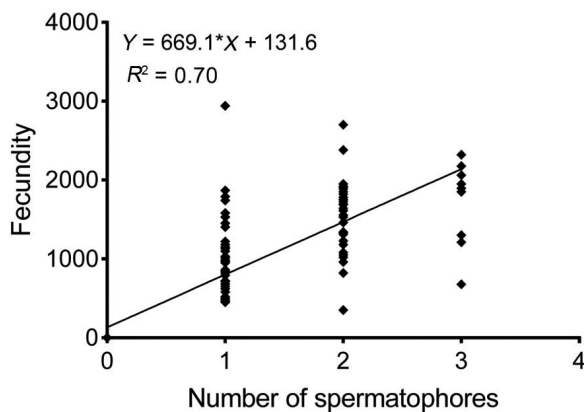


**Figure 2.** Relationship between number of spermatophores and female longevity of *S. frugiperda*.

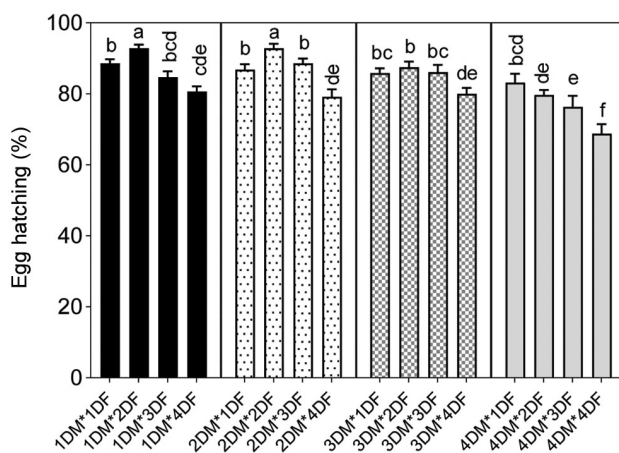


**Figure 3.** Fecundity (number of eggs/female) in different mating combinations of *S. frugiperda*.

compared with other mating treatments except 4 DM × 2 DF combinations. With the increasing age of adults, the oviposition period decreased, and the highest was  $2.89 \pm 0.48$  days by 2 DM × 3 DF, and the lowest was  $0.44 \pm 0.17$  days in 4 DM × 4 DF. It was evident that irrespective of the male age, the oviposition period of 4-day-old females decreased (Table 1). However, the male and female longevity did not show any significant difference due to the crossing of different aged adults, with the highest female longevity of  $11.56 \pm 0.29$  days ( $F = 0.639$ ;  $df = 15$ ,  $P < 0.838$ ) and highest male longevity of  $10.78 \pm 0.43$  days ( $F = 0.468$ ;  $df = 15$ ,  $P < 0.953$ ) (Table 1). The Kaplan–Meier survival analysis revealed the probability of maximum survival of male and female to be up to 13 days. The adult survivorship using log-rank (Mantel–Cox) (male:  $\chi^2 = 7.73$ ;  $df = 15$ ;  $P = 0.934$ , female:  $\chi^2 = 14.55$ ;  $df = 15$ ;  $P = 0.484$ ) and Gehan–Breslow–Wilcoxon test (male:  $\chi^2 = 6.74$ ;  $df = 15$ ;  $P = 0.964$ , female:  $\chi^2 = 10.82$ ;  $df = 15$ ;  $P = 0.765$ ) showed no significant differences in the survival of male and female adults across the different mating treatments (Figures 6 and 7).



**Figure 4.** Relationship between number of spermatophores and fecundity (number of eggs/female) of *S. frugiperda*.



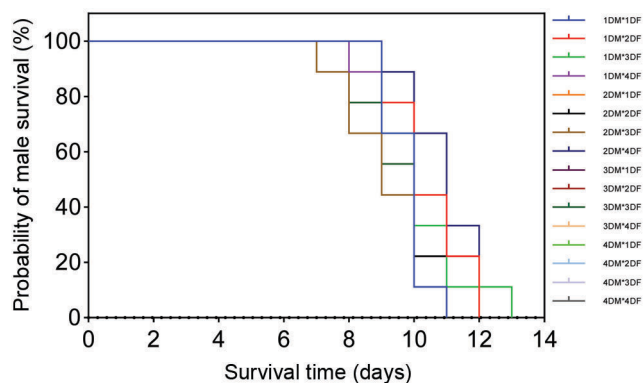
**Figure 5.** Fertility percentage in different mating combinations of *S. frugiperda*.

## Discussion

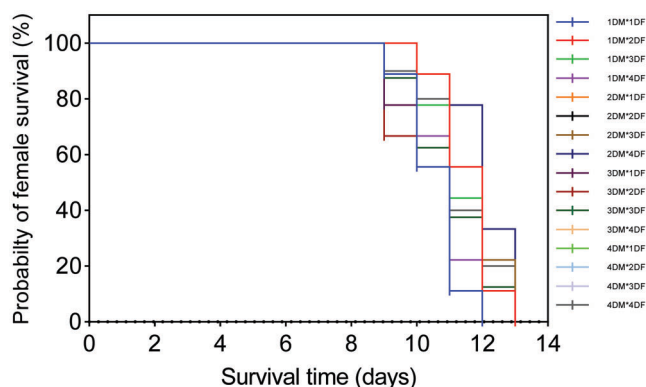
The present study shows that the reproductive performance of different aged adults results in varied reproductive fitness in *S. frugiperda*. Overall, irrespective of male and/or female increasing age, the impact on reproductive fitness showed negative effects. The copulation duration of *S. frugiperda* increased when young males were paired with delayed females (1 DM paired with different aged females) (Table 1). These results are consistent with our previous studies (Ramya *et al.*, unpublished data), indicating that male sexual maturity is the main factor influencing copulation duration. In *Chilo partellus* (Swinhoe), males take 2–3 days to sexually mature and actively participate in fertilization<sup>9</sup>. Likewise, in the majority of *S. frugiperda*, males take a complete scotophase after emergence to sexually mature, and these (males on 2nd scotophase onwards) will take appropriate time for mating (2 DM and 3 DM paired with different aged females) in comparison to 1 DM and 4 DM males (Table 1). The copulation duration decreased when the 4 DM paired with different aged females (Table 1), and a similar trend could be seen in fecundity and fertility.

The copulation percentage showed a significant difference among different mating treatments. The highest percentage of copulation was observed in 2 DM × 3 DF combination with  $99.97 \pm 0.00$ , and the lowest was  $22.23 \pm 14.69$  in 4 DM × 2 DF and 3 DM × 1 DF combinations (Table 1). This again reiterates that the sexual maturity of adults is an important factor which influences the reproductive performance of insects. According to the observations of present and previous studies (Ramya *et al.*, unpublished data), most of the adults *S. frugiperda* sexually mature by 2nd scotophase, and the most feasible mating combination is the 2 DM × 3 DF mating combination. Similar facts about the sexual maturation in *Ectropis obliqua* (Prout) recorded<sup>8</sup> that peak reproductive performance can be achieved if females mate at 1 or 2 days after emergence. Additionally, it is clear from the fact that under lab conditions, *S. frugiperda* females emerge earlier than males, thus resulting in synchronous sexual maturity in both male and female adults. Similar observation with respect to males was recorded in *C. partellus*<sup>9</sup>. The 4-day delay in mating results in a 30–40 reduction in copulation percentage. Our results are consistent with earlier studies on *S. frugiperda* and other lepidopterans<sup>7,30</sup>. Copulation percentage was affected by both male and female age, and we also observed decreased copulation percentage when young females paired with old males and vice versa. This is consistent with the earlier studies on *S. frugiperda* and *Cnephasia jactana*<sup>1,30</sup>.

Mating success is the presence or absence of spermatophores in the bursa copulatrix of the female extracted after death, and the highest mating success was recorded from 2 DM × 3 DF mating combination. *S. frugiperda* is a promiscuous moth<sup>31</sup> with an average mating frequency of females being around 3.7 (Ramya *et al.*, unpublished) in natural conditions. In the present study, the maximum mating frequency



**Figure 6.** Survival analysis (Kaplan–Meier estimation) of male *S. frugiperda* in different mating combinations.



**Figure 7.** Survival analysis (Kaplan–Meier estimation) of female *S. frugiperda* in different mating combinations.

of  $2.33 \pm 0.21$  in 1 DM  $\times$  2 DF mating combination was observed, and it was on par with the results of mating combinations involving 2-day female with different aged males except with 4-day male (4 DM  $\times$  2 DF mating combination). This implies that female age has more impact on the mating frequency, and the highest mating frequency can be achieved only when females are young, which is again directly related to the fecundity and, thereby, the reproductive fitness of the species (Figure 4).

Fecundity and fertility are the fundamental factors that determine the population growth of the species. The physiological age of male and female at the time of mating will have a definite impact on these factors. With the evidence of the present and previous studies, in *S. frugiperda* mating among varying aged males and females in addition to delaying in mating, showed a significant difference in fecundity (Figure 3). With increasing age in either male or female, fecundity shows a decreasing trend, and the age of male has more impact on fecundity in comparison to female (Table 1). Earlier studies on different lepidopteran species like *Epiphyas postvittana* (Walker), *Cydia pomonella* (Linnaeus), *C. jactatana* (Walker)<sup>1,32,33</sup> have reported similar kind of results. Further, we found that increasing age of adults will negatively impact fertility (hatching percentage). The egg hatching was significantly higher in the cases where 2-day females were used in those mating combinations

as compared with other mating treatments, indicating the highest reproductive potential of 2-day females in comparison to others. The aged females (4-day old) mating combinations have reduced fertility than other females, and the same can be seen in aged male (4-day old) mating combinations. The aged adults showed a 10–20% reduction in fertility in comparison to the young adults indicating that egg fertility mainly depends on the age of mating adults. The possible reason behind this is predicted as the degradation of oocytes which ultimately interferes with sperm migration and successful egg fertilization<sup>9,34</sup>. Earlier studies on *S. frugiperda*<sup>28,35</sup> and other lepidopterans<sup>6,36,37</sup> are consistent with our results. Various species have varying effects on adult longevity depending on the age during mating. Delay in mating enhances adult longevity in some species<sup>5,8</sup>, whereas it decreases adult longevity in other species<sup>38,39</sup>, or has no impact in others<sup>4,40</sup>. However, in the present study, we found that the age during the mating of male and female of *S. frugiperda* has no significant difference in longevity among different aged mating combinations. Longevity is the same as in natural conditions, i.e. around 11–12 days, which is also supported by the Kaplan–Meier survival analysis (Figures 6 and 7). This elucidates that the maximum delay of 4 days will not impact the longevity of adults, the natural longevity of *S. frugiperda* is sufficient to complete its reproduction.

## Conclusion

Our basic research revealed that mating among different aged adults results in varied reproductive fitness of *S. frugiperda*. Overall, increasing male and female age endures negative effects on reproductive fitness. When either male or female or both adults were delayed from mating, there was reduced interest in reproductive behaviours like copulation duration, mating frequency and mating success. Fecundity and fertility were also greatly reduced. Therefore, any technique that might delay the *S. frugiperda* breeding may successfully keep the pest under control. The pheromone-mediated mating disruption is one of the important management tactics currently used for FAW management in Western countries; the same technique is at research trials in India. In addition, this technique is a key component used in integrated pest control. In this context, the present study could be useful for effective behavioural management to suppress *S. frugiperda*, thereby affecting its reproductive succession.

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