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Influence of Larval Diet on Reproductive Biology of the Fall Armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera : Noctuidae)

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ABSTRACT

Maize (*Zea mays* L.) is the third most important grain crop, widely cultivated all over the world. A significant proportion *i.e.,* up-to 62 per cent of damage intensity and 34 per cent of yield loss was recorded due to the infestation of fall armyworm, *Spodoptera frugiperda* (NBAIR, 2019). In this study, the influence of natural diet (maize) and artificial diets on the reproductive biology of fall armyworm was studied. The results showed that larval diet significantly affected the mating success in *S. frugiperda (*95.55% on natural diet and 86.66% in artificial diet). However, other reproductive biology parameters such as mating frequency, mating duration, time of mating and egg laying pattern did not differ significantly between natural and artificial diets. With regard to the number of matings, single mating was observed in 35.55 per cent and 28.88 per cent of the moths, respectively on artificial and natural diets. Further, double mating was noticed in 33.33 and 46.66 per cent of the moths in artificial and natural diets, respectively. In this study, single and double mating were more common than mating three or four times. Maximum number of moths initiated mating process at two days after their emergence in both natural diet (58%) and artificial diet (62.85%). Majority of the moths required 40-50 minutes to complete their mating process irrespective of the diet type. Moths laid maximum number of eggs in 3 to 6 days after their emergence and there was no significant difference in the fecundity on artificial diet (771/female) and natural diet (836/female). We conclude that there was no significant difference in reproductive biology of FAW reared on artificial and natural diet.

Keywords : *Spodoptera frugiperda*, Reproductive biology, Artificial diet, Natural diet

MAIZE (*Zea mays* L.) is the third most important grain crop, widely cultivated all over the world. It is popularly known as the 'Queen of Cereals' due to its wider adaptability and highest genetic yield potential among cereal crops (Krishna and Mudalagiriyappa, 2022). Maize plants are infested by more than140 species of insect pests, causing varying degree of damage (Sarup *et al.,* 1987). Major insect pests infesting maize in India are stem borers, shoot fly, Bihar hairy caterpillar, aphid, jassids and newly reported invasive pest, *Spodoptera frugiperda*. FAW a notorious pest has received wide-spread attention in the last three years in terms of its voracity, periodic outbreak in pestiferous scales on key cereals and millets (Nidheesh *et al.,* 2021).

The fall Armyworm, *S. frugiperda* has been classified as a sporadic pest due to its migratory behaviour. It occurs in continuous generation cycles and completes several generations per year. After mating, the female moths lay eggs in several masses of 100-200 eggs, with a fecundity of 1,500 eggs / female. Within a few days of oviposition, young larvae start feeding on the whorl leaves. The larval, pupal and adult periods of *S. frugiperda* are 14, 9 and 10 days, respectively (Montezano *et al.,* 2019).

Although, *S. frugiperda* is such a widespread pest, the information regarding several aspects of its reproductive biology, such as number of matings, mating frequency, duration and time of mating, egg laying pattern, is scanty. These aspects play a critical role in the biology, behaviour and population dynamics of any insect. Hence, the reproductive biology of FAW is likely to be influenced by its diet. Therefore, in this study we aimed to study the influence of diet on the reproductive biology of *S. frugiperda* when fed with an artificial diet and its natural host, maize.

Material and Methods

The present study was conducted under laboratory conditions (26 ± 1°C, 70 ± 10 per cent RH and 14L: 10D h photoperiod) in the Department of Agricultural Entomology, College of Agriculture University of Agricultural Sciences, GKVK, Bengaluru during the year 2021-22.

Maintenance of *S. frugiperda* Culture

The egg masses and larvae of fall armyworm were collected from different maize infested fields. The collected larvae and maize leaves were placed in plastic containers (size - 25 cm in diameter). The containers were covered with muslin cloth and secured using rubber band to prevent larval escape. The larvae-containing containers were kept at room temperature (26±1°C, 70±10 per cent RH and 14L: 10D h photoperiod) until adult moth emergence. Emerged moths were released into the ovipositional cage (35 x 35 x 35cm). The walls of the cage were provided with white paper as a supporting platform for egg laying by the moths and small maize seedlings were also placed inside the cage as substrate for oviposition. A piece of cotton soaked with 10 per cent honey solution was provided as a source food for the adults.

Insect Rearing on different Diets

The study was conducted on two different diets, *i.e.,* artificial diet and natural diet (maize). The newly hatched larvae were released into the separate plastic boxes containing natural diet and artificial diet, prepared (Table 1).

Table 1

Composition of artificial diet for rearing *Spodoptera frugiperda* larvae (Ballal *et al.,*1995)

Groups	Ingredients	Quantity
Fraction A	Chick pea flour	10gm
	Yeast	10gm
	Sorbic acid	1gm
	Casein	30gm
	Methyl para hydroxy benzoate	2gm
	Maize leaf powder	10gm
	Ascorbic acid	3.25gm
	Vitamin capsule	2
	Multivitamin drops	2
	Streptomycin sulphate	0.25gm
	Formaldehyde	2ml
	Distilled water	400ml
Fraction B	Agar-agar	10gm
	Distilled water	400ml

Diet Composition and Preparation

The ingredients of the artificial diet for *S. frugiperda* are listed in Table 1. The ingredients of fraction 'A' were mixed thoroughly in a blender with 400 ml of lukewarm distilled water. Agar-agar (fraction 'B') was weighed separately, dissolved in 400 ml of lukewarm water and boiled in a 1L steel container, cooled to 50^0C. The ingredients of fraction 'A' and 'B' were then added and the mixture stirred well. The mixture was poured into the small plastic vials of size 25 ml and allowed to solidify at room temperature (27-30^0C).

The larvae were reared in group up-to third instar on both natural and artificial diet separately. Then the larvae were transferred individually into small plastic vials of size 25 ml containing artificial diet and natural diet separately in order to avoid larval cannibalism. The natural diet was replaced every day and the artificial diet was replaced once in every five days. The larvae were reared till pupation on respective diets. Later, the pupae were harvested and the emerging adults were used to study the reproductive biology.

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Reproductive Biology of *S. frugiperda* as influenced by the Diets

To study the reproductive biology, pairs of freshly emerged adults of *S. frugiperda* obtained from the respective diets was released into the mating cum oviposition container (small plastic boxes measuring 15 cm in diameter). Small pieces of maize leaves were provided as substrate for oviposition inside the oviposition container and 10 per cent honey solution was provided as adult food. The total number of eggs laid on each day was counted and maize leaves were replaced daily in the ovipositional container. Pairs of male and female moths were released individually in mating cage and parameters such as mating success, frequency of mating, pre-mating period, duration of mating and time of mating were recorded until their natural death. Mating success and frequency of mating were recorded based on the number of spermatophores present in the bursa copulatrix of the female moth.

The data was analyzed using a chi-square test and related samples by Wilcoxon signed rank test. IBM SPSS Statistics version 20 was used to analyse the data.

Results and Discussion

The reproductive characteristics of *S. frugiperda* were studied from adult eclosion to natural death of the moths. Results showed that *S. frugiperda* completed its life cycle on both natural and artificial larval diets. A significant difference was observed in the mating success of *S. frugiperda* between the diets. Mating success rate was significatntly high in moths from natural diet (95.55%) compared to the moths obtained on artificial diet (86.66%) (Table 2). In the current study, multiple mating was observed in *S. frugiperda, i.e.,* the female moth mated with the male moth multiple times (up to 4 times) on both the diets. It was noticed that 35.55 per cent of female moths on artificial diet and 28.88 per cent of female moths on natural diet had one spermatophore per female moth, suggesting single mating in these moths. Further, 33.33 and 46.66 per cent of female moths were found to have two spermatophores on artificial diet and natural diet, respectively suggesting two times mating. Very few females were observed with three and four spermatophores in bursua copulatrix, indicating that there were fewer chances of three and four times of mating on both the diets (Table 3). The study also revealed that a female moth mated with the same male multiple times. Similar mating patterns also been reported in other studies on *S. frugiperda* (Snow and Thelma, 1967). Simmons and Marti (1992) recorded multiple matings in fall armyworm where, on an average male mated 6.7 times and female moths mated 3.7 times during their lifespan. Xu *et al*. (2018) studied multiple matings in *S. litura* and noticed maximum of 8 times mating with the mean number of 6.25 matings when males were given with a new virgin female moth till their death.

Table 2

Influence of larval diet on mating success in *S. frugiperda*

Diet	No. of mated female moths*	Mating success (%)	Chi square test
Natural diet (Maize)	43	95.55	p = 0.01
Artificial diet	39	86.66	

Values within the column are significantly different at 1% level of probability; *n = 45 pairs

The moths of *S. frugiperda* did not mate on the day of emergence; the mating process was initiated on the second day after emergence. The highest mating percentage was observed at two days after emergence, *i.e.,* 58.00 and 62.85 per cent on artificial and natural diet, respectively (Table 4). The results are in accordance with the findings of Simmons and Marti (1992) where they observed frequent matings during the first three nights after their emergence and number of matings decreased with age in *S. frugiperda*. However, according to few studies, maximum mating occurs on the same night of emergence and mating success is dependent on the availability of a partner at the time (Ouye *et al.,* 1965).

Along with the mating status, the duration of mating was also observed. The mating duration was not uniform throughout the mating process. In

TABLE 3

Influence of larval diet on frequency of mating in *S. frugiperda*

No. of matings	Proportion of mating on artificial diet*(%)	Proportion of mating onnatural diet, maize* (%)	Related samples Wilcoxon signed rank rest
1 time mated	35.55 (16)	28.88 (13)	P = 0.89
2 times mated	33.33 (15)	46.66 (21)	
3 times mated	11.11 (5)	8.88 (4)	
4 times mated	6.66 (3)	11.11 (5)	
5 times mated	0 (0)	0 (0)	
Unmated	13.33 (6)	4.44 (2)	

Values within the column are not significantly different at 5% level of probability
Note: Figures in the parenthesis indicates number of pairs
* n = 45 pairs

TABLE 4

Mating status of fall armyworm at different days after emergence

Pre-mating period (Days)	Proportion (%) of mated moths (artificial diet)*	Proportion (%) of mated moths (natural diet, maize) #	Related samples Wilcoxon signed rank rest
2	58 (29)	62.85 (22)	P = 0.67
3	22 (11)	20 (11)	
4	10 (5)	8.5 (3)	
5	10 (5)	8.5 (3)	

Values within the row are not significantly different at 5% level of probability
Note : Figures in the parenthesis indicate number of pairs
* n = 50 pairs #n = 35 pairs

TABLE 5

Influence of larval diets on mating duration in *S. frugiperda*

Duration of mating (Minutes)	Duration of mating on artificial diet* (% proportion)	Duration of mating on natural diet # (% proportion)	Related samples Wilcoxon signed rank rest
< 20	0 (0)	5.71 (2)	P = 0.67
20 - 30	10 (5)	14.28 (5)	
30 - 40	18 (9)	17.14 (6)	
40 - 50	56 (28)	28.57 (10)	
50 - 60	16 (8)	8.57 (3)	
60 - 70	0 (0)	17.14 (6)	
70 - 80	0 (0)	8.57 (3)	

Values within the rows are not significantly different at 5% level of probability
Note: Figures in the parenthesis indicate number of pairs
* n = 50 pairs #n = 35 pairs

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TABLE 6

Influence of larval diets on time of mating in *S. fruigiperda*

Time of mating	Proportion of pairs mated (artificial diet)*	Proportion of pairs mated (natural diet) # (%)	Related samples Wilcoxon signed rank rest
6 am - 8 am	0 (0)	0 (0)	P = 0.14
8 am -10 am	0 (0)	0 (0)	
10 am -12 pm	0 (0)	0 (0)	
12 pm - 2 pm	0 (0)	0 (0)	
2 pm - 4 pm	0 (0)	0 (0)	
4 pm - 6 pm	0 (0)	0 (0)	
6 pm - 8 pm	0 (0)	0 (0)	
8 pm -10 pm	0 (0)	0 (0)	
10 pm -11 pm	0 (0)	0 (0)	
11 pm -12 am	16 (8)	8.5 (3)	
12 - 2 am	28 (14)	28.57 (10)	
2 - 4 am	46 (23)	45.71 (16)	
4 - 6 am	10 (5)	17.14 (6)	

Values within the row are not significantly different at 5% level of probability

Note: Figures in the parenthesis indicates number of pairs mated; * n = 50 pairs #n = 35 pairs

S. frugiperda, the total duration of mating ranged from 20-30 minutes to 50-60 minutes on artificial diet and less than 20 minutes to 70-80 minutes in on natural diet (maize). The mating duration did not vary significantly between moths reared on artificial diet and those reared on natural diet (p = 0.67) (Table 5). Similar results were observed by Simons and Marti (1992) observed, most of the matings (80%) lasted longer than 45 minutes with mean duration of 30 minutes in *S. frugiperda*. Further more, copulation durations in many insect species are known to be influenced by intrinsic and extrinsic factors (Katsuki and Miyatake, 2009).

Time of mating in *S. frugiperda,* was observed from 6 a.m. to 6 a.m. at 2h interval. Moths initiated the mating process between 11 p.m. and 12 a.m. and peak mating was observed between 2 a.m. to 4 a.m. on both the diets. Moths of *S. frugiperda* did not mate during the day time (Table 6). In contrast, Simmons and Marti (1992) observed mating throughout the night and reported that, mating in fall armyworm begins at 21.00 hours and 73-84 per cent of the mating occurred between 22.00 and 03.00 hours. In other related species, *Spodoptera exempta,* mating only occurred between 12.30 and 03.00 hours and males of *S. exempta* take at least 48 hours after emergence for responding to males (Khasimuddin, 1978). According to Fukazawa and Sugino (1972) mating in *S. litura* occurred at 1-2 h after sunset and more than 80 per cent of the pairs copulated before midnight.

TABLE 7

Influence of larval diets on time of oviposition in *S. frugiperda*

Time period	Proportion of eggs laid	
	Artificial diet* (%)	Natural diet, maize#(%)
Day time (6am - 6pm)	8.81 (68)	9.50 (82)
Night time (6pm - 6am)	91.18 (703)	90.49 (781)

Note : Figures in the parenthesis indicates number of eggs/female; * n = 50 pairs # n = 20 pairs

The Egg Laying Pattern in *S. frugiperda*

The female moth laid eggs during both daytime and night time. However, maximum numbers of eggs were deposited in dark conditions on both

artificial diet (91.18%) and natural diet (90.49%). Non-significant difference was found between the diets with regard to the period of egg laying (Table 7). Nocturnal moth's sexual activities (courtship, calling, mating and oviposition) take place during the scotophase. Adult moths eclose at dusk and no matings take place during the night of eclosion (Li *et al*., 2012). Similar oviposition behavior with maximum number of eggs laid during scotophase has been also reported in *Dendrobium punctatus* (Zhau *et al*., 2016).

The oviposition in *S. frugiperda* was noticed on the third day after emergence of moths and it was continued till the eighth day after emergence. Notably, the maximum numbers of eggs were deposited on the third day after emergence in both artificial and natural diet and gradually decreased thereafter. An average of 863 eggs per female moth was observed in natural diet and 771 eggs per female moth in artificial diet (Table 8). Hatching percentage was recorded was high on natural diet (97%) and also on artificial diet (94%). There is no significant difference in the egg laying pattern and hatching percentage when larvae were reared on two different types of diets (Table 8).

TABLE 8

Oviposition pattern in *S. frugiperda* as influenced by the rearing diet

Days after emergence	Proportion of eggs per female moth (%)		Related samples Wilcoxon signed rank rest
	Artificial diet* (%)	Natural diet, maize#(%)	
1	0 (0)	0 (0)	P = 0.12
2	0 (0)	0 (0)	
3	15 (120)	20.85 (180)	
4	28.66 (221)	29.31 (253)	
5	22.82 (176)	24.82 (197)	
6	22.5 (174)	15.17 (131)	
7	7.91 (61)	8.50 (74)	
8	2.46 (19)	3.2 (28)	
9	0 (0)	0 (0)	
Mean no. of eggs per female moth	771	863	
Hatching (%)	94	97	

Values within the row are not significantly different at 5% level of probability; *Note*: Figures in the parenthesis indicates mean number of eggs per female moth; * n = 50 pairs #n = 20 pairs

In conclusion, the results suggested that rearing diets do not significantly alter the reproductive biology paramerters *viz.,* mating behavior, mating duration, egg laying pattern, fecundity and egg hatching in *S. frugiperda*. Therefore, the larvae can be reared either on natural diet or artificial diet in the laboratory depending on the convenience.

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