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Studies on Growth and Developmental Stages of invasive Fall Armyworm *Spodoptera frugiperda* (J.E. Smith) on Maize in India

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ABSTRACT: The present study was designed to understand the FAW biology, growth and development behaviour under tropical conditions. FAW rearing under controlled laboratory conditions under Indian subcontinent conditions is a challenging task as it is a invasive pest emerging as a serious threat for maize cultivation. FAW rearing under laboratory conditions were done during July-September 2021 at Maize research Centre, Hyderabad using a natural maize-based diet under controlled conditions. Field collected FAW egg masses were reared using maize leaf and stalk based diet with controlled temperature at 27 ± 1 °C, 60 ± 5% relative humidity and 12 h day length. The neonates were used for the laboratory studies to document the FAW biology. The study generated most useful insights on FAW growth and developmental stages. The larval stage recorded six instars with longest mean duration of 23.60 days among the other stages of life cycle. The mean duration of egg, pupa and adult stages were recorded 4.00, 8.90 and 8.30 days, respectively.

Keywords: Fall army worm, biology, egg, larva, pupal stages, natural diet and life cycle.

INTRODUCTION

The fall armyworm, *Spodoptera frugiperda* (J.E. Smith) is a polyphagous pest and inflicting huge crop lossesin maize and other major cereal crops (Abrahams *et al*., 2017; Guimapi *et al*., 2022). FAW was first reported in India in 2018. The spread of FAW to different Africa and Asia countries with existing abiotic and biotic production constraints threatening the maize production and productivity (Niassy *et al*., 2021; Sokame *et al*., 2021). In Africa, FAW reported to be causing 21 to 53% yield losses in maize production (Prasanna *et al*., 2018). FAW infestation coupled with abiotic and biotic stresses reported to cause a yield loss of 80% or complete crop failures in maize and sweet corn production (Overton *et al*., 2021; Stokstad, 2017). The yield losses caused by the fall armyworm have threatened food security and the livelihoods of more than 500 million people who depend on maize production and products (Kasoma *et al*., 2021; Macauley and Ramadjita 2015).

In India, the fall armyworm presence was first confirmed in May 2018 by the University of Agricultural and Horticultural Sciences, Shivamogga, Karnataka. Later, transboundary pest moved within the country and to the surrounding countries, *viz*., Bangladesh (December 2018), Myanmar (December 2018), Sri Lanka (January 2019), China (January 2019), Nepal, Thailand (December 2018), South Korea and Japan (July 2019). The temporal spread of FAW within India has been documented from its first report in May 2018 from Karnataka (Suby*et al*., 2020). FAW moved from peninsular India to the North and North East during 2018 and 2019. FAW incidence has been reported from the northern and northwestern parts of the country as well. FAW has severely infested the maize and other major cereal crop production and value chains.

To study the biology and for mass production of the FAW a customized insectary is necessary, while a controlled-environment facility is needed to document biology studies. Proper understanding of FAW developmental stages are crucial in developing proper management strategies. The larval stage is the most destructive stage of pest because of its voracious feeding. Earlier studies documented the insect feeding patterns and ideal abiotic conditions reported to be temperatures of 24 to 31°C, relative humidity of 52 to 88% and a day length of 12 to 14 h for the controlled rearing of FAW from egg or larval samples collected from maize plants (Jin *et al*., 2020; Koffi *et al*., 2020; Laminou *et al*., 2020; Maruthadurai and Ramesh 2019; Walaa, 2020). FAW undergoes holometabolous metamorphosis. Its life cycle includes egg (2–3 days), larvae (total six instars, 13–14 days), pupae (7–8 days) and adults (7–21 days). FAW has approximately 30–40 days during the warm summer months (daily temperature of ~28ºC), and approximately 55 days in cooler temperatures to complete one life cycle (Prasanna *et al*., 2018; Sharanabasappa *et al*., 2018). Fecundity of the female appears to be affected by variations in biotic (different hosts) and abiotic (temperature, humidity, etc.) factors (Sun *et al.*, 2020).

FAW was recently reported in India; there is a lack of information on pest initiation and progression under local conditions. Further, there is an urgency for resistant hybrids in the region to combat the damage caused by fall armyworm. FAW resistance breeding programs depend on the availability of cheap, reproducible methods for mass rearing, infestation and host screening (Montezan *et al*., 2019; Wiseman *et al*., 1966). Knowledge about mass rearing, infestation and development of the pest and standard screening protocols are prerequisites for successful cultivar development and the introgression of FAW-resistant genes into farmer-preferred and locally adapted maize genotypes. Therefore, this study aimed to study the FAW's developmental stages under controlled environment.

MATERIAL AND METHODS

Description of the Study Site: The study was conducted at Maize Research Centre, Rajendranagar, Hyderabad situated at 17°32′N latitude and 78°40′E longitude. This research area falls under the Southern Agro-Climatic Zone of Telangana under a semi-arid tropical climate with an average temperature of 22°C.

Mass rearing of FAW:

Collection of Eggs: 10 egg masses of FAW were collected from unsprayed maize fields of Maize Research Centre, Rajendranagar, Hyderabad. Egg masses were collected using perforated plastic containers from field-grown maize hybrid DHM 117. Fresh eggs were carefully scraped off from the leaf blades and collected into plastic containers. FAW eggs were identified following the description procedure of Deole and Paul (2018) as small, circular masses of mostly white eggs. Sampled FAW eggs were grown in rearing jars, as detailed below.

Laboratory rearing procedure: The field collected egg masses were allowed to hatch in plastic containers containing maize leaves as a diet for newly hatched neonates. Then the larvae were reared in plastic jars containing tender baby corn pieces and tender leaves. The baby corn was washed with 5% sodium hypochlorite and rinsed twice or thrice with water to prevent contamination before being used as feed. The larvae from the third instar were transferred to individual jars covered with the muslin cloth to avoid cannibalism. The eggs and larvae were grown at temperatures of approximately 27 ± 1°C, relative humidity of 60 ± 5%, an average day length of 12 h. and the natural diet replaced for every two days, plastic jars were cleaned with a 5% hypochlorite solution to prevent microbial growth between each successive diet change. The pupae developed were distinguished as loose, oval cocoons that preceded the mature stage of the FAW. The temperatures and relative humidity during the pupal stage were adjusted to 26°C and 70 ± 5% using an internal heating system and humidifier, respectively. These conditions were conducive to pupal development. Male and female FAW pupas were transferred into separate jars for adult emergence. The adult moths in the cage were allowed to mate for subsequent oviposition. FAW moths were supplied with a 5% sugar solution by soaking cotton wool balls in a sugar solution and placing these inside the jars. After mating, the eggs were laid on the muslin cloth. The eggs were collected by using a camel brush. The fresh eggs of the FAW were carefully scraped off from the surface of the muslin cloth using a clean spatula and transferred into new plastic jars possessing tender maize leaves for hatching. New larval neonates that hatched from the eggs were used for further rearing.

Determining the Developmental Stages of FAW. The larvae obtained from above procedure were monitored to document metamorphosis and the developmental stages. Adult insects were allowed to mate and produce a new generation of FAW for further monitoring. The developmental stages of FAW (egg, larva, pupa and adult) and the time taken to complete each stage were recorded by observing the morphological features and behaviour and recording the number of days taken for each stage. FAW eggs were identified based on the description procedure given by Deole and Paul (2018). Similarly, larvae were identified by the procedure given by Luginbill (1928).

RESULTS AND DISCUSSION

FAW growth and development under Indian subcontinent Conditions: Fall armyworm life cycle contains four different stages. They are egg, larva, pupa and adult, and the duration of each stage and their specific features are well defined using the controlled reproduction under specific conditions in India. Developmental studies will enable for accurate identification of the pest and subsequent distinction from other related lepidopteran pests.

Additionally, the sub-stages of egg and larva of FAW life cycle are recorded and summarized in Fig. 2. The FAW egg, larval, pupa and adult moth stages had recorded varying mean durations under the current controlled conditions. The larval stage recorded with six instars having the longest mean duration of 23.60 days. Similarly, the mean duration of egg, pupa and adult moth stages lasted for 4.00, 8.90 and 8.30 days, respectively (Table 1 Duration of Fall army worm life cycle stages under laboratory conditions).

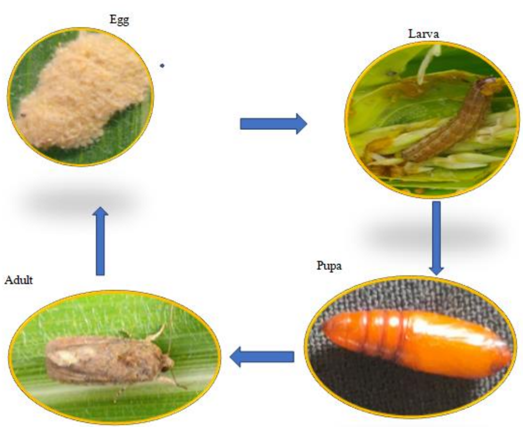

Fig. 1. Life Cycle of Fall army worm.

Within the larval stage comparison, the first instar was the shortest with 2 days duration, followed by the second and third instars with 3 days, fourth instar 4 days, fifth instar 5 days and sixth instar 8 days. Critical transitional phases related to the egg, larva and pupal stages were also recorded (Fig. 2 Description of developmental stages on natural diet (maize leaf) under controlled laboratory conditions). The observed transitional phases included the progressive egg color changes from the blackhead, ecdysis and prepupal phases, which depicted early and late transitions related to the egg, larval and pupal stages, respectively.

Stages	Features	Descriptions	Duration	Appearance
Egg	With three sub-stages distinguishable by color changes from green to cream white to black	Eggs are covered by scales from the female moth. They appear green to grey for 12 h and begin to darken.	**1 day**	
		Cream-white to pink, transitioning into brown	**1 day**	
		Dark egg mass approaching hatching. Egg mass appears grey to black before hatching	**1 day**	
Neonate larva	Immobile, circular, and shiny black protrusions in fur-like mass	Black shiny heads are visible as they emerge from the eggshells.	**1 day**	
Neonate larva	Feeding stage: with six substages distinguishable by body color changes and feeding patterns	Newly hatched larvae on a tender maize stalk remain dormant for about 5 to 6 h.	**2 days**	
		First instar larvae on a maize leaf. Small bodies and shiny blackheads.	**1 day**	
		Second instar larva on a maize leaf with a cream to pale white body and blackhead	**3 days**	

		Third instar larva: light brown, begins to turn green after feeding on leaves	**3 days**		
		Fourth instar larva: dark green or dark brown body	**4 days**		
		Fifth instar larva: well defined brown body with an inverted Y marking on the head	**5 days**		
		Sixth instar larva surrounded by frass. Grey or dark brown body with fully defined segments and head markings.	**8 days**		
	Inactive and compacted body	It stops feeding, and the body becomes short, preparing for pupation. Body segments with defined ridges and markings.	**2 days**		
	Stiff pupal casing with localized circular movements in the head area of the insect	Forms an oval-shaped cocoon using leaf particles. The cocoon gradually changes from green to pink to orange-brown	**9 days**		
Adult	Male moth has conspicuous markings on wings	Female is comparatively plain in appearance with no prominent marks on the wings.	**8 days**		

Fig. 2. Description of developmental stages on natural diet (maize leaf) under controlled laboratory conditions.

Table 1: Duration of Fall army worm life cycle stages under laboratory conditions.

Sr. No.	Batch	Egg	I Instar	II Instar	III Instar	IV Instar	V Instar	VI Instar	Pupa	Adult
1.	Egg mass 1	3	2	3	4	5	3	8	9	8
2.	Egg mass 2	4	3	2	3	4	4	8	10	9
3.	Egg mass 3	5	2	3	4	4	3	7	8	8
4.	Egg mass 4	4	3	3	3	3	4	7	9	8
5.	Egg mass 5	4	2	2	4	3	5	6	9	9
6.	Egg mass 6	5	3	3	3	4	3	8	9	8
7.	Egg Mass 7	4	3	3	4	4	5	7	9	8
8.	Egg Mass 8	4	2	3	4	4	3	8	8	8
9.	Egg Mass 9	3	2	2	3	5	4	7	9	9
10.	Egg Mass 10	4	3	3	3	3	3	7	9	8
Mean		4.00	2.50	2.70	3.50	3.90	3.70	7.30	8.90	8.30

The present study on the growth and development of FAW under laboratory conditions was discussed hereunder.

FAW was successfully reared in plastic containers using field-collected egg masses on natural diet. The present study recorded that average duration of the larval period, including six instars was the longest, with a mean duration of 23.60 days and an average; the egg, pupa and moth stages lasted 4.00, 8.90 and 8.30 days, respectively; however, the average durations of the FAW life cycle stages except the larval stage, differed slightly from the report of Kasoma *et al.* (2022) who reported mean duration of 2.40, 24.3 and 19.7 days for the egg, larva and pupal stages respectively. Similarly, the present results also differed from Montezano *et al*. (2019) who reported mean duration of 2.60, 13.73 and 9.24 days for the egg, larva and pupa stages respectively. Most freshly laid eggs collected from maize fields hatched within an average of four days, but a few egg batches did not hatch this may be probably due to unfavourable laboratory conditions, parasitism or egg masses that trapped the neonates that failed to emerge successfully due to injury as described by Du Plessis *et al*. (2020); Luginbill, (1928). Deole and Paul (2018) reported FAW moth life span as five to seven days under field conditions, which is proximity to the present study report (8.30) under controlled conditions in Indian conditions. Similarly, Prasanna *et al*. (2018); Sharanabasappa *et al*. (2018) studied the biology and reported that FAW undergoes holometabolous metamorphosis. Its life cycle includes egg (2–3 days), larvae (total six instars, 13–14 days), pupae (7–8 days) and adults (7–21 days). FAW has approximately 30–40 days during the warm summer months (daily temperature of ~28ºC), and approximately 55 days in cooler temperatures to complete one life cycle. These differences may be attributable to the laboratory conditions like temperature, relative humidity and photoperiod used in this study, which were significantly different from those used in the earlier studies.

Further, in the present study, six larval instars were observed, as documented in previous studies of Acharya *et al*. (2020); Deole and Paul (2018); Prasanna *et al*. (2018); Sharanabasappa *et al*. (2018); Montezano *et al*. (2019). Although the laboratory conditions in this study enabled the successful observation of all the life cycle stages, a few deviations were observed from the earlier literature. Like, oviposited eggs were mostly heaped rather than layered, as occurs naturally, and most of the pupae appeared fragile compared with those collected from maize fields. The heaping of eggs in the laboratory may be attributed to the effect of the confinement of the female moths within the rearing cages. The fragile appearance could be associated with the absence of soil particles typically used by the insect for developing the pupal casing under field conditions. Fecundity of the female appears to be affected by variations in biotic (different hosts) and abiotic (temperature, humidity, etc.) factors (Sun *et al.*, 2020).

The larval period being the longest stage of the life cycle with 23.60 days, is the most damaging stage of the FAW, within which temperature and diet are very critical factors for growth and development. Lopez *et al*. (2019) reported that FAW larvae required minimum temperatures ranging between 8 and 10°C for survival in Mexico. In South Africa, the minimum temperature for FAW larval survival was reported to be 12°C (Du Plessis *et al*., 2020). Santos *et al*. (2003) observed rapid FAW larval growth and shorter instars under higher temperatures. In this study, the final instar recorded unusually longduration of 7.30 days. Feeding during the larval stage progressively increases, with the first three instars observed to only scrapings, windowpane and punch small holes through maize leaves. In contrast, the fourth to sixth instars are responsible for more severe damage, including ragged appearance, chopping of whorls, eating whole-leaf portions and destroying small plants.

In FAW monitoring and surveillance, farmers should monitor their fields for scrapings, window panes, skeletonized maize leaves, which would indicate the very first presence of FAW because very few lepidopteran species cause such damage (Luginbill, 1928; Prasanna *et al*., 2018). The FAW larva is a gregarious feeder during its last instar stage, usually the sixth instar. Instar stage changes can be determined by the careful monitoring of body colour, body length and head capsule width. Following the larval stage of voracious feeding, the FAW larva moves into a pupal stage. Although the duration of pupation observed in this study was 8.90 days, previous studies have reported a maximum pupation period of 45 days (Luginbill, 1928).

The adult FAW moths were easily distinguishable by their colour patterns, with female moths being dull-coloured compared with the male moths (Capinera, 2002; Deole and Paul 2018; Luginbill, 1928). FAW were dormant during the day and only moved when persistently agitated. They were observed to feed and lay eggs late at night. This agrees with earlier reports by Luginbill (1928), who found that laboratory-reared FAW moths laid eggs after midnight. Both male and female moths were observed to lose vitality progressively.

CONCLUSIONS

The present study documented the salient features of FAW growth and development stages under controlled conditions with few specific observations in egg laying pattern under laboratory conditions. Third instar larvae identification is crucial for screening the germplasm and damage pattern, the present study has given clear photographs and insights for the identification hence, this will useful for resistance breeding programmes.

FUTURE SCOPE

This present study on growth and developmental stages of FAW is useful for mass rearing techniques. Resistance breeding requires mass rearing under laboratory conditions and identification of different larval forms for screening of vast germplasm. So the present study will be very much useful for the resistance breeding programs which are very early stage in the country at present.

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Conflict of Interest. None.

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