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Research Article

Basic Developmental Characteristics of the Fall Armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), Reared under Laboratory Conditions

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The life cycle of the invasive alien insect pest, fall armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith), was studied using a colony established from field-collected larvae. Eggs, neonate larvae, and newly emerged adult moths were used in experiments to investigate the basic biology of the FAW. Adult females laid up to 1184 eggs with a mean of 469 ± 22 eggs per female. The incubation period of eggs and percentage hatchability were 2-3 days and 80–87%, respectively. The mean larval lengths from the first to the sixth instar were 4.63, 6.60, 9.76, 15.86, 25.13, and 27.81 mm, respectively. The mean larval weights were 0.003, 0.019, 0.045, 0.050, 0.060, and 0.067 g, respectively, for the six instars. The mean width of the head capsule of the sixth instar larva was 2.76 mm. The total larval duration throughout the six instar stages was 16–18 days, while the mean pupal weight was 0.25 ± 0.001 g and 0.35 ± 0.011 g for males and females, respectively. The mean pupal length was 14.3 ± 0.16 mm for males and 17.2 ± 0.14 mm for females. Pupal duration ranged from 8 to 14 days, with a mean of 10.35 ± 0.26 days, while the pupal emergence rate ranged from 60 to 94%, with a mean of 80.25 ± 1.28%. The life cycle of males lasted 33–44 days and that of females lasted 36–49 days under laboratory conditions. Adult copulation occurred between 8 and 11 pm, with the peak occurring at 9 pm. This study provides baseline information about the biology of the FAW. Apart from being an important reference point for future research on the FAW, the data provided would aid FAW management decision-making.

1. Introduction

The fall armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), is native to tropical and subtropical regions of the Americas, extending from Argentina to the United States of America [1, 2]. It is a polyphagous insect pest that feeds on several plants including economically important crops like maize, millet, cotton, and soybean [3–6]. It first invaded Africa through Nigeria and São Tomé in early 2016 and has since been regarded as an invasive insect pest in Africa [7]. The spread of the FAW in Africa reached Ghana in April 2016, and by March 2017, it invaded all the administrative regions of the country, causing massive destruction to over 14,000 hectares of maize and sorghum farms worth $163 million [8, 9].

The FAW has, without a doubt, become the most economically important insect pest of cereal crops in Ghana at the moment [10–13]. Current control relies heavily on insecticides including cypermethrin, lambda-cyhalothrin, chlorpyrifos, and emamectin benzoate [14–17]. A recent

study in Ghana has shown that the use of insecticides is the most common control strategy for the FAW [18]. This notwithstanding, across the world including Ghana, biological control of the FAW has been identified as a promising management option [19–22].

A crucial step in achieving biological control of any invasive insect pest is to understand the biology of that pest in its new environment. Once the biology is understood, candidate biological control agents could be scouted for and screened for use. Although the biology of the FAW in its native range is well known, environmental and local cropping conditions can interfere and affect the biology and behaviour of the pest in new environments. Research on the biology of the FAW under various environmental conditions is therefore very important if the effect of these environmental conditions on the development and growth of the pest in Ghana is to be determined. For instance, at temperatures of 20, 25, and 30°C, the average incubation period for the eggs of the FAW were 6.9, 3.4, and 2.1 days, respectively, while the larvae lasted 38.5, 23.7, and 18.6 days, respectively, at these temperatures [23]. The average pupal duration was also 22.5 days at 20°C, 9.4 days at 25°C, and 7.7 days at 30°C, respectively [23]. The average maturation time for the adult female FAW varies and oviposition decreased as the temperature increased from 4.8 days at 20°C to 2.1 days at 30°C [23].

Understanding the biology of the FAW in Ghana will enhance the development of pest-specific management strategies that are cost-effective and environmentally sustainable. Therefore, this study reports the development and growth of the FAW under specific environmental conditions in the laboratory. Knowledge from this study will be useful for the management of the FAW. This in effect will drive the development of an effective pest management regime that suits a particular geographical area.

2. Materials and Methods

The study was conducted at the Radiation Entomology and Pest Management Centre of the Biotechnology and Nuclear Agriculture Research Institute (REPMC-BNARI) of the Ghana Atomic Energy Commission (GAEC). A FAW colony was set up with field-collected larvae from all the agro-ecological zones in Ghana, i.e., Sudan savannah, Guinea savannah, coastal savannah, transitional zone, semi-deciduous rainforest, and high rainforest [24]. Larvae were picked from growing maize plants with a pair of flat Duckbill forceps and placed individually in 3 cm × 5 cm × 4 cm plastic salad containers and covered with a fine mesh net to allow for ventilation before being transported to the insectary. The larvae were fed with freshly cut maize leaves daily and held till pupation. The moisture in the maize leaves was sufficient to hydrate the larvae. Water was sprinkled on the leaves every other day to keep them fresh. Newly pupated FAWs were placed in 5 cm × 5 cm × 10 cm pupal glass containers with net covers at the top till adult emergence. The insectary was maintained at a temperature of 27 ± 2°C, a relative humidity of 75 ± 5%, and a photoperiod of L12 : D12. Upon pupation, 20 pupae consisting of 10 males and 10 females were placed in a 5 cm × 10 cm glass pupal container with a net cover at the top until they emerged as adults. The newly emerged adults were put in a 40 cm × 50 cm × 40 cm insect cage and used to establish a laboratory colony from which experiments were conducted to investigate the biology of the FAW. The cage had a wooden frame placed on a plywood base and was covered on all sides with a net (1 mm^2 mesh size). A cotton ball was soaked with 10% sugar solution only and placed in the cage as food for adult moths. Young potted maize seedlings with heights of about 8–10 cm were placed in the cage to serve as oviposition substrate for female moths. Eggs deposited on the maize seedlings were harvested daily by cutting the part of the maize leaf where the eggs were deposited. The collected eggs were incubated at a temperature of 28°C until hatching.

Newly hatched larvae were placed in 300 ml plastic larval rearing cages with net covering and fed with freshly cut maize leaves daily until pupation. The feed was changed, and enough feed (~20 g) was provided every other day to avoid cannibalism. This continued until the colony was sizeable enough (approximately two thousand individuals) for conducting the experiments. The study was conducted from March to November 2021.

2.1. Egg Duration. Five hundred eggs were divided into five batches, with each batch of one hundred eggs, placed in a labelled sterilized Petri dish, and incubated for three days. The incubated eggs, kept under laboratory conditions of 27 ± 2°C temperature, 75 ± 5% relative humidity, and L12 : D12 photoperiod, were monitored daily until hatching was observed. The number of eggs that hatched in each batch was recorded and used to calculate the percentage hatchability.

2.2. Larval Duration and Development. Forty neonate larvae were placed in a 300 ml larval plastic container with net covering the top. Five replicates of this were set up. The larvae were fed with freshly cut maize leaves every other day and monitored daily until pupation. No artificial diet was used. Changes in the larval length and weight as they progressed from one larval instar to another were recorded. The larval weight was measured with a Sartorius analytical scale (AX124 max 120 g, Sartorius Lab Instruments GmbH & Co. KG, Göttingen, Germany), while the larval length and the head capsule width were measured using a digital Leica stereomicroscope (EZ4 HD, Leica Microsystems, Schweiz, AG, Heerbrugg, Switzerland) fitted with an inbuilt camera for capturing images and with the capability of taking linear measurements with the Leica application software, version 3.4.0. The full-length images of the larvae were captured at a magnification of 20× for larval length measurement, while images of the head capsule were captured at a magnification of 25× for head capsule width measurement. Ten prepupae in each pupal glass container were set for percentage pupation and monitored for 24 hours.

2.3. Pupal Duration and Development. Forty newly formed pupae (1-2 day-old) were put into each of five pupal glass containers and monitored until adult moth emergence. The

number of days from the onset to the end of pupation was recorded and used to calculate the pupal duration and the percentage pupal emergence. The pupal length (Figure 1) and weight were also recorded using the digital Leica stereomicroscope and a Sartorius analytical balance, respectively.

2.4. Adult Longevity. Fifty adult male and 50 female (1M: 1F) moths were collected with an aspirator and released into separate adult cages. A cotton ball was soaked with 10% sugar solution and placed in each cage as feed. The number of male and female adults in each cage that died was recorded each day until the last moth in each cage died. These data were used to indicate of adult longevity.

2.5. Adult Starvation. The period an unfed newly emerged adult lived until it died was used as a proxy for its energy reserve. Unfed newly emerged adult male ($n = 50$) and female ($n = 50$) moths were released into adult cages. The number that died was recorded on a daily basis.

2.6. Adult Coupling Time. Forty newly emerged adults were paired (male and female) in cages with dimension 30 cm × 20 cm × 30 cm and monitored for copulation activities such as courtship and mounting of the female by the male. The period and exact time of the courtship and mounting behaviours were recorded daily for three days.

2.7. Female Fecundity. Newly emerged adults ($n = 40$) were set up as couples in cages (30 cm × 20 cm × 30 cm) for 48 hours and fed with 10% sugar solution. After 48 hours of copulation, the number of egg masses per female and the number of eggs per egg mass were recorded. All experiments were replicated four times and over different generations.

2.8. Data Analysis. Fall armyworm life stages (egg to adult mortality), preoviposition period, oviposition period, adult survival after starvation (energy reserve), and mortality were analyzed using descriptive statistics. Data on the larval duration, larval length, and head capsule width were subjected to one-way analysis of variance at 95% confidence intervals and a 0.05 significance level (Minitab v.17, Minitab, State College, PA, USA). Means that were found to be significantly different were compared using Tukey's pairwise mean separation test (Minitab). Moreover, Fisher's pairwise LSD comparison was used to separate mean head capsule width. The two-sample *t*-test (Minitab) was used to compare male and female adult lifespans in the maize leaf-fed and starved (energy reserve) tests.

3. Results

3.1. Eggs. Adult female moths laid their eggs in clusters or masses. Some of the eggs were in single layers (Figure 2(a)), while others were in multiple layers (Figure 2(b)) and were pale green when freshly laid (Figure 2).

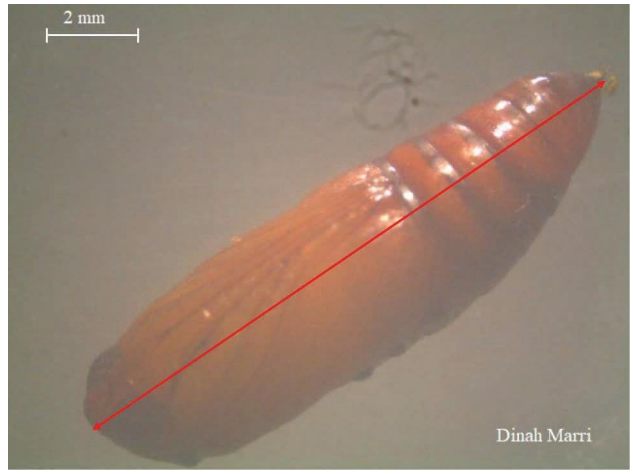

Figure 1: Fully formed pupa of *Spodoptera frugiperda* showing a straight-line measurement of the length.

The eggs turned cream in colour as they matured and finally to black just before hatching. Several egg masses were laid by individual female FAW, and the number of eggs per egg mass varied. Females laid up to 1184 eggs over their lifetime with high percentage hatchability. It took eggs a maximum of three days to hatch (Table 1).

3.2. Larvae. Newly emerged first instar larvae were greenish with a black head capsule (Figure 3(a)). The second instar larvae turned greenish brown after three to four days (Figure 3(b)). The third instar larvae were brownish with three lateral lines (Figure 3(c)), while the fourth to sixth instar larvae were similar to one another (Figures 3(d)–3(f)), being dark brown with lateral and dorsal lines. They also had elevated spots on the body with spines on them. The fourth to sixth instar larvae also had four dots at the end of the last abdominal segment. Close visual examination of the epidermis of the larvae indicated a rough surface that did not feel rough to touch. The head capsule of the sixth larval instar was reddish, with the frontal part of the head capsule having a white inverted Y-shaped line (suture).

The head capsule (Figure 4) width increased as the larvae progressed from younger instars to older instars (Table 2). Similarly, the larval length and weight also increased with the progression of the larval instars (Table 2). Percentage pupation was 82.9%.

3.3. Pupae. When larvae attained maturity, they stopped feeding (prepupal stage) and spun a cocoon around themselves. The cocoon was attached to the tissue paper laid at the bottom of the larval container and remnants of the maize leaf tissue provided as food. The newly formed pupae were greenish but progressively turned brownish and dark brown before finally turning black prior to emergence (Figure 5).

Female pupae were slightly heavier than male pupae and weighed 0.25–0.45 g. The male pupae weighed 0.20–0.30 g. The pupal length ranged from 1.3 to 1.6 cm for males and 1.5 to 1.9 cm for females. Pupal duration ranged from 8 to 14 days, and percentage emergence was 60–94% (Table 3).

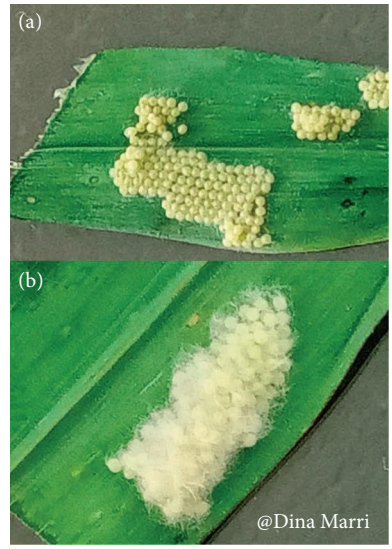

Figure 2: Egg masses of *Spodoptera frugiperda* deposited on the underside of maize leaves in single layers (a) and multiple layers (b)

Table 1: Female *Spodoptera frugiperda* fecundity, egg hatchability, and incubation period under insectary conditions.

Parameters	Mean ± std	Range	
		Minimum	Maximum
No of egg masses/female	6 ± 2	2	11
No of eggs/egg mass	88 ± 4	29	237
No of eggs/female	469 ± 2	117	1184
% hatchability	85 ± 2	80	87
Incubation period (days)	2.75 ± 0.15	2	3

Figure 3: Larval stages of the fall armyworm: (a) first instar, (b) second instar, (c) third instar, (d) fourth instar, (e) fifth instar, and (f) sixth instar.

3.4. Adults. Morphologically, the forewing of the male adult was grey and brown with white patches at the apical regions. It also had a circular spot at the centre of the forewing. The female had uniform greyish brown forewings. Both male and female adults had silver white hind wings with a narrow dark border (Figure 6).

Figure 4: Head capsule of *Spodoptera frugiperda*.

Table 2: Mean head capsule width, larval length, and weight of *Spodoptera frugiperda* larvae.

Larval instars	Head capsule width (mm)	Larval length (mm)	Larval weight (g)
1	—	4.63	0.003
2	0.33	6.60	0.019
3	0.48	9.76	0.045
4	0.86	15.86	0.050
5	1.84	25.13	0.060
6	2.76	27.81	0.067

Figure 5: Pupae of *Spodoptera frugiperda* showing the different stages of pupation. P = prepupa; 1–7 = pupal day 1 to day 7.

Copulation in adult FAWs occurred a few hours after emergence. The copulation occurred mainly between 8 pm and 11 pm, with the peak copulation occurring at 9 pm (Figure 7).

The preoviposition and oviposition periods ranged from 2–3 days and 2–4 days, respectively. Female fall armyworms lived relatively longer than their male counterparts without food. Moreover, at the later stage of starvation, i.e., the 3rd to 5th day, more females survived than males (Figure 8).

Adult fall armyworms were short-lived. Male longevity was 2 – 6 days, while female longevity was 2 – 9 days (Figure 9). A few minutes after emergence, FAW moths moved around in search of food and then mated. Female moths then laid eggs and died off within days.

4. Discussion

The development of an insect species is influenced by the conditions of the local environment within which it occurs [25–27]. For instance, it has been established that temperature is an important factor that influences insect development [28–30]. Generally, higher temperatures speed up and increase the rate of growth of insects [31, 32]. Knowledge of the biology of an invasive insect species in a local environment is therefore essential for the effective management of that species in that environment [33].

In this study, it was observed that the fecundity of the FAW was relatively high and comparable to that of previous studies conducted under similar conditions [27, 34–36]. Moreover, just like the established life cycle of the FAW, each larva in this experiment went through six instar stages. The colour and the head capsule width recorded for the

Table 3: *Spodoptera frugiperda* pupal duration and development.

Pupal parameters		Sample size (n)	Mean ± std. dev	Range	
				Minimum	Maximum
Pupal weight (g)	Male	50	0.25 ± 0.001	0.20	0.30
	Female	50	0.35 ± 0.011	0.25	0.45
Pupal length (cm)	Male	50	1.45 ± 0.016	1.3	1.6
	Female	50	1.72 ± 0.014	1.5	1.9
Pupal duration (days)		100	10.35 ± 0.29	8	14
% emergence		100	80.25 ± 1.281	60	94

Figure 6: Adult male (left) and female (right) *Spodoptera frugiperda*.

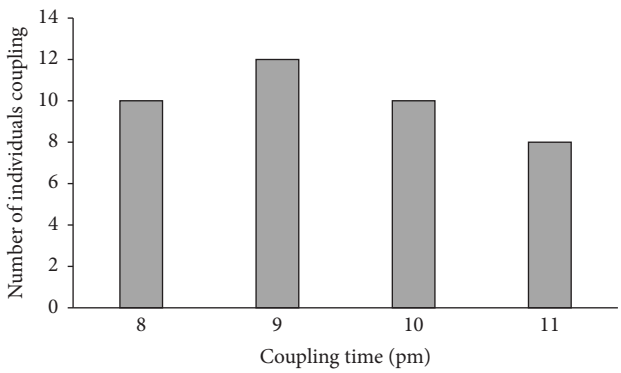

Figure 7: Copulation time for adult fall armyworm moths.

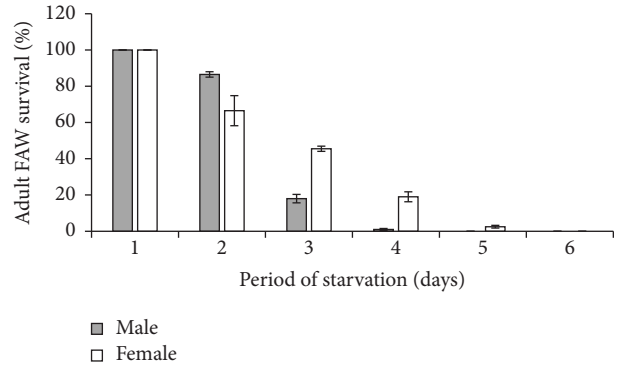

Figure 8: Energy reserve (survival without food) of adult fall armyworms.

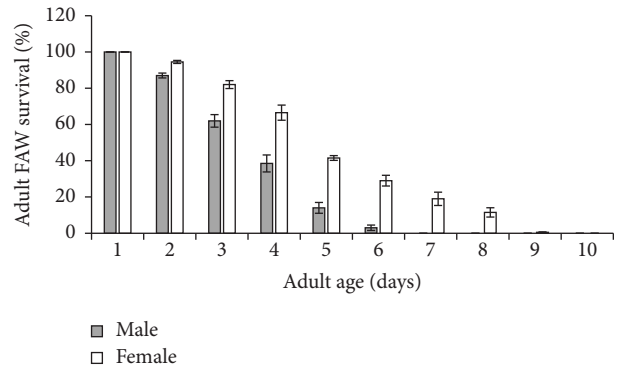

Figure 9: Longevity of male and female adult fall armyworms.

FAW larvae were also similar to findings from earlier studies [37].

Furthermore, the larval duration recorded was only 16 to 18 days probably due to the relatively high temperature under which the study was conducted. In a similar study under similar environmental conditions, it was reported that the total larval duration of the FAW was 15 to 19 days [38]. Other studies conducted at lower temperatures (e.g., [27]) have however reported longer larval durations. Given that the larval stage of the FAW is where physical damage occurs [27, 28, 39], the stage is noted to be most destructive. Additionally, it has been established that the amount of food consumed by the FAW larvae increases as larvae mature [19]. In view of these, long larval durations can result in a high rate of loss of the plant leaf surface area and subsequently affect photosynthesis and carbon dioxide absorption [40]. This can potentially result in low yields and, in some cases, complete yield loss [41, 42].

While this study found the pupal duration to be 8–14 days, previous studies under laboratory conditions (26 ± 2°C, 75–80% RH, and L12 : D12 photoperiod) found the mean pupal duration to be 9–12 days [43]. Moreover, the mean pupal duration in this study was similar to what has been recorded in studies conducted under similar environmental conditions [27, 44]. Previous studies have shown that the diet of insects can influence the duration of their developmental stages [44]. Knowledge of the developmental period of the FAW is therefore useful for the development and implementation of effective management strategies for the pest.

Adult morphological characteristics in this study were similar to those reported in previous studies [45]. Generally, adult FAWs have a relatively short lifespan. A few minutes after emergence, the moths move around in search of food

and mating partners. After mating and oviposition by females, both male and female moths die within days [46]. In this study, females had relatively short preoviposition and oviposition periods and lived relatively longer than their male counterparts. Even when starved, females lived longer than males [47]. This could be because male insects use more carbohydrates due to their activities, i.e., courtship rituals, sexual activity, feeding, and mating [48, 49]. Insects rely on their reserved energy for all their activities when they are not feeding [50].

Findings from this study have demonstrated that the life cycle of male FAWs is relatively shorter than that of females. This is in line with previous studies that reported a total life cycle of 30–40 days. The generation period for FAW is likely to reduce with the current worldwide phenomenon of global warming [51], resulting in more generations per year.

In conclusion, this study provides baseline information about the basic biology of the FAW, an alien invasive insect pest in Ghana. To the best of our knowledge, this is the first comprehensive report on the biology of the FAW in a laboratory condition in Ghana. This insect has become a major pest of maize, an important staple food crop in Ghana. Establishing the biology of this pest will be useful to the sustainable management of the FAW in Ghana. The FAW is likely to be more devastating over time as global temperatures rise. To forestall this potential devastating occurrence, compatible integrated pest management approaches are needed at the early stages of FAW infestation. Knowledge of the biology of the FAW will play a vital role in this. When new strategies based on the biology of the FAW are used to manage it, the frequency of insecticide application will be reduced and consequently reduce the risk of insecticide resistance.

Data Availability

The data and material used in this manuscript are available on request from the first author.

Disclosure

An earlier version of this manuscript has been presented as a preprint in Research Square and can be found at https://www.researchsquare.com/article/rs-1993286/v1.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Michael Osae and Maxwell Kelvin Billah conceived the research. Dinah Marri, Samuel Adjei Mensah, and Maxwell Kelvin Billah collected the data. Samuel Adjei Mensah and John Abraham analyzed the data. Dinah Marri wrote the first draft. Daniel Ashie Kotey and John Abraham reviewed the first draft of the manuscript. All the authors have reviewed and approved the final manuscript before submission for publication.

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