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LIFE CYCLE AND MORPHOMETRICS OF FALL ARMYWORM (*Spodoptera frugiperda*) (LEPIDOPTERA: NOCTUIDAE) ON MAIZE CROP

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ABSTRACT

Fall armyworm (FAW) (*Spodoptera frugiperda* J.E. Smith) is a polyphagous pest, native to North and South America and is invasive in Eurasia and Africa. Life cycle and morphometric studies with the invasive population of FAW in Nepal were conducted under laboratory conditions in Chitwan, Nepal. Gravid females laid an average of 156.25 eggs per egg batch, with an average of 979.43 eggs during their whole lifetime. The average pre-oviposition period was 3.75 days, oviposition period was 3 days, the larval developmental period was 16.31 days, the pupal period was 9.69 days, and the whole life cycle (egg to egg) averaged 33 days. The average length of each instar larva from I to VI instars was 1.5, 3.6, 7.1, 11.6, 18.5, and 34.4 mm, respectively. Head capsule widths for each instar were 0.35, 0.47, 0.8, 1.37, 2.11, and 2.7 mm, respectively for instars first through sixth. Pupal and adult emergence rates were 98.9 and 97.2%, respectively, with a male to female ratio of 1:1.3. Larval mortalities of the I, II, and III instars were 70, 66, and 12%, with no mortality in the IV, V and VI instars. Adult longevity was 20.73 days for males and 22.78 days for females. These findings are useful to design an integrated management protocol of the fall armyworm.

Keywords: Biology, Invasive, Maize, Morphometrics, *Spodoptera frugiperda*

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INTRODUCTION

Fall armyworm (FAW), *Spodoptera frugiperda* (JE Smith), (Lepidoptera: Noctuidae) is native to tropical and subtropical parts of the America (Capinera, 2017). In the United States, this species is a late season pest, hence the name is called fall armyworm (Prasanna et al., 2018). An invasive population of this species was reported in West and Central Africa and in the islands of Sao Tome and Principe in 2016 (Goergen et al., 2016; Devi, 2018). In India, it was first detected in Karnataka State in 2018 (Sharanabasappa et al., 2018) and then spread throughout India (Kalleshwaraswamy et al., 2018). In Nepal, it was first reported in 2019 (Bajracharya et al., 2019) and now is widespread, causing high (43%) losses in maize fields (GC et al., 2019).

While fall armyworm is polyphagous (Casmuz et al., 2010), the population in Africa and Asia prefers maize; however, it is known to feed on more than 353 crops (Tendeng et al., 2019) or wild plant species (Montezano et al., 2018;), including maize, sorghum, rice, potato, cotton, vegetable crops, peanut, Bermuda grass (CABI, 2017, Padhee et al., 2019, Sparks, 1979). Maize and sorghum are the preferred hosts in some parts of the world (Chimweta et al., 2020, Srikanth et al., 2018). It is a sporadic pest, in part, due to its migratory behavior (Hardke et al., 2015), give that it can fly 100-150 km per night (Johnson, 1987). Diagnostic features useful to farmers for identification of this pest include a white inverted 'Y' band on the larva's head, distinct black spots on the body, and four 'dots' that form a rectangle on the eighth abdominal segment (Capinera, 2002, Davis and William, 1992, Kalleshwaraswamy et al., 2018). Crop losses from FAW in maize crops vary from 22 to 67% in different parts of the world (Day et al., 2017; De Groote et al., 2020). Maize farmers in Nepal had been experienced high levels of FAW damage since this pest's invasion (GC et al., 2019). To develop strategies for FAW management, information on its biology, ecology, and migratory patterns is needed (Harrison, 2019). This study was carried out in Nepal, under laboratory conditions. The detailed laboratory life cycle and morphometrics study were first conducted in Nepal as well as in other South Asian countries. This study will provide a first-hand data on FAW life cycle and morphological information of FAW to the entomologist as well as to the IPM practitioners and specialists working in these regions.

MATERIALS AND METHODS

FAW colony management

FAW laboratory colony was maintained in rectangular screen cages (71 cm height, 46 cm width) at the Department of Entomology of Agriculture and Forestry University. The colony was started by collecting eggs found on maize leaves (cv. Rampur Composite) from a pesticide-free maize field of National Maize Research Program (NMRP), at Rampur, Nepal from October to November 2020. After the field-collected eggs hatched, pieces of pesticide-free fresh maize leaves (cv. Rampur

Composite) were provided as food for the larvae, and leaves were replaced daily. Individual larvae were reared in Petri-dishes (5 cm diameter) until adult emergence. Newly emerged adults (four pairs) from these field-collected eggs were used to create the rearing colonies, by releasing these adults (P_1 generation) into the same rectangular screen cages (90 cm height x 30 cm length x 45 cm breadth). Each rectangular screen cage was contained two 45 cm tall maize plants (cv. Rampur Composite) each in plastic pots (26 cm diameter x 20 cm height) filled with potting mix (normal compost, loamy soil, and sand mixture, at 1:1:1 ratio). Six such rectangular screen cages were maintained for the colony management, continuation, and the source of the FAW eggs used in our experiments. Both insect rearing and the experiments were carried out at 26^0C, 75% RH, and 16L: 8D (Light and Dark) photoperiod.

Life history parameters for Nepalese FAW population

The goal of these observations was to determine values for various life history parameters for the Nepalese population of FAW for comparison to FAW in other regions. Specifically, we measured (1) the number of eggs/mass, (2) hatching period, (3) the larval and pupal developmental times, (4) larval mortality, (5) the adult pre-oviposition period, (6) adult longevity, (7) duration of the whole life cycle (egg to egg), (8) the adult emergence rate and (9) the adult sex ratio, as follows:

(1) The number of eggs/mass was determined by removing the moth scales covering the eggs with the help of camel hair brush (11 cm) and counted the eggs with a hand lens (10 x 18 mm).

(2) The duration of the egg stage was determined by selecting freshly laid egg masses, along with the maize leaf. These egg masses were transferred into Petri dishes (9 cm diameter) with slightly moistened filter paper on the bottom and the eggs observed daily to determine the day of hatch.

(3) The larval and pupal developmental times were determined by transferring 100 newly hatched larvae from the above step individually onto maize leaf pieces (4 cm length x 3 cm width) that were then placed individually into covered Petri-dishes (5 cm diameter) with slightly moistened filter paper at the bottom. The moistened filter paper and maize leaf pieces in the Petri dishes were replaced at two-day intervals for the young larvae (I and II instars) but was done daily for the older larvae. Larvae that died were replaced immediately with extra larvae from a cohort of similarly handled larvae maintained in the insect rearing colony. Individual VI^{th} instar larvae were transferred into small glass jars (7.5 cm high and 6 cm diameter), one-third of which was filled with slightly moist sandy soil, and the mouth of the jar was covered by a fine mesh net. A piece of maize leaf (20 cm x 18 cm) was placed in the jar over the sand with the larva. The leaf was changed daily until the larva pupated. From these data the average larval developmental period (all instars combined) was calculated. Similarly, the average pupal duration was calculated from the time of pupation until adult emergence.

(4) For the larval mortality study, fresh neonates (n =100) were reared in a tray. Number of dead larvae were counted until I instar larvae molted to the II instar. Again, a fresh lot of II instar larvae (n =100) of same cohorts were taken from the FAW larval colony, and mortality information was taken until II instar molted to the III instar. The same process was repeated for III, IV, V, and VI instar larvae. III to VI larval instars were individually studied by keeping them in each Petri-dish to avoid FAW cannibalism.

(5) The adult pre-oviposition period was observed from the freshly emerged paired (male and female) adults released into the rectangular screen cage along with maize plant and observed the days required for the first laid egg mass from the date of adults released.

(6) The adult longevity was recorded by feeding 10% honey solution to the newly emerged adults.

(7) Duration of the whole life cycle (egg to egg),

(8) The adult emergence rate,

(9) The adult sex ratio was also recorded during the life cycle study.

Morphometric information and measurements

Morphological features of FAW life stages were carefully observed. For the morphometric measurement, freshly collected egg masses along with the maize leaves were kept on a rectangular tray (length-32.4 cm, breadth-26.8 cm, and height-6.2 cm) over a slightly moistened filter paper. When eggs hatched, single I instar larva was kept individually in 100 Petri dishes. Fifty samples (n = 50) were taken for the morphometric information for each instar larva, pupa, and adult. The length of the I and II instar larvae and the head capsule widths for 50 larvae of each instar were measured using a binocular microscope (GE-48, Unilab microscope, 100-265 V, 50 Hz, 3 W lamp) with using ocular and stage micrometers. Other larval stages (III to VI instars), pupa and adult measurements were taken using a straight plastic ruler (30 cm L x 3 cm W and 0.2 cm thickness). Live larval length was measured by slightly relaxing their body on the next day of molting with the help of plastic ruler (15 cm scale). Length and width of wing span were measured by a plastic ruler (see above). Digital weighing balance (SF-400 c, electronic compact scale) was used to measure the weight of VI stage larvae, pupa, and adults. Digital hygrometer (HTC-1) was used to measure the temperature (^{0}C) and relative humidity (RH%) of the laboratory during study time.

RESULTS AND DISCUSSION

Life history parameters for Nepalese FAW population

Values for all life history parameters measured for the Nepalese FAW population are presented in Table 1. The average duration of the egg stage was 3 days. Similar

results were also reported by Motezano et al. (2019). The total larval period was 16.31 days with durations of I, II, III, IV, V and VI instars at 2.98, 2.90, 1.98, 2.19, 2.63, and 3.63 days, respectively. Similar larval duration is reported by the study conducted by Ahir et al. (2020). However, this larval duration is influenced by host type of FAW and prevailing rearing conditions in a laboratory (Dahi et al., 2020). Larval mortality was 70%, 66% and 12% for the I, II and III instars, respectively but zero mortality was recorded in IV, V and VI larval instars. Higher mortality in early three stages and zero mortality in later stages indicates that laboratory reared larvae need more careful handling in colony management. But in open field conditions, it is suggested to manage in early stages, this could be more effective and economic while pest management (Debora et al., 2017). Pupal duration of the FAW was 8 to 13 days. Similar range of pupal duration (8-13 days) was also reported by Sharanabasappa et al. (2018). Pupation rate was 98.8%. The average adult pre-oviposition period was 3.75 days. Adult longevity was 20.73 days for males and 22.87 days for females on 10% honey solution diet. But the adult male lived an average of 5.24 days and adult female up to 6.9 days in absence of food material. The duration of the whole life cycle (egg to egg) was 33.75 days. The adult emergence rate was 97.2%. The adult male to female sex ratio was 1:1.30. Ahir et al. (2019) also reported 1:1.30 sex ratio of the FAW.

The adult emergence rate was 97.22% (Table 1). However, biology and ecology of FAW is influenced by growing temperature, relative humidity, host, artificial diet (Lekha et al., 2020).

Table 1. Life history parameters of fall armyworm, *Spodoptera frugiperda* in maize crop

Stages	**Mean (days) ± SE**
Pre-oviposition period (n= 20)	3.75 ± 0.099
Larval stage duration (n= 100)	16.31 ± 0.205
I instar	2.98 ± 0.037
II instar	2.90 ± 0.039
III instar	1.98 ± 0.020
IV instar	2.19 ± 0.044
V instar	2.63 ± 0.076
VI instar	3.63 ± 0.048
Pupa (n= 100)	9.69 ± 0.145
Total life cycle (egg to egg) (n= 100)	33.75 ± 0.441
Adult longevity (fed on 10% honey sol^{n}) (n= 30)	
Male	20.73 ± 0.51
Female	22.87 ± 0.26
Other biological parameters	
Fecundity (n = 30): 979.43 ± 24.086 (range 713-1166)	
Male: Female sex ratio (n = 180): 1: 1.3	

Morphometric information and measurements

Egg

The freshly laid eggs by gravid female were globular, pale green in color for a day, turned into a golden yellow, and later changed to a black color before hatching. Egg masses were covered with grey scaly hairs. Similar morphological features of FAW eggs were also reported in earlier studies (Deshmukh et al., 2021).

Larva

There were six larval instars. The first instar larvae were greenish in color with a black head, II instar larvae were greenish brown, III instar larvae were brownish with three dorsal and lateral white lines, IV to VI instar larvae were brownish black with a white inverted "Y" shape marking on its head. The last abdominal segment has four dots arranged in a square.

The average length of each instar (I to VI) was 1.51 mm (1.41-1.7 mm), 3.56 mm (3.18-3.66 mm), 7.12 mm (6.4-7.8 mm), 11.6 mm (9.4 -13.5 mm), 18.51 mm (15.8-22.5 mm), and 34.39 mm (28.3-38.8 mm), respectively (Table 2). The width of head capsule of each instar was 0.35, 0.47, 0.8, 1.37, 2.11, and 2.7 mm, respectively (Table 2). Montezano et al. (2019) suggested width of head capsule of FAW varies according to the instars and this study findings also suggested same results saying head capsules are smaller in early instars which is increased with the increasing instars.

Table 2. Morphometric measurements (larval instars length and head capsule width of each instar) of FAW, *Spodoptera frugiperda* in maize crop

Larval instars (n= 50)	Length (mm) ± SE	Width of head capsule (mm) ± SE
I Instar	01.51 ± 0.013	0.35 ± 0.001
II Instar	03.56 ± 0.017	0.47 ± 0.003
III Instar	07.12 ± 0.052	0.80 ± 0.004
IV Instar	11.60 ± 0.181	1.37 ± 0.006
V Instar	18.51 ± 0.212	2.11 ± 0.013
VI Instar	34.39 ± 0.351	2.70 ± 0.013
Pupa	15.27 ± 0.122	-

Pupa

Pupa was reddish brown and oval. The pupa possessed a cremaster with two spines on its last segment. The average weight of single pupa was 18.9 mg (15-24 mg), with an average length and width of 15.25 mm (14-16.4 mm) and 4.41 mm (4-5 mm), respectively.

Adult

The forewing of the male is shaded with gray and brown with a triangular white patch at the apical region and a circular spot at the center of the wing, while the forewing of the female is uniform grayish brown to a fine mottling of gray and brown. The hind wing is silver, white with a narrow dark border in both male and female. These characters are similar as reported earlier (Sharanabasappa et al., 2018).

Table 3. Morphometric measurements (spread of wingspan, and weight of larva, pupa and adults) of FAW, *Spodoptera frugiperda* in maize crop

Adults (spread wingspan, mm) (n= 50) ± SE	
Male	14.16 ± 0.119 (length); 33.62 ± 0.085 (width)
Female	16.8 ± 0.099 (length); 34.32 ± 0.101 (width)
VI Larval instar pupal stage, and adult weight (mg) (n = 50) ± SE	
VI instar	38.16 ± 0.49
Pupa	18.90 ± 0.29
Male	2.84 ± 0.053
Female	3.32 ± 0.108

The length and width (wings spread) were measured as 14.16 mm and 33.62 mm (male), and 16.8 mm and 34.32 mm (female), respectively (Table 3). The average weight (mg) of individual of VI instar, pupa, adult male, and adult female was 38.16, 18.90, 2.84 and 3.32, respectively. The wingspan of male and female was measured as 3.5 and 3.2 cm, respectively.

CONCLUSION

Life cycle and morphometric parameters of FAW were studied to understand the behavior of the pest, which will ultimately help to design an effective management strategy. The average incubation period was 3 days with an average fecundity of 979.43 eggs, larval (I to VI stage) and pupal development period was 14-25 days and 8-13 days, respectively. Adult longevity was 5.24 days (for male) and 6.9 days (for female) in absence of food and water, but longevity was increased by four-fold when 10% honey solution was fed (20.73 days for male and 22.78 days for female). High mortality was found in early instar larvae at 77% (I instar), 67% (II instar), 12% (III instar), and no-mortality in IV, V and VI instars. Pupation and adult emergence rate were 98.8 and 97.22%, respectively, with male to female ratio 1:1.3. The result of this study is useful to design a FAW management strategy.

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