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Occurance, taxonomic identification and life cycle of fall armyworm; *Spodoptera frugiperda* (J.E. Smith) in Sri Lanka

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Abstract

Fall armyworm (*Spodoptera frugiperda* J.E. Smith) is an alien, invasive voracious pest attacking mainly maize crop and about 80 other plant species. It is native to the tropical regions of the western hemisphere from the United States to Argentina. Recent occurrence of this pest was reported in African continent and in India in years 2016 and 2018, respectively. The first reporting of fall armyworm in Sri Lanka was in October, 2018 from Iddapola, Mahaoya in Ampara district though it was first reported as an unknown pest damage. Samples were collected and the pest were reared in the laboratory in insect cages till the adult stage and based on morphological features it was suspected to be armyworm. Mated adults were transferred to new insect cages and the second generation adults and sixth instar larvae were identified based on taxonomic features and confirmed as fall armyworm, *S. fruigiperda*. The life cycle study indicated that the incubation period of eggs ranged 2-3 days, the larval period 14-19 days and the pupal period between 9 -12 days. Thus, the total life cycle of male and female fall armyworm reported in Sri Lanka is 32-43 days and 34-46 days, respectively.

Key words: Fall armyworm, Life cycle, Sixth instar larvae, *Spodoptera frugiperda*, Taxonomy

Introduction

The Fall Army Worm (FAW), *Spodoptera frugiperda* (J.E. Smith) is the larval life stage of a fall armyworm moth. Taxonomically, *S. frugiperda* belongs to genus Spodoptera of family Noctuidae of the order Lepidoptera (https://bugguide.net/node/view/851197). The term "armyworm" refers to several species, often describing the large-scale invasive behavior of the larval stage of the species. FAW is regarded as a pest and can damage and destroy a wide variety of crops causing heavy economic damages. Its scientific name derives from the Latin word '*frugiperda*', which refers the 'lost fruit' is an indication of the ability of the species to destroy crop (https://bugguide.net/node/view/851197). The pest is known to damage over 80 plant species, with a particular preference for maize, a main staple crop of people around the world. It causes major damage to economically important cultivated grasses such as maize, rice, sorghum and sugarcane and also damage vegetable crops and cotton. Because of its propensity for destruction, the behavior of FAW and its control have been studied in detail. The pest appears to be diverging into two species and also showing cannibalistic behavior.

The FAW is native to the tropical regions of the western hemisphere from the United States to Argentina, and is widely distributed in Eastern, Central North and South regions of America. It overwinters successfully only in Southern Florida and Southern Texas in the United States. Generally it cannot overwinter below freezing temperatures (Meagher and Nagoshi, 2004; Murúa *et al*., 2009). FAW is a strong flier and disperses long distances annually during the summer months. It is recorded from virtually all states east of the Rocky Mountains. However, as a regular and serious pest, its range tends to be mostly the Southeastern states. FAW invaded the African continent in the year 2016, where it was first reported in Nigeria, and West and Central Africa, threatening both Africa and Europe (IITA, 2016). It has been repeatedly intercepted at quarantine in Europe. In Africa it causes significant damage to maize crops and has high potential for further spread and causing high economic damage (https://www.cabi.org/isc/datasheet/29810). It has been invasive in Africa since 2016 and rapidly spread across 44 countries in sub-Saharan Africa. In April 2017, in response to the continuing spread of FAW in Africa, Department for International Development (DFID) and Centre for Agriculture and Bioscience International (CABI) rapidly published a summary of the presence of pest and possible

future distribution in the continent, while summarizing information on its impacts and control measures. In 2018, it began to spread widely in India (Hodal, 2018; IITA, 2018; ICAR-NBAIR, 2018). Scientists from the College of Agriculture at the University of Agricultural and Horticultural Sciences (UAHS) confirmed the arrival of the pest in maize fields within campus grounds in Shivamogga, in the state of Karnataka, southern India. Both morphological and molecular techniques confirmed the pest as FAW. Then, it has also been recorded in Thailand in 2018, Bangladesh in 2019, China in 2019 and Myanmar in 2019 (IPPC, 2018; IPPC, 2019).

Larvae cause damage by consuming foliage. Young larvae initially consume leaf tissue from one side, leaving the opposite epidermal layer intact. By the second or third instar, larvae begin to make holes in leaves, and eat from the edge of the leaves inward. Feeding in the whorl of maize plants often produces a characteristic row of perforations in the leaves. Their damage appears as ragged-edged holes on leaves and at later stages cause damage to tassels and/or ears. Severe feeding may give the appearance of hail damage, where the crop is affected in three ways: plant stand reduction, direct damage, and leaf defoliation.

Though eggs are laid in clusters ranging from 55 to 888 on the under or upper surface of the maize leaf, base of the plant and also in whorls. The larval densities are usually reduced to one to two per plant when larvae feed in close proximity to one another, due to cannibalistic behavior. Older larvae cause extensive defoliation, often leaving only the ribs and stalks of corn plants, or a ragged, torn appearance (Pit Pitre, H. N. and D.B. Hogg, 1983). Marenco *et al.* (1992) studied the effects of fall armyworm injury to early vegetative growth of sweet corn in Florida and reported that the early whorl stage was least sensitive to injury, the mid whorl stage intermediate, and the late whorl stage was the most sensitive. Further, they noted that mean densities of 0.2 to 0.8 larvae per plant during the late whorl stage could reduce the yield by 5 to 20%.

Pannuti *et al*. (2015) studied larval feeding behavior and reported that FAW prefer young (vegetative stage) leaf tissue compared to leaf tissue of mature plants for growth and survival. When plants are in reproductive stage larvae tend to settle and feed in the ear zone, particularly on the silk tissues though silk is not very suitable and results in poor

larval growth. Larvae feeding on the corn kernels display the fastest rate of development. Further, tassel tissue may be suitable for initial feeding, perhaps until the larvae locate the silk and ears. However, feeding only on tassel tissue is suboptimal.

The present paper reports the first incidence and taxonomic identification of *S. frugiperda* in Ampara district of Sri Lanka.

Materials and Methods

First incidence in Sri Lanka

An infestation of a new larval insect pest, which was not observed before in the maize (*Zea maize*) cultivations in Mahaoya area was first reported to Plant Protection Service of the Department of Agriculture at Gannoruwa in October 2018 by Assistant Director of Agriculture, Mahaoya based on complain made by Agriculture Instructor (AI) and two farmers. Considering the potential risk of FAW infestation, the affected fields were visited and further investigations were made.

One of the affected farms was located in Iddapola, Mahaoya, planted with hybrid maize variety Pacific 987 in an acre of land. About 30% of the plants had been destroyed by the pest where the young leaves were eaten up fully. Another farm where hybrid variety American Super was grown in land extent of an acre damage was comparatively low as it was in early stage of infestation. Age of the crop of the two farms was almost the same (about 25 days old). The damage of the two affected farms resemble the damage of *Spodoptera litura*, which is already present in the country. However, it was also noted that the damage to the leaf canopy of the above two fields was much severe.

Identification and laboratory rearing

Maximum possible number of specimens were collected and brought to Plant Quarantine Unit laboratory, Gannoruwa. The specimens were inspected under the microscope and identification was done based on morphological characters and found that the larvae were fall armyworm (*S. frugiperda*) larvae. Larvae and eggs specimens were collected from the same fields and brought to the Plant Quarantine Unit Laboratory, Gannoruwa and reared them in insect cages. During rearing maize leaves were provided

as feeding material and replaced every other day till the larvae and eggs reached the adult stage.

The adult colony was maintained by providing the adults with 10% solution of bee honey and allowed to mate inside the cages. The mated adults were then transferred to new insect cages provided with 3-4 weeks old maize plants to enable them to lay eggs. After hatching, the larvae were reared till the adult stage, feeding them with maize leaves and replacing them every other day. Photographs were taken on both adults and larvae to compare. The sixth instar larvae and adults (both males and females) were subjected to taxonomic identification comparing them with taxonomic characters described in literature (CABI, 2019, https://www.cabi.org/isc/fallarmyworm).

Maintenance of laboratory colony

The emerged adults were also used to establish a laboratory colony reared with maize leaves (Hybrid, Pacific 780) to study the life cycle. The larvae were reared using circular (90 x 40 mm dia.) insect breeding dishes containing the pieces of maize leaves and maintained at 28±2 ^{0}C , 75 to 80% RH. The adults were kept in a plastic oviposition cages (25 x 15 cm diameter) lined with paper towels as oviposition substrate, inside the insect cage (100 x 100 cm). In each oviposition cage, a pair of male and female adults was released. The adults were fed with 10% honey solution soaked on cotton pads hanged inside the cages and cotton pads were replaced daily. Eggs were collected and kept in circular insect breeding dishes for hatching. The eggs were then examined at 12 hr intervals till hatching. After hatching, the larvae (n = 30) were reared individually in containers on fresh maize leaf pieces as food. The feeding materials were changed daily.

Data recording

Containers were daily checked and larval head capsule exuviae were removed after each ecdysis and measured the width of head capsule at the widest point of the vertex using an ocular micrometer and stored in 70% ethanol. The width of head capsule and the number of larval moults (exuviae) were used to determine the number of larval instars. These studies were confirmed by a Dyar's rule, which states that width of head capsule grows in geometrical progression (Hatchinson *et al*., 1997). About 30 larval samples of different stages were taken for Morphometric studies.

Male and female adults when emerged were reared in a rearing cage (30 x 30 x 45 cm) for longevity study. Oviposition was studied by confining ten newly emerged pairs each in one litre plastic boxes. Eggs were counted and removed daily until the female of the pair died. The larval and pupal period, sex ratio, and adult emergence were also observed. Pre-oviposition, oviposition, post-oviposition periods and fecundity were also recorded.

Results and Discussion

Researchers around the world have reared *S. frugiperda* in laboratories for the purpose of conducting studies on its biology and behavior. Cannibalistic behavior of its larvae is considered as a challenge for most researchers. Cannibalism has been well documented in field (Sarmento *et al.*, 2002; Silva, 1999) and under laboratory (Zenner-de-Polania *et al*., 2009; Goussain *et al*., 2002) conditions, discouraging to rear *S. frugiperda* in groups. Incidence of cannibalism of larval *S. frugiperda* on maize under field conditions was investigated using filed cages. Cannibalism was found to account for approximately 40% mortality when maize plants were infested with two or four fourth-instar larvae over a 3-day period (Chapman *et al*., 2000). In the present study, the larvae brought to the laboratory from Iddapola field and colonies maintained in cages with adequate food supply too showed that there was decrease in number of larvae before pupation and later observed cannibalism among larvae. Therefore, larvae were reared in individual cages as a way to decrease the pre-imaginal mortality (Vilarinho *et al*., 2011; Busato *et al*., 2006). Photographs taken on specimens from the laboratory reared FAW colonies and compared with the descriptions and photographs of FAW available in literature (Simmons and Wiseman, 1993) for taxonomic identification of the FAW. Both male and female adults and larvae at sixth instar stage were compared.

Morphological and taxonomic characters of *S. frugiperda* larvae

Debora *et al.* (2017) and Visor (2017) described that the larval stage of FAW usually consist of six instars, and the head capsule widths were about 0.35, 0.45, 0.75, 1.3, 2.0, and 2.6 mm, and length of the larvae were about 1.7, 3.5, 6.4, 10.0, 17.2, and 34.2 mm, in six instars respectively. According to their description, the young larvae are greenish with a black head, the head turning orangish in the second instar, but particularly

in the third instar, the dorsal surface of the body becomes brownish, and lateral white lines begin to form. Fourth to sixth instars the head become reddish brown in colour, mottled with white and the brownish body forms white sub-dorsal and lateral lines. A comparison of specific characteristics of sixth instar larvae between descriptions given in literature and those observed in the present study are given in Table 1.

Distinguishable characteristics of larvae reared in the laboratory are given in Figure 1 (b-d) which tally well with descriptions of the characters of larvae described in literature (Debora *et al.*, 2017; Brambila, 2013; Visor, 2017) confirming the specimens from Sri Lanka are that of FAW larvae in sixth instar stage.

Table 1. Comparison of specific characters of sixth instar larva of *S. frugiperda*

Character	Literature	Iddapola sample
Inverted "Y" in head capsule	Present Fig. 2 (a)	Present Fig. 2 (b)
Dorsal spots on second last body segment	Present Fig. 2 (a)	Present Fig. 2 (c)
Broad pale band along top of the body and dark side stripping	Present Fig. 3 (a)	Present Fig. 2 (d)
Skin smooth under magnification of 25 power without microspines	Yes 6-9 cm in length	Yes 5-9 cm in length
Young larvae greenish/yellowish, 6-9 cm in length Mature larvae	Brownish 30-36 cm	Brownish 29-35 cm

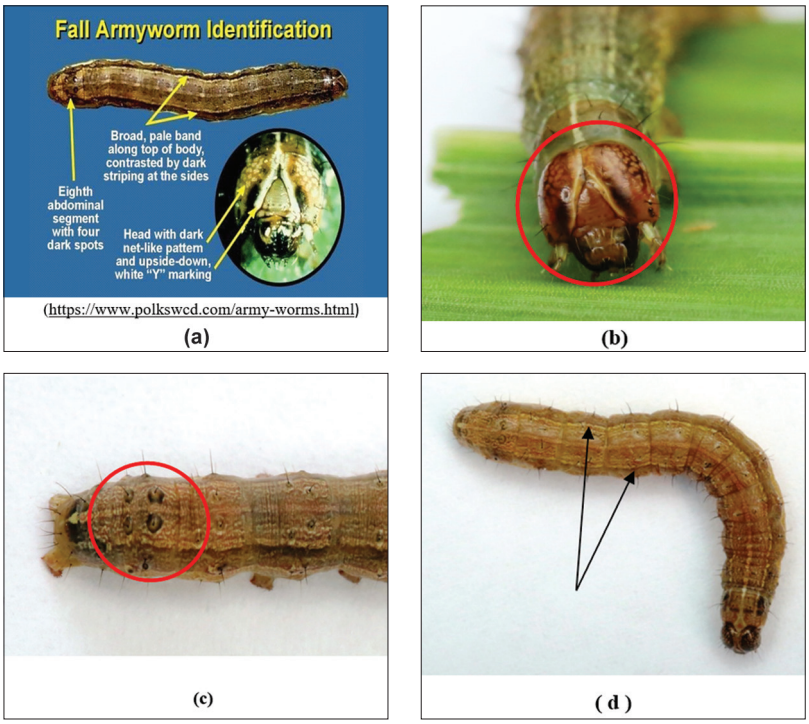

Figure 1. Specific characters present on dorsal surface of *S. frugiperda* larvae (a) and larvae collected from Iddapola, Sri Lanka (b, c & d)

Adult identification characters of S. *frugiperda*

The moths of *S. frugiperda* show a wingspan of 32 to 40 mm. The adult FAW carries dark gray, mottled forewings with light and dark splotches. These observations are similar to the description of Debora *et al.* (2017). There were white and brown transverse lines and markings on the forewings but they were not as contrasting as in other *Spodoptera* species. Brown background color of the forewings is varying from grayish brown to rust brown. The male and female are distinct in their forewing characters, especially the wing markings (Table 2). When the species that have contrasting forewing markings are considered, *S. frugiperda* is much smaller than those species. The hind wing is iridescent silver-white with a narrow dark border in both sexes. Figures 2 and 3 clearly shows the similarities of all the taxonomic characters of adult male and female reared in the laboratory during the present study and those described in the literature.

Adult FAW are nocturnal, and are most active during warm, humid evenings. After a pre-oviposition period of three to four days, the female normally deposits most of her eggs during the first four to five days of life, but some oviposition occurs for up to three weeks. Duration of adult life is about 10 days on average, with a range of about seven to 21 days.

Table 2. Characteristics of male and female *S. frugiperda*

Male (Figure 3)	Female (Figure 4)
1. Upper half of the wing in dark shades of grey and brown and lower half to post medial line or beyond pale grey or brown	1. Front wing is with dark brownish gray lines that are usually indistinct
2. An oblique white bar in anterior apical corner	2. Orbicular spot obliquely elongate, narrowly outlined in black with a pale rim around the dark centre
3. Orbicular spot oblique, pale brown and narrowly outlined by dark scales, outer margins sometimes confluent with oblique pale shade which usually extends from the costa to CU1. Reniform indistinct with a white spot beneath it	3. Hindwing is translucent white with partial narrow black border
4.Ventral surface of thorax dark grey	4. Veins often dark distally
5. Forewings shaded with gray and brown colours, with triangular white spots at the tip and towards the center of the wing	5. Forewings are less distinctly marked, ranging from a uniform grayish brown to a fine mottling of gray and brown

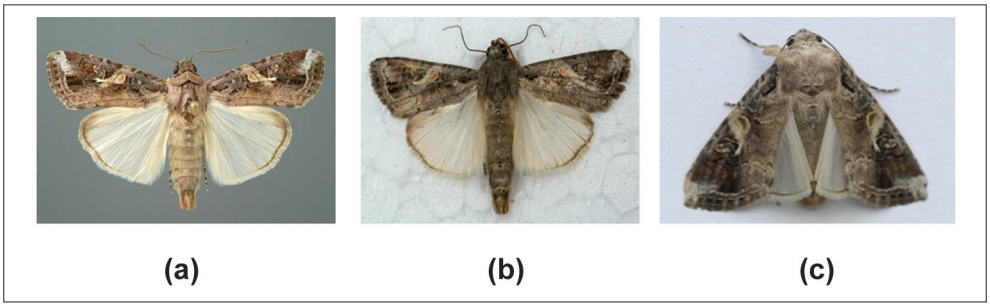

Figure 2. Typical adult male fall armyworm, *Spodoptera frugiperda* (J.E. Smith). Photograph by Lyle J. Buss, University of Florida (a) and specimen from Wilthalawa, Iddapola, Mahaoya, Sri Lanka (b & c)

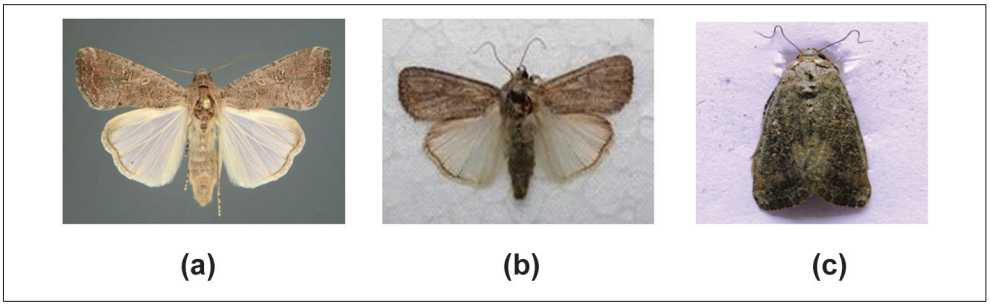

Figure 3. Typical adult female fall armyworm, *Spodoptera frugiperda* (J.E. Smith). Photograph by Lyle J. Buss, University of Florida (a) and specimen from Wilthalawa, Mahaoya, Sri Lanka (b & c)

Taxonomic characters of forewings of male *S. frugiperda*

The forewing of the male *S. frugiperda* adult was unique and has several characteristics specific to the Fall armyworm as listed below.

- Reniform spot indistinct, partially outlined in black, with a small, sideways v shaped marking (see arrow and circle 1 of Figures 4 and 5) .
- Orbicular spot light brown, oval, oblique, and not as narrow as in the species with strongly contrasting transverse lines (circle 2 of Figures 4 and 5).
- Row of small, black, hour-glass shaped markings near apical margin of the wing (circle 3 of Figures 4 and 5).

Thus, the forewing characteristics of locally reared male adults (Figure 5) totally tally with those cited in literature confirming that local specimens are that of *S. frugiperda.*

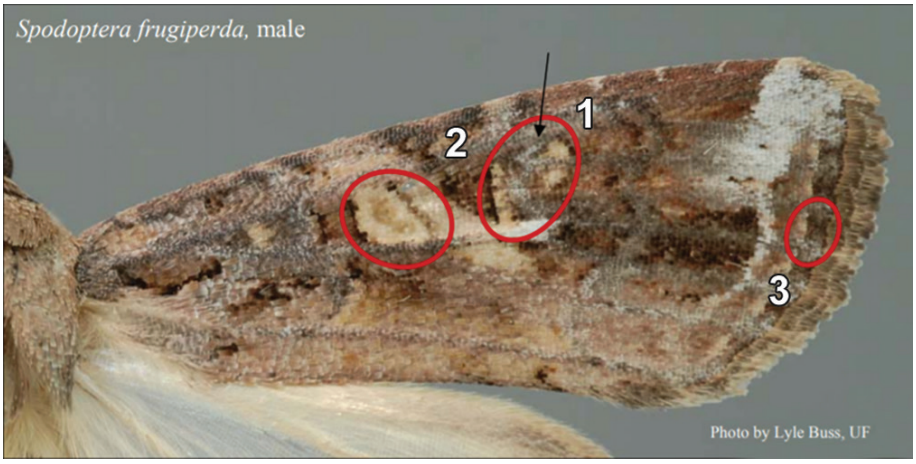

Figure 4. Taxonomic characters of forewing of typical adult male fall armyworm, *Spodoptera frugiperda* (J.E. Smith). Photograph by Lyle J. Buss, University of Florida

Figure 5. Taxonomic characters of forewing of adult male fall army worm, *S. frugiperda* obtained from larvae collected from Wilthalawa, Iddapola, Mahaoya, Sri Lanka

Study of the life cycle of *S. frugiperda* under laboratory conditions

The life cycle under local environmental conditions was studied using moths reared under laboratory conditions.

Eggs:

Gravid female laid eggs in clusters ranged from 92 to 200 on the lower or upper surface of the maize leaf, base of the plant and also in whorls. The eggs were dorso-ventrally flattened, initially these were pale green for one day and turned to golden yellowish and ultimately turned to black before hatching. The female covered the egg mass with a layer of scales and this gave moldy appearance. Incubation period ranged from 2-3 days with a mean of 2.5 days (Table 3).

Larva:

First instar larvae (Figure 6) were greenish with a black head and turned greenish brown in the second instar (Figure 7). The third instar was brownish with three dorsal and lateral white lines (Figure 8). Fourth to the sixth instars were brownish black and had three white dorsal lines and alight lateral line (Figure 1 d). Black tubercles were found dorsally on the body bearing spines. The front area of head carried a white inverted "Y" line (Figure 1 a).

Table 3. Life cycle of *Spodoptera frugiperda* reared on maize leaf (n=30) under laboratory conditions

Stages	Mean ± SD (Days)	Range (Days)
Incubation period	2.30±0.50	2.00 – 3.00
Larval period	15.4±1.45	14.00-19.00
Pupal period	10.20 ± 1.32	9.00- 12.00
Total life cycle (egg to adult)		
Male	37.30 ± 5.10	32.00-43.00
Female	40.20 ± 4.90	34.00-46.00

Figure 6. First instar larvae

Figure 7. Second instar larvae

Figure 8. Third instar larvae

Figure 9. Pupa

Each larva passes through six distinct instars over a period of 14 -19 days (Table 4). The final and the longest larval instar was sixth instar and it takes 4-6 days before it enters pre-pupal period. The head capsule width at different larval stages in five moults was found to be 0.34±0.01, 0.48±0.01, 0.81±0.02, 1.22±0.05 and 1.96±0.06 mm, respectively. The larval period extending to about 14 - 30 days under cool climates was reported by Pitre and Hogg (1983) in Georgia province in USA.

Table 4. Larval growth stages and head capsule width at different larval stages

Growth stage Laval stage	Mean± SD (Days)	Range (Days)
Instar I	2.10±0.49	2.00- 3.00
Instar II	2.20±0.40	2.00-3.00
Instar III	2.00±0.00	2.00
Instar IV	2.00±0.00	2.00
Instar V	2.20±0.49	2.00-3.00
Instar VI	4.30±0.50	4.00-6.00
Larva- Head capsule width	**Mean ± SD (mm)**	**Range (mm)**
Ist moult	0.32 ± 0.01	0.32- 0.35
IInd moult	0.44 ± 0.01	0.47- 0.52
IIIrd moult	0.71 ± 0.02	0.80-0.85
IVth moult	1.12 ± 0.05	1.17–1.30
Vth moult	1.76 ±0.06	1.87 - 2.05

Pupa:

After the sixth instar the full-grown larva stop feeding, turned greenish to the bright brown colour, which is called pre-pupal period. After the pre–pupal period larvae drops to the ground for pupation under the soil. The fully grown pupa is shown in Figure 9. Duration of the pupal period was about 9 to 12 days under local conditions (Table 3). Similarly, Débora *et al*. (2017) studied the pupal period of *S. frugiperda* on maize leaves and reported to be 8.54 days. Pupal sexing can be done by looking at the genital opening. The distance between genital opening and anal slot can be used to distinguish the female and male pupa where the distance is longer in females than in males.

Adult:

Adult emergence occurs after 25 -34 days after egg laying. The male moths live for about 7-9 days whereas the female moths live about 9 -12 days period. Fore wing of male is shaded with gray and brown, with triangular white patch at the apical region and circular spot at the center of the wing (Figure 6). The fore wings of females are grayish brown to a fine mottling of gray and brown and uniform (Figure 4). The hind

wing was similar in both male and female moths and is silver-white in colour with a narrow dark border. The morphological characters of adult described here are similar as reported in literature earlier (Oliver and Chapin, 1981). Pre-oviposition, oviposition and post oviposition period ranged from 3-4, 2-3 and 4-5 days, respectively. Under laboratory conditions each female laid 835 to 1169 eggs with an average of 1064.80 and very high hatchability (95-98%) (Table 5). The total life cycle of male and female ranged from 32-43 and 34-46 days, respectively (Table 3). The average wingspan of female was 3.20 cm with a range of 3.00 to 3.4 cm and it was 3.25 cm with a range of 3.00 to 3.50 cm in male.

Table 5. Reproductive biology of *Spodoptera frugiperda*

Stages	Mean ± SD	Range
Pre oviposition period (days)	3.4 ± 0.493	3.00-4.00
Oviposition period (days)	2.6 ± 0.40	2.00-3.00
Post oviposition period (days)	4.10 ± 0.46	4.00-5.00
Fecundity/female (Number)	1004.80 ± 153.53	835.00-1167.00
Egg hatchability (%)	93.60 ± 1.43	95.00-98.00
Male adult longevity (days)	7.90 ± 0.75	7.00-9.00
Female adult longevity (days)	10.60 ± 0.87	9.00-12.00

The life cycle study clearly provides basic information about the biology and external morphology of FAW recently invaded in Sri Lanka. The pest spread to entire maize growing areas in Sri Lanka within a short period as it is migratory in nature. The pest may shift to other hosts and survive in the absence of maize thereby maintaining their population throughout the year. Immediate focus of research could be on reproductive biology, host preference and host plant resistance, diapause, strains, natural enemies and migration of FAW.

Conclusion

Based on the larval and adult taxonomic and morphological characters it could be confirmed that the specimens collected from Iddapola Mahaoya, Sri Lanka is that of fall armyworm, *Spodoptera frugiperda*. Further confirmation using molecular technology may be carried out for re-confirmation, if necessary. The life cycle study indicated that the

incubation period, larval period and pupal period of the pest ranged between 2-3 days, 14-19 days and 9 -12 days, respectively under local conditions, whereas the total life cycle of male and female fall armyworm was 32-43 days and 34-46 days, respectively.

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