

LIFE CYCLE, MORPHOMETRY AND NATURAL ENEMIES OF FALL ARMYWORM, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) ON *Zea mays* L. IN THE PHILIPPINES

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ABSTRACT

The recent introduction of fall armyworm, *Spodoptera frugiperda* (J.E. Smith) into the country which was first detected in the Cagayan Valley, the major corn growing area, has the potential to cause yield losses, in the absence of effective control measures. This paper sought to describe briefly the development stages of *S. frugiperda* and determine its life history parameters on a locally bred variety of corn (var. Lagkitan), as a new food source, being a newly introduced pest. It also determined the morphometrics of the different life stages; larval damage, pupation and behavior, including its natural enemies collected from the field in several provinces in the Philippines. A short development time and high reproductive rate of the pest was demonstrated when fed with young leaves of corn under laboratory conditions.

Key words: bionomics, morphology, biological control, entomopathogens, parasites, predators

INTRODUCTION

Corn is the most important staple cereal crop grown by small farm holders in sub-Saharan Africa (Macauley 2015), of which, 90% of production is used as food (Igyuve 2018; Pandey 1988). In the Philippines, however, corn is used in industries and major component of animal feed (Philippine Statistics Authority 2019).

The fall armyworm, *Spodoptera frugiperda* (J.E. Smith), is the most important noctuid pest in North and South America and has recently become an invasive pest in Africa (Montezano et al. 2018; Igyuve et al. 2018). *S. frugiperda* is a polyphagous pest that attacks corn, sorghum, sugarcane, forage grasses, turf grasses, rice, cotton, and peanuts. It is a voracious pest in its countries of invasion (Knipling 1980; Pashley 1986; Lu and Adang 1996 as cited by Levy et al. (2002). Recently, Montezano et al. (2018) reported 353 plant species as hosts in the Americas, distributed in 76 families, mainly Poaceae (106 species), Asteraceae (31 species) and Fabaceae (31 species).

As a polyphagous pest, *S. frugiperda* consumes almost any plant parts of its hosts. On young corn, larvae feed on the surface of leaves leaving only white papery patches, called window panes. Older larvae consume more tissues, with stronger mandibles, cut large portions of plant tissues with high silica content and includes seedlings, foliage, tassels, cobs, husks, and developing kernels (Pogue 2002; Brown and Dewhurst 1975, as cited by Goergen et al. 2016).

S. frugiperda is considered an outbreak pest with remarkable dispersal capacity, as part of its life history strategy (Johnson 1987). In its annual migration, it is able to expand from its endemic area in the warmer parts of the New World over more than 2000 km across the entire US up to Canada in

the North and reaching the northern parts of Argentina and Chile in the South (Pair et al. 1986). The pest has become a new invasive species in West and Central Africa where outbreaks were reported for the first time in early 2016 (Goergen et al. 2016). It is also reported in India and several Asia Pacific countries including China, Taiwan (CABI 2019) and Philippines (IPPC 2019; Navasero et al. 2019).

S. frugiperda is composed of two sympatric and morphologically identical strains: the corn strain, feeding on corn, sorghum, and other large grasses and the other, rice strain, feeding on rice, Bermuda grass and other small grasses. In the America's, cryptic host strains have been previously distinguished through differences in protein composition, nuclear DNA restriction fragment length polymorphism (RELP) (Pasley et al. 1985; Lu et al. 1992; Lu and Adang 1996); through amplified fragment-length polymorphic (AFLP) to detect both differences of the FAW strains as well as hybrids (Mc Michael and Prowell 1999), and mitochondrial protein cytochrome oxidase I (COI) (Maas and Sanjur, 1996) and using PCR-RFLP of COI gene (Levy et al. 2002). Strain distribution of fall armyworm populations which invaded Sub-Saharan Africa was reported by Nagoshi et al. (2019).

The biology of *S. frugiperda* had been reviewed by Sparks (1979). This was expanded by Andrews (1998) including distribution, economic importance, seasonal abundance, host plants, life history as well as natural, cultural, genetic, and chemical controls of FAW. These vary depending on the larval development in particular hosts and temperature of the country.

CABI listed several species of natural enemies of *S. frugiperda* (<http://www.cabi.org/isc/datashet/29808>) and includes *Bacillus cereus*, *B. huringiensis*, *B. thuringiensis aizawa*, *B. tentomocidus*, *Bt galleriae*, *Bt kurstaki*, *Bt dendrolimus*, *Bt thuringiensis*, *Beauveria bassiana*, *Eocanthecona furcellata*, nucleopolyhedrosis virus (NPV), *Orius* spp., *Steinernema* spp., *Telonemus* spp., *Trichogramma* spp..

S. frugiperda was detected in Cagayan Valley and nine more provinces across the Philippines and 66 municipalities and 17 cities, mostly in Luzon Island by the Bureau of Plant Industry (Navasero et al. 2019). This was reported to the International Plant Protection Convention (IPPC) for the period covering four months (June 20 to October 28, 2019) by BPI. Its high dispersal capacity, as reported in its countries of origin, led government workers and growers to request for appropriate and sustainable methods of control.

This study sought to investigate some basic aspects of the biology of *S. frugiperda* as an essential prerequisite for laboratory mass production, to produce ample supply of test insects for behavioural and efficacy evaluation of entomopathogens. Specifically, it aimed to: 1) know the life cycle on a native variety of corn; 2) describe the changes in the color patterns and other morphological features during development; 3) determine morphometry at specific growth stages; 4) record natural enemies of *S. frugiperda*.

MATERIALS AND METHODS

Test Insect. The identity of fall armyworm *S. frugiperda* larvae, collected from a farmer's field in Barangay Patel, Gonzaga, Cagayan was established through morphological (Navasero et al. 2019a) and molecular analyses, and was found to belong to the rice strain (Caoli et al. 2019). These were brought to the laboratory for sorting, rearing and holding. Some larvae were reared until pupation using corn leaves (variety Lagkitan). Eggs were held in plastic plates lined with two-ply tissue paper until the black-head stage and when about to hatch these were placed on pans for further rearing using corn leaves until pupation. Pupae were kept in cages and after emergence, adults were allowed to mate and lay eggs. These served as parental stock for mass rearing *S. frugiperda* in the laboratory. All set-ups were kept under laboratory conditions at 27-29 °C, 70 to 80% RH and 12D: 12L photoperiod.

Life cycle study. An adult male and a female were confined in pair in cylindrical mylar plastic oviposition cage. Ten pairs were prepared in this manner. The cylinder was covered above and below and along the sides with pieces of wax paper as oviposition substrate. They were provided with cotton soaked in 10% sugar solution as food source. From the progeny of these parental stock, 60 neonates (newly-hatched) larvae were individually transferred to fresh corn leaves and reared in plastic plates until pupation. The morphological features of the different instars were observed and recorded along with 1) Incubation period; 2) Development period from the first instar to the sixth instar 3) Pupal period and 4) Post-developmental periods: a. Pre-oviposition period (the time adult female emerged to the time the first mass of eggs is laid b. Oviposition period (egg laying period) c. Post oviposition period (the time female stopped laying eggs till death; d) Longevity of male and female adults - the time from adult emergence till their death; e) Fecundity - number of egg-masses and number of eggs per egg-mass laid in the lifetime of adult females; f) Hatchability of eggs - taken by counting the number of neonates that hatched from all egg-masses laid by a female in her lifetime. All periods of observations were taken in days.

Morphometry of the different life stages. Digital measurements and images of the eggs, 1st, 2nd and 3rd instar larvae were obtained using a Carl Zeiss binocular stereoscope Stemi 305 with a built-in Labscope version 2.8.1, and camera wirelessly connected to a tablet. The measurement of 4th, 5th, and 6th instar larvae, pupae and adults were measured using a digital caliper. Egg diameter and height were also taken. The length and width of the larvae were measured as well as the width of the head capsule. The male and female pupae were likewise measured from the tip of the head down to the tip of abdomen and the widest width of the body. Male and female adults were pinned, wings expanded and dried. The wing expansion of the forewings was measured and body length from the tip of the head to the tip of the abdomen were taken. The measurement of each stage was done by using ten individuals for each stage.

Statistical analysis. Analysis of variance (ANOVA) for duration of different larval instars, larval development, pupal period and total development time of male and female *S. frugiperda* were performed using Proc GLM procedure for unbalanced data in SAS. The experimental design was Completely Randomized Design in which the treatment factor (male and female) had different numbers of replicates. Tukey-Kramer method was used for mean separation test. Differences in means were determined using diffogram (called the mean-mean scatter plot by Hsu 1996).

RESULTS AND DISCUSSION

Life cycle of *S. frugiperda*. *S. frugiperda* went through the egg, six larval instars, and pupal stage before becoming an adult (Fig. 1). Description of each developmental stage is presented below.

Egg. Dome-shaped and dorso-ventrally flattened, white to yellow, and laid singly but in mass or cluster, in several layers, one on top of the others. On corn (variety Lagkitan) female deposited eggs in clusters and covered them with cottony-gray scales. However, in confinement, female deposited eggs also in cluster but these are loosely covered with cottony gray scales. Eggs turned dark or black when about to hatch.

Larvae. First instar is yellowish but appeared dark to the naked eye due to brownish-black setae and black head. The base, called pinacula, of each seta is prominent, arranged on a line in the thoracic segments, trapezoidal in the first to the seventh abdominal segment and square on the eighth. The pinacula are conspicuous as early as the first instar when viewed under a microscope but become more prominent and bigger as the larva grows up to the 6th instar larval stage. This character is distinct to the species. The larvae molted five times when reared on corn. Color of matured larvae ranges from dark green to purplish green, or brown. The most common color was purplish green, with a lighter dorsal median stripe and a darker stripe along each side. The sixth instar stopped feeding for a day or two (pre-

pupa) before turning into a pupa (Fig. 1). The face has a white to yellow inverted “Y” line compound of the ecdysial line and fronto-clypeal suture.

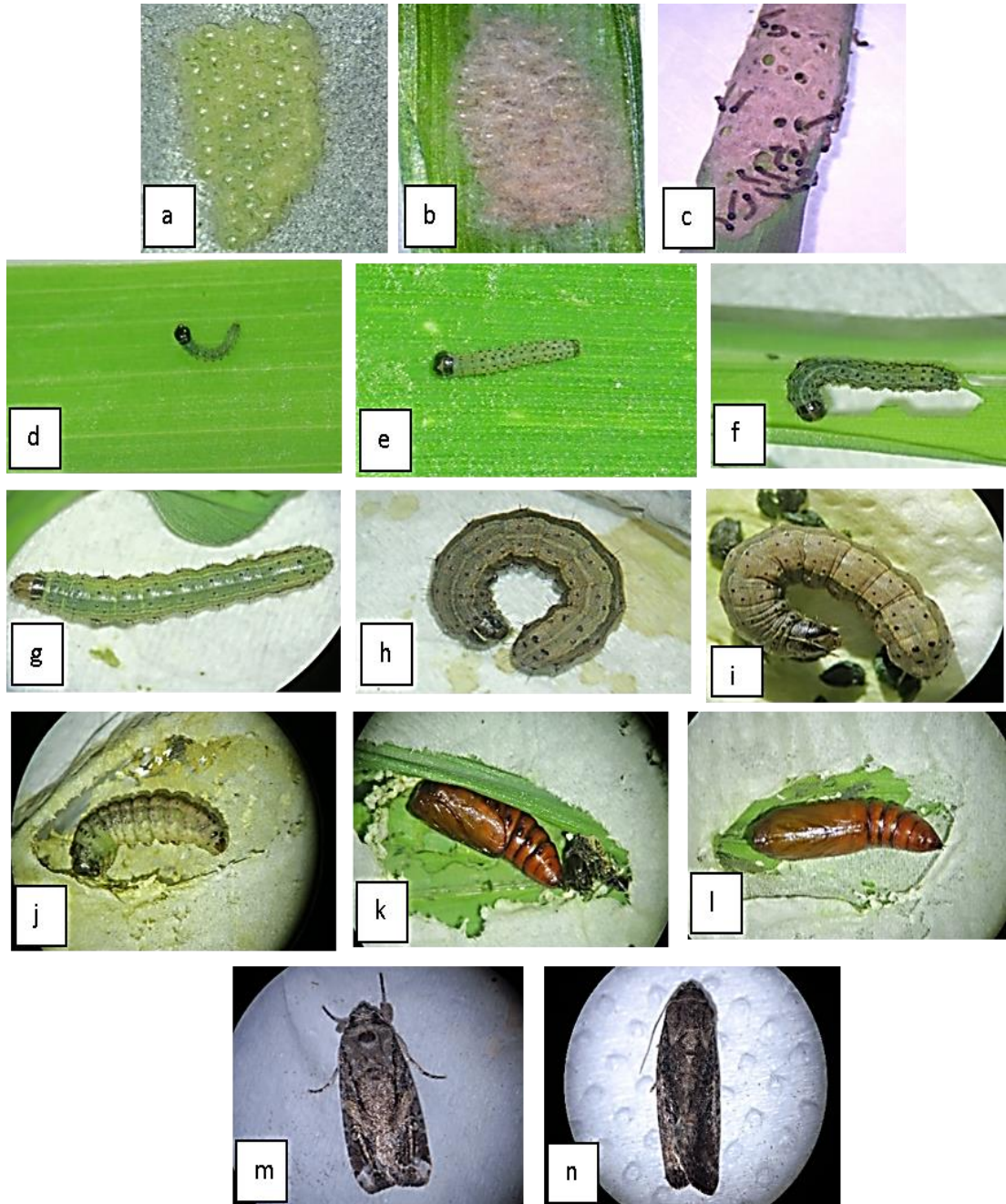


Fig. 1. Developmental stages of *Spodoptera frugiperda*: Eggs laid on a wax paper (a), on corn leaf (b), on stalk of corn starting to hatch, (c); First instar larva on a corn leaf (d); Second instar larva (e); Third instar larva (f); Fourth instar larva (g); Fifth instar larva (h); Sixth instar larva (i); Pre-pupa (j); Male (k) and Female (l) pupae; Male (m) and Female (n) adults.

Feeding damage of the first three larval instars is limited to the leaves which is characterized by window panes; while the older instars fed on whorl, tassel and ear (Fig. 2).

Pupa. In the laboratory, pupation occurs in between corn leaf cuttings, below the tissue paper lining and sides of the plastic plate. Pupae are obovate, initially whitish - green turning brown and darkened nearing adult eclosion.

Both sandy-clay and clayey-sand soils were the most suitable for pupation and adult emergence (Ramirez-Garcia et al. 1987). These soil conditions are found in several places in the Philippines with known history of volcanic eruption like in Central Luzon, Bicol Region, SOCSKSARGEN and Negros provinces.

Adult. Sexual dimorphism exists in the color markings on the forewings (Fig. 2). Forewings of male were grayish brown to rust brown with conspicuous triangular white patch at the apical regions; reniform spot faint, partially but lined in black; orbicular spot light brown, oval and oblique; with a row of small markings near the apical margins. The forewings of female were uniformly grayish brown to rust brown but darker than the male. In both sexes, the hindwings are silvery-white with brownish apical borders.

Our results conform within the range reported in other countries (Andrews, 1998; Sparks, 1979). In Mexico, for instance, larval development takes 20 days in midsummer and 35d in September on corn; respectively 17 days and 15 days on sorghum and corn; respectively 13 days and 22 days on corn and artificial diet. In Surinam, larval development is typically 15 days, may be extended to 22 days or shortened to 9.6 days. In Peru, larval development at 27°C on corn was 18.9 days. In Brazil, it was 21-28 days. In Cuba, 24 days (first instar to adult) on corn, and respectively 26, 28, and 29 days on sorghum, *Stizolobium deeringianum* and *Pennisetum purpureum*. Pupal period was 9, 10, or 11 days to complete development. Females laid a mean of 1,500 or 1,600 eggs, with a mean oviposition period of 9.2 days. Females laid more eggs on corn than on inter-planted sorghum

Morphometry of the different life stages. Measurements (mm) of each developmental stages are presented in Table 1. The dome-shaped egg measures about 0.39mm in diameter and 0.29mm in height. The width head capsule of the six larval instars were as follows: 0.22, first instar; 0.35mm, second instar; 0.61mm, third instar; 0.95mm, fourth instar; 1.46mm, fifth instar; 2.03mm, sixth instar. The increase in the width of the head capsules of the larvae followed a J-shaped curve, where there was a slower increase for the first, second and third instar and dramatic increase starting from the fourth to the sixth instars. The same trend was also observed on the length and width measurements of the body at each instar.

The male pupa measures on the average 16.15mm and the female 15.28mm. The males are generally bigger than females although the general trend in arthropods is that males are smaller.

Adult male body length measures 13.33mm with a wing expanse of 32.66mm. The male is bigger than the female but the difference was not significant.

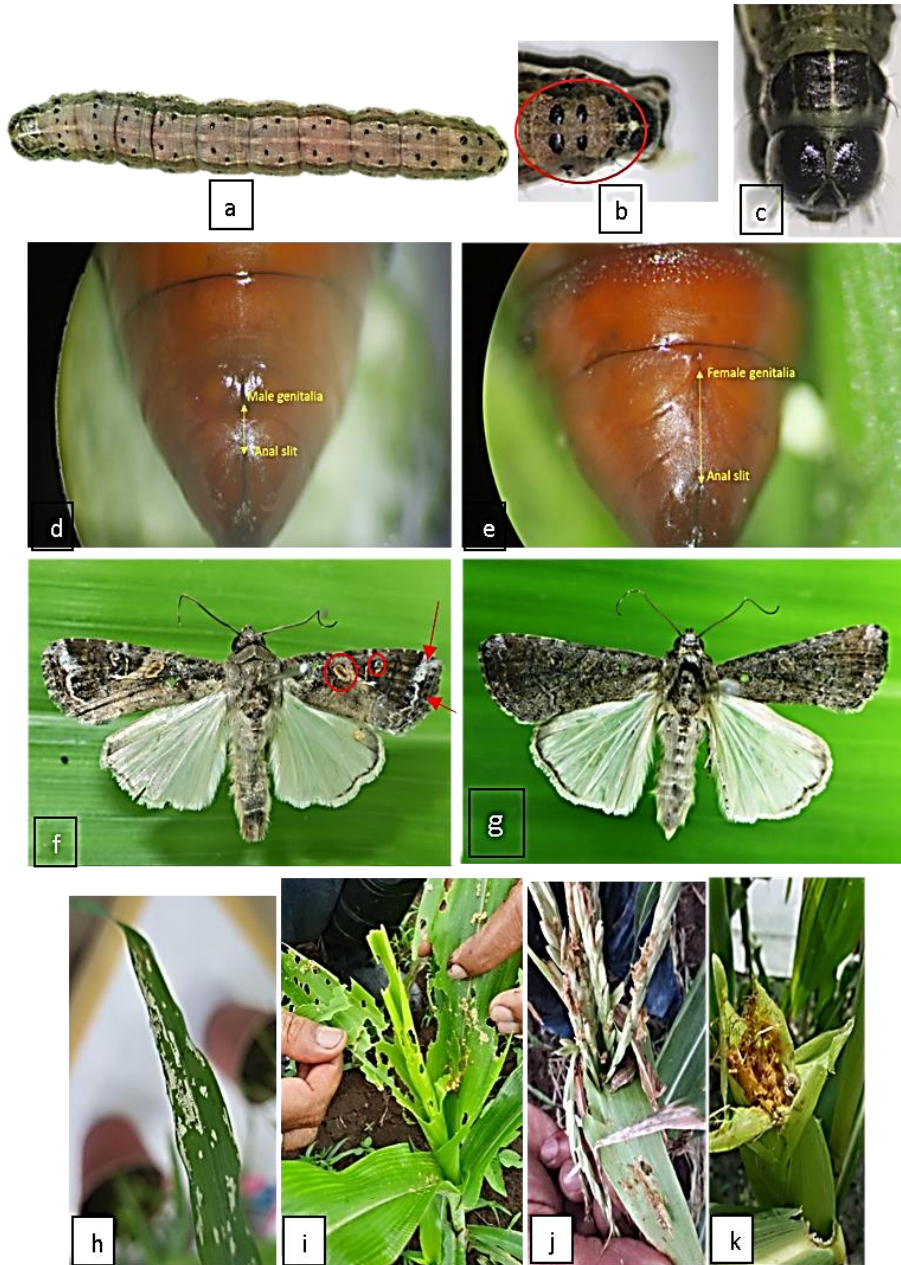


Fig. 2. Distinguishing characters of *Spodoptera frugiperda*: a) Sixth instar larva showing the arrangement of pinacula and close-up of the four pinacula arranged in a square (b); c) head showing inverted white “Y”; d) male and e) female pupae showing short distance between the male genitalia and anal slit but longer in female; f) male and g) female adults; and characteristic feeding damage of larvae- h) window pane due to 1st, 2nd and 3rd instar, i) on whorl; j) tassel and (k) ear.

Table 1. Measurements (mm) of the eggs, larval instars, pupae and adults of *Spodoptera frugiperda* reared on corn under laboratory conditions.

Developmental Stage	Parameter	Mean±SD	
Egg	Width	0.39±0.01	
	Thickness	0.29±0.20	
Larva			
1 st instar	Head capsule	Width	0.22±0.00
	Body	Length	1.77±0.09
		Width	0.23±0.30
2 nd instar	Head capsule	Width	0.35±0.01
	Body	Length	2.79±0.35
		Width	0.35±0.05
3 rd instar	Head capsule	Width	0.61±0.03
	Body	Length	7.41±0.58
		Width	0.89±0.28
4 th instar	Head capsule	Width	0.95±0.50
	Body	Length	14.57±2.09
		Width	1.86±0.18
5 th instar	Head capsule	Width	1.46±0.08
	Body	Length	21.25±1.47
		Width	2.99±1.47
6 th instar	Head capsule	Width	2.03±0.07
	Body	Length	30.79±3.14
		Width	3.82±0.26
Pupa			
Male	Length	16.15±0.43	
	Width	4.93±0.17	
Female	Length	15.28±0.67	
	Width	4.81±0.25	
Adult			
Male	Length of body	Length of body	13.30±0.77
	Wing expanse	Wing expanse	32.66±1.43
Female	Length of body	Length of body	12.20±0.44
	Wing expanse	Wing expanse	32.81±1.60

Mean developmental duration. Table 2 shows the mean development time of FAW fed on young leaves of corn (variety Lagkitan) under laboratory conditions. The egg, first, second and fifth instar larvae of both sexes had similar durations; they are about one and a half times as long as those of the third and fourth instar larvae. The sixth instar larvae are about twice longer than the other stages; total larval period is slightly longer in males (14.73d) than in females (14.4d). Interestingly female pupal duration (8.5d) was significantly shorter than the male pupa (9.0d). Total development periods for the

male was 25.73 days and 24.9 days for the female, although the difference was not significant. Females emerged earlier than males. Generally, males complete development ahead of females in arthropods.

The development of the noctuid *Spodoptera frugiperda* was studied in the laboratory at constant temperatures ranging from 15 to 30°C and LD 12:12. The development time of the various stages decreased with an increase in temperature. On the other hand, the survival rate tended to increase with an increase in temperature (Ramirez-Garcia et al. 1987).

Post development of *S. frugiperda* reared on *Z. mays*. The pre-oviposition (3.5 days) and post-oviposition (2.81d) periods were similar, but slightly shorter than the duration of the oviposition period (3.69d). Longer pre-oviposition period of *S. frugiperda* indicates longer sexual maturation of females. However, in other species of armyworm, a shorter pre-oviposition period of 2.3 days was recorded on *S. exempta* which went into outbreak and damaged corn and other crops in Southern Luzon (Navasero et al. 2011); 2 days for *S. exigua*, on onion and other crops in Central Luzon (Navasero et al. 2019b).

Fecundity and longevity of adult females and males. Fertile females laid eggs ranging from 800 to 1,639 eggs (mean of 1,033) throughout their lifetime; fertile eggs accounted to 73.9% of eggs laid. Males lived slightly longer than females (respectively 10.18 and 9.82 days). Shorter male longevity is a general pattern in arthropods.

Table 2. Durations (in days) of the different developmental stages of male and female *Spodoptera frugiperda* fed on leaves of corn under laboratory condition.

Developmental Stage	Sex	
	Male	Female
	Mean±SD	Mean±SD
Egg	2-3	
Larva		
First instar	2.28±0.45a	2.40±0.49a
Second instar	2.20±0.40a	2.30±0.54a
Third instar	1.45±0.60a	1.37±0.49a
Fourth instar	1.49±0.72a	1.50±0.73a
Fifth instar	2.31±0.57a	2.20±0.48a
Sixth instar	3.95±0.73a	3.75±0.85a
Pre-Pupa	1.08±0.28a	1.10±0.31a
Total larval period	14.73±1.72a	14.40±1.90a
Pupa	9.00±0.70b	8.50±0.86a
Total developmental period (Egg-Adult)	25.73±1.75a	24.90±1.83a

Nature of damage. In Gonzaga and Solana, Cagayan, damage by FAW were all observed from open pollinated varieties of corn in mid-whorl to late whorl among the different growth stages. Similarly, in Ilocos Norte, Zamboanga Del Sur, Zamboanga del Norte and Polomolok, South Cotabato, FAW larvae were collected from open pollinated corn in mid- to late-whorl stages. In General Santos City, South Cotabato, 2nd to 3rd instar larvae were found on 12-day old seedlings. At about 6-7 days from egg-laying to 2nd or 3rd instar, oviposition had occurred at only around 2-3 days after seedling emergence.

Natural enemies of *S. frugiperda*. Initial inventory of natural enemies of *S. frugiperda* recovered from larvae collected from Cagayan and Ilocos Norte and experimental fields in the University of the

Philippines Los Baños, Laguna include a Mermithid nematode, two entomopathogenic fungi, *Metarhizium* sp. and *Beauveria* sp., and two parasitoids, *Chelonus* sp. and *Charops brachypterum* Gupta and Maheswary (Fig. 3). These were new records of natural enemies of FAW in the country. Other workers had also documented similar observations in Africa (Cruz et al. 2018; Sisay et al. 2018; Kenis et al. 2019) and in India (Shylesa et al. 2018; Chormule et al. 2019) which have their own native species of *Spodoptera*.

A total of 172 species of parasitoids and parasites of FAW were reported in the Americas and Caribbean Basin (Molina-Ochoa et al. 2003). Ten species were found parasitizing the pest, including two egg parasitoids, one egg-larval, five larval and two larval-pupal parasitoids. The most abundant parasitoids in both countries were two Braconidae: the egg-larval parasitoid *Chelonus bifoveolatus* and the larval parasitoid *Coccygidum luteum*. Parasitism rates were determined in three Ghanaian regions and averages varied from 0% to 75% between sites and from 5% to 38% between regions. These data provide an important baseline for the development of various biological control options. The two egg parasitoids, *Telenomus remus* and *Trichogramma* sp. can be used in augmentative biological control and investigations should be conducted to assess how cultural practices can enhance the action of the main parasitoids, *C. luteum* and *Ch. bifoveolatus*, in the field. Understanding the parasitoid complex of *S. frugiperda* in Africa is also necessary before any development of classical biological controls involving the introduction of parasitoids from the Americas (Agboyi et al. 2020).

In Florida, parasites destroyed 63% of each of the first four FAW instars, and *C. insularis* emerged from 71% of the parasitized larvae (Ashley et al. 1982).

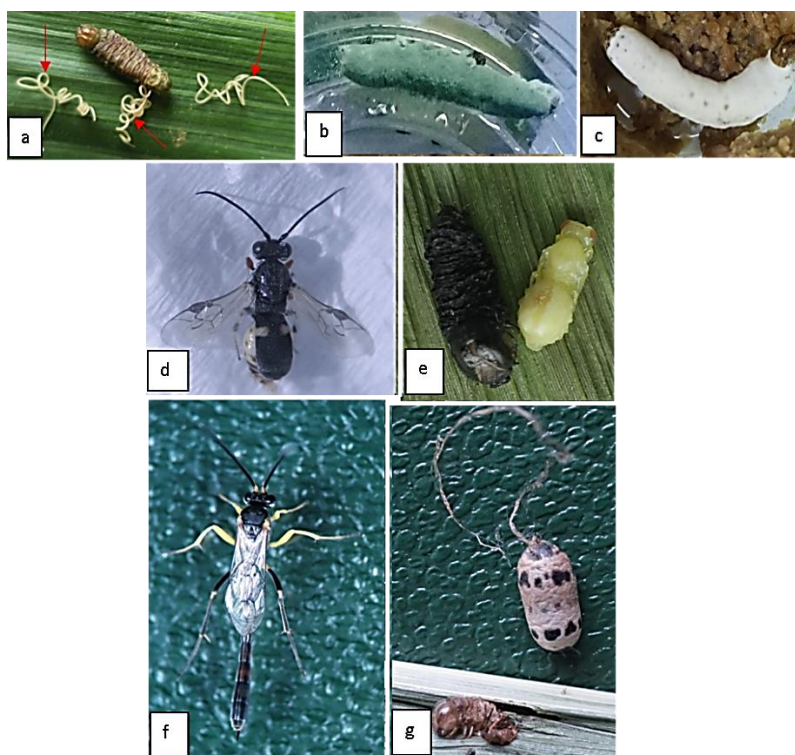


Fig. 3. Mermithid nematode (a, with arrows), *Metarhizium* sp. (b), *Beauveria* sp. (c), *Chelonus* adult (d), pupa of *Chelonus* (e), *Charops brachypterum* Gupta and Maheswary adult (f), and pupa of *Charops brachypterum* Gupta and Maheswary (g).

CONCLUSION AND RECOMMENDATION

The life history and morphometrics data generated from this study report that *S. frugiperda* could develop successfully on a native corn variety (Lagkitan). The short developmental time and high fecundity suggest the potential of this species as a threat to corn production in the Philippines. Initial inventory of natural enemies of *S. frugiperda* include a Mermithid nematode, entomopathogenic fungi and hymenopterous parasitoids, all new records for the country. A continuous inventory of natural enemies of FAW must be conducted to determine their impact on the population of *S. frugiperda*. Subsequent works should evaluate the effect of the pest directly on corn plants and effects of entomopathogens, under controlled and field conditions.

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