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Research Article Biologycycle and Natural Enemies of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in Maize Crops in Cote D'ivoire

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Abstract

Background and Objective: In Cote d'Ivoire, maize is the second most important cereal crop after rice. However, fall armyworm, which is now the most important pest of this plant, is found throughout Africa and Cote d'Ivoire. This study aimed to study the biological cycle of *S. frugiperda* and to identify natural enemies and entomopathogenic fungi to counter the spread of this pest in maize crops in Cote d'Ivoire. **Materials and Methods:** Regular monitoring of maize plots set up in three agroecological zones was carried out. This allowed to collect dead larvae of the pest, to capture predators of the pest and to collect live larvae of the pest for rearing in the laboratory. **Results:** The results showed that the complete biological cycle of *S. frugiperda* reared at a temperature of 24-26 °C and relative humidity of 60-63% is approximately 54 days, with an average of about 39.79 ± 4.59 days. The sex ratio is in favour of the males which were more numerous than the females. The identification of natural enemies revealed the presence of four main predators which are *Doru* sp. (Dermaptera: Forficulidae), Hymenoptera Sphecidae and Heteroptera Reduviidae *Rhynocoris rapax* and *Rhynocoris segmentarus*. The culture of dead larvae on PDA medium allowed isolation and identification of entomopathogenic fungi *Penicillium* sp., *Metarhizium* sp., and *Fausarium* sp., causing the death of this pest in the plots. **Conclusion:** The life cycle of *Spodoptera frugiperda* is about 40 days and four main predators and three entomopathogenic fungi of fall armyworm have been identified in Cote d'Ivoire.

Key words: Fall armyworm, biological cycle, predatory insects, entomopathogenic fungi, provitamin A, maize, relative humidity

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Native to tropical Central America, maize, Zea mays belongs to the Gramineae or Poaceae families like rice, millet and sorghum¹⁻³. It is the number one grain produced in the world and now feeds nearly one billion people on earth. Since the 16th century, maize cultivation has been established in Cote d'Ivoire and West Africa² and is the main cereal crop involved in feeding people in West Africa. In addition, in Cote d'Ivoire, maize is also second only to rice among the cereals grown in the country³.

It provides about 15% of the energy needs of the Ivorian population. Maize is considered as the ideal food for provitamin A for areas with poor access to fortified food⁴. It is also the most cereal used to compose food for cattle³.

Despite these many advantages, maize cultivation is strongly hindered by attacks of many insects, the main ones being *Sesamia calamistis* (Lepidoptera: Noctuidae), *Eldana saccharina* (Lepidoptera: Pyralidae), *Busseola fusca* (Lepidoptera: Noctuidae)⁵ and fall armyworm *Spodoptera frugiperda* JE Smith,1797 (Lepidoptera: Noctuidae) which was first reported in the African continent in January, 2016⁶. This latter causes enormous damage to growing corn, resulting in huge yield losses ranging from 15-73%⁷.

However, control of this insect is difficult due to its resistance to the products used. To carry out an integrated or biological environmentally friendly and efficient control of fall armyworm, a better knowledge of this pest and its natural enemies is important and needed. It is in this perspective that this study was conducted to study the life cycle of fall armyworms and then searching for natural enemies.

MATERIALS AND METHODS

Study sites: The work was carried out in three agro-ecological zones in Cote d'Ivoire: the Southern, the Central and the Northern part of the country. The study was realized in Cote d'Ivoire from January-December, 2020. This study was conducted in the Laboratory of Entomology of La Mé for the rearing of fall armyworm, in the Laboratory of Entomology of Nangui Abrogoua University for identification of natural

enemies and the Laboratory of Plant Pathology of CNRA-Bouaké for isolation and identification of entomopathogenic fungi. Three maize plots were set up in the South at CNRA research station in La Mé, three plots in the Centre at CNRA food crop research station in Bouaké and three plots in the North at CNRA maize, millet and sorghum research station in Ferkessédougou. The geographical coordinates of these sites are presented in red colour in Table 1.

Experimental design: The design used was a completely randomized block design. Three plots of 0.5 ha each were selected for each agro-ecological zone. Each plot was subdivided into three blocks. In each block, a total of 12,000 seeded maize plants were regularly monitored. In these plots, daily observations were made. Predators were collected as well as dead larvae of fall armyworm *Spodoptera frugiperda*. Live larvae of fall armyworms were also captured for rearing in the laboratory.

Evaluation of some biological parameters: The study of the life cycle consisted of determining the biological parameters such as the developmental stages (egg, larva, adult), the total duration of development and the sex ratio of these insects. Collected larvae were studied in the laboratory in rearing boxes at temperatures ranging from 24-26°C and relative humidity ranging from 60-63%. Ten pairs of adults (Male×Female) were placed in rearing boxes, to follow their evolution until oviposition and from oviposition to the last larval stage.

Duration of the pre-oviposition period: The average duration of pre-oviposition (Dp), expressed in days, is the time between the emergence of the adult and the first oviposition (Pp). It is obtained according to the following formula:

$$Dp = \frac{\sum tiji}{\sum si}$$
, with $ti = Pp - T$

Ti: Time from emergence to first egg-laying

ji : Number of females

Table 1: Geographical coordinates of study sites	
CNRA	
Research station of La Mé	Latitude 5°26' N
	Longitude 3°50' W
Research station on food crops of Bouaké	Latitude 7°44' N
	Longitude 5°04′ W
Research station on maize, millet and sorghum of Ferkessédougou	Latitude 9°35'37" N
	Longitude 5°11'50" W

Life span of the different larval stages: Upon hatching, the first larval stages were placed separately in boxes containing the corn leaves. These leaves were renewed regularly to feed the larvae until the emergence of the adults. Each larval stage was monitored daily until moulting. The dates of moult were recorded and used to calculate the life span of each developmental stage. The average duration of the transition from one larval stage (Dlx) to the next is the time between a moult corresponding to a given larval stage and the next. It was calculated according to the following formula:

$$Dlx = \frac{\sum \sin i}{\sum ni}$$

si : Time between one moult and the next ni : Number of larvae of stage x

III . Nulliber of larvae of stage x

Average life span of the adult: The average life span (Dv) of the adult is the time between the date of the adult's emergence (De) and the date of its death (Dm). It is evaluated as follows :

$$Dv = \sum \frac{Dm - De}{Ni}$$

with Ni: Number of adults.

Developmental cycle length: The average Developmental Cycle length (Dc) is the period between egg-laying and adult emergence (the period between the date of observation of the 1st egg and the date of observation of the 1st immature adult).

$$Dc = \frac{\sum pi Ni}{Ni} pi = Di + Dl_1 + Dl_2 + Dl_3 + Dl_4 + Dl_5 + Dl_6 + Dp$$

- pi : Different stages of development
- Ni : Number of adults
- Di : Duration of egg incubation
- DI_1 : Duration of 1st larval stage L1
- Dl₂ : Duration of 2nd larval stage L2
- Dl₃: Duration of 3rd larval stage L3
- Dl₄ : Duration of 4th larval stage L4
- Dl₅: Duration of 5th larval stage L5
- Dl₆: Duration of 6th larval stage L6
- Dp : Duration of pre-oviposition

Determination of the sex ratio: The sex ratio is the ratio of the number of males to the number of females in a sexually

reproducing species for a generation or in the offspring of an individual. It has been calculated according to the following formula:

$$Sex-ratio = \frac{Nm}{Nf}$$

Nm = Number of males Nf = Number of females

Capture and identification of predatory insects of fall armyworm: In each plot, regular monitoring was carried out. This allowed us to capture predators using a mowing net. Then they were put in contact with FAW to see how effective their predation was on this maize pest. The identification of these predators was made by careful observation of certain morphological characteristics such as the colour, head, abdomen, thorax and wings of the insects. Identification or determination keys of Poutouli⁸ and Willsch *et al.*⁹ were used for the completion of this study.

Collection of dead larvae and identification of entomopathogenic fungi of fall armyworm: Samples of 10 dead S. frugiperda larvae were collected from each of the following three sites, La Me, Bouake and Ferkessedougou. A total of 30 dead larvae were collected and placed in jars containing 70% alcohol for better preservation. The larvae were sent to the phytopathology laboratory of the Food crop research station of CNRA in Bouake, to isolate and identify possible entomopathogenic fungi responsible for their death. Isolation of fungi from dead larvae was done according to the modified method of Zulfitri et al.¹⁰. The dead larvae were first disinfected with 1% sodium hypochlorite for 30 sec and rinsed 3 times for 3 min with sterile distilled water. They were then wiped in sterile blotting paper. The disinfected larvae were placed in sterile Petri dishes on PDA medium and incubated in the dark at room temperature until abundant mycelial fluff appeared. Then mycelial fluff was inoculated into new sterile Petri dishes on PDA medium. a classic rich medium for the isolation of fungi. Petri dishes were placed in a dark incubation chamber at room temperature $(28\pm2^{\circ}C)$ for 3 days. Mycelial colonies obtained from the seeded fluff were transplanted on a PDA medium for purification. Each isolate consists of the mycelial colony obtained from the dead larvae. The colour and appearance of the thallus of each isolate were noted. Fragments of mycelium were then observed with a camera-exit light microscope (brand: Axio Lab. A1 ZEISS) to identify the fungi using the identification key of Sharma et al.¹¹.

Data analysis: The results obtained were subjected to a descriptive analysis using SAS 9.4 software. This allows to determine the means, standard deviations, minimums and maximums of the various biological parameters studied.

RESULTS

Biological cycle of *S. frugiperda*: The life cycle of *Spodoptera frugiperda* is composed of four main stages which are (1) The egg, (2) The larva, (3) The nymph and (4) The adult as in all holometabolous insects or insects with complete metamorphosis.

Pre-oviposition: Pre-oviposition lasts 2-3 days. The average is about 2.5 ± 0.52 days.

Eggs: The eggs are laid in clusters and covered with protective silk on the underside of the corn leaf. They are spherical, whitish or greyish. Figure 1a, b show eggs of different colourations of fall armyworm. The average hatching time is 3.87 ± 1.12 days (Table 2).

Larva: The L1 larvae are greenish-yellow with a blackish head. However, the old larva is dark green. There is an inverted "Y" visible on the head. Longitudinal lines are present on the back, with four black dots on the back of the back (Fig. 2). The duration of the larval stage ranges from 15 to 25 days, with an average of 19.31 ± 3.47 days (Table 2).

Chrysalis: The chrysalis is brown (Fig. 4). The average time from pupation to the emergence of the adult moth was 7-13 days. The average duration was 10.20 ± 1.78 days (Table 2).

Adult: The forewings are greyish. On the forewings of the male, there is a pale oblique band towards the middle of the wing and a white spot at the apex (Fig. 3). Adult life span varies from 1-11 days with an average of 10.00 ± 0.92 days (Table 2).

Sex ratio: The sex ratio obtained in this study is in favour of males. Indeed, out of 20 insects that reached the adult stage, the sex ratio was 1.5, i.e., 12 males and 8 females.

Development stages	Minimum duration (days)	Maximum duration (days)	Mean±Standard deviation (days)
Eggs	2	5	3.87±1.12
1st larval stage	3	5	3.84±0.14
2nd larval stage	1	3	1.45±0.50
3rd larval stage	1	2	1.52±0.51
4th larval stage	3	4	3.23±0.61
5th larval stage	2	3	2.55±0.60
6th larval stage	5	8	6.72±1.11
Total duration of larval stages	15	25	19.31±3.47
Pupal stage	7	13	10.20±1.78
Life span of adult	1	11	10.00±0.92
Total	25	54	39.79±4.59

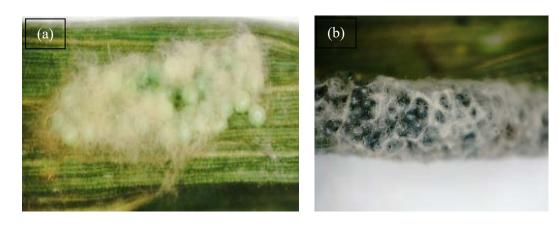


Fig. 1(a-b): Eggs of *S. frugiperda*

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(a) Whitish eggs laid in a laboratory and (b) Greyish eggs collected in a trial plot in Ferkessedougou

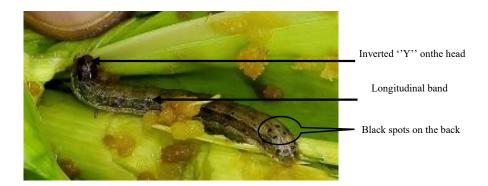


Fig. 2: Aged larva of *S. frugiperda* in the horn of a maize plant

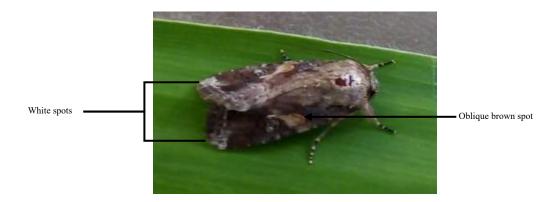


Fig. 3: Male adult of *S. frugiperda*

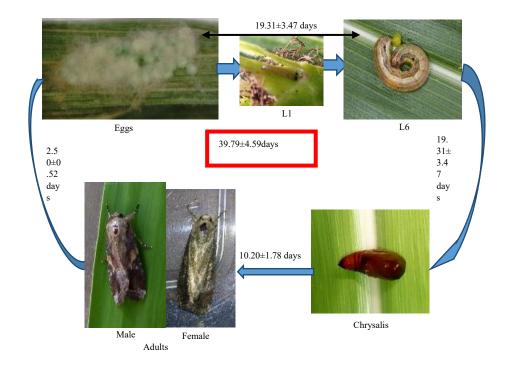


Fig. 4: Biological cycle of fall armyworm *Spodoptera frugiperda* (Lepidoptera: Noctuidae)



Fig. 5(a-d): Main predators captured in the three agro-ecological zones

(a) *Doru* sp. (Dermaptera: Forficulidae), (b) *Rhynocoris rapax* (Heteroptera: Reduviidae), (c) Hymenoptera Sphecidae and (d) *Rhynocoris segmentarus* (Heteroptera: Reduviidae)

The complete developmental cycle of *S. frugiperda* is a maximum of 54 days, with an average of 39.79 ± 4.59 days (Table 2, Fig. 4). The duration of the different developmental stages and the complete life cycle of the fall armyworm are recorded in Table 2 below.

Natural enemies collected

Predators of the armyworm encountered: Four main predators of fall armyworm *S. frugiperda* were caught in the trial plots in the agroecological zones surveyed (Fig. 5a-d). There are:

- *Doru* sp. (Dermaptera: Forficulidae) captured in the three zones surveyed (South, Centre, North) on the experimental plots (Fig. 5a)
- *Rhynocoris rapax* (Heteroptera: Reduviidae) encountered in the plots of the research station of CNRA of La Me (Fig. 5b)
- The hymenoptera sphecidae captured on the plots of the research station of Food Crops of CNRA of Bouake and the research station of Maize, millet and sorghum of CNRA of Ferkessedougou (Fig. 5c)

Rhynocoris segmentarus (Heteroptera: Reduviidae) which was captured on the plots of the research station of Maize, Millet and Sorghum of CNRA of Ferkessedougou (Fig. 5d)

Entomopathogenic fungi identified: Three entomopathogenic fungi were isolated from dead larvae of fall armyworm collected in the field. There are *Penicillium* sp., *Metarhizium* sp. and *Fusarium* sp. (Fig. 6-8).

Penicilliumsp.: The shape of the colonies is round. The colony of *Penicillium* sp., is green, round with a powdery surface. Its mycelial growth is more or less rapid on the PDA culture medium (Fig. 6a). Microscopic appearance showed the presence of aseptate mycelium with smooth, branched conidiophores that have a brush-like shape, on which the phialides are borne by the tips of the branches. In turn, they bear hyaline or brightly coloured conidia when en masse. They are spherical and assembled in chains(Fig. 6b-c).

Metarhizium sp.: In mass, the colonies show an olive-green colouration (Fig. 7a-b). Their microscopic appearance shows

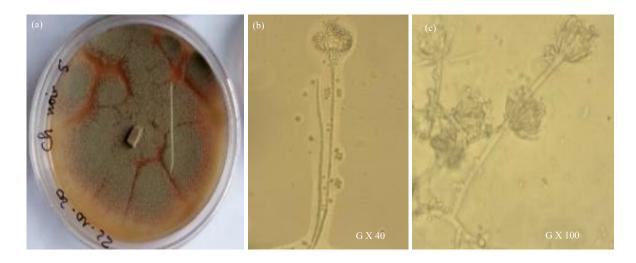


Fig. 6(a-c): Morphological characters of *Penicillium* sp. (a) Macroscopic view and (b-c) Microscopic view

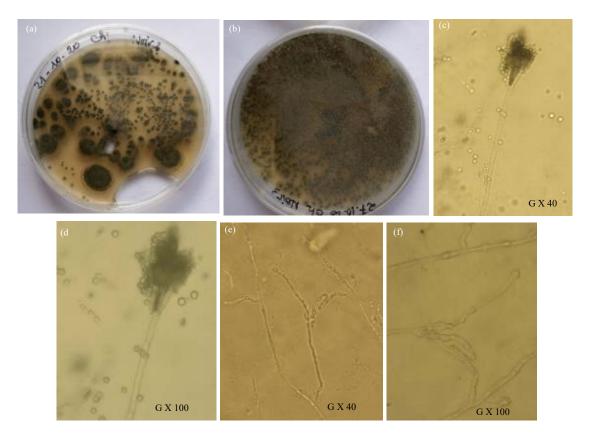


Fig. 7(a-f): Morphological characters of *Metarhizium* sp. (a-b) Macroscopic view and (c-f) Microscopic view

that fruiting is in the form of phialides giving rise to conidia or phialospores. Phialides may be isolated, in pairs or groups, produced from a basal chain. They are compacted into long ovoid or even cylindrical columns (Fig. 7c-f). **Fusarium sp.:** This species forms white fluffy colonies, which then remain white or turn purplish-red (Fig. 8a-b). The main morphological characteristic of *Fusarium* is the presence of fusiform, septate macroconidia. Phialides are short and broad

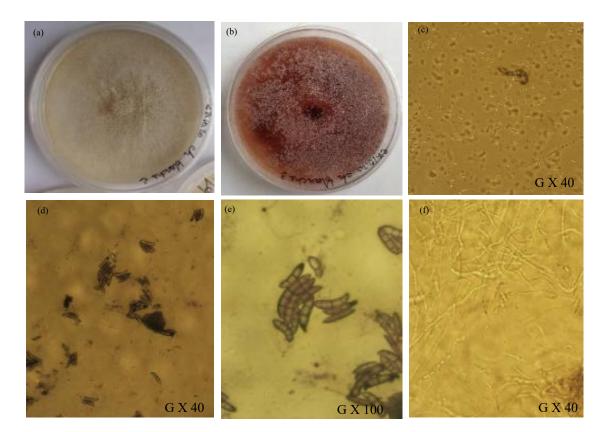


Fig. 8(a-f): Morphological characters of *Fusarium* sp. (a-b) Macroscopic view and (c-f) Microscopic view

and form on the aerial mycelium. Micro conidia are abundant (Fig. 8c), curved, fusiform and septate (Fig. 8d-e) while chlamydospores are intercalated or terminal and formed by the mycelium or conidia (Fig. 8f).

DISCUSSION

Collected *Spodoptera frugiperda* caterpillars were reared in the laboratory on a maize leaf substrate at a temperature of 24-26°C and a humidity of 60-63%. The results showed whitish or greyish coloured eggs, six larval stages with a visible inverted "Y" on the head for stages 3-6. The chrysalis is brown, the adult emerging with white spots on the wingtips and brown spots in the middle of the wings. The morphological characteristics of the developmental stages correspond to those described by Prada *et al.*¹², Navasero and Navasero¹³ and FAO¹⁴.

In this study, the duration of pre-oviposition was on average 2.52 ± 0.52 days. This is within the range of 2-3 days, corresponding to that observed by FAO¹⁴. Also, the average complete development cycle of the insect was 39.79 ± 4.59 days. Sharanabasappa *et al.*¹⁵ reported that the

complete life cycle of males and females of fall armyworm is 32-43 days and 34-46 days, respectively.

During our study, many natural enemies of fall armyworms were encountered. These were predators on the one hand and entomopathogenic fungi on the other hand. Concerning predators, Doru sp. (Dermaptera: Forficulidae), Rhynocoris rapax, Rhynocoris segmantarius belonging to the family of Reduviidae and orders of Heteroptera and one Hymenoptera Sphecidae were captured. According to Prasanna et al.¹⁶, Doru luteipes is one of the most important natural enemies of fall armyworm. It feeds on eggs and larvae of S. frugiperda. The results also showed that the Reduviidae R. rapax and R. segmentarius are good predators of fall armyworms. The majority of Reduviidae are so-called generalist predators as they feed on a wide range of prey. Except for the subfamily Triatominae, which are haematophagous, all Reduviidae are insect predators. Their prey can there fore be caterpillars, aphids, bugs, Diptera, beetles, Hymenoptera, etc.⁶, which explains the predation of *S. frugiperda* by these species of Reduviidae during our study. The Hymenoptera Sphecidae observed was described by Eroğlu and Yuksel¹⁷ as a parasitoid that captures various insects or spiders, paralysis them with its venom and transports them to its nests, which are of ten built in the soil, wood, hollow stems or various crevices. Entomopathogenic fungi were isolated from dead larvae of fall armyworms. The presence of these fungion fall armyworm would be due to the presence of moulds on the maize plants for fungi such as Penicillium sp. and on pupae, in the soil as is the case of *Metarhizium* sp. and *Fusarium* sp. which are found in the soil. These fungi infect many insects, thus reducing their populations. According to many authors such as Er et al.¹⁸ and Wang et al.¹⁹, these fungi have also been isolated from Thaumatopoeidae lepidopteran Thaumetopoea pityocampa (Schiff.) and fall webworm, *Hyphantria cunea* (Drury) (Lepidoptera: Arctiidae and proved to be effective against this insect. This is similar to our study. Fall armyworm is a dangerous pest in maize cultivation. The recognize of the life cycle and natural enemies of this insect appear as an advantage for cultivators. Natural enemies help to fight against this pest. it a method of respecting the environment and is not dangerous for users. However, this method can belimitedbecauseeachnaturalenemyisconfrontedwithenviro nmentalfactorswhich can impact its action.

CONCLUSION

Some biological parameters were investigated during this study. The results showed that the complete life cycle of armyworm *S. frugiperda* averaged 39.79 ± 4.59 days. Four main predators of the pest were recorded in the three agroecological zones. These were *Doru* sp. (Dermaptera: Forficulidae), *Rhynocoris rapax* (Heteroptera: Reduviidae), a Hymenoptera Sphecidae and *Rhynocoris segmentarus* (Heteroptera: Reduviidae). Three entomopathogenic fungi were isolated from the dead larvae of *S. frugiperda*. These are *Penicillium* sp., *Metarhizium* sp. and *Fusarium* sp. It is, therefore, necessary to extend the study to other ecological zones but also to test the pathogenicity of these fungi towards fall armyworm.

SIGNIFICANCE STATEMENT

This study discovers the life cycle duration and some natural enemies such as predatory insects and entomopathogenic fungi of the fall armyworm in maize crops. This can be beneficial for both maize cultivators and other crops as it is a polyphagous pest. These predators and entomopathogenic fungi of this pest could be used for effective and environmentally friendly control of fall armyworms. This study will help the researcher in the decision making regarding the use of biological control against this pest. Thus, a new theory on the joint use of predators, entomopathogenic fungi and chemicals can be developed.

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