OCCURRENCE AND DISTRIBUTION OF FALL ARMYWORM, SPODOPTERA FRUGIPERDA (LEPIDOPTERA: NOCTUIDAE) AND OTHER MOTHS ON MAIZE IN GHANA

By

DJIMA KOFFI

ID: 10600839

A THESIS SUBMITTED TO THE UNIVERSITY OF GHANA, LEGON IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF PHILOSOPHY (M.PHIL.) DEGREE IN ENTOMOLOGY.

AFRICAN REGIONAL POSTGRADUATE PROGRAMME IN INSECT SCIENCE
(ARPPIS) UNIVERSITY OF GHANA, LEGON, ACCRA, GHANA
AUGUST, 2018

* JOINT INTER-FACULTY INTERNATIONAL PROGRAMME FOR THE TRAINING OF ENTOMOLOGISTS IN WEST AFRICA

COLLABORATING DEPARTMENTS:

ANIMAL BIOLOGY AND CONSERVATION SCIENCE

(SCHOOL OF BIOLOGICAL SCIENCES)

AND

CROP SCIENCE

(SCHOOL OF AGRICULTURE)

COLLEGE OF BASIC AND APPLIED SCIENCES

DECLARATION

I hereby declare that this thesis is the result of the original work personally done by me for the award of a Master of Philosophy Degree in Entomology at the African Regional Postgraduate Programme in Insect Science (ARPPIS), University of Ghana, Legon. All the references to other people's work have been duly acknowledged and this thesis has not been submitted in part or whole for the award of a degree elsewhere.

Signature DJIMA KOFFI (STUDENT)	Date
Signature DR. ROSINA KYEREMATEN (PRINCIPAL SUPERVISOR)	Date
Signature DR. VINCENT Y. EZIAH (CO-SUPERVISOR)	Date
SignatureDR. SABINE HÄNNIGER (CO-SUPERVISOR)	Date
Signature DR. MAXWELL KELVIN BILLAH (ARPPIS COORDINATOR)	Date

ABSTRACT

The fall armyworm, Spodoptera frugiperda (Lepidoptera: Noctuidae) is an exotic polyphagous insect pest originating from the Americas first reported in Ghana in 2016 on maize. To improve its management and control in Ghana which is poorly known, 72 Agricultural Officers and 187 farmers were interviewed with structural questionnaires on their perceptions and field practices on the management of the pest. Damage on maize plants was determined in maize farms from different localities of the ten regions of Ghana. The larvae that had infested the maize farms were collected and identified, and the infestation levels as well as their distributions were determined. The natural enemies of the fall armyworm infesting or attacking the larvae were identified, and parasitism rate was calculated on the collected larvae. Strains of fall armyworm and their hybrids were then molecularly identified, and their distribution mapped. From the respondents, S. frugiperda was reported to be a key pest of maize but only the larval stage could be identified. The pest population increased during the cropping seasons but its occurrence throughout the year was not observed by most of the responders (65.25%). To manage the pest, 73.26% of the farmers used insecticides. Out of a total of 110 farms inspected during the study, 62.43% were sprayed with chemicals, 46.85% of maize plants were damaged by S. frugiperda, Leucania sp, Chilo partellus, Anicla infesta, Alpenus maculosa, Eldana saccharina and Sesamia calamisti (other moths infesting maize plants). The damage was higher in the major cropping season than the minor cropping season with infestations of 0.41 and 0.40 larva per infested plant, respectively. Spodoptera frugiperda was the most abundant representing 95.43% of all the collected pests. Pheidole megacephala (Fabricius) (Hymenoptera: Formicidae), Haematochares obsuripennis Stål and Peprius nodulipes (Signoret) (Heteroptera: Reduviidae) were the predators that preyed on the larvae. Seven species of fall armyworm parasitoids emerged from the larvae: Chelonus cf. maudae Huddleston, *Cotesia* sp., *Coccygidium* sp., *Meteoridea testacea* (Granger) and *Bracon* sp. (Hymenoptera: Braconidea); *Anatrichus erinaceus* Loew (Diptera: Chloropidae) and an undetermined species of Tachnidae (Diptera) with 3.58% parasitism rate. All the fall armyworm collected and identified belonged to the corn strain. There was no rice strain on the samples on the samples collected and identified.

DEDICATION

With the Protection and Grace of God, I have been able to successfully complete the MPhil study and research. I therefore wish to dedicate this firstly to God and then to my beloved mother, Yawa Ogouragba and my beloved father, Yawou Odjougnan, my sisters (Bella, Collette, Emefa) and my brothers (Komi, Ismael) for their unflinching supports.

ACKNOWLEDGMENTS

This work would not have been accomplished without the support and assistance of many people to whom I express my gratitude and thanks.

My appreciation goes first of all to the German Academic Exchange Service (DAAD) for the scholarship that I was awarded to pursue this M.Phil. Programme.

I am most thankful to Dr. Rosina Kyerematen, Dr. Vincent Y. Eziah and Dr. Sabine Hänniger for accepting without any hesitation to supervise this work from the beginning to the end. I really appreciate their great scientific contributions which have made this work more meaningful.

I express my sincere gratitude to all the ARPPIS lecturers, for the quality of training they provided me. My acknowledgement especially goes to the coordinator, Dr. Maxwell Kelvin Billah for his pieces of advice and encouragement. Many thanks to Prof. Kwame Afreh -Nuamah and Drs. Millicent Cobblah and Ken Okwae Fening for the time they spent to discuss with me and encouraged me in various ways to complete this programme and this research work. I am also grateful to the Laboratory technicians of ARPPIS for their assistances.

My profound gratitude goes also to Prof. John L. Capinera from University of Florida, United States of America (USA), Prof. Astrid Grost from University of Amsterdam, Netherlands, for their advice and orientation they provided me for my research work. Many thanks to Dr. Robert L. Meagher and Dr. Rodney Nagoshi from Center for Medical, Agricultural and Veterinary Entomology, USDA-ARS, Gainesville, Florida, the United States of America for their collaboration and assistance they provided me during my research work. With deep sense of respect, I especially wish to acknowledge the effort and sacrifice of Prof. David Heckel, director of the Department of Entomology, Max Planck Institute for Chemical Ecology, Jena, Germany and his staff for accepting me in their department for the molecular part of my research work. I

appreciate their kindness and the relationship of friendship we have built up during the time I spent with them. Another deep sense of respect goes to Dr. Goergen Georg from International Institute for Tropical Agriculture, Cotonou, Benin and his institution, IITA, for accepting to identify the natural enemies associated with *S. frugiperda*.

I really appreciate the huge help I received from Dr. Agyeman and Daniel from West Wfrican Center for Crop Improvement (WACCI), University of Ghana, Legon. My sincere appreciation goes to Mr. Otu Benjamin Owusu and all Agricultural Biotechnology laboratory staff specially Mr. Raphael Efoe for the training they gave me in their lab.

Great thanks to Prof. Koffi Koba and Prof. Minikpo Jean Sogbedji from University of Lomé, Togo for their kind support and encouragement for my study. Special thanks to Dr. Komivi S. Akutse from Internationa Centre of Insect Physiology and Ecology (ICIPE), Kenya for his interest in my academic welfare. Thanks to Mr. Medetissi Adom and Mr. Ayaovi Agbessenou, they believed in me and encouraged me to participate in the ARPPIS program.

I am most thankful to all my classmates namely Abundance Itohan Osseratin, Kokouvi Delanyo Adenka, Derrick Verschii Tanka, Favour Morrah Chinelo, Komlanvi Amouzou, Lilian Chiamaka Ezemuoka, Oguenetega Sunday, Samuel Riely Aravessochey. We spent unforgettable toil and joyful times together during our sojourn and study in Ghana.

I am very grateful to my parents, relatives and friends for their moral support and their prayers for me. Finally and above all, I give praise to the Almighty God for His protection and blessings.

TABLE OF CONTENTS

DECLARATION	i
DEDICATION	iv
ACKNOWLEDGMENTS	V
TABLE OF CONTENTS	vii
LIST OF PLATES	xi
LIST OF FIGURES	xii
LIST OF TABLES	xiv
LIST OF APPENDICES	xvi
CHAPTER ONE	1
GENERAL INTRODUCTION	1
1.1. Background information	1
1.3. Objectives	5
1.3.1. Main objectives	5
1.3.2. Specific objectives	5
CHAPTER TWO	6
LITERATURE REVIEW	6
2.1. Introduction to the fall armyworm	6
2.2. Distribution of fall armyworm	7
2.3. Biology and Ecology of FAW	7
2.3.1. Egg	8
2.3.2. Larva	9
2.3.3. Pupa	10
2.3.4. Adult	11

2.4. Str	ains and hybrids of fall armyworm	12
2.5. Alt	ternative host plants	13
2.6. Da	mage caused by FAW	13
2.7. Na	tural enemies	15
2.8. Ma	anagement of FAW	16
2.8.1.	Sampling	16
2.8.2.	Insecticides	17
2.8.3.	Cultural techniques	18
2.8.4.	Host plant resistance	18
2.8.5.	Biological control	19
2.9. FA	W similar moths with economic important	20
CHAPTER	THREE	22
MATERIAI	LS AND METHODS	22
3.1. Stu	ıdy areas	22
3.1.1.	Geographic description of study area	22
3.1.2.	Distribution of agricultural areas in Ghana	23
3.2. Su	rvey	23
3.2.1.	Zones and localities of survey	23
3.2.2.	Period of survey	30
3.2.3.	Interviews of agricultural actors	30
3.2.4.	Survey on the distribution of the fall armyworms and similar moths	31
3.2.5.	Survey on the infestations of maize farms	32
3.2.6.	Survey of FAW larval natural enemies	32
3.3. Re	aring	33

3.3.1. Reari	ing design	33
3.3.2. Conse	ervation and preservation of specimens	34
3.4. Identificat	tion of FAW, similar moths and natural enemies	35
3.4.1. Ident	ification through the damage caused	35
3.4.2. Morp	phological identification of the pests	36
3.4.3. Natur	ral enemies Identification	38
3.4.4. Fall a	armyworms' strains an hybrids identification	39
CHAPTER FOUR.		45
RESULTS		45
4.1. Knowledge a	nd perception on FAW and farmers' practices for management	45
4.1.1. Profile of	f the respondents	45
4.1.2. Knowled	ge on occurrence of fall armyworm	46
4.1.3. Farmers	applied control methods for the fall armyworm	49
4.2. Infestation an	nd distribution of FAW and similar moths in Ghana	51
4.4. Natural enen	nies of the FAW in Ghana	65
4.5. Molecular ch	aracterization of fall armyworm samples from Ghana	72
4.5.1. Fall arm	yworm strains distribution based on mitochondrial marker	72
4.5.2. Fall arm	yworm Strain distributions based on the mitochondrial and Z-linked mar	kers 73
4.5.3. Addition	al Tpi test	75
CHAPTER FIVE		77
DISCUSSION		77
5.1. Perceptions of	of the agricultural players and the farmers practices on fall armyworm	77
5.2. Infestation a	nd distribution of the fall armyworm and other moths	78
5.3. Field control	and damage of the fall armyworm on maize crop	80

5.4. Natural enemies of the larva of FAW	80
5.5. Molecular characterization of FAW samples from Ghana	83
CHAPTER SIX	85
GENERAL CONCLUSIONS AND RECOMMENDATIONS	85
6.1. Conclusion	85
References	87
APPENDICES	106

LIST OF PLATES

Plate 1: Description of the fall armyworm; a- male, b- female, c- larva8
Plate 2: A mass of the fall armyworm eggs showing scales between the eggs9
Plate 3: Fall armyworm larval stage showing different instars; a- first instar, b-second instar, c-
third instar, d- fourth instar, e- fifth instar, f- sixth instar
Plate 4: Fall armyworm pupation; a- in the soil, b- in the debris
Plate 5: Fall armyworm adults showing; a- male, b- female
Plate 6: Damage caused by the fall armyworm on maize; a- leaves, b- whorl, c-ear14
Plate 7: Rearing bottle; a- type "voltpack" 11x8.5x5.5 cm3, b- aerated bottle, c- ready bottle.33
Plate 8: Rearing procedure; a- larval stage, b- pupa stage, c- adult stage34
Plate 9: Preserving of specimens; a- drying of adults, b- conserving of adults, c- conserving of
larvae with ethanol 70%, d- conserving of natural enemies with ethanol 70%35
Plate 10: Damage of FAW on maize plant; a- on the leaves, b- on the tassel, c- on the ear36
Plate 11: Last instar of FAW larva showing the main features
Plate 12: Typical fall armyworm adult; a- male, b- female
Plate 13: Strains identifications with mitochondrial gene COI and sex chromosome gene Tpi (A)
PCR digested with Msp1 and with Sac1; (B) the ten different consensual references SNPs to
identify the Tpi-strains; (C) the final decision on strain and hybrid identifications based on the
results of COI and Tpi42

LIST OF FIGURES

Figure 1: Ghana map showing the different localities of survey
Figure 2: Quadrant designs on the farms; a- rectangle and square farm, b- Trapeze farm, c- triangle
farm
Figure 3: Management methods adopted by farmers to reduce the fall armyworm infestation50
Figure 4: Distribution of FAW and other moths per farm at different maize stages in Ghana, 2017;
FAW portion in red and other moths in pink
Figure 5: Distribution of the FAW and other moths per maize farm in the ten regions of Ghana,
2017; FAW is colored in red and other moths in pink; 1-Greater Accra Region, 2- Central Region,
3- Western Region, 4- Ashanti Region, 5- Eastern Region, 7- Brong Ahafo Region, 8- Northern
Region, 9- Upper West Region, 10- Upper East Region
Figure 6: Damage levels on different stages of maize during the two cropping season64
Figure 7: Distribution of damage across the country during the two cropping seasons65
Figure 11 : Fall armyworm mitochondrial Cytochrome Oxidase Subunit1 (COI) gene distribution:
orange color represent COI-CS and blue color COI-RS whereas the orange dots are the sampling
sites ; 1. Greater Accra Region, 2. Central Region, 3. Western Region, 4. Ashanti Region, 5.
Eastern Region, 6. Volta Region, 7. Brong Ahafo Region, 8. Northern Region, 9. Upper West
Region, and 10. Upper East Region
Figure 12 : Situation report of FAW strain with mitochondrial COI marker and sex chromosome
Z-link Tpi marker; orange color represent COI-CS Tpi-C, green color shows COI-RS Tpi-C, pale
color represent COI-CS Tpi-R, the COI-RS Tpi-R is usually colored with bleu but not present in
the samples whereas the orange dots are the sampling sites; 1. Greater Accra Region, 2. Central

Region, 3. Western Region, 4. Ashanti Region, 5. Eastern Region, 6. Volta Region, 7. Bror	ng Ahafo
Region, 8. Northern Region, 9. Upper West Region and 10. Upper East Region	75

LIST OF TABLES

Table 1: Sample sites with date, region, locality, coordinates, and maize stage in the farm 24
Table 2: The socio-demographic characteristics of the respondents
Table 3: Occurrence of fall armyworm on maize plant
Table 4: Host plants of fall armyworm in Ghana on regional basis in 201747
Table 5: Infestation levels on maize reported by the respondents
Table 6: Distribution of FAW and other moths on maize plant during the major cropping season
Table 7: Distribution of FAW and other moths on maize plant during the minor cropping season
Table 8: Distribution of FAW and other moths on maize plant in 2017 cropping seasons 54
Table 9: Distribution of FAW and other moths on maize plant on maize stages during the major
cropping season
Table 10: Distribution of FAW and other moths on maize plant on maize stages during the
minor cropping season
Table 11: Distribution of FAW and other moths on maize plant on maize stages during the
major cropping season
Table 12: Pesticides with active ingredients, trade name and rate of application per hectare
recommended by PPRSD and distributed by the Government of Ghana to tackle the FAW 60
Table 13: Damage levels and field practices to manage the FAW during the major cropping
season
Table 14: Damage levels and field practices to manage the FAW during the minor cropping
season
Table 15: Damage levels and field practices to manage the FAW during the major cropping
season 63

Table 16: Species and sites of natural enemies of <i>S. frugiperda</i> in Ghana, 2017
Table 17: Natural enemies of the fall armyworm natural enemies collected from the field 69
Table 18: Sites hosting the natural enemies of the fall armyworm during the major cropping
season
Table 19: Sites where the natural enemies were collected in the minor cropping season 72

LIST OF APPENDICES

Appendix 1: COI loading gel with Msp1 and Sac1:
Appendix 2: Site of action of different digestive enzymes and mode of action of different primers
Appendix 3: PCR carried with Tpi and product run in the gel to test the quality of DNA before
sequencing process. It gives approximately the quality of the sequencing results. 1- lower quality
of sequencing, 2- good quality of sequencing, 3- very low quality of sequencing, 4- very high
quality of sequencing
Appendix 4: Layout of COI and Tpi analysis110

CHAPTER ONE

GENERAL INTRODUCTION

1.1. Background information

The fall armyworm (FAW), *Spodoptera frugiperda* originated from the Americas, where, it has been considered as a generalist pest. It spreads during the warm seasons from tropical and subtropical regions of the Americas Northwards to Canada and Southwards to Argentina. Because FAW has no diapause, during the winter, an annual migration occurs throughout the Eastern part of the United States from the overwintering areas to tropical and subtropical regions of the Western hemisphere (Nagoshi *et al.*, 2017). A continental and seasonal migration of the pest therefore occurs every year, so far from Canada to Argentina (Mitchell *et al.*, 1991; Nagoshi and Meagher, 2008). The whole life cycle duration of *S. frugiperda* depends on the season and varies according to the weather from 30 days in summer to 90 days in winter (Luginbill, 1928; Capinera, 2000), however, the larval stage is the harmful stage for field crops and occurs on over 80 host plants but mostly on grass species (Ashley *et al.*, 1989).

The fall armyworm has been sub-divided into two strains based on larval host plant preference (Pashley, 1986; Pashley et martin, 1987). The two strains are morphologically undistinguishable but genetically identifiable into corn strain and rice strain (Nagoshi *et al.*, 2017 and 2018). The corn strain feeds primarily on corn (*Zea mays L.*) and other large grasses while the rice strain is mainly collected from rice (*Oryza sativa L.*) and various lower forage and native grasses species (Pashley, 1986). This distinguiction may be based on the initial stage of speciation, when the two strains may have been host races in which interbreeding was reduced due to host preference differences (Diehl and Bush, 1984; Groot *et al.*, 2010; Hänniger, 2015). For success on pest management, the two strains distribution has to be determined because they differ in their

susceptibility to chemical and biological agents (Nagoshi et al., 2007). The rice-strain larvae are more susceptible to transgenic Bacillus thuringiensis (Bt) Berliner cotton and to the insecticides diazinon and carbaryl, whereas the corn-strain is more susceptible to carbofuran (Pashley and Martin, 1987, Adamczyk et al., 1997). It has also been demonstrated that several Bermudagrass (Cynodon dactylon L. Pers.) cultivars showed different types of resistance to the two fall armyworm strains (Pashley an Martin, 1987, Quisenberry and Whitford, 1988, Jamjanya et al., 1990). Several control methods have been applied against the fall armyworm. Trap and lure combinations have been tested for monitoring; the standard bucket trap with a green canopy, yellow funnel, and white bucket was the most effective (Meagher, 2001). The cultural method and host plant resistance had been successful tools strategies to control the fall armyworm. The corn antibiosis and non-preference have been demonstrated to be the key mechanisms of host plant resistance. The fall armyworm resistant hybrids have successfully produced greater yields compared to susceptible hybrids at similar infestation levels (Wiseman et al., 1981 and 1983; Sparks, 1986; Wiseman and Davis, 1990). In biological control, about 53 species parasites that attack fall armyworm globally have so far been reported (Ashley, 1979; Sparks, 1986). In periods of outbreaks of the pest, conventional chemical insecticides have been used for successful fall armyworm control but usually necessitate the use of insecticides at the upper range of their labeled rates (Adamczyk and Sumerford, 2000). Once a fall armyworm population became established in a field, two chemical insecticide applications were reported to be often needed for successful management (Sullivan et al., 1999).

As *S. frugiperda* adults have a long distance flight behavior (100 km in a single night), it has widely dispersed in the Americas and recently invaded several Sub-Saharan Africa countries with the potential of rapid dispersion in all of the Eastern Hemisphere (Goergen *et al.*, 2016; Nagoshi

et al., 2017, 2018). The importance of feeding on crops, of reproducing and the long-distance flight behaviors exhibited by the fall armyworm make the pest significant threat to African agriculture (Nagoshi et al., 2017). In Africa, agricultural productivity has been already threatened by several pest infestations (Ruttan, 2005) and large numbers of larval armyworms are also plaguing on various crops of economic importance in many countries of tropical Africa (Rose et al., 2000). The introduction of the fall armyworm in 2016 is a challenge to African agriculture. The pest is seen by African farmers as a key pest and the most destructive pest for maize production. This is of high significance because, maize is the most consumed cereal with increasing importance in western Africa, where around 7.5 million hectares of the crop is reported to be grown (CIMMYT, 2001). In West Africa, maize is grown in all ecological zones from the humid forest to the Sudan savannah, and from sea level to the highest altitude. It has diversified uses, including food, animal feed and industrial uses, but over 70% of maize is grown for human consumption (CIMMYT, 2001). In some regions, maize becomes a business crop, farmers can keep maize in store for an extended period in order to sell it when market prices rise (Compton et al., 1998).

In Ghana, agriculture is the largest sector of the economy and the highest contributor to the nation's Gross Domestic Product, GDP (ISSER, 2010), employing about 44.7% of the country's labor force of persons older than 15 years in 2015 (MoFA, 2016). The demand for maize increases yearly due to the rising population and urbanization as well as growing poultry and fish sectors. For example the consumption of maize increased marginally from 38.4 kg in 1980 to 43.8 kg in 2011 per capita (MoFA 2010; MoFA 2012) while maize used for the poultry industry as feed, was estimated to have grown by 10% annually between 2000 and 2009 with the possibility to surpass 540,000 million tons in proper ration use (Hurelbrink and Boohene, 2011). The agricultural sector in recent time in Ghana also faces to a new challenge with the severe outbreak of *S. frugiperda*

that was considered as a very destructive pest during the agricultural cropping seasons due to its wide host range and geographical distribution (Knipling, 1980). Its severe outbreak usually observed at the beginning of the wet season, that follows a long period of drought (Goergen *et al.*, 2016). Several African countries as well as Ghana rely on the farm during the wet season for agriculture. The severe outbreak of fall armyworm in Ghana affects the economies of farmers and the country at large by causing yield losses that affect GDP as well as the farmers. The recent estimation in 2017 published by the Centre of Agricultural Bioscience International (CABI) on Sub-Saharan Africa countries indicates that 13.5 million tons of maize, that were valued at \$3 billion, were at risk during the 2017-2018 maize production year. This estimation is equivalent to over 20 percent of total maize production for the region.

1.2. Justification

In Ghana, the presence of the pest was observed in 2016 but the general and severe outbreak was reported by the Ministry of Food and Agriculture (MoFA) at the beginning of the cropping season of 2017. The infestation was severe on maize field populations. Workshops were organized nationally and were extended internationally on the biology, ecology and management of the pest and farmers also tried to control the pest by spraying insecticides. Maize, which is the preferred host plant for the pest, is the principal staple crop produced and consumed by most Ghanaian families. It is produced predominantly by smallholder resource poor farmers under rain-fed conditions and across the county because it is well adapted and grows in most of the ecological zones of Ghana. However, the yield of maize production in Ghana is lower (1.7 t/ha) than the expected yield that was estimated at 6.0 t/ha (MoFA, 2011). The low yield recording on maize production is a result of drought, low soil nutrients, striga (*Striga sp*), diseases and pests infestations. Maize production is also limited by: low maize plant populations in the farms,

inappropriate planting time, and inadequate control of weeds, limited use of agricultural inputs (especially fertilizer and improved seeds) as well as untimely application of adequate quantities of fertilizers, inadequate drying and storage facilities leading to high post-harvest losses, and poor market access, and lack of financial credit. The damage on maize in Ghana is caused by stem and ear borers as well as defoliators (lepidopterans). The introduction of the FAW in 2016 has affected over 18,000 hectares (ha) of farmlands in Ghana, causing the country to lose an estimated of \$ 64 million and thus compelled the Government of Ghana to allocate \$4million to purchase insecticides only for the FAW management.

For suitable and long-term management of FAW in Ghana, the situation of the pest in the field has to be known. Therefore, this study was carried out to provide information on the occurrence and distribution of fall armyworm in all the ten regions of Ghana.

1.3.Objectives

1.3.1. Main objectives

The purpose of this study was to gather information on the occurrence and distribution as well as the management of the fall armyworm (FAW), *Spodoptera frugiperda* by analyzing the perception and the knowledge as well as the field practices of the farmers and also to determine its natural enemies of the pest in Ghana.

1.3.2. Specific objectives

To fulfil these objectives, the following questions:

- To investigate on the perceptions and the knowledge of agricultural players on fall armyworm and practices applied by the farmers to manage the invasive pest in Ghana;
- To assess the composition, distribution and infestation level of the fall armyworm and other moths in maize farms in Ghana

- To determine natural enemies associated with the fall armyworm in Ghana.

CHAPTER TWO

LITERATURE REVIEW

2.1. Introduction to the fall armyworm

The fall armyworm (FAW), Spodoptera frugiperda (JE Smith, Lepidoptera: Noctuidae) is a holometabolous insect whose larval stage can destroy a wide variety of crops with large economic damage. Frugiperda derives from the Latin fructus (fruit) and perditus (lost) because this species has the ability to destroy crops (Ashley, 1982) while leads to yield reduction. The fall armyworm is native to the tropical regions of the Western hemisphere, occuring from Canada to Argentina. It is a strong flier, disperses long distances annually from overwinter regions to tropical and subtropical areas of Americas. It has been considered since 1928 by Luginbill as a primary pest of corn or maize and prefers grass species as hosts, but it can also occur on other economically important plants, such as cotton and soybean (Luginbill, 1928; Bass, 1978; Pitre, 1979; Young, 1979; Pitre and Hogg, 1983). The fall armyworm presents a sympatric speciation that has been studied and described and it appears to be diverging into two strains, the corn and rice strains (Groot et al., 2010). The corn strain prefers corn or maize and other large grasses and the rice strain prefers the rice and other small grasses.

Maize is however the most attacked crop by the fall armyworm whose populations increase importantly with migration from one region to another. Its densities vary tremendously from year to year and place to place due to the increase of acreage of maize production in traditional agricultural regions. With its introduction to Africa, several Sub Saharan Africa countries have registered the severe infestation in the maize field populations with the occasional attacks on other crops with economic importance (Goergen *et al.*, 2016; Nagoshi *et al.*, 2017).

2.2. Distribution of fall armyworm

The fall armyworm, *Spodoptera frugiperda* (J.E. Smith, 1797) is a destructive insect pest for the Americas. The high variation of the weather in the Americas makes the fall armyworm that, has no diapause, to migrate seasonally and continentally so far from Canada to Argentina (Mitchell *et al.*, 1991; Nagoshi and Meagher, 2004a, 2004b, 2008, Nagoshi *et al.*, 2012). In Eastern of the United States, annual migrations of adults occur from Northeastern towards Southeastern (Nagoshi *et al.*, 2017).

Unfortunately, it was recently report in several African countries (Goergen *et al.*, 2016; Nagoshi *et al.*, 2017, 2018) including Ghana. From the period of the invasion of fall armyworm, severe devastations have been observed on maize farms in Africa (Nagoshi *et al.*, 2017). It was firstly reported from Benin, Nigeria, Sao Tome and Principe, and Togo in early 2016 (Goergen *et al.*, 2016). In April 2017, it affected Ghana, South Africa, Tanzania, Kenya, Mozambique, DRC, and suspected in Burkina Faso, Cote d'Ivoire, Sierra Leone, Senegal, Ethiopia, Zimbabwe, and many Sub Saharan African countries. At the end of year 2017, most of Sub Saharan African countries were invasive by fall armyworm. In 2018, all the Sub Saharan African countries were invasive excepted Lesotho (FAO, 2019). It has recently spread to Asia, being confirmed in India, Yemen, Thailand, Myanmar and Sri Lanka. Because of trade and the moth's strong flying ability, it has the potential to spread further (Nagoshi *et al.*, 2017; IITA, 2018; ICAR-NBAIR, 2018; IPPC, 2018, 2019; FAO, 2019).

2.3. Biology and Ecology of FAW

The adult of the fall armyworm wing size can measure 32 to 40 mm (Capinera, 2000), however, there is sexual dimorphism. The male has brown forewings with patterns that distinct white white spots on them (Plate 1a). The female forewings are gray without important patterns (Plate 1b). The

head of the last instar larvae are dark with an inverted white Y-shaped mark (Plate 1c). The larva becomes progressively browner during its development and develops white lengthwise line and dark spots with spines. The fall armyworm's life cycle vary and depend on the natural conditions, it can be completed in 30 days during summer, and 60 days during the spring and autumn seasons; in the winter, it can prolong between 80 to 90 days (Luginbill, 1928; Capinera, 2000). The number of generations in a year varies based on climate and natural conditions, but in her life span a female will typically lay about 1,500 eggs (Luginbill, 1928).



Plate 1: Description of the fall armyworm; a- male, b- female, c- larva

2.3.1. Egg

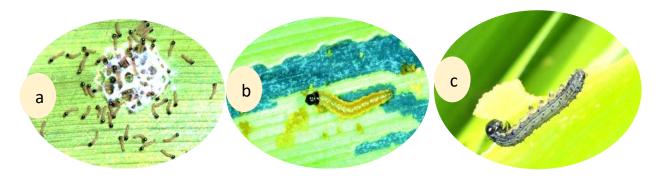
The dome-shaped egg has flattened base and curves upward to a broadly rounded point at the apex (Luginbill, 1928; Capinera, 2000). The egg measures about 0.4 mm in diameter and 0.3 mm in height (Sparks, 1979). One female can lay up to 2000 eggs grouped in masses of 100 to 200 eggs in layers, but most eggs can be spread over a single layer attached to foliage. The grayish scale layer covers the eggs (Plate 2). The eggs hatch at the same time between two and five days after oviposition (Luginbill, 1928; Capinera, 2000).



Plate 2: A mass of the fall armyworm eggs showing scales between the eggs

2.3.2. Larva

The fall armyworm larval instars differ slightly in physical appearance and pattern (Plate 3), this stage takes from 12 to 18 days in tropical natural conditions. Old larvae measure 38 to 51mm in length (Ashley, 1980). Young larvae have greenish color with a black head (Plate 3a) and turn to orangish in the second instar (Plate 3b). The third instar has brownish dorsal surface of the body and lateral white lines begin to form (Plate 3c). In the fourth to the sixth instars the head is reddish brown, mottled with white, and the brownish body bears white sub dorsal and lateral lines (Plate 3d, 3e, 3f).



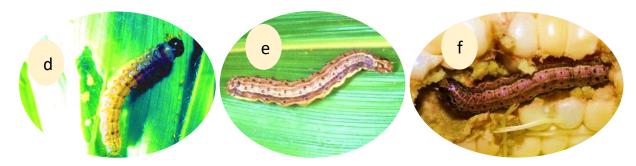


Plate 3: Fall armyworm larval stage showing different instars; a- first instar, b-second instar, c-third instar, d- fourth instar, e- fifth instar, f- sixth instar.

2.3.3. Pupa

The pupa is the less active stage of the fall armyworm. To avoid external attacks and impact of environmental factors, the last larval instar gets in the soil where pupation normally takes place at a depth of 2 to 8 cm (Plate 4) (Sparks, 1979). The last larval instar constructs a loose cocoon by tying together particles of soil with silk (Plate 4a). If the soil is too hard, larvae web together leaf debris and other material to form a cocoon on the soil surface (Plate 4b). The pupa is reddish brown in color, and measures 14 to 18 mm in length and about 4.5 mm in width (Sparks, 1979). The duration of the pupal stage is about 7 to 9 days under laboratory condition. The pupal stage of the fall armyworm cannot withstand protracted periods of cold weather. For example, Pitre and Hogg (1983) reported on winter survival of the pupal stage in Florida, and found 51% survival in southern Florida, but only 27.5% survival in central Florida, and only 11.6% survival in northern Florida.

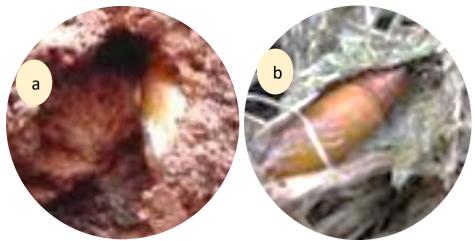


Plate 4: Fall armyworm pupation; a- in the soil, b- in the debris

2.3.4. Adult

The triangular white spots are observed at the tip and near the center of the forewings of the male moth (Plate 5a) while the female has forewings less distinctly marked, ranging from a uniform grayish brown to a fine mottling of gray and brown (Plate 5b). The hind wing is iridescent silverwhite with a narrow dark border in both sexes. Duration of adult life is estimated at an average of 10 days, with a range of about 7 to 21 days.

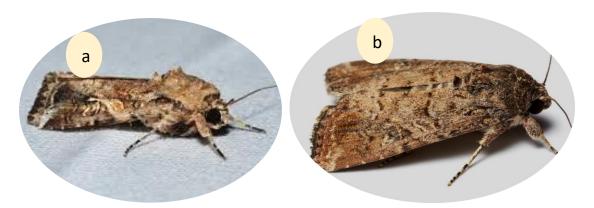


Plate 5: Fall armyworm adults showing; a- male, b- female

The eggs of the fall armyworm are laid on the tender and hidden parts of the host plants. The new larvae are found on the host plants leaves and on the new emergent tissues. On maize, the larva get in the whorl where, it feeds and causes the damage to the plant. Larvae of fall

armyworm is usually exposed to the natural factors that reduce its population. The later instar larva, get into the soil, where, the pupation takes place. The emerged adults are active in the night, early in the morning, and later in the evening, when, they migrate, feed and mate. The fall armyworm is subdivided into two strains that are morphological identic but have preference for the host plants.

2.4. Strains and hybrids of fall armyworm

The two strains are identified as corn strain, mainly collected on maize, sorghum, and other large grasses fields, and rice strain mainly collected on rice, Bermudagrass and other small grass species (Pashley 1986 and 1988; Nagoshi et al., 2007; 2017; 2018 and Groot et al., 2010). The two strains are physiologically different and can be distinguished through molecular markers (Nagoshi and Meagher, 2003). The two strains may also differ in their host preference, behavior, and pesticide susceptibility (Pasheley et al., 1995, Prowell et al., 2004 and Groot et al., 2010). Whether, the both strain have invaded or been introduced into Africa is still unclear. In the first report, Nagoshi et al. (2017) identified two individuals as rice strains. Recently, when characterizing populations and the dispersion patterns in Togo, Sao Tome and Principe (Western Africa), Burundi, Democratic Republic of the Congo (Central Africa), Kenya and Tanzania (East Africa), only corn strain were identified (Nagoshi et al., 2018). The mitochondrial marker that was used for the first Togo samples was compromised and made this result uncertain (Nagoshi et al., 2018). The study comparative the fall armyworm populations from Togo show similarities to populations collected from the Florida and Greater Antilles (Nagoshi et al., 2017). This study showed also that only one genotype of the four types of corn strains had been found in Togo samples and that genotype referred to Florida type. It is therefore important to do more collections to determine the status of the strains and their hybrid in African countries.

2.5. Alternative host plants

The fall armyworm (FAW) mostly occurs on grasses but has a very wide host range with over 80 plants were reported by Ashley *et al.* (1982). The most frequently attacked host plants are maize, sorghum, bermudagrass, and other grasses (Pashley and Martin, 1987). When the larvae are very numerous, they defoliate the preferred plants, acquire an "armyworm" habit and disperse in large numbers, consuming nearly all vegetation in their path. The injured field crops are frequently maize, millet, sorghum, rice, ryegrass, alfalfa, barley, Bermudagrass, buckwheat, cotton, clover, oat, peanut, sugarbeet, Sudangrass, soybean, sugarcane, timothy, tobacco, and wheat spp (Pashley and Martin, 1987). Among the crops, maize is regularly damaged, but the others are occasionally attacked. The damage sometimes occurred on apple, grape, orange, papaya, peach, strawberry and a number of flowers (Ashley *et al.*, 1982). Weeds known to serve as hosts include bentgrass, *Agrostis* sp.; crabgrass, *Digitaria* spp.; Johnson grass, *Sorghum halepense*; morning glory, *Ipomoea* spp.; nutsedge, *Cyperus* spp.; pigweed, *Amaranthus* spp.; and sandspur, *Cenchrus tribuloides* (Ashley *et al.*, 1980).

2.6. Damage caused by FAW

The larvae of the fall armyworm attack and thus cause the loss of cereal and other crops file in important economic crop as well as the pasture losses that affect farmers and Nations' economies. Fall armyworm was considered as a cosmopolitan pest for the Americas maize production (Wiseman *et al.*, 1966). Larvae feed on tender tissues including leaves, flowers and cobs by leaving damage symptoms (Plate 6). The early larvae feed on soft leaf tissue from one side, leaving the opposite epidermal layer intact (Plate 6a). The second or third instar, larvae make small holes in leaves, and eat from the edge of the leaves inward. Feeding in the whorl of maize often produces a characteristic row of perforations in the leaves (Plate 6b). Then, when larvae take age, they begin

making holes on the leaf and the last instars, fourth to sixth, are able to destroy small plants and trip larger ones (Cruz, 1995). The newly hatched larvae feed vertically on the same plant or disperse horizontally to adjacent plants and the density of larvae varies according to their stage of development. Due to cannibalistic behavior, the density is usually reduced to one or two per plant when larvae feed in close proximity to one another. Older larvae cause extensive defoliation, often leaving only the ribs and stalks of corn plants, or a ragged, torn appearance. Marenco *et al.* (1992) studied the effects of fall armyworm injury to early vegetative growth of sweet maize in Florida. They reported that the early whorl stage was least sensitive to injury, the mid whorl stage intermediate, and the late whorl stage was most sensitive to injury. Furthermore, they noted that mean densities of 0.2 to 0.8 larva per plant during the late whorl stage could reduce yield by 5 to 20 percent. Larvae burrow into the growing point, destroying the growth potential of plants, or clipping the leaves. In maize, they sometimes burrow into the ear, feeding on kernels (Plate 6c).



Plate 6: Damage caused by the fall armyworm on maize; a- leaves, b- whorl, c-ear The studies on larval feeding behavior by Pannuti *et al.* (2015) showed that, young (vegetative stage) leaf tissue is suitable for growth and survival, because on more mature plants the leaf tissue become hard and unsuitable. Larvae tend to settle and feed in the ear zone, and particularly on the silk tissues, but silk is not very suitable for growth. Larvae attaining the maize kernels display the fastest rate of development. Although the closed tassel was suitable with respect to survival, it

resulted in poor growth, thus, tassel tissue may be suitable for the initial feeding, perhaps until the larvae locate the silk and ears, but feeding only on tassel tissue is suboptimal. The fall armyworm therefore, reduces yield of maize as high as 34% (Carvalho, 1970; Cruz and Turpin, 1982; 1983, Williams and Davis, 1990; Willink *et al.*, 1991; Cruz *et al.*, 1996). Reported losses vary according to the stage of the plant attacked; however, the relationship between stages of attack and yield loss is complex because the loss may also depend on the infestation levels and vary from area to area, crop variety to crop variety and even between adjacent fields with different agronomic practices.

2.7. Natural enemies

Survival and reproduction of the FAW are favored by the cool, wet springs followed by warm and humid weather in farms. The high rate of multiplication observed on the FAW allows it sometimes, to escape suppression by its natural enemies. Although the FAW has many natural enemies, few act effectively enough to prevent crop injury. These natural enemies include parasitoids, predators and pathogens. The wasp parasitoids most frequently reared from larvae in the United States are *Cotesia marginiventris* (Cresson) and *Chelonus texanus* (Cresson) (both Hymenoptera: Braconidae), species that are also associated with other noctuid species. The most abundant fly parasitoids of the FAW are *Archytas marmoratus* (Townsend) (Diptera: Tachinidae), however, the dominant parasitoid often varies from place to place and from year to year. Luginbill (1928) and Vickery (1929) described and pictured many of the fall armyworm parasitoids.

The predators of the fall armyworm are general predators that attack many other caterpillars. The most important are various ground beetles (Coleoptera: Carabidae); the striped earwig, Labidura Labiduridae); riparia (Pallas) (Dermaptera: the spined soldier bug, **Podisus** maculiventris (Say) (Hemiptera: Pentatomidae); and insidious flower bug, Orius the insidiosus (Say) (Hemiptera: Anthocoridae). The vertebrates such as birds, skunks, and rodents

also consume the FAW larvae, pupae and adults readily. Predation may be quite important, as Pair and Gross (1984) demonstrated 60 to 90 percent loss of pupae to predators in Georgia.

Numerous pathogens, including viruses, fungi, protozoa, nematodes, and a bacterium have been associated with the fall armyworm, but only a few cause epizootics. Among the most important are the *Spodoptera frugiperda* nuclear polyhedrosis virus (NPV), and the fungi *Entomophaga aulicae*, *Nomuraea rileyi*, and *Erynia radicans*. Despite causing high levels of mortality in some populations, disease typically appears too late to alleviate high levels of defoliation.

2.8. Management of FAW

Fall armyworm infestation levels that produce economic damage vary with a given crop and the stage of development. There are guidelines-or thresholds of sorts for some crop and growth stages: seedling plants may be economically damaged by larval of first to sixth instars per row foot of soybeans, sorghum, maize, and southern peas but later stages can tolerate one or more caterpillars per plant. After three weeks of growth, rice can tolerate 19-29 larvae per square foot (Navas, 1974). These values need to be validated in field research, particularly early in the annual cycle with predation, parasitization, disease, and spotty larval distribution within a field as variables. Other variables that should be considered before implementing control measures are: the number of egg masses on a given amount of foliage since larvae will disperse to cover an area 20 foot in the radius from a single egg mass and are subject to parasitism by *Chelonus texanus* (Luginbill, 1928); quantity of feeding (Wiseman *et al.*, 1966); and the percentage of whorls having visible damage.

2.8.1. Sampling

Caterpillars of the FAW can be sampled in the farms to determine their density or rate of infestation. Moth populations can be sampled with pheromone and trap combinations; the latter is more efficient. Pheromone traps should be suspended at canopy height, preferably in maize during

the whorl stage. Catches are not necessarily good indicators of density, but indicate the presence of moths in an area. Once moths are detected it is advisable to search for eggs and larvae. A search of 20 plants in five locations or 10 plants in 10 locations is generally considered to be adequate to assess the proportion of plants infested (Capinera, 2000). Sampling to determine larval density often requires large sample sizes, especially when larval densities are low or larvae are young, so it is not often used.

2.8.2. Insecticides

Insecticidal control of fall armyworm is often necessary to protect many crops (Luginbill, 1950; Straub and Hogan, 1974; Bass, 1978). As crops develop, the height and density of the canopy become a limiting factor for efficient insecticidal control because it is difficult to obtain canopy penetration with spray materials. Control of FAW larvae has so far been achieved by using a systemic insecticide applied at planting (Kuhn et al., 1975). This is particularly true in areas having high FAW populations, thus requiring protection of seedlings to assure retention of sufficient foliage for growth. Control in the later stages of growth of these crops is obtained with ground or air applications. In the case of low growing crops, control can be achieved by using a tractor or high clearance sprayer to make broadcast applications since feeding larvae are usually found on the terminal growth. On the other hand, with metering into irrigation systems, limitation on the volume of water that can be used is eliminated and maximum coverage is obtainable. Young et al. (1972) indicate that oil and particulate solutions (flowable and wettable powders) are best applied by irrigation. Other methods of control include applying granular formulations to the whorl or on the terminals. Multiple applications (a daily schedule during silking) to sweet corn will result in larval and adult suppression (Young et al. 1972). Baits and ultra-low volume formulations have been used and are alternatives to the use of large quantities of a water carrier (Harrell et al., 1977),

however, some resistance to insecticides has been noted, with resistance varying regionally. Foster (1989) reported that keeping the plants free of larvae during the vegetative period reduced the number of sprays needed during the silking period. The grower practice of concentrating the sprays at the beginning of the silking period instead of spacing the sprays evenly provided little benefit.

2.8.3. Cultural techniques

Early planting and/or early maturing varieties is the most important cultural practice, employed widely in the Americas. Early harvest allows many corn ears to escape the higher armyworm densities that develop later in the season (Mitchell, 1978). Reduced tillage seems to have little effect on fall armyworm populations (All, 1988), although delayed invasion by moths of fields with extensive crop residue has been observed, thus delaying and reducing the need for chemical suppression (Roberts and All, 1993).

2.8.4. Host plant resistance

A prerequisite to resistance studies is a method of classifying or measuring damage. Generally, such classifications involve some sort of visual rating scale, e.g., 0-3, 0-5, and 0-10, but the 0-9 rating scale seems to be used more recently and mostly separates relative differences. Wiseman et al. (1966) were the first to develop a visual rating scale for fall armyworm damage. Wiseman et al. (1966) were also among the firsts to capitalize on an outbreak of fall armyworm and to detect differences among corn genotypes by counting the number of nodes damaged. Other researchers use the amount of leaf damage in percentages, such as 0 = 0-10% area damaged, etc. Rating systems should be developed for rapidity, at least those used in the screening phases of testing. When high levels of only resistant or susceptible genotypes exist, rating techniques must be refined.

Bertels and Ardry (1956) stated that the most promising genetic sources of resistance to the fall armyworm in Brazil were corn varieties with the "Amargo" character. Horovitz (1960) was unsuccessful in Venezuela in his search for a variety or an individual plant with resistance to the fall armyworm. In the United States, Ditman and Cory (1936) reported that there were differences among corn strains in injury of infestation by the fall armyworm. Dicke (1977) also, reported tests in Virginia that showed northern inbreds were generally more subject to fall armyworm attack on husk, ear and shank than other lines having southern maize in their parentage. In the Director's Report of the Mexican Agricultural Program (Anonymous, 1959), 'Guerrero 169,' 'Guerrero 115,' 'Cuba 30,' and 'Yucatan 15' were reported as the varieties least affected by the fall armyworm. In addition, varieties resistant to the fall armyworm among 81 lines tested at Tepalcingo were 'Coastal Tropical Flints,' 'Antigua 2D,' and 'Antigua 8D' from the island of Antigua, and 'Zapalote Chico' varieties from the dry coastal region of Oaxaca and Chiapas (Anonymous 1965). Generally, all resistance characters presently known can be traced to 'Antigua' corns and to similar 'Coastal Tropical Flints' from Antigua and the coastal tropical areas.

2.8.5. Biological control

The FAW, *Spodoptera frugiperda*, is a key pest causing yield reductions in maize production systems (Cruz *et al.*, 2010). In Brazil, biological control via the release of natural enemies such as egg parasitoids has been proposed. The use of natural enemies, particularly parasitoids, has shown promising results in reducing damage from insect pests (Mills *et al.*, 2000; Mills, 2010). Favorable results in biological control use have facilitated research opportunities to develop new commercial insectaries, and various natural enemies are commercially available for inundative release in pest management (Cruz *et al.*, 2013; Van Lenteren, 2000).

The release in a timely manner and appropriate density is imperative for successful pest management. Furthermore, interactions between the natural enemy community and fall armyworm population dynamics in maize fields must be considered in developing an integrated pest management (IPM) program for this crop (Wyckhuys and O'Neil, 2006). This approach has been shown to reduce pest population densities, resulting in less plant injury and yield loss. It is believed that conservative measures taken for biological control in organic systems may promote the survival and the performance of the natural enemy in the area (Wyckhuys and O'Neil 2006).

Despite progress in biological control worldwide, this alternative is not widely used compared to chemicals. Its efficiency depends on several factors, including the kind, quality and suitability of the biological agent, released number, method and the time of release, and the complex interactions between the parasitoid, the target pest, culture, and environmental conditions (Ables *et al.*, 1979; Knutson, 1998).

Insects captured in pheromone traps have been used as a tool in IPM decision-making and to initiate chemical control and natural enemy releases (Ameline and Frérot, 2001). A trap containing the *S. frugiperda* sex pheromone is considered the best method to determine if a maize crop requires insecticide treatment (Cruz *et al.*, 2012) and can also be used to ascertain the most suitable parasitoid release time. The trap detects moth arrival in the target area, and consequently, female parasitoid release can proceed at the appropriate time.

2.9. FAW similar moths with economic important

There are some other species of the moths whose larvae seem FAW larva-like or cause the similar damage as the fall armyworms. These similar moths include: the maize defoliators and stem and ear borers.

2.9.1. Maize defoliators

The moths that defoliate the maize plants include: *Spodoptera spp.*, *Leucania* sp, *Anicla infesta*, *Alpenus maculosa*. The females lay their eggs in the young tenderer leaves of maize plant and the young hatcher larvae feed by dispersing vertically on the leaves of the same plant or horizontally to the adjacent plants (Pashley, 1986). The early stages of the maize are the most infested by the Lepidoptera defoliators of maize. The latest infestation on the maize plant occur on the reproductive parts.

2.9.2. Maize stem and ear borers

There are several species of moths that bore the maize stems and ears worldwide. The most reported in Africa and in Ghana are *Sesamia calamistis*, *Busseola fusca* (Lepidoptera: Noctuidae), *Eldana saccharina*, *Chilo partellus* (Lepidoptera: Pyralidae) (Abu, 1986). They infest the maize farms during the first eight weeks of plant developmental stages and bore the stems or cause "dead heart" and the late infestation lead to the damage on maize ear that yielding to drastic loss in maize production (Bowden, 1956, 1976, Girling, 1980 and Bosque-Perez, 1995). The yield reduction by the stemborers was significant, the range was estimated from 10 to 100% in West Africa (Usua, 1968). Due to the important damage and yield loss caused on maize by the stembrers, some farmers did not planting during the minor cropping season (Gounou, *et al.*, 1993).

CHAPTER THREE

MATERIALS AND METHODS

3.1. Study areas

3.1.1. Geographic description of study area

The study was conducted in Ghana, located on the Southern coast of West Africa, between latitudes 4° 44' N and 11° 11'N and longitudes -3° 11' W and 1° 11' E (MoFA, 2016). It is bordered with Togo to the East, with Cote d'Ivoire to the West with Burkina Faso to the North, and the Gulf of Guinea to the South. The country's elevation variation depend on the physical appearance of localities; the highest point, Mount Afadjato has 880 m above sea level. Two rainy seasons occur in the South from April to July and from September to November while, the North has only one rainy season from April to September (Breisinger, 2008). The annual rainfall varies and ranges from 1,100 mm in the North to 2,100 mm in the Southwest. The highest temperatures are usually recorded in March and the lowest in August. Ghana shows a low and sandy coastline with scrubs, rivers and streams. The central and West of Ghana extended from the coastline to Northward is covered by a tropical rain forest belt with hills, streams, and rivers. The scattered grassy plains, low bush, park-like savanna are mainly observed in the North of the country (Breisinger, 2008). The country is divided into six agro-ecological zones including - Sudan Savannah Zone, Guinea Savannah Zone, Forest Transitional Zone, Deciduous Forest Zone, Rain forest Zone and Coastal Savannah Zone (MoFA, 2016).

The farms were randomly chosen based on their accessibility and their size within Greater Accra Region, Central Region, Western Region, Eastern Region, Volta Region, Ashanti Region, Brong-Ahafo Region, Northern Region, Upper West, and Upper East.

3.1.2. Distribution of agricultural areas in Ghana

From Ghana's total land area of 23,884,245 hectares, about 13,600,000 ha (56.94%) are suitable for agricultural purposes but only 6,421,450 ha (47.22%) areas are under cultivation (MoFA, 2016). These areas are divided into three main agriculture zones. (i) Western, Eastern, Ashanti, Brong-Ahafo and Volta Regions fall under Forest Vegetation Zone. (ii) Upper East, Upper West and Northern Regions under Northern Savannah Vegetation Zone and (iii) Central, Greater Accra and the south parts of Volta Regions under Coastal Savannah (MoFA, 2016).

According to the FAO (2004), the Northern Savannah Zone grows rice, millet, sorghum, yam, tomatoes, cotton, mango, and ostrich considered as the largest agriculture zone where. In Coastal Savannah, rice, maize, cassava, vegetables, sugar cane, mangos and coconut, sweet potato, and soybean crops are grown. Cocoa, coffee, oil palm, cashew, rubber, plantain, banana, and citrus are mostly grown in the forest zone where rainfall is plentiful. The annual area ('000ha) planted for major food crops in 2015 include cocoa (1,717.44), cassava (917), maize (880), yam (430), oil palm (425.60), plantain (363), groundnut (336), rice (233), sorghum (228), cocoyam (200), cowpea (163), millet (162), soya bean (86) (MoFA, 2016).

3.2. Survey

3.2.1. Zones and localities of survey

Maize is expected to be produced in all the localities in Ghana. To gather the information and establish the occurrence and distribution of the FAW, different localities within the ten administrative regions of Ghana were randomly chosen for agricultural players' interviews, farm inspections, and larvae and associated natural enemies' collections (Fig. 1). The surveys were carried out without taking in account the varieties of maize since the farms' owners were note always be in the farms; however, farms were geo-referenced with GPS (table 1).

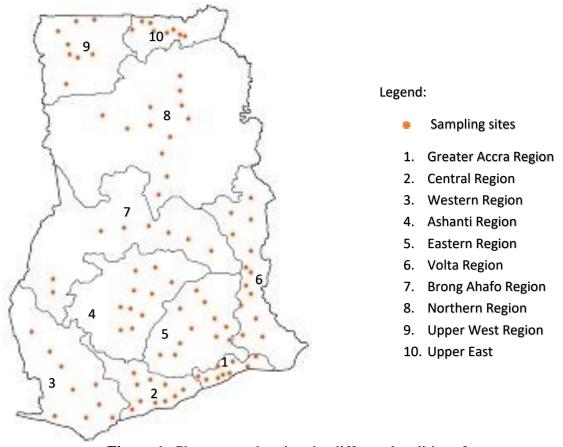


Figure 1: Ghana map showing the different localities of survey

Table 1: Sample sites with date, region, locality, coordinates, and maize stage in the farm

Date	Region	Locality	Coordinates (L/l)	Maize stage
22.06.2017	Greater Accra	UG farm	5°66'03"/-0°19'36"	8-10 leaves
22.06.2017	Greater Accra	Okponglo	5°63'75"/-0°17'46"	Mature
22.06.2017	Greater Accra	37 MH	5°59'59"/-0°18'54"	Tasseling
14.10.2017	Greater Accra	Legon	5°38'52"/-0°10'9"	10 - 12 leaves
14.10.2017	Greater Accra	Afuaman	5°63'43"/-0°32'99"	6 - 8 leaves
14.10.2017	Greater Accra	Danfa	5°75'82"/-0°16'73"	10 - 12 leaves
14.10.2017	Greater Accra	Adenta	5°71'53"/-0°15'94"	Tasseling

Table 1, cont': Sample sites with date, region, locality, coordinates, and maize stage in the farm

Date	Region	Locality	Coordinates (L/l)	Maize stage
15.10.2017	Greater Accra	Sebrpor	5°72'74"/0°00'01"	4 - 6 leaves
15.10.2017	Greater Accra	Afienya	5°79'77"/0°01'30"	4 - 6 leaves
15.10.2017	Greater Accra	Sege	5°88'86"/0°36'50"	Tasseling
15.10.2017	Greater Accra	Bator	5°93'55"/0°37'56"	8 - 10 leaves
24.06.2017	Central	Cap Coast	5°11'14"/-1°25'47"	Tasseling
24.06.2017	Central	Elmina	5°10'02"/-1°29'99"	8-10 leaves
24.06.2017	Central	Atiabardi	5°09'72"/-1°40'03"	6-8 leaves
07.10.2017	Central	Elmina	5°7'22"/-1°16'11"	Tasseling
07.10.2017	Central	Ayensu	5°5'40"/-1°25'45"	Tasseling
07.10.2017	Central	Kayee	5°5'60"/-1°30'44"	10 - 12 leaves
08.10.2017	Central	Abura Dunkwa	5°31'23"/-1°16'96"	8 - 10 leaves
08.10.2017	Central	Wineba	5°35'26"/-0°62'27"	10 - 12 leaves
08.10.2017	Central	Twifo Praso	5°59'23"/-1°53'46"	4 -6 leaves
24.06.2017	Western	Komenda	5°08'70"/-1°48'88"	4-6 leaves
24.06.2017	Western	Badukrom	5°12'68"/-1°55'04"	Tasseling
24.06.2017	Western	Takoradi	4°80'85"/-1°71'53"	Tasseling
07.10.2017	Western	Dunkwa	5°6'39"/-1°37'58"	8 - 10 leaves
07.10.2017	Western	Aggrey Krom	5°3'34"/-1°39'26"	8 - 10 leaves
07.10.2017	Western	Mufriso	5°10'25"/-2°1'90"	6 -8 leaves
07.10.2017	Western	Bogoso	5°58'43"/-1°99'20"	4 - 6 leaves

Table 1, cont': Sample sites with date, region, locality, coordinates, and maize stage in the farm

Date	Region	Locality	Coordinates (L/l)	Maize stage	
07.10.2017	Western	Asankragwa	5°79'29"/-2°50'27"	Mature	
08.10.2017	Western	Ankwaaso	6°12'08"/-2°21'78"	6 -8 leaves	
08.10.2017	Western	Awaso	6°24'54"/-2°27'49"	8 - 10 leaves	
08.10.2017	Western	Kojina	6°42'24"/-2°69'58"	Tasseling	
25.06.2017	Ashanti	Obuasi	6°47'32"/-1°59'22"	Mature	
25.06.2017	Ashanti	Kumasi	6°11'52"/-1°60'19"	Tasseling	
25.06.2017	Ashanti	KNUST	6°68'40"/-1°57'28"	Tasseling	
29.08.217	Ashanti	Buaso	6°59'40"/-1°13'83"	6 -8 leaves	
29.08.217	Ashanti	Agogo Aburkyi	6°83'71"/-1°08'50"	Tasseling	
29.08.217	Ashanti	Agogo North	6°41'32"/-1°8'37"	4 - 6 leaves	
29.08.217	Ashanti	Agogo Dukurser	6°91'74"/-0°97'86"	Mature	
30.08.217	Ashanti	Bosukwakwa	7°9'54"/-1°24'0"	4 - 6 leaves	
30.08.217	Ashanti	Atoso	7°59'43"/-1°36'60"	4 - 6 leaves	
30.08.217	Ashanti	Ejura	7°24'14"/-1°20'44"	4 - 6 leaves	
30.08.217	Ashanti	Juanou	7°19'13"/-1°37'33"	8 - 10 leaves	
25.06.2017	Brong-Ahafo	Gawso	6°81'78''/-2°47'80''	4-6 leaves	
25.06.2017	Brong-Ahafo	Sunyani	7°31'85"/-2°29'65"	Tasseling	
25.06.2017	Brong-Ahafo	Techiman	7°54'67"/-1°91'24"	10-12 leaves	
31.08.217	Brong-Ahafo	Techiman	7°22'43"/-2°12'15"	4 - 6 leaves	
31.08.217	Brong-Ahafo	Nkoranza	7°32'29"/-1°42'38"	6 - 8 leaves	

Table 1, cont': Sample sites with date, region, locality, coordinates, and maize stage in the farm

Date	Region	Locality	Coordinates (L/l)	Maize stage	
31.08.217	Brong-Ahafo	Dandwa	7°76'88"/-1°34'95"	4 - 6 leaves	
31.08.217	Brong-Ahafo	Sampa	7°65'63"/-1°04'49"	4 - 6 leaves	
31.08.217	Brong-Ahafo	Garadima	7°42'43"/-0°50'4"	6 - 8 leaves	
31.08.217	Brong-Ahafo	Kwame Danso	7°43'19"/-0°38'32"	6 - 8 leaves	
26.06.2017	Northern	Yapei	9°12'96"/-1°14'99"	4-6 leaves	
26.06.2017	Northern	Tamale	9°37'27"/-0.87'48"	8-10 leaves	
26.06.2017	Northern	Savelugu	9°63'37"/-0°81'68"	8-10 leaves	
01.09.2017	Northern	Branam	8°0'53"/-2°3'36"	10 - 12 leaves	
01.09.2017	Northern	Signakura	8°42'41"/-2°18'10"	10 - 12 leaves	
01.09.2017	Northern	Kilompobile	9°40'47"/-2°47'12"	10 - 12 leaves	
01.09.2017	Northern	Sawla	9°15'56"/-2°25'15"	10 - 12 leaves	
03.09.2017	Northern	Libga 1	9°60'65"/-0°47'11"	Mature	
03.09.2017	Northern	Libga 2	9°36'11"/-0°50'25"	6 - 8 leaves	
03.09.2017	Northern	Libga 3	9°58'60"/-0°84'30"	Tasseling	
03.09.2017	Northern	Kukpehi	9°24'59"/-0°57'73"	Mature	
03.09.2017	Northern	Sanga	9°25'55"/-0°56'56"	Tasseling	
26.06.2017	Upper East	Navrongo	10°88'28"/-1°05'05"	4-6 leaves	
26.06.2017	Upper East	Bolga 1	10°86'77"/-0°86'26"	4-6 leaves	
26.06.2017	Upper East	Bolga 2	10°84'45"/-0°77'08"	4-6 leaves	
02.09.2017	Upper East	Pina	10°52'21"/-1°46'6"	Tasseling	

Table 1, cont': Sample sites with date, region, locality, coordinates, and maize stage in the farm

Date	Region	Locality	Coordinates (L/l)	Maize stage	
02.09.2017	Upper East	Banu	10°81'.31"/-1°61'79"	Mature	
02.09.2017	Upper East	Awenia	10°50'12"/-1°23'30"	Tasseling	
02.09.2017	Upper East	Chuchuliga	10°47'38"/-1°32'15"	Mature	
02.09.2017	Upper East	Doba konta	10°50'19"/-1°0'9"	Tasseling	
02.09.2017	Upper East	Pusu-Namongo	10°50'16"/-1°0'6"	Tasseling	
02.09.2017	Upper East	Wulugu	10°35'12"/-1°0'13"	Mature	
02.09.2017	Upper East	Gbimsi	10°24'20"/-0°47'11"	Tasseling	
27.06.2017	Upper West	Tumu	10°89'74"/-1°93'84"	None	
27.06.2017	Upper West	Ping	10°62'22"/-2°43'36"	4-6 leaves	
27.06.2017	Upper West	Wa	10°07'76"/-2°39'21"	4-6 leaves	
01.09.2017	Upper West	Wa	10°3'95"/-2°29'22"	6 - 8 leaves	
01.09.2017	Upper West	Busa1	10°3'55"/-2°29'22"	Tasseling	
01.09.2017	Upper West	Busa 2	10°3'61"/-2°27'02"	Millet 7 weeks	
01.09.2017	Upper West	Fian	10°42'34"/-2°47'32"	10 - 12 leaves	
01.09.2017	Upper West	Lilixia	10°48'43"/-2°11'13"	Tasseling	
01.09.2017	Upper West	Tumu	10°52'24"/-1°98'06"	Mature	
29.06.2017	Eastern	Kpong	6°13'25"/0°07'40"	Mature	
29.06.2017	Eastern	Sosseese	6°00'96"/-0°04'38'	Tasseling	
29.06.2017	Eastern	Somanya	6°09'30"/-0°01'25"	Mature	
05.11.2017	Eastern	Amanase	5°98'55"/-0°41'81"	10 - 12 leaves	

Table 1, cont': Sample sites with date, region, locality, coordinates, and maize stage in the farm

Date	Region	Locality	Coordinates (L/l)	Maize stage	
05.11.2017	Eastern	Bunso	6°28'57"/-0°42'51"	10 - 12 leaves	
05.11.2017	Eastern	Adawso	6°52'55"/-0°25'26"	8 - 10 leaves	
05.11.2017	Eastern	Hukunya	6°12'46"/-0°21'98"	Tasseling	
06.11.2017	Eastern	Atimpoku	6°18'39"/-0°08'49"	6 - 8 leaves	
06.11.2017	Eastern	Kpong	6°7'46"/-0°43'26"	10 - 12 leaves	
06.11.2017	Eastern	Mampon	5°90'96"/-0°06'98"	8 - 10 leaves	
06.11.2017	Eastern	Pokrom Nsabaa	5°81'97"/-0°17'64"	6 - 8 leaves	
02.07.2017	Volta	Adome	6°25'71"/0°14'23"	10-12 flowers	
02.07.2017	Volta	Apeguso	6°38'99"/0°25'37"	Tasseling	
02.07.2017	Volta	Agove	6°50'02"/0°37'09"	10-12 leaves	
07.11.2017	Volta	Tsrefe	6°51'43"/0°51'65"	10 - 12 leaves	
07.11.2017	Volta	Matse	6°67'78"/0°48'69"	8 - 10 leaves	
07.11.2017	Volta	Vane	6°81'38"/0°42'76"	Tasseling	
07.11.2017	Volta	Gbi-wegbe	7°09'82"/0°46'94"	6 -8 leaves	
07.11.2017	Volta	Hohoe	7°16'56"/0°49'27"	10 - 12 leaves	
08.11.2017	Volta	Jasikan	7°39'46''/0°47'54''	6 - 8 leaves	
08.11.2017	Volta	Kadjebi	7°51'58"/0°48'32"	6 - 8 leaves	
08.11.2017	Volta	Papase	7°73'19"/0°54'53"	10 - 12 leaves	
08.11.2017	Volta	Abrubrua	7°93'75"/0°56'92"	6 - 8 leaves	
08.11.2017	Volta	Kwanta	8°29'57"/0°53'50"	8 - 10 leaves	

3.2.2. Period of survey

Surveys were conducted during the two cropping seasons of 2017 (June-July and August-November). The major cropping season covers the period of May-August with full agricultural production in North and South while there is a minor cropping season in the South from August to November.

3.2.3. Interviews of agricultural actors

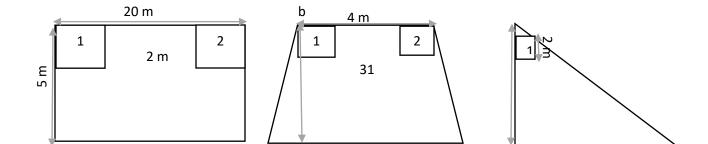
The interviews covered both the Agricultural Officers (extension and phytosanitary officers) and farmers randomly selected within the ten regions of Ghana. It was done to provide the information on actors' perceptions and knowledge on FAW and farmers practices to control the invasive pest. About, 259 agricultural actors were interviewed; 72 Agricultural Officers and 187 farmers (8 and 10 in Greater Accra Region, 8 and 18 in Central Region, 6 and 17 in Western Region, 7 and 12 in Eastern Region, 8 and 16 in Volta Region, 12 and 18 in Ashanti Region, 8 and 10 in Brong-Ahafo, 5 and 44 in Northern Region, 4 and 17 in Upper West Region, 6 and 22 in Upper East Region) were randomly administered with the questionnaires. The questionnaires were designed on the (i) profile of respondents; (ii) knowledge and perceptions on FAW based on the stage identification of the pest known, alternative host crops, stage of the crop attacked, critical phase of the crop to the pest attack, period of occurrence and increase of populations during the year, and level of damages; then (iii) control measures applied in the field including: types of control, period of starting control applications, time of the day that control is applied, effectiveness of controls, equipment used, techniques applied.

All the agricultural officers were interviewed in English using structured questionnaires. The farmers were interviewed either in the farms or in the villages using appropriate local languages (varies from locality to locality) with the help of the local Agricultural Officers or the translators.

On average, the interviews took 20-35 minutes. To analyze the information generated from the respondents, for each question, number of interviewees who gave similar answers were put together per administrative region and also nationwide. Percentage values of the number of responses to a specific question were calculated based on the number of total responses. Farmers who did not respond to a particular question were excluded from the calculation.

3.2.4. Survey on the distribution of the fall armyworms and similar moths

Larvae of S. frugiperda and other moths were collected from all maize growth stages of farms visited. The other moths are the moths that morphologically seem-like FAW or cause damages in the field maize. The two development phases of maize plant, vegetative and reproduction were targeted for this study. The vegetative phase was subdivided into four stages: vegetative 4-6 leaves (V_{4-6}) , vegetative 6-8 leaves (V_{6-8}) , vegetative 8-10 leaves (V_{8-10}) and vegetative 10-12 leaves (V_{10-1}) 12) and the reproductive phase into two stages: tasseling and maturation. A stratified random sampling technique was developed for sampling. The total and quadrants acreages as well as coordinate system of the farms were defined with a GPS. Farms were divided into five quadrats each representing 5% of the total farm area inside which inspections and collections were carried out. The quadrant positions depended on the shape of the farm (Fig. 2). The larvae of FAW and similar moths were collected from five infested plant-stands inside each quadrant. Data was collected from different localities of Ghana and grouped into ten regions and into six maize stages by cropping season. The collected larvae were classed by group (FAW or similar moths) and counted according to farm and their mean calculated based on the region and maize stage. The total number of FAW or other moth larvae per infested plant as well as relative abundances (RA) of the pests were also determined.



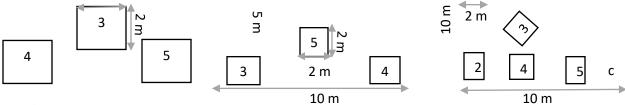


Figure 2: Quadrant designs on the farms; a- rectangle and square farm, b- Trapeze farm, c- triangle farm

3.2.5. Survey on the infestations of maize farms

With the guides of both Agricultural Officers and farmers, the type of control practiced on each inspected farm was determined. Two control methods were practiced against the FAW in the field, chemical and pheromone traps. Inspected farms were classified based on the practices on the field: chemical application farms trap and lure combination farms, and no control farms. Infestation data was collected from five quadrants of each farm (described above) and pooled together for each farm. Twenty standing maize plants were randomly selected from each quadrant and the infested ones were counted. The numbers of the infested plants from the five quadrants were gathered, and the infestation rates were determined per farm. The data from the farms were grouped according to the period of collection (major or minor cropping seasons) and grouped into regions and into maize stages.

3.2.6. Survey of FAW larval natural enemies

For the present study only natural enemies that are associated with the larval stage of the FAW were collected from the field. Also collected were other arthropods preying on the larvae of the FAW. The associated parasitoids emerged during the rearing of the larvae in the laboratory. From each site approximately 11 ± 7 larvae were collected and put together in the rearing bottles with the diet before being identified and separating individually into rearing bottles in the laboratory. The sites where the natural enemies were found were separated and grouped by region. Larvae that died due to unknown factors were counted and the mortality due to the unknown factor calculated.

The number of larvae hosting the parasitoids was determined, and parasitism was calculated based on the works of Van Driesche (1983), Pair *et al.* (1986) and Crisostomo-Legapi *et al.* (2001). The natural enemies were grouped and counted by species to determine their relative abundance according to Canal Daza (1993), Molina-Ochoa *et al.* (2001 and 2004).

3.3. Rearing

Spodoptera frugiperda larvae were placed individually into rearing plastic bottles and fed with fresh maize leaves and kept in the laboratory under natural conditions of temperature ($25 \pm 4^{\circ}$ C), relative humidity ($81\pm13\%$), pressure (1010 hpa to 1020 hpa). Some larvae died during the rearing period due to natural death and unknown effects. Some were reared till adulthood or till the emergence of parasitoids.

3.3.1. Rearing design

The conical transparent plastic rearing bottles (upper base 11 cm, lower base 8.5 cm and heights 5.5 cm) were used to rear larvae (Plate 7a). To allow air to enter into the bottle, the cover was cut and replaced by a transparent back net (Plate 7b). To absorb moisture generated by larvae or other sources, tissue was put at the base of each rearing bottles (Plate 7c).

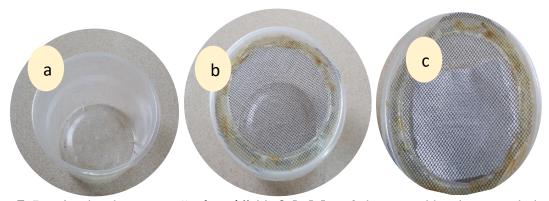


Plate 7: Rearing bottle; a- type "voltpack" 11x8.5x5.5 cm3, b- aerated bottle, c- ready bottle.

Individually, larvae were placed in the rearing bottle with maize leaf and tissue (Plate 8a). Tissue and maize-leaf were removed from the rearing bottles when larvae pupated (Plate 8b). The cotton

soaked in honey was used to feed the adults when it emerged (Plate 8c). The soaked cotton of honey served as food for the adult.

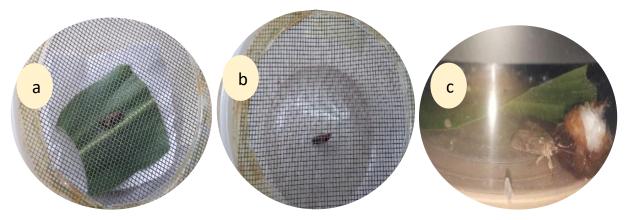


Plate 8: Rearing procedure; a- larval stage, b- pupa stage, c- adult stage

3.3.2. Conservation and preservation of specimens

The dead moths were dried inside of the same type of rearing bottles (Plate 9a) at room temperature. They completely dried in three days and preserved in tissue envelopes (Plate 9b). Some larvae (Plate 9c) as well as the natural enemies (Plate 9d) were preserved in 70% ethanol. All the samples were preserved in the Laboratory air conditions but the duration varies and depends on the period of collection; however, it ranged between three to eight months.

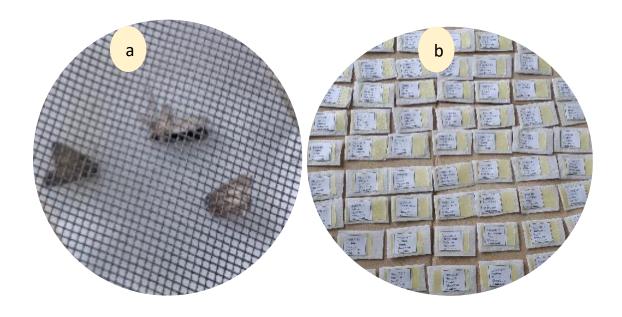




Plate 9: Preserving of specimens; a- drying of adults, b- conserving of adults, c- conserving of larvae with ethanol 70%, d- conserving of natural enemies with ethanol 70%

3.4. Identification of FAW, similar moths and natural enemies

To determine the distribution patterns of the FAW, its similar moths and its natural enemies, the morphological identification was done. Molecular characteristic, helped to determine the strains of the FAW occurring in Ghana.

3.4.1. Identification through the damage caused

The fall armyworm is assigned to some host crops on which it feeds; the prefered host crop is the maize plant. The FAW larvae feed on all stages of maize plant. This insect is present all year round around and occasionally extremely numerous. Feeding damage from caterpillars occurs first on leaves (Plate 10a), deep within the whorl, on leaves and in the newly forming green tassel (Plate 10b). The whorl stage of the corn shows ragged feeding damage and masses of sawdust-like excrement. Fall armyworm larvae eat into the side of corn ears, leaving behind excrements and a large whole (Plate 10c). They also feed in the tip, making a mess of the kernels. It usually emerges near the base of the ears, leaving round holes 3/16 inch (4.8 mm) in diameter in the shucks, however, there are many other moths' species whose larvae cause similar damage as the FAW

larvae. Morphological identification of specimens collected from the field should therefore be helpful for the pest distributions.

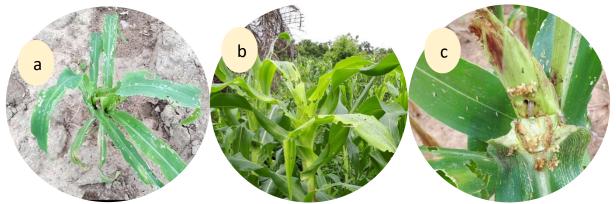


Plate 10: Damage of FAW on maize plant; a- on the leaves, b- on the tassel, c- on the ear.

3.4.2. Morphological identification of the pests

The morphological identification of the FAW and its similar moths started during the collection on the farms. All the caterpillars which caused similar damage as described above were collected from the field for identification.

It is easy to identify the larva of the FAW from the others due to characteristic marks and spots on larger larva. Marks that are often used for identification include the inverted Y mark on the head region and the four larger spots on the second last segment. The most common lines and spot are indicated below (Plate 11).

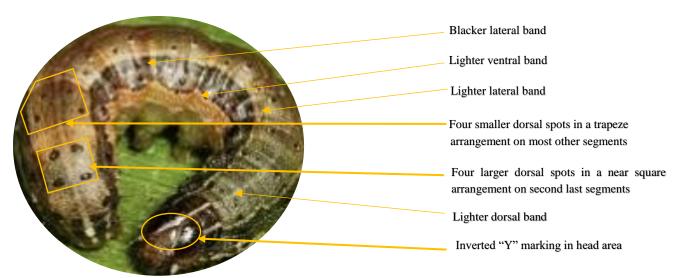


Plate 11: Last instar of FAW larva showing the main features.

The male moth of the FAW has forewing generally gray and brown, with triangular white spots at the tip and near the center of the wing (Plate 12a). The forewings of females are less distinctly marked, ranging from a uniform grayish brown to a fine mottling of gray and brown (Plate 12b). The hind wing is iridescent silver-white with a narrow dark border in both sexes.

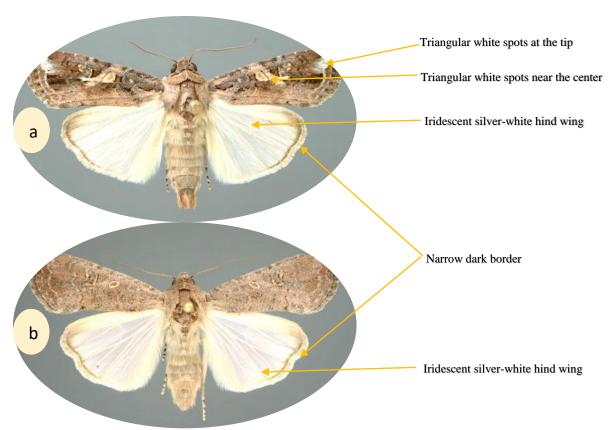


Plate 12: Typical fall armyworm adult; a- male, b- female

Dichotomous keys were used to identify the pests. A dichotomous key is a method for determining the identity of organism using a series of choices that lead to the correct identification of the specimen.

3.4.3. Natural enemies Identification

Both parasitoids that emerged from the samples were recorded every 24 hours and the predators collected from the fields were put into 70% ethanol for their preservations. The collected natural enemies were sent to International Institute of Tropical Agriculture (IITA), Biodiversity Resource Center, 08 BP 0932 Tri Postal Cotonou, Republic of Benin (Report Number 16/17 of 17.Dec. 2017 and 04/18 of 28. Feb. 2018 by G. Goergen). It is possible that some end-parasitoids died inside the larvae during the rearing however, no dissection was done to examine the presence of the dead parasitoids in the dead larvae or pupae due to the lack of logistics.

3.4.4. Fall armyworms' strains an hybrids identification

DNA extraction

Two methods of DNA extraction were used: the Chelex method and Zymo-spin column method. The Chelex method is a fast method and can extract 96 samples simultaneously; hence this method was given preference. As this method did not yield sufficient amounts of DNA for all samples, some samples had to be extracted with the Zymo-spin method, following Nagoshi et al. (2017). For the Chelex method, a leg of each specimen (larvae or adults of FAW) was homogenized in 300µl Chelex of a 10% Chelex 100 solution (BIO-RAD Laboratories, Germany) using a tissue lyser. Samples were centrifuged shortly at room temperature before being boiled at 95°C for 30 min and frozen overnight at -20°C. The frozen samples were centrifuged at room temperature for 30 min with 4,000 rpm. The supernatants were filtered with Zymo-Column III by spinning. For the Zymo-spin method, one quarter of a specimen was put into a 2ml microcentrifuge tube with 1.5ml of phosphate buffered saline (PBS, 20mM sodium Phosphate, 150mM of NaCl, pH 8.0) and homogenized in tissue lyser (10 min at 30Hz). The homogenized tissue was pelleted by centrifugation at 6000 x g for 5 min at room temperature and the supernatant was discarded by pipetting. The pellet was suspended in 800µl of Genomic lyser buffer (Zymo Research, Germany) with 0.5% v/v beta-mercaptoethanol before being incubated in the heating block at 55°C for 5-30 min. Debris was removed by 3 min centrifugation at 10,000 x g. 600µl of the supernatant was transferred into a Zymo-Spin III column (Zymo Research, Germany) in a collection tube then centrifuged at 10,000 x g for 1 min. The Zymo-column was transferred to a new collection tube before adding 200µl of DNA Pre-Wash Buffer to the spin column (Zymo Research, Germany) and centrifuged at 10,000 x g for one minute. 500µl of g-DNA Wash Buffer was then added into the spin column and centrifuged two times at the same speed and maximal speed for one minute each.

The spin column was transferred to a clean 1.5 ml micro centrifuge tube and 50µl of water was added onto spin column, incubated at room temperature for 2-5 min and then centrifuged at 22,500 x g for 30 seconds to elute the DNA.

PCR amplification

PCR amplification of the mitochondrial COI gene was performed in a 20μl reaction mix containing 10.92μl of H₂O, 2.0μl of 10x mi-Tac buffer, 1μl of 10mM fw primer, 1μl of 20mM rv primer, 2.0μl of 2mM dNTPs, 0.08μl of mi-Taq (5 U/μl) polymerase (Metabion International AG) and 3μl of extracted genomic DNA. The PCR was performed in the program at 94°C for 2 min, followed by 35 cycles of 94°C for 1 min, 56°C for 1 min, 72°C for 1 min and a final segment of 72°C for 10 min. The amplification of the COI region used the pair primers COI-58F (5'-GGAATTTGAGCAGGAATAGTAGG-3') as forward primer and JM-77R (5'ATCACCTCCWCCTGCAGGAT-3') as reverse primer (Nagoshi *et al.*, 2006; Unbehend *et al.*, 2013).

The PCR product was digested at 37 °C for 1 hour with MspI and SacI restriction enzymes. For MspI digest, 8μl PCR product was mixed with 1μl NEB Cutsmart Buffer, 0.5μl H₂O, 0.5μl MspI enzyme and for SacI digest, 8μl PCR product was mixed with 1μl NEBufferTM 1.1; 0.5μl H₂O, 0.5μl SacI enzyme. The corn strain has one MspI (5'...C'CGG...3') cutting-site (C-600) and the rice strain has one SacI (5'...GAGCT'C...3') cutting-site (R-260).

Because the mitochondrial DNA tested by COI is inherited from the mother, the Z-linked sex chromosome was also tested on the samples. The combination of the two results should then give the real strain status of samples. The Z-linked sex chromosome test was conducted by sequencing DNA with the *Triose Phosphate Isomerase* (Tpi). The PCR amplification for sequencing was

therefore carried out with forward Tpi-282 and reverse Tpi-850. Amplification of the Tpi gene segment used the primers 282F (5'-GGTGAAATCTCCCCTGCTATG -3') and 850R (5'- AATTTTATTACCTGCTGTGG -3') was performed with the same procedure of performing

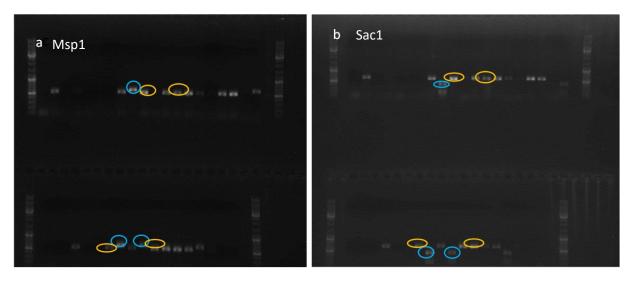
PCR with COI described above with the program 94°C for 2 min, followed by 35 cycles of 94°C

for 1 min, 50°C for 1 min, 72°C for 1 min and a final segment of 72°C for 10 min.

Strain identifications and data analysis

After being digested, each COI PCR product was mixed with 2µl of loading dye and spun down before being loaded on 1% of agarose gel and run at 130 V. The cut corn strain with Msp1, runs faster in the agarose gel than the uncut rice strain (Plate 13A) and the same process applied for the samples digested with Sac1 that cut rice strain (Plate 13A). The Tpi PCR products were sangersequenced. The sequences were analyzed using ten single nucleotide polymorphisms (SNPs) (Nagoshi 2010) where the nucleotides of corn strain differ from the nucleotides of rice strain. Consensus limit is normally fixed at 70% of the presence of the references SNPs. Under this limit, the sample test is defined as Tpi-intermediaries (Tpi-int) that is subdivided into two: Tpi-int under Tpi-C and Tpi-int under Tpi-R (Plate 13B). A sample diagnosed as corn strain for both markers was considered as a pure corn strain (CS) and a sample diagnosed as rice strain for the two markers was also considered a pure rice strain (RS). A sample with the diagnosis COI corn strain and Tpi rice strain was considered as a corn-rice (CR) hybrid, i.e. originating from a corn strain mother and a rice strain father. A sample with the diagnosis COI rice strain and Tpi corn strain was considered as a rice-corn (RC) hybrid, i.e. originating from a rice strain mother and a corn strain father.

Α



В

74	95	173	174	184	185	253	352	355	370
T	T	A	T	С	T	T	C	T	С
T	(-)	A	T	(-)	T	(-)	C	T	T
T	(-)	A	T	(-)	T	T	(-)	T	(-)
(-)	C	G	(-)	T	C	(-)	T	C	(-)
C	C	(-)	A	T	(-)	C	T	(-)	T
C	C	G	A	T	C	C	T	C	T
	T T T (-)	T T T (-) T (-) C C	T T A T (-) A T (-) C G C C (-)	T T A T T (-) A T T (-) A T (-) C G (-) C C (-) A	T T A T C T (-) A T (-) T (-) A T (-) (-) C G (-) T C C (-) A T	T T A T C T T (-) A T (-) T T (-) A T (-) T (-) C G (-) T C C C (-) A T (-)	T T A T C T T T (-) A T (-) T (-) T (-) A T (-) T T (-) C G (-) T C (-) C C (-) A T (-) C	T T A T C T T C T (-) A T (-) T (-) C T (-) A T (-) T T (-) C C G (-) T C (-) T C C C (-) A T (-) C T	T T A T C T T C T T (-) A T (-) T (-) C T T (-) A T (-) T T (-) T (-) C G (-) T C (-) T C C C (-) A T (-) C T (-)

C

Tpi-Strain	Final decision on the strain
С	Corn-Strain
R	Hybrid Corn-Rice
С	Hybrid Rice-Corn
R	Rice-Strain
	C R

Plate 13: Strains identifications with mitochondrial gene COI and sex chromosome gene Tpi (A) PCR digested with Msp1 and with Sac1; (B) the ten different consensual references SNPs to identify the Tpi-strains; (C) the final decision on strain and hybrid identifications based on the results of COI and Tpi.

3.5. Statistical analysis

The number of larvae per infested plant was calculated using the formula $Np = \frac{Lc}{Pi}$, where:

Np: number per infested plant;

Lc: number of a species

Pi: is the number of infested plants sampled.

Relative abundance (RA) was calculated using the formula: $RA = \frac{Ni}{Nt} \times 100$ (Canal Daza, 1993;

Molina-Ochoa et al., 2001 and 2004) with:

Ni: number of individuals of species i,

N_t is the total number of all individuals collected.

The numbers of farms with a specific type of control were grouped by region or by maize stage and percentages were calculated using the formula $p = \frac{nc*100}{Nf}$. Where:

P: percentage of the type of control (chemical or trap and lure or free control);

Nc: number of the farms with specific control application;

Nf: total number of the farms in a given region or a given maize stage.

The infestation rates from the farms in a given region or a given maize stage were added and the mean was calculated. The infestation rate was calculated by using the general formula of estimating the rate where, $R = \frac{ni*100}{Nn}$ with:

R: rate;

Ni: numbers of infested plants;

Np: total number of counted plants.

The mean of infestation rates was calculated using the general formula, $\mu = \frac{\sum_{0}^{i} R}{N}$, where:

μ: mean;

 $\sum_{i=0}^{n} R$: sum of infestation rate in the region or stage;

N: total number of inspected farms in the region.

The numbers of all larvae, of fall armyworm and of other similar moths were ranged by cropping season as well as total numbers and grouped into regions and into maize stages. Data were finally

submitted to analysis of variance and means separated using Duncan test with arcsine ARSIN (SQRT(X)) transforming X percentage in GenStat Twelfth Edition GenStat Procedure Library Release PL20.1.

CHAPTER FOUR

RESULTS

4.1. Knowledge and perception on FAW and farmers' practices for management

4.1.1. Profile of the respondents

A total of 259 agricultural actors was associated in this study. Among them, 72 were Agriculture Officers and 187 were farmers. The respondents (35.91%) were below 30 years, 31.27% between the age of 31 and 40 years, 21.62% between of 41 and 50 years and 11.20% above 50 years. 81.94% of the Agricultural Officers had a minimum of five years of service experiences, and 84.49% of farmers had a minimum of ten years of farming experience (Table 2).

Table 2: The socio-demographic characteristics of the respondents

Character	Number of respondents	%
Respondents		_
Agricultural officers	72	27.79
Farmers	187	72.21
Age		
Under 30 years	93	35.91
31 - 40 years	81	31.27
41 - 50 years	56	21.62
Above 50 years	29	11.20
Year of service of Agricultural Officers		
Under 5 years	13	18.06
6 – 10 years	21	29.17
11 - 20 years	27	37.50
21 - 30 years	11	15.28
Year of Farmers experience		
Under 10 years	29	15.51
11 - 25 years	95	50.80
26 – 40 years	49	26.20
Above 40 years	14	7.49

4.1.2. Knowledge on occurrence of fall armyworm

All the interviewed persons knew and could identify the fall armyworm (FAW) larval stage. They described the larva by associating it to the type of damage it caused on the maize plant. Only nine (12.5%) of Agricultural Officers had already seen the FAW eggs and could identify them. The other FAW stages were not known to the respondents. About 65.25% of the respondents did not observe the FAW larvae throughout the year against 34.75% who did it. The fall armyworm populations went up twice a year, the first from May to June and the second from August to September (Table 3).

Table 3: Occurrence of fall armyworm on maize plant

Variable	No of respondents	%
Infested parts of the plant		
Leaves	259	100
Apical meristem	259	100
Flowers	52	20.08
Fresh cobs	82	31.66
Critical stage of maize for FAW infestation		
Pre-flowering	163	62
Flowering	61	23.55
Post-flowering	35	13.51
Presence of FAW year around		
Yes	90	34.75
No	169	65.25

Table 3, contd: Annual incidence of fall armyworm on maize

Variables	No of respondents	%
Period of increased on number of FAW during the		
year		
January to April	0	0
May	72	27.80
June	93	35.91
July	0	0
August	43	16.60
September	51	19.69
October-December	0	0

The respondents also pointed out that FAW was the key pest of the year that attacked mainly maize (*Zea mays*) farms (reported by 100% of the respondents) in Ghana but was reported to occasionally attack other crops (Table 4).

Table 4: Host plants of fall armyworm in Ghana on regional basis in 2017

Region	Maize	Rice	Onion	Tomato	Cowpea	Eggplant	Millet	Sorghum
Greater Accra	18	3	1	0	1	0	0	0
Central	26	5	2	0	0	0	0	0
Western	23	5	1	0	1	0	0	0
Eastern	19	6	3	2	2	2	0	0
Volta	27	8	4	3	0	2	0	0
Ashanti	30	11	5	5	7	0	0	1
Brong Ahafo	18	7	0	0	4	3	0	1

Table 4 contd: Host plants of fall armyworm in Ghana on regional basis in, 2017

Region	Maize	Rice	Onion	Tomato	Cowpea	Eggplant	Millet	Sorghum
Northern	49	16	0	0	5	0	3	3
Upper West	21	0	0	0	3	0	7	4
Upper East	28	0	0	0	1	2	5	5
Total of respondents	259	61	16	10	24	9	15	14
%	100	23.6	6.18	3.86	9.27	3.47	5.79	5.41

The phenology of maize production was subdivided into different stages classified into three phases: preflowering, flowering and post-flowering. All the different stages of maize plants were reported by the respondents to be attacked by the novel invasive pest, and all the three phases of maize plants were seen to be critical for the FAW attack but the most reported phase was the pre-flowering (62.93%), followed by the flowering phase (23.55%) and the post-flowering phase (13.25%). Generally, for the maize production in 2017-2018, 56.76% of the respondents reported that the infestations were high, whereas, 23.55% reported that the infestations were very high, and 19.69% classified the infestations as medium (Table 5).

Table 5: Infestation levels on maize reported by the respondents

Region	Medium		Higl	h	Very high		
	No	%	No	%	No	%	
Greater Accra	3	1.16	12	4.63	3	1.16	
Central	6	2.32	13	5.02	7	2.70	
Western	4	1.54	12	4.63	7	2.70	
Eastern	5	1.93	11	4.25	3	1.16	
Volta	3	1.16	19	7.34	5	1.93	
Ashanti	5	1.93	17	6.56	8	3.09	
Brong Ahafo	6	2.32	9	3.47	3	1.16	
Northern	11	4.25	26	10.04	12	4.63	
Upper West	4	1.54	12	4.63	5	1.93	
Upper East	4	1.54	16	6.18	8	3.09	

4.1.3. Farmers applied control methods for the fall armyworm

Due to the new status and the severe damage caused by the fall armyworm, chemical insecticides were the most applied by the farmers (73.26%) to manage this introduced pest but the lured traps were also used (Fig. 3). Different chemicals from different families were sprayed to control the FAW on the field. These insecticides were purchased and distributed by the Ghana Government: Eradicot T (*Maltodextrin*, 50 mL/15 L H₂O/ha), Ema star 112 EC (*Emamectin+ Benzoate+ Acetamiprid*, 20 mL/15 L H₂O/ha), Control 5WDG and Ataka Super EC (*Emamectin+Benzoate*, 30 g/15 L H₂O/ha and 75 mL/15 L H₂O/ha, respectively), Bypel 1 and Agoo (*Bacillus thuringiensis*, 15 g/15 L H₂O/ha and 50 g/15 L H₂O/ha, respectively), Pyrinex Quick 256 EC (*Chlorpyrifos +Deltametrin*, 70 mL/15 L H₂O/ha), Viper 46 EC (*Acetamiprid+Indoxacarb*, 40 mL/15 L H₂O/ha), Adepa (*Ethyl palmitate*, 100 mL/15 L H₂O/ha), Super

top and K-Optimal EC (*Lambda-cyhalothrin+Acetamiprid*, 30 mL/15 L H₂O/ha and 50 mL/15 L H₂O/ha, respectively), Thunder 145 OD O-TEQ (*Imidacloprid+Bectacyflutherine*, 50 mL/15 L H₂O/ha), Galil 300 SC (*Imidacloprid+Bifenthrin*, 15 mL/15 L H₂O/ha) and Chemaprid (*Acetamiprid+Cypermethrin*, 100 mL/15 L H₂O/ha). Some insecticides were purchased by the farmers in the local markets, the most commonly used of them included: Condifor, Attack, Topcork, Agrigold, Neemplus, Neemazal, Postine, Dimekin, KD215, Emater, Furadan, Eradicat, Emacot.

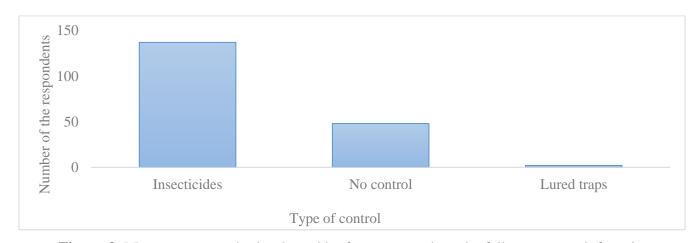


Figure 3: Management methods adopted by farmers to reduce the fall armyworm infestation. These insecticides were mostly reported (88.32%) to be effective, however, the strategy of insecticide applications differed from farmer to farmer. About 53.48% (74/137) of the farmers started spraying after observing the pest in the farm while 46.52% started two weeks after the maize germination. Many farmers (65.39%) targeted the whorl of the plant; whereas, 21.49% of the farmers targeted the maize leaves and 13.13% all the emergent parts of the maize plant. Most of the farmers (58.62%) sprayed both in the morning and in the evening whereas, 19.68% and 13.7% of the farmers sprayed only in the morning or only in the evening, respectively. The weekly spraying of the maize farms is the most reported (42.26%), however, the frequency of spraying varied and depended on the infestation level. The farmers sprayed between 8 to 13 days (23.36%), every 14 days (23.36%) or after 14 days (8.03%). The farmers applied only the insecticides distributed by the Government (47.06%), only purchased from the local markets (31.02%) or insecticides from the Government and from the local markets (23.53%).

4.2. Infestation and distribution of FAW and similar moths in Ghana

4.2.1. Infestations and distributions of fall armyworm on maize in the regions

Major cropping season in the South and beginning of season in the North (April – July)

During the major cropping season, the number of larvae collected per farm differed significantly (F=0.04, df=29, P=0.05) among the regions. The highest (14 larvae per farm) was collected from Ashanti Region and the lowest (6 larvae per farm) from Upper East Region. About 0.41 larvae per infested plant was determined that was high (0.55) in Ashanti Region and low (0.24) in Upper East Region (Tables 6).

The FAW larvae per farm were significantly (F=0.04, df=29, P=0.05) different among the regions, Greater Accra Region recorded the highest (13 larvae per farm), and Upper East and Eastern regions registered the lowest (6 larvae per infested farm). About (0.39) of the FAW larva per infested plant was determined with the highest (0.53) recorded from Greater Accra Region and the lowest (0.24) from Upper East and Eastern regions. The relative abundance of FAW was 94.54% nationwide with the lowest, 66.67% in the Eastern Region (Table 6).

Other moths included: *Leucania* sp, *Anicla infesta*, *Alpenus maculosa*, *Eldana saccharina and Sesamia calamistis* and were collected from Ashanti, Brong Ahafo, Eastern and Volta regions. Their number statically (F=0.01, df=29, P=0.05) differed among the regions. Eastern Region recorded the high number (3 larva per farm). Their number per infested plant was 0.02 with the highest (0.05 larva per infested plant) in Eastern Region. These moths recorded a relative abundance of 5.46% with the highest, 33.33% in the Eastern Region (Table 6)

Table 6: Distribution of FAW and other moths on maize plant during the major cropping season

Region	AL/F	AL/P	FAW/F	FAW/P	RA FAW	OM/F	OM/P	RA OM
Greater Accra	13.33	0.53	13.33	0.53	100	0	0	0
Central	11.67	0.47	11.67	0.47	100	0	0	0

Western	9.33	0.37	9.33	0.37	100	0	0	0
Ashanti	13.67	0.55	13	0.52	95.12	0.67	0.03	4.87
Brong-Ahafo	12.67	0.51	12.33	0.49	97.37	0.33	0.01	2.63
Northern	10.33	0.41	10.33	0.41	100	0	0	0
Upper East	6	0.24	6	0.24	100	0	0	0
Upper West	6.33	0.25	6.33	0.25	100	0	0	0
Eastern	9	0.36	6	0.24	66.67	3	0.12	33.33
Volta	9.67	0.39	8.33	0.33	86.21	1.33	0.05	13.79
Mean	10	41%	10	39%	95	1	2%	5

AL: all larvae; F: farm; FAW: fall armyworm; P: plant; RA: relative abundance; OM: other moths

Minor cropping season in the South and end season in the North (August-November)

The number of larvae collected was 10 per farm but differed significantly (F=0.03, df=79, P=0.05) among the regions. The highest (15 larvae per farm) was collected from Northern Region and the lowest (6 larvae per farm) from Upper West Region. All the sampled larvae was 0.40 per infested plant with the highest (0.60) in the Northern Region and the lowest (0.25) in the Upper West Region (Tables 7).

The numbers of the FAW larvae per farm were significantly (F=0.01, df=79, P=0.05) different among the regions, Northern Region recorded the highest (15 larvae per farm) and Upper West Region the lowest (6 larvae per farm). The FAW per infested plant was 0.38 with the highest (0.60) recorded from Northern Region and the lowest (0.25) from Upper West and Greater Accra regions. The relative abundance of FAW was 96% nationwide and the lowest (73%) in Greater Accra Region (Table 7).

Other moths were collected only from Greater Accra, Central, Eastern, Volta and Northern regions and included: *Leucania* sp, *Chilo partellus, Anicla infesta, and Sesamia calamistis*. Their number differed significantly (F=0.1, df=79, P=0.05) among the regions. Greater Accra Region recorded the high number

(2) larvae per farm. They were 0.1 per infested plant with the highest (0.9) in Greater Accra Region. These moths recorded a relative abundance of 4% with the highest, 27% in Greater Accra Region (Table 7).

Table 7: Distribution of FAW and other moths on maize plant during the minor cropping season

Region	AL/F	AL/P	FAW/F	FAW/P	RA FAW	OM/F	OM/P	RA OM
Greater Accra	8.38	0.34	6.13	0.25	73.13	2.25	0.09	26.87
Central	9	0.36	8.86	0.35	98.41	0.14	0.01	1.59
Western	9.88	0.40	9.88	0.40	100	0	0	0
Ashanti	13.57	0.54	13.57	0.54	100	0	0	0
Brong-Ahafo	8.67	0.35	8.67	0.35	100	0	0	0
Northern	15	0.6	14.89	0.60	99.26	0.11	0.01	0.74
Upper East	11	0.44	11	0.44	100	0	0	0
Upper West	6.13	0.25	6.13	0.25	100	0	0	0
Eastern	9.63	0.39	9.13	0.37	94.81	0.5	0.02	5.19
Volta	9.1	0.36	8.3	0.33	91.21	0.8	0.03	8.79
Mean	10	40%	10	39%	96	1	2%	4%

AL: all larvae; F: farm; FAW: fall armyworm; P: plant; RA: relative abundance; OM: other moths

The 2017 cropping seasons (April-November)

The number of all the moth larvae collected per farm were significantly (F=0.02, df=109, P=0.05) different among the regions. Ashanti Region recorded the highest (14 larvae per farm) and the lowest (6 larvae per farm) in the Upper West Region. All the collected larvae were 0.40 per infested plant with the highest 0.54 in the Ashanti Region and the lowest 0.25 in the Upper West Region (Table 8).

Statistically, the number of the FAW collected per farm were significantly (F=0.04, df=109, P=0.05) difference among the ten regions. The highest (13 larvae per farm) collected in the Ashanti Region and

the lowest (6 larvae per farm) in the Upper West Region. The FAW were 0.39 per infested plant with the highest 0.53 in Ashanti Region and the lowest 0.25 in Upper West Region. The relative abundance of the FAW was 95% ranged between 81% in the Eastern Region and 100% in the Western, Upper West and Upper East regions (Table 8).

Other moths recorded a significant (F=0.01, df=109, P=0.05) difference in the number of the larvae per farm among the ten regions. These moths were 0.2 per infested plant with the highest 0.5 in the Greater Accra. The relative abundance of the other moths was 5% with the highest (19%) in the Eastern Region (Table 8).

Table 8: Distribution of FAW and other moths on maize plant in 2017 cropping seasons

Region	AL/F	AL/P	FAW/F	FAW/P	RA FAW	OM/F	OM/P	RA OM
Greater Accra	10.85	0.43	9.73	0.39	89.64	1.13	0.05	10.36
Central	10.33	0.41	10.26	0.41	99.31	0.07	0.01	0.69
Western	9.60	0.38	9.60	0.38	100	0	0	0
Ashanti	13.62	0.54	13.29	0.53	97.55	0.33	0.013	2.45
Brong-Ahafo	10.67	0.43	10.5	0.42	98.44	0.17	0.01	1.56
Northern	12.67	0.51	12.61	0.50	99.56	0.06	0.01	0.44
Upper East	8.5	0.34	8.5	0.34	100	0	0	0
Upper West	6.23	0.25	6.23	0.25	100	0	0	0
Eastern	9.3125	0.3725	7.5625	0.3025	81.21	1.75	0.07	18.79
Volta	9.38	0.38	8.32	0.34	88.63	1.07	0.04	11.37
Mean	10	40%	10	39%	95	1	2%	5

AL: all larvae; F: farm; FAW: fall armyworm; P: plant; RA: relative abundance; OM: other moths

4.2.2. Infestation and distribution of fall armyworm on maize stages

Major cropping season in the South and beginning of season in the North (April-July)

During the major cropping season, the infestation of all the moths on the maize plants was significantly (F=0.03, df=23, P=0.05) different among the stages. The highest number (14 larvae per farm) of all the moth larvae per farm was collected from the farms at the tasseling stage and the lowest (7 larvae per farm) from the farms at the vegetative 6-8 leaves stage. The highest rate (54% of larva per infested plant) was recorded on the farms at the tasseling stage and the lowest (28% of larva per infested plant) on the farms at the vegetative 6-8 leaves stage (Table 9).

The number of the fall armyworm was significantly (F=0.03, df=23, P=0.05) different among the maize stages. The highest (13 larvae per farm) was collected in the farms at the tasseling stage and the lowest (7 larvae per farm) in the farms at the vegetative 6-8 leaves stage. Apart of the maize farms at the last stages (tasseling, 98% RA and mature, 56% RA) the young stages recorded 100% RA of FAW (Table 9).

The young maize stages (vegetative 4 to 12 leaves stages) did not host the other moths. These moths were collected mostly in mature stages of maize with 4 larvae per farm and recorded therefore, the highest rate (14%) of these moths per plant a well as the highest RA (44%) (Table 9).

Table 9: Distribution of FAW and other moths on maize plant on maize stages during the major cropping season

Maize stage	AL/F	AL/P	FAW/F	FAW/P	RA FAW	OM/F	OM/P	RA OM
4-6 leaves	8.98	0.36	7.75	0.31	86.32	0	0	0
6-8 leaves	7	0.28	7	0.28	100	0	0	0
8-10 leaves	12	0.48	12	0.48	100	0	0	0
10-12 leaves	11.67	0.47	11.67	0.47	100	0	0	0
Flowering	13.56	0.54	13.33	0.53	98.36	0.22	0.01	1.64

Mature 8 0.32 4.5 0.18 56.25 3.5 0.14 43.75

AL: all larvae; F: farm; FAW: fall armyworm; P: plant; RA: relative abundance; OM: other moths

Minor cropping season in the South and end of the season in the North

In the minor cropping season, the distribution of all the moths on the maize plants was significantly (F=0.04, df=59, P=0.05) different among the stages. The highest number (12 larvae per farm) of all the moth larvae per farm was collected from the farms at the vegetative 8-10 leaves stage and the lowest (8 larvae per farm) from the farms at the vegetative 4-6 leaves stage. The highest rate (48%) was recorded on the farms at the vegetative 8-10 leaves stage and the lowest (29%) on the farms at the vegetative 4-6 leaves stage (Table 10).

The number of FAW was statically (F=0.04, df=59, P=0.05) different among the maize stages. The highest (12 larvae per farm) was collected in the farms at the vegetative 8-10 leaves stage and the lowest (7 larvae per farm) in the farms at the mature stage. The rate of FAW per plan was high (47%) at the stage vegetative 8-10 leaves and low (27%) in mature stage. The lowest RA (68%) was registered in the farm at the mature stage (Table 10).

Other moths' species were found from the farms at the vegetative 8-10 leaves stage and the highest (3 larvae per farm) was recorded in the farms at the mature stage. The mature stage also recorded the highest (1.28%) RA (Table 10).

Table 10: Distribution of FAW and other moths on maize plant on maize stages during the minor cropping season

Maize stage	AL/F	AL/P	FAW/F	FAW/P	RA FAW	OM/F	OM/P	RA OM
4-6 leaves	7.36	0.29	7.36	0.29	100	0	0	0
6-8 leaves	10.27	0.41	10.27	0.41	100	0	0	0
8-10 leaves	11.9	0.48	11.8	0.472	99.16	0.1	0.004	0.03
10-12 leaves	9.27	0.37	9	0.36	97.12	0.27	0.01	0.12
Flowering	10.33	0.41	10.22	0.41	98.92	0.11	0.01	0.04
Mature	9.75	0.39	6.63	0.27	67.95	3.13	0.13	1.28

AL: all larvae; F: farm; FAW: fall armyworm; P: plant; RA: relative abundance; OM: other moths

2017 cropping seasons (April-November)

During the two cropping seasons of 2017, the numbers of all the moth larvae were statistically (F=0.03, df=83, P=0.05) different among the maize stages. The highest (12 larvae per farm) was collected in the farms at the vegetative 8-10 leaves stage and lowest (8 larvae per farm) in the farms at the vegetative 4-6 leaves stage. The maize farms at the vegetative 8-10 leaves stage recorded the highest rate (48%) larva per infested plant and the lowest rate (30% larva per infested plant) was found in the farms at the vegetative 4-6 leaves stage (Table 11).

The number of the FAW in the maize farms at different stages was significantly (F=0.02, df=83, P=0.05) different among the maize stage. The FAW number was high (12 larvae) in the farms at the vegetative 8-10 leaves stage and low (6 larvae) in the farm at the mature stage. The highest rate (48% larva per infested plant) was collected at the vegetative 8-10 leaves stage and the lowest rate (30% larva per infested plant) in the farms at the vegetative 4-6 leaves stage. The relative abundance of FAW was lower (63%) in the farms at the mature stage than the other stages (Table 11).

A significant (F=0.01, df=83, P=0.05) different was also recorded in the number of other moths collected from the maize farms at different stages. The highest (3 larvae per farm) were collected in the farms at the

mature stage but these moths were not collected from the farms at the early stages (vegetative 4-8 leaves). The highest (37%) RA of the other moths was recorded on the mature stage (table 11).

Table 11: Distribution of FAW and other moths on maize plant on maize stages during the major cropping season

Maize stage	AL/F	AL/P	FAW/F	FAW/P	RA FAW	OM/F	OM/P	RA OM
4-6 leaves	7.56	0.30	7.56	0.30	100	0	0	0
6-8 leaves	8.63	0.35	8.63	0.35	100	0	0	0
8-10 leaves	11.95	0.478	11.9	0.476	99.58	0.05	0.42	0.01
10-12 leaves	10.47	0.42	10.33	0.41	98.73	0.13	1.27	0.01
Flowering	11.94	0.48	11.78	0.47	98.60	0.17	1.40	0.01
Mature	8.88	0.36	5.56	0.22	62.68	3.31	37.32	0.13

AL: all larvae; F: farm; FAW: fall armyworm; P: plant; RA: relative abundance; OM: other moths

The infestation of the FAW in the farms during the major cropping season was not significantly difference from the infestation during the minor cropping season (Major 10 vs minor 10; F=0.1, df=19, P=0.05). The relative abundances of the FAW were also similar during the two cropping seasons (Major 95% vs minor 96%; F=0.4, df=19, P=0.05). The other moths infested the maize farms from the vegetative 8-10 leaves and increase with the age of the plant (Fig. 4). The highest numbers of the other moths were recorded in the farm from Greater Accra, Central and Volta regions (Fig. 5).

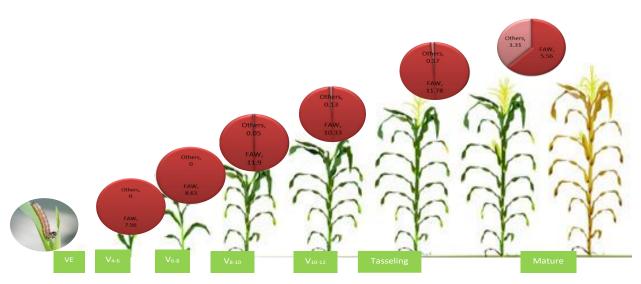


Figure 4: Distribution of FAW and other moths per farm at different maize stages in Ghana, 2017; FAW portion in red and other moths in pink

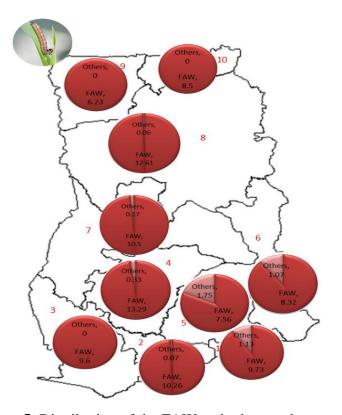


Figure 5: Distribution of the FAW and other moths per maize farm in the ten regions of Ghana, 2017; FAW is colored in red and other moths in pink; 1-Greater Accra Region, 2- Central Region, 3- Western Region, 4- Ashanti Region, 5- Eastern Region, 7- Brong Ahafo Region, 8- Northern Region, 9- Upper West Region, 10– Upper East Region

4.3. Field practices and damage levels of fall armyworm on maize

4.3.1. Insecticides distributed by the government of Ghana

According to the Plant Protection and Regulatory Services Directorate (PPRSD), the Government of Ghana had purchased and distributed different types of pesticides to the farmers (Table 12). The aim of this distribution followed the assumption that, rotating ingredients by spraying products from different groups consecutively can control the fall armyworm over a long period. Due to the outbreak and the level of spreading observed for this insect, the Government of Ghana spent about GhC16 millions to purchase insecticides and train agricultural actors on early detection and control of the FAW, however, the insecticides were not distributed to the farmers early enough before the severe outbreak in the country. PPRSD recommended that a spray gang should be deployed to affected areas under the supervision of Metropolitan, Municipal and District Assemblies (MMDAs).

Table 12: Pesticides with active ingredients, trade name and rate of application per hectare, recommended by PPRSD and distributed by the Government of Ghana to tackle the FAW

Nº	active ingredients	trade name	rate of applications
1	Maltodextrin	Eradicot T	50 mL/15 L water
2	Emamectin Benzoate+Acetamiprid	Ema Star 112EC	20 mL/15 L water
3	Emamectin Benzoate	Control 5WDG Ataka Super EC	30 g/15 L water 75 mL/15 L water
4	Bacillus thuringiensis (Bt)	Bypel 1 Agoo	15 g/15 L water 50 g/15 L water
5	Chlorpyrifos+Deltamethrin	Pyrinex Quick 256EC	70 mL/15 L water
6	Acetamiprid + Indoxacarb	Viper 46EC	40 mL/15 L water
7	Ethyl palmitate	Adepa	100 mL/15 L water
8	Lambda- cyhalothrin+Acetamiprid	Super top K-Optimal EC	30 mL/15 L water 50 mL/15 L water
9	Imidacloprid + Betacyflutherine	Thunder 145 OD O-TEQ	50 mL/15 L water
10	Imidacloprid+Bifenthrin	Galil 300SC	15 mL/15 L water
11	Acetamiprid+Cypermethrin	Chemaprid	100 mL/15 L water

4.3.2. Similarity on the level of maize plants damage among the regions in Ghana

Major cropping season in the South and beginning of the season in the North (April-July)

During the major cropping season, the insecticides were applied on 46.67% of the farms and 53.33% farms were not controlled with insecticides. The damages on the plants were high (57.3% of maize plants) and the damage levels in the farms are similar (F=0.04, d.f.= 29, P=0.05) among the regions, however, the damage varied from 31.67% in the Upper East Region, where, 66.67% of the farms were sprayed to 82.33% in the Volta Region with the same rate of sprayed farms (Table 13).

Table 13: Damage levels and field practices to manage the FAW during the major cropping season

Region	% damaged plants	% sprayed farms	% lured trap farms	% no control farms
Greater Accra	72.33	66.67	0	33.33
Central	57	33.33	0	66.67
Western	62,33	33.33	0	66.67
Ashanti	81	33.33	0	66.67
Brong-Ahafo	77	66.67	0	33.33
Northern	57.67	33.33	0	66.67
Upper East	31.67	66.67	0	33.33
Upper West	35	33.33	0	66.67
Eastern	79	33.33	0	66.67
Volta	82.33	66.67	0	33.33
Mean	57.3	46.67	0	53.33

Minor cropping season in the South and end of the season in the North (August-November)

In the minor cropping season, 66.12% of the farms were sprayed with insecticides. The farms from Ashanti and Western regions were the most sprayed (75% of sampled maize farms each region). The lowest rate

(55.56% sprayed farms) was registered from the Northern region. The lured traps were tested on two farms to monitor the fall armyworm infestation in Brong Ahafo Region. Despite, the severe outbreak of the fall armyworm, 32.22% of the farms did not receiving control measures. The damage on the maize plants was 40.65% and mere similar (F=0.4, d.f.= 79, P=0.05) among the regions but ranged from 30.13% in the Upper East Region, where, 62.5% of the farms were sprayed to 68.68% in the Northern Region, where, 55.56% of the farms were sprayed (Table 14).

Table 14: Damage levels and field practices to manage the FAW during the minor cropping season

Region	% damaged plants	% sprayed farms	%lured trap farms	% no control farms
Greater Accra	31.25	62.5	0	37.5
Central	44	71.43	0	28.57
Western	41.75	75	0	25
Ashanti	47.88	75	0	25
Brong-Ahafo	45.83	66.67	16.67	16.67
Northern	68.78	55.56	0	44.44
Upper East	30.13	62.5	0	37.5
Upper West	32.83	60	0	40
Eastern	30.5	62.5	0	37.5
Volta	33.5	70	0	30
Mean	40.65	66.12	1.67	32.22

The two cropping season of 2017 (April-November)

In Ghana, 62.43% of the sampled maize farms were sprayed in 2017 with the highest rate (72.72%) in the Ashanti Region and the lowest (50%) in the Northern Region. Only 1.11% of the sampled farms received the installation of the lured traps and 36.46% of the sampled farms did not receive control measures. The

damage on the plants was 46.85% nationwide and similar (F=0.4, d.f.= 109, P=0.05) across the regions (Table. 15).

Table 15: Damage levels and field practices to manage the FAW during the major cropping season

Region	% damaged plants	% sprayed	% lured trap farms	% no control farmers
		farm		
Greater Accra	42.45	66.67	0	33.33
Central	47.9	60	0	40
Western	47.36	63.64	0	36.36
Ashanti	56.91	72.72	0	27.27
Brong-Ahafo	56.22	66.67	11.11	22.22
Northern	66	50	0	50
Upper East	32.57	63.64	0	36.36
Upper West	30.55	57.14	0	42.86
Eastern	43.73	54.55	0	45.45
Volta	44.77	69.23	0	30.77
Mean	46.85	62.43	1.11	36.46

4.3.3. The damage on different maize stages varied between the two cropping seasons

In the major cropping season, the damage on the maize plants were similar (F=0.1, d.f=23, P=0.05) among the maize stages, however, during the major cropping season, the damage on the maize plant was significantly (F=0.04, d.f.=59, P=0.05) different among the different maize stages. The lowest damage (30.55% per plant) was registered on maize plants at the vegetative 4-6 leaves' stages where 90% of the farms were sprayed with insecticides. The highest damage (49.38%) was recorded on the maize mature stage where 62.5% of the farms were sprayed with insecticides. When the results of the two cropping

seasons were combined, the damage levels were similar (F=0.4, d.f.= 83, P=0.05) among the maize stages (Fig. 6).

The damage on maize plants across the regions was significantly (F=0.7, d.f.= 19, P=0.05) different between the two cropping seasons. It was higher (57.3% damaged plants) during the major cropping season than the minor cropping season (40.65% damaged plants) (Fig. 7) and the level of insecticide applications was lower rates (46.67% sprayed farms) during the major cropping season than the minor cropping season (66.12% sprayed farms).

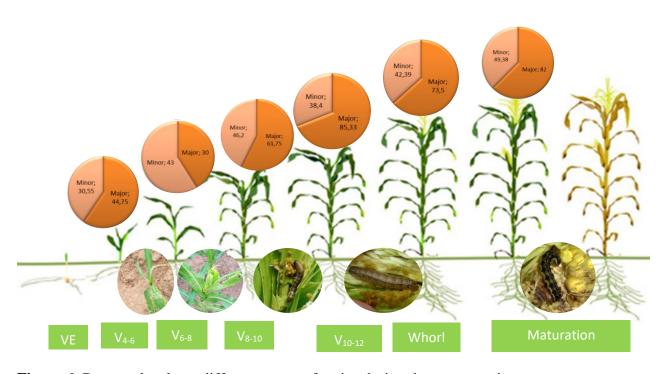


Figure 6: Damage levels on different stages of maize during the two cropping season

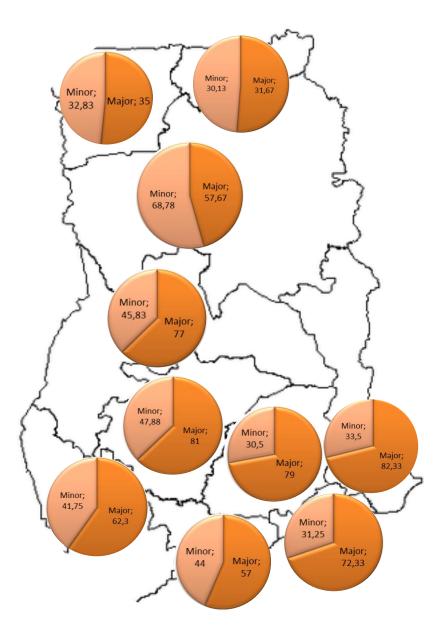


Figure 7: Distribution of damage across the country during the two cropping seasons.

4.4. Natural enemies of the FAW in Ghana

Fall armyworm larvae were collected from several maize fields across Ghana. The natural enemies of the fall armyworm occurred in 16.98% (18/106) of the inspected sites (Table 16), out of which eight sites were sprayed with insecticides. A total of 1062 *S. frugiperda* larvae were collected of wich 38 were parasitized yielding a parasitism rate of 3.58% ranging from 0.83% in the Volta Region to 7.83% in Northern Region.

Table 16: Species and sites of natural enemies of *S. frugiperda* in Ghana, 2017

Region	Sites	Coordinate N/W	Species (Order: Family)
Greater	Legon	5°38'52"/-0°10'9"	Coccygidium sp., Bracon sp.,
Accra			Chelonus cf. maudae
			Huddleston (Hymenoptera:
			Braconidae)
	Sege	5°88'86"/0°36'50"	Meteoridea testacea (Granger)
			(Hymenoptera: Braconidae)
Central	Ayensu	5°5'40"/-1°25'45"	Pheidole megacephala (F)
			(Hymenoptera: Formicidae), C.
			cf. maudae
Western	Dunkwan		Pheidole megacephala (F),
		5°6'39"/-1°37'58"	Meteoridea testacea (Granger)
Ashanti	Kumasi	6°11'29"/-1°60'95"	C. cf. maudae
			Anatrichus erinaceus Loew
	KNUST	6°68'05"/-1° 57'89"	(Diptera: Chloropidae), P.
			megacephala
	Agogo Aburkyi	6°83'71"/-1°08'50"	C. cf. maudae
Brong			
Ahafo	Ejura	7°24'14"/ -1°20'44"	C. cf. maudae

Table 6, contd: Species and sites of natural enemies of *S. frugiperda* in Ghana, 2017

Region	Sites	Coordinate N/W	Species (Order: Family)
			Bracon sp., Cotesia sp., C. cf.
Eastern	Kpong	6°13'54"/0°07'01"	maudae and A. erinaceus,
			Haematochares obsuripennis
			Stål and Peprius nodulipes
			(Signoret) (Heteroptera:
			Reduviidae), and P.
			megacephala
Volta	Agove	6°50'23"/0°37'91"	C. cf. maudae
	Tamale	9°37'76"/-0°87'84"	Cotesia sp. and Coccygidium
			sp.
NI41			
Northern	0 1	006227027 00073042	
	Savelugu	9°63'78"/-0°87'84"	C. cf. maudae
	Kilompobile	9°40'47"/-2°47'12"	Undetermined sp. (Diptera:
			Tachinidae)
	Kukpehi	9°24'59"/-0°57'73"	Coccygidium sp.
	Sanga	9°25'55"/-0°56'56"	Coccygidium sp.
	Bolga1	10°87'77"/-0°86'63"	A. erinaceus
Upper East	Bolga2	10°84'54"/-0°77'81"	Bracon sp. and C. maudae

Seven species of parasitoids were identified: *Chelonus* cf. *maudae* Huddleston, *Cotesia* sp., *Coccygidium* sp., *Meteoridea testacea* (Granger) and *Bracon* sp. (Hymenoptera: Braconidae); *Anatrichus erinaceus* Loew (Diptera: Chloropidae) and an unidentified Tachnidae species (Plate 14).



Plate 14: Natural enemies of Spodoptera frugiperda collected in 2017 in Ghana

The two most abundant parasitoids were *C. maudae* and *Coccygidium* sp. with the relative abundance of 28.95% and 23.68% of parasitoids, respectively. Parasitism rates were 1.04% and 0.85% for *C. maudae* and *Coccygidium* sp., respectively. *Chelonus maudae* was the most dispersed parasitoid, found in seven of the inspected sites (Table 17). Three species of predators were identified: *Pheidole megacephala* (F) (Hymenoptera: Formicidae), *Haematochares obsuripennis* Stål and *Peprius nodulipes* (Signoret) (Heteroptera: Reduviidae). The predator most abundant and most dispersed nationwide was *P. megacephala* with the relative abundance of 45.95% collected from four of the inspected sites (Table 17). Larval mortality due to unknown factors was 63.75%, and ranged from 55.34% in the Eastern Region to 3.83% in the Greater Accra Region (Fig. 8). All regions hosted natural enemies of fall armyworm except

for the Upper West Region. Only 32.67% of larvae collected from the fields reached the adult stage and ranged from 20.56% in the Greater Accra Region to 44.12% in the Upper West Region (Fig. 8).

Table 17: Natural enemies of the fall armyworm natural enemies collected from the field

	Number	Number		Relative	National
Species of NE	of sites	of NE*	Parasitism rate	abundance	dispersion
Cotesia sp.	3	4	0.38	10.53	2.83
Coccygidium sp.	4	9	0.85	23.68	3.77
Bracon sp.	4	6	0.56	15.79	3.77
C. maudae	7	11	1.04	28.95	6.60
A. erinaceus	3	3	0.28	7.89	2.83
Tachinidae	1	2	0.19	5.26	0.94
M. testacea	1	3	0.28	7.89	0.94
P. megacephala	4	17	-	45.95	3.77
H. obsuripennis	1	11	-	29.73	0.94
P. nodulipes	1	9	-	24.32	0.94

^{*}Natural enemies; the national dispersion was determined basing on the number of the sites with a specific natural enemy over the total maize farm inspected.

Major cropping season in the South and beginning of the season in the north

The collection of fall armyworm during the major cropping season from May to July included 29 farms from the ten regions, but the fall armyworms' natural enemies were recorded only in eight farms representing 27.59% of the sites. Parasitism rate of $17.44 \pm 3.41\%$ was recorded from the eight sites which hosted natural enemies and ranged from 6.25% (1/16) in KNUST, Ashanti Region to 60% (3/5) in Bolga 2, Upper East Region (Table 18). A total of 306 larvae were collected from the field and only 12 were

parasitized representing 3.92% of parasitism rate nationwide. Larval mortality due to unknown factors was 67.32% and ranged from 50% recorded in the Upper East Region to 85% in the Greater Accra Region (Fig. 9). Even if parasitoids were not found in the Greater Accra, Central, Western, Brong Ahafo and Upper West regions, a mean of 3.92% (12/306) of larval parasitism per region was recorded ranging from 3.45% in the Volta region to 22.22% in the Upper East region (Fig. 9). Adult emergence was 28.79% and ranged from 15% in the Greater Accra Region to 44.44% in the Eastern Region.

Table 18: Sites hosting the natural enemies of the fall armyworm during the major cropping season

Locality	Maize	Insecticide	Larvae	Number larvae	Parasitism
	stage	application	Collected	parasitized	rate
Kumasi	Flower	Yes	14	1	7.14
KNUST	Flower	No	16	1	6.25
Tamale	V8-10	No	9	3	33.33
Savelugu	V8-10	No	15	1	6.67
Bolga2	V4-6	No	5	3	60
Bolga1	V4-6	Yes	8	1	12.5
Kpong	Mature	Yes	4	1	25
Agove	V10-12	No	9	1	11.11

Minor cropping season in the South and end of the season in the North

collections of the minor cropping season were carried out from the end of August to November in 77 farms from the ten regions. Larval natural enemies of fall armyworm were recorded in 10 sites representing 12.99% of sampled farms. The parasitism rates ranged from 8.33% recorded in Ayensu, Central Region to 60% in Agogo Aburkyi, Ashanti Region (Table 19). The sites, Agogo Aburkyi, Legon,

Kpong and Sanga were not sprayed with insecticides and had the highest parasitism rate, 60%, 55.56%, 33.33% and 23.81%, respectively (Table 19). During this season, 756 larvae of *S. frugiperda* were collected and 26 larvae were parasitized representing a parasitism rate of 3.44%. Larval mortality due to unknown factors was 62.17%, ranging from 53.06% in the Upper West Region to 67.35% in the Upper East Region. Parasitoids were not found in the Upper West, Upper East and Volta regions during this season, however, the regional parasitism rate was 3.57%, ranging from 1.59% in the Central Region to 8.96% (6/67) in the Greater Accra Region. Adult emergence was 34.26% during this season and ranged from 23.88% in the Greater Accra Region to 44.9% in the Upper West Region.

The parasitism rate was highest in the sites where parasitoids were collected during the minor cropping season than the parasitism rate in the sites where the parasitoids were collected during the major cropping season (major $17.44 \pm 3.40\%$ vs. minor $24.97 \pm 6.19\%$; F= 1.14; d.f.= 19; p=0.30), but when the parasitism rates were grouped into the regions, no significant difference was recorded between the two cropping seasons (major $4.72 \pm 2.33\%$ vs. minor $3.21 \pm 0.90\%$; F= 0.36; d.f.= 19; p=0.55). The larval mortality due to unknown factors was high during the two seasons but higher during the major cropping season than the minor cropping season (major $65.59 \pm 3.47\%$ vs. minor $61.78 \pm 6.1.50\%$; F= 1.02; d.f.= 19; p=0.33), however, the emergence rate of *S. frugiperda* adults was higher during the minor season than the major season (major $30 \pm 3.47\%$ vs. minor $35 \pm 1.5\%$; F= 0.32; d.f.= 19; p=0.47).

Table 19: Sites where the natural enemies were collected in the minor cropping season

	Maize	Insecticide	Larvae	Number larvae	Parasitism
Locality	stage	application	Collected	parasitized	rate
Legon	V10-12	No	9	5	55.56
Sege	Flower	Yes	8	1	12.5
Ayensu	Flower	Yes	12	1	8.33
Dunkwan	V8-10	No	18	3	16.67
Ejura	V4-6	No	12	1	8.33
Agogo Aburkyi	Flower	Yes	5	3	60
Kilompobile	V10-12	Yes	17	2	11.76
Kukpehi	Mature	Yes	18	2	11.11
Sanga	Flower	No	21	5	23.81
Kpong	V10-12	No	9	3	33.33

4.5. Molecular characterization of fall armyworm samples from Ghana

4.5.1. Fall armyworm strains distribution based on mitochondrial marker

The mitochondrial gene Cytochrome Oxidase Subunit 1 (COI) was partially amplified and the PCR product digested with Msp1 and Sac1 restriction enzymes, resulting in strain-specific size polymorphisms. The analysis of the band sizes revealed that the corn strain variant of the gene (COI-CS) was more represented than the rice strain variant of the gene (COI-RS) in Ghana. 78.96% of the total samples were COI-CS while only 21.04% were COI-RS. The strain distribution was comparable between the regions, yet Greater Accra, Northern, Western, Ashanti and Upper East showed the highest COI-CS percentage with 85.71%, 84.62%, 83.33%, 82.5%, 81.21% respectively (fig 11). The highest COI-RS ratios were

recorded with 28.57%, 28.07%, 26.92%, 23.08% and 21.21% in Brong Ahafo, Volta, Upper West, Eastern and Central regions, respectively (Fig. 11).

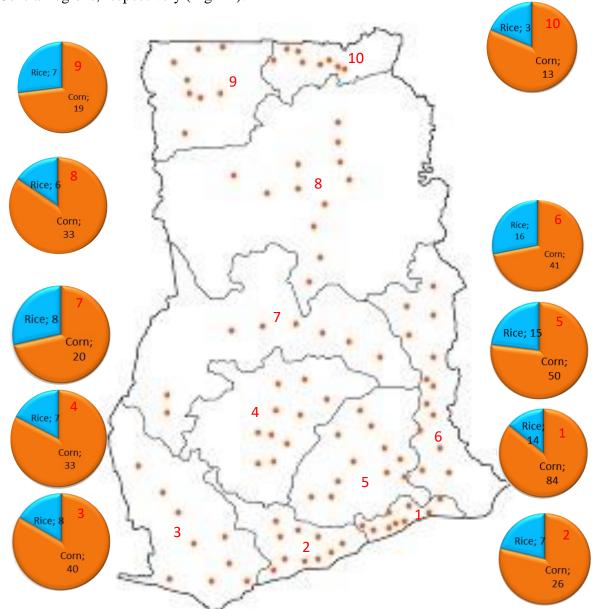


Figure 8: Fall armyworm mitochondrial Cytochrome Oxidase Subunit1 (COI) gene distribution: orange color represent COI-CS and blue color COI-RS whereas the orange dots are the sampling sites; 1. Greater Accra Region, 2. Central Region, 3. Western Region, 4. Ashanti Region, 5. Eastern Region, 6. Volta Region, 7. Brong Ahafo Region, 8. Northern Region, 9. Upper West Region, and 10. Upper East Region

4.5.2. Fall armyworm Strain distributions based on the mitochondrial and Z-linked markers

As the COI is a mitochondrial gene marker and mitochondria have always been inherited by the mother, an additional marker is needed to determine hybridization between the strains. For this, the Z-linked sex

chromosome *Triose Phosphate Isomerase* (Tpi) gene was sequenced and 10 strain-specific SNPs were analyzed. The pure corn strain (COI-CS Tpi-C) was diagnosed in the majority of the samples, 74.47% (63/85). In the Upper East Region, 90.91% (10/11) of the samples were pure corn strains, representing the highest rate, while in Western Region 50% (3/6) of the samples were pure corn strains, representing the lowest rate. Pure rice strain (COI-RS Tpi-R) was not found in the samples from Ghana. Only one cornrice hybrid (COI-CS Tpi-R, the offspring of female corn-strain and male rice-strain) representing 1.17% was found in the Western Region where it represents 16.67% (1/6) of the Western samples. A total of 22.36% rice-corn hybrids (COI-RS Tpi-C, the offspring from rice strain female and corn strain male) were found. They were present in all the regions of Ghana in few numbers. The Upper West, Volta and Western regions recorded 33.33% representing 4/12, 2/6 and 2/6, respectively (Fig. 12).

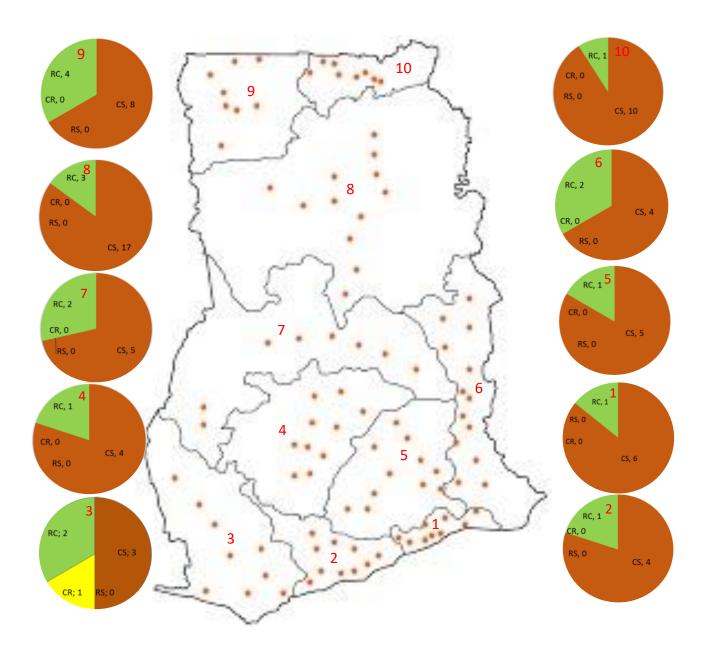


Figure 9: Situation report of FAW strain with mitochondrial COI marker and sex chromosome Z-link Tpi marker; orange color represent COI-CS Tpi-C, green color shows COI-RS Tpi-C, pale color represent COI-CS Tpi-R, the COI-RS Tpi-R is usually colored with bleu but not present in the samples whereas the orange dots are the sampling sites; 1. Greater Accra Region, 2. Central Region, 3. Western Region, 4. Ashanti Region, 5. Eastern Region, 6. Volta Region, 7. Brong Ahafo Region, 8. Northern Region, 9. Upper West Region and 10. Upper East Region

4.5.3. Additional Tpi test

When it became apparent, that the pure rice strain was not present in the subset of samples that were diagnosed with the Tpi marker, the Tpi marker was sequenced in all COI-RS samples, thus 53 samples

were added. Additionally, 43 COI-CS samples were added to the sequencing plate, and it revealed that, the corn strain was represented in all the ten regions of Ghana and so for the hybrid rice-corn. However, the rice-strain still being absent on the samples and the hybrid corn-rice was represented only in two regions: Greater Accra and Western.

CHAPTER FIVE

DISCUSSION

5.1. Perceptions of the agricultural players and the farmers practices on fall armyworm

The age structure of the respondents indicated a young age bracket for the agricultural players. This structure is an asset for a sustained training on the management of the fall armyworm which causes the damage to the crops is known and can be identified by all of them.

The respondents had knowledge of the larval stage though the pest is newly introduced and knew that only the larvae cause damage on maize crop. Indeed, the other stages of the fall armyworm were not known by the respondents because the adult stage of the fall armyworm is active at night (Gorgen et al., 2016; Nagoshi et al., 2017 and 2018) and can also be active in the late evening or early in the morning (Hardke, 2015) while pupation takes place in the soil (Sparks, 1979) and the eggs mass is hatched in the inferior hidden parts of the tendered maize leaves (Capinera, 2000). Many respondents reporting that the occurrence of the fall armyworm was not year round was limited due to the period they were reporting, during the maize growing season, and inducts that the pest may occur occasionally on many other annually crops as well as the vegetables (Ashley, 1979; Pashley, 1986). Elsewhere, the fall armyworm does not diapause (Mitchell, 1991; Nagoshi and Meagher, 2008; Nagoshi et al., 2017) and then can occur throughout the year (as Ghana) where the natural conditions of weather are favorable and food is available (Nagoshi and Meagher, 2004a; Nagoshi et al., 2017). Due to the severe outbreak exhibited by the pest in Africa (Goergen et al., 2016) and the high damage levels observed by most of the respondents, the fall armyworm is seen as a key and most destructive pest of maize plants as was noted in the Americas, where it has a preference for the maize crop but also feeds on forage grasses as well as many other plant species (Luginbill, 1928; Sparks, 1979, 1986; Wiseman et al., 1983; Pitre and Hogg 1983; Nagoshi and Meagher, 2004; Goergen et al., 2016). Some crops with the important economics as maize, millet

and rice are mostly produced and serve as food for the fall armyworm (Bass, 1978; Pitre, 1979; Young, 1979; Pitre and Hogg, 1983) that favors the pest multiplication and leads to the severe outbreak during the cropping seasons. The severe damage observed on the farms is also due to the ability of the pest to feed on all stages of the maize plant but the pre-flowering phase of maize plant was the most reported to be critical to fall armyworm attack. This observation was also corroborated by Cruz (1995), who reported that the fall armyworm has the ability to feed mainly in the whorl of young maize plants up to 45 days old.

To control the fall armyworm, most of the Ghanaian farmers interviewed, applied insecticides to maize as recommended by Luginbill (1928) to control fall armyworm in the production of maize, sorghum, millet, cowpea, rice as reported by Straub and Hogan (1974) and Bass, (1978) in the severe outbreak of the pest.

5.2. Infestation and distribution of the fall armyworm and other moths

The differences observed in the infestation levels on maize farms during the two cropping seasons across the ten regions of Ghana was due most probably to different factors and the new wide distribution of the pest subjected it differing climatic conditions (temperature, moisture, and soil type). The environmental factors influencing its development and survival, as well as genotype, agricultural practices, crop phenology, and plant maturity may contribute to the dynamics of the pest population in a given farm (Harrison, 1984; Pair *et al.*, 1986; Barfield & Ashley, 1987; Simmons, 1992; Riggin *et al.*, 1993).

The high infestation levels recorded for the fall armyworm may be due to its status as an introduced of the pest that enjoys the favorable conditions in the country (temperature, food and lack of many natural enemies) for its high multiplication. The unpreparedness of the agricultural sector for the outbreak of the fall armyworm, caused an unusually high level of infestation during both major

and minor cropping seasons. Elsewhere, the fall armyworm populations tend to be fluctuated with seasonal shifts in rainfall with the lowest populations recorded during the dry seasons (Mitchell *et al.*, 1991). The severe outbreak coincides therefore with the onset of the wet season, generally when the new cropping season follows a long period of drought (Goergen *et al.*, 2016) as the case of the major cropping season in Ghana. The fluctuation observed on the infestation levels between the two seasons can also be the effects of insecticide applications that were applied in fewer farms during the major cropping season than the minor season. Indeed, the management of the fall armyworm has been mainly effected through the use of insecticides (Cook *et al.*, 2004).

The fall armyworm prefers the tender tissues of the maize plant and therefore infests the crop at the young stages whereas, the other moths prefer the well-developed maize plants (old stages) where, they bore the stem or feed on new formed ears. The population of the fall armyworm increases in the farms when the plants grow and drop down when food become rare (plant tissues become hard, at the mature stage). Indeed, the early instars of fall armyworm feed on leaves at the site of the egg mass then dispersing vertically within the plant canopy, as well as horizontally to adjacent plants in the search of the food (Ali et al., 1989; 1990). At this stage of the pest, the feeding becomes important and migration is regular and rapid; that increase the number of damaged plant and reduce therefore, the number of larva per infested plant as well as the chance to find the larvae during the collection. In contrast, the population of other moths increases from the vegetative 8-10 leaves stage peaking at the mature stage due to the ability of some species (S. sesamia, E. saccharina, C. partellus and A. maculosa) to feed either on the stem and leaves or on the old stages of the plant as well as in the ears. In this context, the movement of the larvae of the fall armyworm and other moths is critical to effectively apply pest management strategies (Ross and Ostlie, 1990; Spangler and Calvin, 2001; Paula-Moraes et al., 2012).

5.3. Field control and damage of the fall armyworm on maize crop

Due to the new status of the fall armyworm, different methods including the lured traps, were applied to manage the pest, however, the lured traps were not effective as a control method since the damage per plant went up to 62% on the farms that received the lured traps. They may hold only the potential for predicting ahead of time where and when infestations of FAW might develop (Meagher and Mitchell, 2000).

The high infestation reported during the major cropping season caused major damage on the maize. This made the farmers increase the amount of insecticide sprayed on maize farms during the minor cropping season because to suppress the fall armyworm larvae population, multiple applications of insecticides is needed (Young *et al.*, 1972; Kuhn *et al.*, 1975). This practices and other natural factors (climate, season's factors) reduce the infestation of the fall armyworm on the field as well as the damage caused to the maize plant.

Similarity damage levels found on the farms at different maize stages as well as from the ten regions despite the different levels of infestation can be explained by the methodology using of assessing damage. The methodology was based on the count of a plant when a part (leaf, stem, and ear) presents a single symptom of damage, however, a larva can attack a plant and move from one plant to the adjacent as described in 5.2.

5.4. Natural enemies of the larva of FAW

Parasitism rates ranged from 6.25% in the KNUST site, Ashanti Region to 60% in Bolga 2 site, Upper East Region and Agogo Aburkyi, Ashanti Region. The site with the lowest as well as the sites with highest parasitism rates were not sprayed with chemical insecticides. The variations can be attributed to natural factors and cultural practices that can negatively or positively affect population of the natural enemy (Kogan *et al.*, 1999). The average parasitism rate across the

country was lower than previous findings in the Americas (8.1%, Ordònez-Garcia *et al.*, 2015; 13.8%, Molina-Ochoa *et al.*, 2004; 15.5%, Wheeler *et al.*, 1989; 18.3%, Murŭa *et al.*, 2009; 28.3%, Meagher *et al.*, 2016; 35%, Rios-Velasco *et al.*, 2011). This low parasitism rate of *S. frugiperda* by its parasitoids is due to the fact that it is a new pest introduced in Ghana. It needs Mass rearing of introduced parasitoids in laboratories in Africa with field releases are needed to increase the parasitism of the pest. Since the parasitism rate was higher during the minor (second) cropping season than the major (first) one, it is highly believed that parasitism on the fall armyworm will increase in the next years. However, the high application of chemical insecticides would negatively affect the natural enemies. The parasitoids must therefore be preserved (Pair *et al.*, 1986; Molina-Ochoa *et al.*, 2004) by using selective systemic insecticides with the outbreak of the pest (Figueiredo *et al.*, 2006).

Chelonus maudae was the most abundant parasitoid among the total collected larvae. Chelonus spp. are typical of egg-larval solitary koinobiont endoparasitoids that attack noctuidae and pyralidae (Marsh, 1978; Virla et al., 1999; Murúa et al., 2009) by ovipositing into host eggs (Pierce and Holloway, 1912; Rechav and Orion, 1975). The parasitized host larvae exhibit reduced growth rates and weight compared to unparasitized larvae (Ables and Vinson, 1981; Ashley et al., 1983). In the Western Hemisphere, Chelonus spp. appear to be the most geographically dispersed parasitoid of fall armyworm (Ashley et al., 1982; 1983; Meagher et al., 2016) and were reported to be present in 12 countries of the Caribbean, South and Central America (Molina-Ochoa et al., 2003). In many areas, Chelonus spp. were reported to be the most common species collected (Wheeler et al., 1989; Cortez-Mondaca et al., 2010, 2012; Rios-Velasco et al., 2011; Estrada-Virgen et al., 2013). Chelonus maudae was found in seven of the ten regions (Greater Accra, Central, Eastern, Volta, Ashanti, Brong Ahafo and Northern) and was recorded in all

agroecological zones of Ghana (Coastal Savana, Evergreen, Equatorial Forest, Transition zone and Guinea Savannah). These results suggest that this species is adapted to all sub-Saharan African agroecological zones. Chelonous maudae can therefore be a good biological agent for biological control of fall armyworm in Africa, however, the most efficient biological control programs for the fall armyworm are ones that use and amplify several parasitoid species rather than programs that rely on an individual natural enemy species (Riggin et al., 1993). Therefore, we believe that adding New World parasitoids that are known to attack the fall armyworm (classical biological control) plus preserving the parasitoids that are already active in Africa (conservation biological control), will contribute to reducing pest populations. The other active parasitoids include: Cotesia sp. that is a koinobiont endoparasitoid that attacks lepidopteran larvae (Whitfield, 1997; Quicke, 1997). This genus was also reported to be a parasitoid of fall armyworms in the Americas (Ashley et al., 1983; Meagher et al., 2016). It can parasitize both eggs (Ruberson and Whitfield, 1996) and the first and second instar larvae (Loke et al., 1983). It was reported to be a successful parasitoid of a major maize pest in West Africa and in the Mediterranean countries (Kaiser et al., 2017). Coccygidium sp. as all members of this genus is an internal koinobiont parasitoids of larval Noctuidae (Sharkey et al., 2009). Bracon sp. is an idiobiont ectoparasite that attacks larvae with hidden behavior like cereal stem borers and cereal store products borers (Tobias, 1986). Meteoridae testacae is a gregarious endoparasitoid that was defined as an egg-larval parasitoid of Lepidoptera (Achterberg, 1993). The grass fly, Anatrichus erinaceus is widespread in Africa but its parasitic status report is controversial (Upadhayay et al., 2001), however, it is documented as a parasitoid of sugarcane borers. Most Tachnidae species are parasitoids. In Africa, they are usually collected from the cereal stem borer larvae.

Pheidole megacephala, the most abundant and most dispersed predator, prefers humid forest habitats (Burwell et al., 2012; Hoffmann et al., 1999; Wilson, 2003). When introduced into a new area, P. megacephala expanded its range and invaded the forest interiors where it attacked and displaced other introduced and naturally occurring ant species (Burwell et al., 2012; Hoffmann, 1998), but the efficacy of P. megacephala as a biological agent is a challenge due to its generalist behavior and ecological disaster. However, it is a good predator with an efficient nest mate recruitment that enables the species to dominate baits and to retrieve prey too large for single workers to carry (Dejean et al., 2007 and 2008). Phedole megacephala must therefore be protected as a complementary biological agent of S. frugiperda in forest zones as well as the other two species of predators: H. obsuripennis and P. nodulipes which were also found in the Equatorial Forest of Eastern Region. The two Reduviids that were praying on FAW larvae were also found in the collection of true bags sampled from Lama Forest in southern Benin without detail information on the behavior of the two species (Attignon, 2004).

5.5. Molecular characterization of FAW samples from Ghana

The fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) has two strains, corn and rice strains that are morphologically identical but genetically differ one from another (Pashley 1986). The two strains have different behaviors on the choice of the preferred host plants. The corn strain is mostly reported on corn (maize) and other larger grasses while rice strain is usually reported on rice and other small grasses, however the two strains can co-occur on the same host plant. In the pool collection from corn-strain host plants in the Americas, 51% of the collected samples were corn strain while 26% were rice strain (Nagoshi *et al.*, 2018). In contrast, the collections on maize from different localities of the ten regions of Ghana did not contain the rice strain. These results demonstrate that the fall armyworm rice strain is probably not occurring on

maize in Ghana compared to the origin of the pest, the Western Hemisphere or is poorly represented in the country.

While the corn strain is the most represented strain, its representation is still lower than expected. With the absence of pure rice strains in the samples, we'd expect only corn strains to be collected from the field, as the formation of hybrids needs both strains. The representation of hybrids in the samples may hint towards the rice strain occurring probably in other host plants in adjacent areas to maize farms. An alternative possibility is that rice corn hybrids rather than pure rice strain individuals were introduced to Ghana and the observed hybrids resulted from mating of pure corn strain individuals with hybrids. In this case, the offspring (rice strain) from the two parents' hybrids may not survive. To follow up on these hypothesis, additional samples from different host plants in Ghana need to be analyzed.

The higher representation of the rice corn hybrids than the hybrid corn rice may be due to the isolation barriers in the field, because there are three main prezygotic isolation barriers in *S. frugiperda*: habitat isolation, behavioral through strain-specific sexual pheromone communication and behavioral isolation through strain-specific timing of reproduction (Groot *et al.*, 2010 and Hänniger, 2015).

CHAPTER SIX

GENERAL CONCLUSIONS AND RECOMMENDATIONS

6.1. Conclusion

The fall armyworm is an insect pest that originated from the Americas where, it occurs mainly on maize crop before being introduced to several African countries includes Ghana in 2016. In Ghana, the larval stage is the most known and can be identified by the agricultural. It is populations increase during the cropping seasons (May-July and August-September in Ghana). The pest attacks all stages of the maize plant but the pre-flowering phase is reported to be the most critical to fall armyworm attack.

Insecticides are the most used control method applied by Ghanaian farmers to manage and control the fall armyworm, however, the percentage of the farms that received insecticide applications was higher during the minor cropping season than the major cropping season. The fall armyworm infestation was generally high in Ghana but higher during the major cropping season than the minor season. The infestation level of fall armyworm varies across the ten regions and different maize stages, whereas, the other moths were more collected from Eastern and the adjacent regions. These other moths were collected on maize plants from vegetative 8-10 leave stage peaking at the mature stage. The damage was also high but similar across the ten regions and different maize stages. Some natural biological agents were controlling the fall armyworm larvae in the farms. Seven species of parasitoids emerged from the fall armyworm larvae and caused 3.58% of parasitism but the most abundant and dispersal parasitoid is *C. maudea*. Three species of predators that were preying on fall armyworm were identified.

The two strains of fall armyworm that are morphologically similar and can be identified only by molecular characterization were expected to be present in the samples. Surprise, only corn strain (highly represented) and the two hybrids (rice-corn and corn-rice) were found in Ghana samples.

6.2. Recommendations

To perform the fall armyworm management, farmers need to be trained more on the biology and ecology of the pest to perform mechanical control. The insecticides should be all tested and registered before being distributed to the farmers. The biological agents (especially *Chelonus* spp.) should be mass reared and released in the field during the dry season when insecticides are low applied on the farms to suppress the pest population before the cropping seasons. A program of the population dynamics of the fall armyworm must be established in Ghana. The maize yield loss caused by the fall armyworm should be examined in some years to determine annual losses. Many samples should be carried out on different host plants to determine the status of rice strain in Ghana. For success on pest management in this period of outbreak of fall armyworm, where insecticides are the most used in Ghana, carbofiran should be used to control the corn strain (Pashley *et al.*, 1987, Adamczyk *et al.*, 1997).

References

- Ables, J.R., Jones, S.L., Morrison, R.K., House, V.S., Bull, B.L., Bouse, L.F., Carlton, J.B. 1979.

 New developments in the use of Trichogramma to control lepidopteran pests of cotton.

 Proc. 1979 Cotton Prod. Res. Conf. National Cotton Council, Memphis, pp 125–127.
- Ables, J.R., Vinson, S.B. 1981. Regulation of host larval development by the egglarval endoparasitoid *Chelonus insularis* (Hym.: Braconidae). Entomophaga. 26: 453–458.
- Abu. J.F. 1986. A review of stem borers and their control in Ghana. Ghana Journal of Science. 14:107-113.
- Achterberg, C.V. 1993. Illustrated key to the subfamilies of the Braconidae (Hymenoptera; Ichneumonoidea). Zoological Verh Leiden. 283:1-189.
- Adamczyk, J.J., Jr., Holloway, J.W., Leonard, B.R., Graves, J.B. 1997. Susceptibility of fall armyworm collected from different plant hosts to selected insecticides and transgenic Bt cotton. Journal of Cotton Science. 1: 21-28.
- Adamczyk, J.R., Sumerford, D.V. 2000. Increased tolerance of fall armyworms (Lepidoptera: Noctuidae) to Cry1Ac d-endotoxin when fed transgenic Bacillus thuringiensis cotton: Impact on the development of subsequent generations. Florida Entomologist. 84: 1–6.
- Ali, A., Luttrell, R.G., Pitre, H.N., Davis, F.M. 1989. Distribution of fall armyworm (Lepidoptera: Noctuidae) egg masses on cotton. Environ. Florida Entomologist. 18:881–885.
- Ali, A., Luttrell, R. G., Pitre, H. N. 1990. Feeding sites and distribution of fall armyworm (Lepidoptera: Noctuidae) larvae on cotton. Environmental Entomology. 19:1060–1067.
- Ameline, A., Frérot, B. 2001. Pheromone blends and trap designs can affect catches of *Sesamia nonagrioides* (Lepidoptera: Noctuidae) males in maize fields. Journal of Application Entomology 125:15–18.

- All, J.N. 1988. Fall armyworm (Lepidoptera: Noctuidae) infestations in no-tillage cropping systems. Florida Entomologist. 71: 268-272.
- Anonymous. 1959. Director's annual reports of the Mexican Agricultural Program, September 1, 1957-August 31, 1958. The Rockefeller Foundation, New York. p 225.
- Anonymous. 1965. The Rockefeller Foundation program in the agricultural sciences. Annual report, 1964-1965. The Rockefeller Foundation, New York. p 262.
- Ashley TR. 1979. Classification and distribution of fall armyworm parasites. Florida Entomologist. 62: 114–122.
- Ashley, T.R., Mitchell, E.R. Leppla, N.C., Grissell, E.E 1980. Parasites attacking fall armyworm larvae *Spodoptera frugiperda* in late planted field corn. Florida Entomologist 63: 136–142.
- Ashley, T.R., Waddill, W.H., Mitchell, E.R., Rye, J. 1982. Impact of native parasites on the fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae), in Sout hFlorida and release of the exotic parasite, Eiphosoma vitticole (Hymenoptera: Ichneumonidae). Environmental Entomology 11: 833–837.
- Ashley, T.R., Barfield, C.S., Waddill, V.H., Mitchell, E.R. 1983. Parasitization of fall armyworm larvae on volunteer corn, bermudagrass, and paragrass. Florida Entomologist. 66: 267–271.
- Ashley, T.R., Wiseman, B.R., Davis, F.M. Andrews, K.L. 1989. The fall armyworm: a bibliography. Florida Entomologist. 72: 152–202.
- Attignon, E.K. 2004. Invertebrate diversity and ecological role of decomposer assemblages in Natural and plantation forests in southern Benin. Erlangung der Würde eines Doktors der Philosophie vorgelegt der Philosophisch-Naturwissenschaftlichen Fakultät der Universität Basel. p129.

- Bass, M.H. 1978. Fall armyworm: Evaluation of insecticides for control. Auburn University of Agricultural Experimentation Station, Leaflet 93:7.
- Barfield, C.S. Ashley, T.R. 1987. Effects of corn phenology and temperature on the life cycle of fall armyworm, Spodoptera frugiperda (Lepidoptera: Noctuidae). Florida Entomologist. 70: 110-116.
- Bertels M., Ardrez. 1956. Pragas do milho metados de defina. Inst. Agron. Sul. (Pelotas.) Bol. Tec. 16: 1-18.
- Bosque-Perez, N.A. 1995. Major insect pests of maize in Africa: Biology and control. IITA

 Research Guide 30. Training Program; International Institute of Tropical Agriculture

 (IITA), Ibadan. Nigeria 30 p Second edition.
- Bowden, J. 1956. New species of African boring Agrotidae (Lepidoptera): Bulletin of Entomological Research. 47: 415-428.
- Bowden, J. 1976. Stem borer ecology and strategy for control. Annual Applied Biology. 84: 107-134.
- Breisinger, C. 2008. Agriculture for Development in Ghana New Opportunities and Challenges, IFPRI. Discussion Paper 00784, Washington D.C.
- Burwell, C.J., Nakamura, A., McDougall, A., Neldner, V.J. 2012. Invasive African big-headed ants, *Pheidole megacephala*, on coral cays of the southern Great Barrier Reef: distribution and impacts on other ants. Journal of Insect Conservation. 16: 777–789. doi: 10.1007/s10841-012-9463-6.
- CABI. 2017. New report reveals cost of fall armyworm to farmers in Africa, provides recommendation for control. CABI, UK.

- Canal Daza, N.A. 1993. Especies de parasitoides (Hymenoptera: Braconidae) de moscas-dasfrutas (Diptera: Tephritidae) em quatro locais do estado do Amazonas. Tesis de Maestría ESALQ/USP; Piracicaba, Sao Paulo, Brasil.
- Capinera, J.L. 2000. Fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Insecta: Lepidoptera: Noctuidae). The University of Florida, Institute of Food and Agricultural Sciences. (UF/IFAS), Gainesville, Florida.
- Carvalho, R.P.L. 1970. Danos, Ilutuação de população, controle e comportamento de Spodoptera Irugiperda (J.E. Smith, 1794) susceptibilidade de dilerentes genótipos de milho em condições de campo. (Piraci aba: Imprensa ESALQ) PhD Thesis, pp 170.
- CIMMYT 2001. 1999/2000. World maize facts and trends: Meeting world maize needs: Technological Opportunities and priorities for the public sector. Centro Internaciaonal the Mais y Trigo. P 25.
- Compton, J.A.F., Floyd, S., Magrath, P.A., Addo, S., Gbedevi, S.R. Agbo, B. Bokor, G. Amekupe, S., Motey, Z., Penni, H. & Sumi, S. 1998. Involving grain traders in determining the effect of post-harvest insect damage on the price of maize in African markets. Crop Protection. 17, 483-489.
- Cook, D.R., Leonard, B.R., Gore, J. 2004. Field and laboratory performance of novel insecticides against armyworms (Lepidoptera: Noctuidae). Florida Entomologist. 87, 433–439.
- Cortez-Mondaca, E., Armenta-Cárdenas, I., Bahena-Juárez, F. 2010. Parasitoids and percent parasitism of the fall armyworm (Lepidoptera: Noctuidae) in southern Sonora, Mexico. Southwestern Entomologist. 35: 199–203.

- Cortez-Mondaca, E., Pérez-Márquez, J., Bahena-Juárez, F. 2012. Natural biological control of fall armyworm (Lepidoptera: Noctuidae) in maize and sorghum, in northern Sinaloa, Mexico. Southwestern Entomologist. 37: 423–428.
- Crisóstomo-Legaspi, J., French, J.V., Garza Zuniga, A., Legaspi, B.C. 2001. Population dynamics of the citrus leafminer, Phyllocnistic citrella (Lep.: Gracillariidae), and its natural enemies in Texas and México.Biological Control. 21:84–90.
- Cruz, I., Figueiredo, M.L.C., Oliveria, C.A., Vasconcelos, A.C. 1999. Damage of *Spodoptera frugiperda* (Smith) in different maize genotypes cultivated in soil under three levels of aluminium saturation. International Journal of pest management. 45 (4): 293-296.
- Cruz, I., Turpin, F.T. 1982. Eleito da Spodoptera Irugiperda em dilerentes estágios de crescimento da cultura de milho. Pesquisa Agropecuária Brasileira, 17, 355 359.
- Cruz, I., Turpin, F.T. 1983. Yield impact of larval infestation of the fall armyworm Spodoptera Irugiperda (J. E. Smith) to mid-whorl growth stage of corn. Journal of Economic Entomology, 76, 1052-1054.
- Cruz, I. 1995. A lagarta-do-cartucho na cultura do milho. Embrapa/CNPMS. Circular Técnica 21, p. 45.
- Cruz, I., Oliveira, L.J., Oliveira, A.C., Vasconcelos, C. A. 1996. Eleito do nível de saturação de alumínio em solo ácido sobre os danos de *Spodoptera Irugiperda* (J. E. Smith) em milho. Anais Sociedade Entomológica do Brasil, 25, 293-297.
- Cruz, I., Figueiredo, M.L.C., Silva, R.B., Foster, J.E. 2010. Efficiency of chemical pesticides to control Spodoptera frugiperda and validation of pheromone trap as a pest management tool in maize crop. RBMS 10:107–122.

- Cruz I, Figueiredo, M.L.C., Silva, R.B., Silva, I.F., Paula, C.S., Foster, J.E. 2012. Using sex pheromone traps in the decision-making process for pesticide application against armyworm (*Spodoptera frugiperda* [Smith] [Lepidoptera: Noctuidae]) larvae in maize. International Journal of Pest Management. 58:83–90.
- Cruz, I., Figueiredo, M.L.C., Matoso, M.J. 2013. Production de Trichogramma avec la pyrale de la farine (Anagasta kuehniella). In: de Almeida RP, Ivan C (eds) Technologie de production de Trichogramma spp. pour la lutte biologique contre les lépidoptères-ravageurs. Embrapa, Brasília, pp 13–75.
- Dejean, A., Moreau, C.S., Kenne, M., Leponce, M. 2008. The raiding success of *Pheidole megacephala* on other ants in both its native and introduced ranges. Comptes Rendus Biologies 331: 631–635. doi: 10.1016/j.crvi.2008.05.004.
- Dejean, A., Moreau, C.S., Uzac, P., Le Breton, J., Kenne, M. 2007. The predatory behavior of *Pheidole megacephala*. Comptes Rendus Biologies 330: 701–709. doi: 10.1016/j.crvi.2007.06.005.
- Dicke, F.F. 1977. The most important corn insects. In George F. Sprague (ed.) Corn and corn improvement. Agronomy. 18:501-90.
- DiehL, S.R., Bush, G. L. 1984. An evolutionary and applied perspective of insect biotypes. Annual Reviews of Entomology. 29: 471-504.
- Ditman, L.P., Cory, E.N. 1936. The corn earworm. Maryland Agric. Exp. Stn. Bull. 399:77-90.
- Estrada-Virgen, O., Cambero-Campos, J., Robles-Bermudez, A., Rios-Velasco, C., Carvajal-Cazola, C., Isiordia-Aquino, N., Ruiz-Cancino, E. 2013. Parasitoids and entomopathogens of the fall armyworm *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in Nayarit, Mexico. Southwestern Entomologist. 38: 339–344.

- FAO. 2004. Scaling soil nutrient balances. FAO Fertilizer & Plant Nutrition Bulletin No. 15. Rome.
- FAO, 2019. FAO Statement on Fall Armyworm in Sri Lanka. Rome, Italy: FAO. http://www.fao.org/srilanka/news/detail-events/en/c/1177796/
- Figueiredo, M.L.C., Martins-Dias, A.M.P., Cruz, I. 2006. Relationship between fall armyworm and their natural biological control agents in the maize crop. Pest Agropec Brasil. 41: 1693-1698.
- Foster, R.E. 1989. Strategies for protecting sweet corn ears from damage by fall armyworms (Lepidoptera: Noctuidae) in southern Florida. Florida Entomologist. 72: 146-151.
- Girling, D.J. 1980. As a crop pest in Ghana. Tropical Pest Management. 26: 152-156.
- Goergen, G., Kumar, P.L., Sankung, S.B., Togola, A., Tamò, M., 2016. First report of outbreaks of the fall armyworm *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera, Noctuidae), a new alien invasive pest in West and Central Africa. PLoS One 11 (10), e0165632. http://dx.doi.org/10.1371/journal.pone.0165632.
- Groot, A., Marr, M., Heckel, G.D., Schöfl, G. 2010. The roles and interactions of reproductive isolation mechanism in fall armyworm (Lepidoptera: Noctuidae) host strains. Ecological Entomology. 35 (1): 105-118.
- Gounou, S., Schultness, F., Shenover, T., Hammond, W.N.O., Braima H., Olaleye I., Cudjoe A.R., Adjakloe, R., Antwi, K. K. 1993. Stem and ear borers in Ghana: Plant Health Management Division Research Monograph. International Institute of Tropical Agriculture. Ibadan, Nigeria.

- Hänniger, S. 2015. Chasing sympatric speiation: The relative importance and genetic basis of prezygotic isolation barriers in diverging populations of *Spodoptera frugiperda*. PhD thesis, University of Amsterdam, the Netherlands. P17.
- Hardke J.T,G. M. Lorrenz III, B.R. Leonard. 2015. Fall Armyworm (Lepidoptera: Noctuidae)

 Ecology in Southeastern Cotton. J. Integ. Pest Mngmt. 6(1): 10; DOI: 10.1093/jipm/pmv009.
- Harrell, E.A., Young, J.R., Hare, W.W. 1977. Insect control on late planted sweet corn. Journal of Economic Entomology. 70:129-131.
- Harrison, F. P. 1984. Observations of the infestation of corn by fall armyworm (Lepidoptera: Noctuidae) with reference to plant maturity. Florida Entomologist. 67: 333-339.
- Hoffmann, BD. 1998. The big-headed ant *Pheidole megacephala*: A new threat to monsoonal northwestern Australia. Pacific Conservation Biology 4: 250–255.
- Hoffmann, B.D., Andersen, A.N., Hill, G.J.E. 1999. Impact of an introduced ant on native rain forest invertebrates: *Pheidole megacephala* in monsoonal Australia. Oecologia (Berlin) 120: 595–604. doi: 10.1007/pl00008824.
- Horovitz, S. 1960. Trabajos en morcha sobre resistencia a insectos en el maiz Agronomic Tropic. 10:107-14.
- Hurelbrink, R., and G. Boohene. 2011. Ghana Feed Sector Assessment Report. Report for ACDI/VOCA.
- ICAR-NBAIR, 2018. Spodoptera frugiperda (J. E. Smith). Insects in Indian Agrosystems. ICAR-National Bureau of Agricultural Insect Resources (NBAIR), India. http://www.nbair.res.in/insectpests/Spodoptera_frugiperda.php.

- IITA, 2018: Fall armyworm has reached the Indian subcontinent! Ibadan, Nigeria: IITA. http://www.iita.org/news-item/fall-armyworm-has-reached-the-indian-subcontinent.
- IPPC, 2018. First detection of Fall Army Worm on the border of Thailand. IPPC Official Pest Report, No. THA-03/1. FAO: Rome, Italy. https://www.ippc.int.
- IPPC, 2019. First Detection Report of the Fall Armyworm *Spodoptera frugiperda* (Lepidoptra: Noctuidae) on Maize in Myanmar. IPPC Official Pest Report, No. MMR-19/2. Rome, Italy: FAO. https://www.ippc.int.
- ISSER. 2010. The state of the Ghanaian economy in 2009, Institute of Statistical Social and Economic Research (ISSER), University of Ghana, Legon, Ghana. pp218.
- Jamjanya, T., Quisenberry, S.S., Croughan, S.S., Story, R.N. 1990. Comparison of bermudagrass lines grown in different cultural conditions and the effect on screening for all armyworm (Lepidoptera: Noctuidae) resistance. Journal of Economic Entomology. 83: 585-590.
- Kaiser, L., Fernandez-Triana, J., Capdevielle-Dulac, C., Chantre, C., Bodet, M., Kaoula, F., Benoist, R., Calatayud, P., Dupas, S., Herniou, E.A., Jeannette, R., Obonyo, J., Silvain, JF., Le Ru, B. 2017. Systematics and biology of Cotesia typhae sp. n. (Hymenoptera, Braconidae, Microgastrinae), a potential biological control agent against the noctuid Mediterranean corn borer, Sesamia nonagrioides. ZooKeys @@: @-@. https://doi.org/10.3897/zookeys.@@.13016.
- Knipling, E.F. 1980. Regional management of the fall armyworm a realistic approach? Florida Entomologist. 63: 468-480.
- Knutson, A. 2009. The fall armyworm pest of pasture, hayfields and small grains. AgriLife Extension. Texas A&M System, Dallas, TX.

- Kogan, M., Gerling, D., Maddox, J.V. 1999. Enhancement of Biological Control in annual agricultural environments. In: Bellows T, Fisher T, editors. Handbook of Biological Control, pp. 789-818. Academic Press.
- Kuhn, C.W., Jellum, M.D., All, J.N. 1975. Effect of carbofuran treatment on corn yield, maize chlorotic dwarf and maize dwarf mosaic virus diseases and leafhopper populations. Phytopathology. 65:1017-1020.
- Loke, W.H., Ashley, T.R., Sailer, R.I. 1983. Influence of fall armyworm, *Spodoptera frugiperda*, (Lepidoptera: Noctuidae) larvae and corn plant damage on host finding in Apanteles marginiventris (Hymenoptera: Braconidae). Environemental Entomology. 12: 911–915.
- Luginbill P. 1928. The fall armyworm. US Deptment of Agriculture Technology Bulletin. 34:1–91. 2.
- Luginbill, P.A. 1950. Habits and control of the fall armyworm. USDA Farmers' Bull. 1990. U.S. Gov. Print Office.
- Marenco, R.J., Foster, R.E., Sanchez, C.A. 1992. Sweet corn response to fall armyworm (Lepidoptera: Noctuidae) damage during vegetative growth. Journal of Economic Entomology 85:1285–1292.
- Marsh, PM. 1978. Tha braconid parasites (Hymenoptera) of Heliothis species (Lepidoptera: Noctuidae). Proceedings Proceedings of the Entomological Society of Washington 80: 15-36.
- Meagher, R.N., Nuessly, G., Nagoshi, R.N., Hay-Roe, M.M. 2016. Parasitoids attacking fall armyworm (Lepidoptera: Noctuidae) in sweet corn habitats. Biological Control. 95: 66-72.
- Meagher Jr, R.L. 2001. Collection of fall armyworm (Lepidoptera: Noctuidae) adults and nontarget Hymenoptera in different colored unitraps. Florida Entomology. 84: 77-82.

- Meagher Jr, R.L., Mitchell, E.R. 2000. Collection of fall armyworm (Lepidoptera: Noctuidae) using selected pheromone lures and trap designs. Journal of Entomological Science. 36: 135–142.
- Mills, N.J. 2010. Egg parasitoids in biological control and integrated pest management. In: Cônsoli FL, Parra JPR, Zucchi RA (eds) Egg parasitoids in agroecosystems with emphasis on Trichogramma. Springer, London, pp 389–411.
- Mills, N.J., Pickel, C., Masfield, S., McDougall, S., Buchner, R., Caprile, J., Edstron, J., Elkins, R., Hasey, J., Kelley, K., Krueger, B., Olson, B., Stocker, R. 2000. Mass releases of Trichogramma wasps can reduce damage from codling moth. California Agriculture. 56:22–25.
- Mitchell, E.R. 1978. Relationship of planting date to damage by earworms in commercial sweet corn in north central Florida. Florida Entomologist. 61: 251-255.
- Mitchell, E.R., McNeil, J.N., Westbrook, J.K., Silvain, J.F., Lalanne-Cassou, B., Chalfant, R. B., Pair, S.D., Waddill, V. H., Sotomayor-Rios, A., Proshold, F. L. 1991. Seasonal periodicity of fall armyworm, (Lepidoptera: Noctuidae) in the Caribbean basin and Northward to Canada. Entomology Science. 26 (1): 39-50.
- MoFA, Ministry of Food and Agriculture. 2010. Agriculture in Ghana: Facts and Figures. Accra, Ghana: Ministry of Food and Agriculture, Statistics, Research, and Information Directorate.
- MoFA, Ministry of Food and Agriculture. 2011. Statistics, Research and Information Directorate (SRID), "Agriculture in Ghana: Facts and Figures (2010)".
- MoFA, Ministry of Food and Agriculture. 2012. Agriculture in Ghana: Facts and Figures. Accra: Ministry of Food and Agriculture, Statistics, Research, and Information Directorate.

- MoFA Ministry of Food and Agriculture. 2016. Agriculture in Ghana. Stastistics, Research and information Directorate, Accra, Ghana, p4.
- Molina-Ochoa, J., Hamm, J.J., Lezama-Gutiérrez, R., López-Edwards, M., González-Ramírez, M., Pescador-Rubio, A. 2001. A survey of fall armyworm (Lepidoptera: Noctuidae) parasitoids in the Mexican states of Michoacán, Colima, Jalisco, and Tamaulipas. Florida Entomologist. 84:31–36.
- Molina-Ochoa, J., Carpenter, J.E., Heinrichs, E.A., Foster, J.E. 2003. Parasitoids and parasites of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in the Americas and Caribbean Basin: an inventory. Fla. Entomol. 86: 254–289.
- Molina-Ochoa, J., Carpenter, J.E., Lezama-Gutiérrez, R., Foster, J.E., González-Ramírez, M., Ángel-Sahagún, C.A., Fariías-Larios, J. 2004. Natural distribution of hymenopteran parasitoids of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) larvae in México. Florida Entomologist. 87:461–472.
- Murúa, M.G., Molina-Ochoa, J., Fidalgo, P. 2009. Natural distribution of parasitoids of larvae of the fall armyworm, Spodoptera frugiperda, in Argentina. 17pp. Journal of Insect Science 9:20.
- Nagoshi, R.N. 2010. The fall armyworm Triose Phosphate Isomerase (Tpi) gene as a marker of strain identity and interstrain mating. Annuall Entomological Society of America. 103: 283-292.
- Nagoshi, R.N., and Meagher, R.L. 2003. FR tandem repeat sequence in fall armyworm (Lepidoptera: Noctuidae) host strains. Annual Entomological Society of America. 96: 329-335.

- Nagoshi, R.N., and Meagher, R.L. 2004a. Seasonal distribution of fall armyworm (Lepidoptera: Noctuidae) hoststrains in agricultural and turf grass habitats. Environmental Entomologist. 33: 881-889.
- Nagoshi, R.N. and Meagher, L.R. 2004b. Behavior and distribution of the two fall armyworm host strains in Florida. Florida Entomologist. 87(4): 440-445.
- Nagoshi, R.N., Meagher, R.L., Adamczyk, J.J., Braman, S.K., Brandenburg, R.L., and Nuessly, G. 2006. New restriction fragment length polymorphisms in the cytochrome oxidase I gene facilitate host strain identification of fall armyworm (Lepidoptera: Noctuidae) populations in the Southeastern United States. Journal of Economic Entomology. 99: 671–677.
- Nagoshi, R.N., Silvie, P., Meagher, L.R., Lopez, J., Machado, V. 2007. Identification and Comparison of Fall Armyworm (Lepidoptera: Noctuidae) Host Strains in Brazil, Texas, and Florida. Annal of Entomological Society of America. 100(3): 394-402.
- Nagoshi, R.N., Meagher, R.L. 2008. Review of fall armyworm (Lepidoptera: Noctuidae) genetic complexity and migration. Florida Entomologist. 91(4): 546–554.
- Nagoshi, R.N., Koffi, D., Agboka, K., Tounou, K.A., Banerjee, R., Jurat-Fuentes, J.L., Meagher, L.R. 2017. Comparative molecular analyses of invasive fall armyworm in Togo reveal strong similarities to populations from the eastern United States and the Greater Antilles. PLoS ONE 12(7): e0181982.
- Nagoshi, R.N., Goergen, G., Tounou, K.A., Agboka, K., Koffi, D., Meagher, L.R. 2018. Analysis of strain distribution,, migratory potential, and invasion history of fall armyworm populations in northern Sub-Saharan Africa. Scientific Reports. 8: 3710.
- Navas, D. 1974. Fall armyworm in rice. Tall Timbers Res. Bulletin No. 6:99-166.

- Ordóñez-García, M., Rios-Velasco, C., Berlanga-Reyes, D.I., Acosta-Muñiz, C.H., Salas-Marina, M.A., Cambero-Campos, O.J. 2015. Occurrence of natural enemies of Spodoptera frugiperda (Lepidoptera: Noctuidae) in Chihuahua, Mexico. Florida Entomologist. 98, No. 3.
- Pair, S.D., Gross, Jr.H.R. 1984. Field mortality of pupae of the fall armyworm, *Spodoptera frugiperda* (J.E. Smith), by predators and a newly discovered parasitoid, *Diapetimorpha introita*. Journal of the Georgia Entomological Society. 19: 22-26.
- Pair, S.D., Raulston, J.R., Sparks, A.N. Martin, P.B. 1986. Fall armyworm (Lepidoptera: Noctuidae) parasitoids: differential spring distribution and incidence on corn and sorghum in the Southeastern United States and Northeastern Mexico. Environmental Entomology. 15: 342–348.
- Pannuti, L.E.R., Baldin, E.L.L., Hunt, T.E., Paula-Moraes, S.V. 2015. On-plant larval movement and feeding behavior of fall armyworm (Lepidoptera: Noctuidae) on reproductive corn stages. Environmental Entomology. 45: 192-200.
- Pashley, D.P. 1986. Host associated genetic differentiation in fall armyworm: a sibling species complex? Annual Entomological Society of America. 79: 898-904.
- Pashley, D.P. 1988. Quantitative genetics, development, and physiological adaptation in host strains of fall armyworm. Evolution. 42: 93-102.
- Pashley, D.P., Martin, J.A. 1987. Reproductive incompatibility between host strains of the fall armyworm (Lepidoptera: Noctuidae). Annual. Entomological Society of America. 80:731–733.

- Pashley, D.P., Hardy, T.N., Hammond, A.M. 1995. Host effects on developmental and reproductive traits in fall armyworm strains (Lepidoptera: Noctuidae). Annual Entomological Society of America. 88: 748-755.
- Paula-Moraes, S.V., Hunt, T.E., Wright, R.J., Hein, G.L., Blankenship, E.E. 2012. On-plant movement and feeding of western bean cutworm (Lepidoptera: Noctuidae) early instars on corn. Environmental Entomology. 41: 1494–1500.
- Pierce, W.D., Holloway, T.E. 1912. Notes of the biology of *Chelonus texanus* cress. Journal of Economic Entomology. 5: 425–428.
- Pitre, H.N. 1979. Fall armyworm on sorghum: other hosts. Bull. 876. Mississippi Agriculture and Forest Experimentation Station. Mississippi State, MS.
- Pitre, H.N., Hogg, D.B. 1983. Development of the fall armyworm on cotton, soybean and corn.

 Journal of Georgia Entomological Society. 18:182-194.
- Pitre, H.N., Mulroony, J.E., Hogg, D.B. 1983. Fall armyworm (Lepidoptera: Noctuidae) oviposition: crop preference and egg distribution on plants. Journal of Economic Entomology. 76: 463-466.
- Prowell, D.P., McMichael, M., Silvain, J. F. 2004. Multilocus genetic analysis of host use, introgression, and speciation in host strains of fall armyworm (Lepidoptera: Noctuidae).

 Annual Entomological Society of America. 97: 1034-1044.
- Quicke, D.L.J. 1997. Parasitic Wasps. Chapman & Hall, London, pp470.
- Quisenberry, S.S., and Whitford, F. 1988. Evaluation of bermudagrass resistance to fall armyworm (Lepidoptera: Noctuidae): influence of host strain and dietary conditioning. Journal of Economic Entomology. 81: 1463-1468.

- Rechav, Y., Orion, T. 1975. The development of the immature stages of *Chelonus inanitus*. Annual Entomological Society of America. 68: 457–462.
- Riggin, T.M., Espelie, K.E., Wiseman, B.R. and Isenhour, D.J. 1993. Distribution of fall armyworm (Lepidoptera: Noctuidae) parasitoids on five corn genotypes in South Georgia. Florida Entomologist. 76: 292-302.
- Rios-Velasco, C., Gallegos-Morales, G., Cambero-Campos, J., Cerna-Chávez, E., Rincón-Castro, Del., Ma, C., Valenzuela-García, R. 2011. Natural enemies of the fall armyworm *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in Coahuila, México. Florida Entomology. 94: 723–726.
- Roberts, P.M., All, J.N. 1993. Hazard for fall armyworm (Lepidoptera: Noctuidae) infestation of maize in double-cropping systems using sustainable agricultural practices. Florida Entomologist0. 76: 276-283.
- Rose, D.J.W., Dewhurst, C.F., Page, W.W. 2000. The African armyworm handbook. 2nd ed. Chatham: Natural Resources Institute, University of Greenwic.
- Ross, S.E., Ostlie, K.R. 1990. Dispersal and survival of early instars of European corn borer (Lepidoptera: Pyralidae) in field corn. Journal of Economical Entomology 83: 831–836.
- Ruberson, J.R., Whitfield, J.B., 1996. Facultative egg-larval parasitism of the beet armyworm, Spodoptera exigua (Lepidoptera: Noctuidae) by Cotesia marginiventris (Hymenoptera: Braconidae). Florida Entomology. 79: 296–302.
- Ruttan, V.W. 2005. Scientific and technical constraints on agricultural production: prospects for the future. Proceedings of the American Philosophical Society. 149: 453-68.

- Sharkey, M.J., Yu, D.S., Noort, S.V., Seltmann, K., Penev, L. 2009. Revision of the Oriental genera of Agathidinae (Hymenoptera, Braconidae) with an emphasis on Thailand including interactive keys to genera published in three different formats. ZooKeys. 21: 19-54.
- Simmons, A.M. 1992. Effects of constant and fluctuating temperatures and humidities on the survival of *Spodoptera frugiperda* pupae (Lepidoptera: Noctuidae). Florida Entomologist. 76: 333-340.
- Spangler, S.M., Calvin, D.D. 2001. Vertical distribution of European corn borer (Lepidoptera: Crambidae) egg masses on sweet corn. Environmental Entomology. 30: 274–279.
- Sparks, A. 1979. A review of the biology of the fall armyworm. Florida Entomologist. 62: 282–287.
- Sparks, A.N. 1986. Fall armyworm (Lepidoptera: Noctuidae): Potential for area-wide management. Florida Entomology. 69: 603–614.
- Straub, R.W., H.J. Hogan. 1974. Feasibility of fall armyworm, *Spodoptera frugiperda* (J. E. Smith), control on late-planted dent corn. N.Y. Food Life and Scientific Bulletin. No. 49. 4 p.
- Sullivan, M.J., Turnipseed, S.G., D. Robinson. 1999. Insecticidal efficacy against a complex of fall and beet armyworms and soybean looper in South Carolina cotton, pp. 1034–1036. In Proceedings, 1999 Beltwide Cotton Conferences, 3–7 January 1999, Orlando, FL. National Cotton Council, Memphis, TN.
- Tobias, V.L., 1986. Agathidinae: 276-291. In: Medvedev, G.S. (ed.). Opredelitel nasekomych Evropeiskoi tchasti SSSR 3, Perepontchatokrylye 4.— Opr. Faune SSSR 145:1-501, figs 1-263. Translation 1995: 480-507.— Lebanon, U.S.A.

- Unbehend, M., Hänniger, S., Meagher, L.R., Heckel, G.D., Groot T.A. 2013. Pheromonal Divergence between Two Strains of *Spodoptera frugiperda*. Journal of Chemistry Ecology. 39:364–376.
- Upadhyay, R.K., Mukerji, K.G., Chamola, B.P. 2001. Biocontrol Potential and its Exploitation in Sustainable Agriculture. Insect Pests Springer (India) Private Ltd. New Delhi. 2: pp434.
- Usua, E. J. 1968. The biology and ecology of *Busseola fusca* and *Sesamia* spp. in South Western Nigeria. Distribution and population studies. Journal of Economic Entomology. 61:830-833.
- Van Driesche, R.G. 1983. Meaning of Percent Parasitism in studies of insect parasitoids. Environmental Entomology. 12:1611–1621.
- Van Lenteren, J.C. 2000. Success in biological control of arthropods by augmentation of natural enemies. In: Gurr G, Wratten S (eds) Biological control: measures of success. Kluwer Academic Publishers, The Netherlands, pp 77–103.
- Vickery, R.A. 1929. Studies of the fall armyworm in the Gulf coast region of Texas. USDA Technical Bulletin 138. 63 pp.
- Virla, E.G., Colomo, MV., Berta, C., Valverde, L. 1999. El complejo de los parasitoides del "gusano cogollero" del maíz, Spodoptera frugiperda, en la República Argentina (Lepidoptera: Noctuidae). Neotrópica. 45: 3-12.
- Wheeler, G.S., Ashley, T.R., Andrews, K.L. 1989. Larval parasitoids and pathogens of the fall armyworm in Honduran maize. Entomophaga 34: 331–340.
- Whitfield, J.B. 1997. Subfamily Microgastrinae. In: Wharton RA, Marsh PM, Sharkey MJ (Eds)

 Manual of the New World genera of the family Braconidae (Hymenoptera): Special

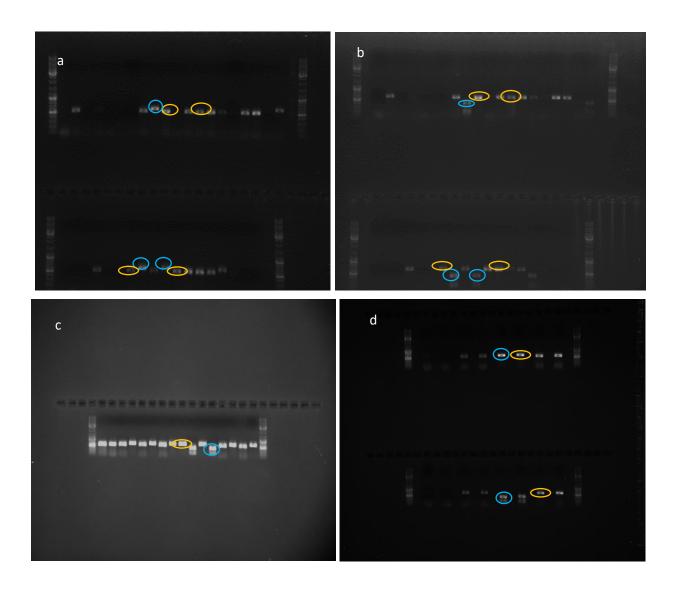
 Publication No. 1. International Society of Hymenopterists, Washington D.C. 333–364.

- Williams, W.P. and Davis, F.M. 1990. Response 01 corn to artificial infestation with fall armyworm and southwestern corn borer larvae. Southwestern Entomology, 15, 163-166.
- Willink, E., Osores, V.M., Costilla, M.A. 1991. EI gusano 'cogollero' dei maiz. Avance Agroindustrial sv, 3-7.
- Wilson, E.O. 2003. *Pheidole* of the New World: A dominant, hyperdiverse ant genus. Harvard University Press, Cambridge, pp 794.
- Wiseman, B.R., Painter, R.H., Wassom, C.E. 1966. Detecting corn seedling differences in the greenhouse by visual classification of damage by the fall armyworm. Journal of Economic Entomology. 59:1211-1214.
- Wiseman, B.R., Williams, W.P., Davis, F.M. 1981. Fall armyworm: Resistance mechanisms in selected corns. Journal of Economic Entomology. 74: 622–624.
- Wiseman, B.R., Davis, F.M., Williams, W.P. 1983. Fall armyworm: Larval density and movement as an indication of non-preference in resistant corns. Protection Ecology. 5: 135–141
- Wiseman, B.R., Davis, F.M. 1990. Plant resistance to insects attacking corn and grain sorghum. Fla. Entomol. 73: 446–458.
- Wyckhuys, K.A.G., O'Neil, R.J. 2006. Population dynamics of *Spodoptera frugiperda* Smith (Lepidoptera: Noctuidae) and associated arthropod natural enemies in Honduran subsistence maize. Crop Protection. 25:1180–1190.
- Young, J.R., HarrelL, E.A., Hare, W.W. 1972. Mortality of adult corn earworms treated with insecticidal formulations in sweet corn fields and in the laboratory. Ibid. 65:786-9.
- Young, J.R. 1979. Fall armyworm: Control with insecticides. Florida Entomologist. 62:130-133.

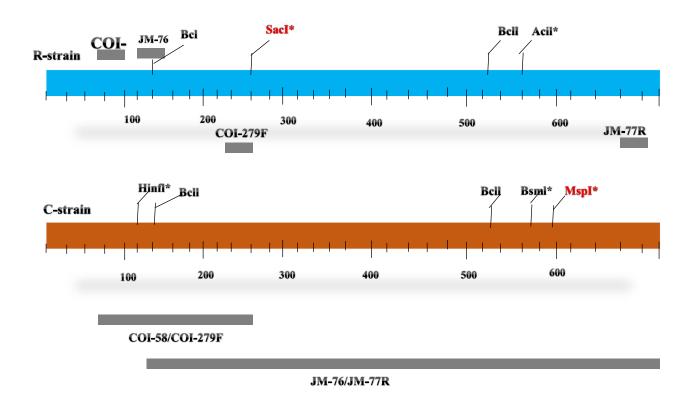
APPENDICES

Appendix 1: COI loading gel with Msp1 and Sac1:

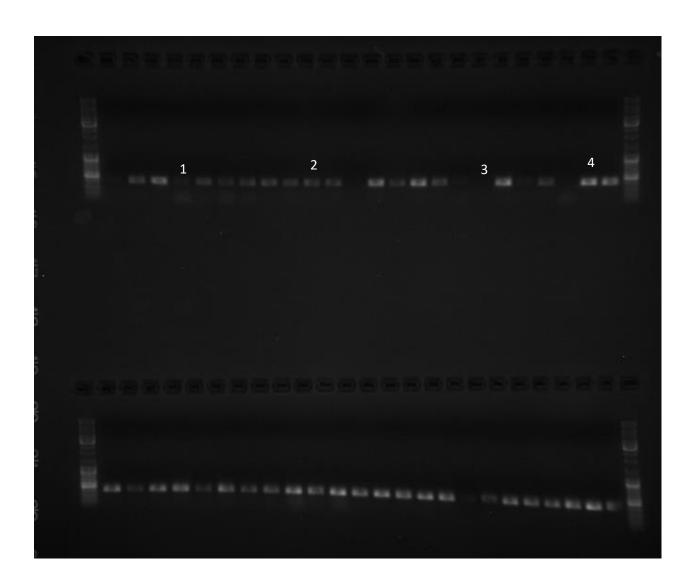
a- PCR product digested with Msp1enzyme which cuts DNA sequence of corn strain of *S.frugiperda* at about 600 pb and reduce the length of the sequence. When the digest product with Msp1 is run in the gel, the shorter sequence is faster than the entire sequence. The faster ones are the corn strain samples (in yellow circles) and the uncut sequences are slower running and are rice strain samples (in blue circles). b- PCR product digested with Sac1 enzyme, this enzyme cuts DNA sequence of rice strain at about 260 pb separating the sequence into two chains and leaves corn strain DNA uncut. When run in the gel the two cut sequence are faster than uncut DNA and make the two strains sample results clear. (a and b) are the same samples in separated gel, (c) is one row of Msp1 and Sac1 samples combined and (d) is one gel with different row of Msp1 samples above and Sac1 below.



Appendix 2: Site of action of different digestive enzymes and mode of action of different primers.



Appendix 3: PCR carried with Tpi and product run in the gel to test the quality of DNA before sequencing process. It gives approximately the quality of the sequencing results. 1- lower quality of sequencing, 2- good quality of sequencing, 3- very low quality of sequencing, 4- very high quality of sequencing.



Appendix 4: Layout of COI and Tpi analysis

Sites	MSP1	Sac1	Tpi	Strain	sex	Tpi	Tpi	Tpi	Final									
			74	95	173	174	184	185	253	352	355	370			129	140	180	Decision
G4GAm4	С	С	T	T	G	A	С	T	T	С	T	С	С	na	_			С
G1GAm3	С	C	T	T	A	T	С	T	T	C	T	C	С	na	C	G	C	C Africa
G3GAm5	С	C	T	T	A	T	C	T	T	C	T	C	C	na	C	G	C	C Africa
G4GAm8	R	R	T	T	A	0	С	T	T	C	T	C	RC	F	C	G	C	RC Africa
G7GAm8	С	C	T	T	A	T	C	T	T	C	T	C	C	na	C	G	C	C Africa
G1Cm2	С	C	T	T	R.	W.	C	T	G	C	T	C	С	M	C	G	C	С
G2Cm4	С	C	T	T	A	T	C	T	T	C	T	C	C	na	C	G	C	С
G3Cm5	С	C	T	T	G	0	C	T	T	C	T	C	C	na	C	G	C	С
G2Cm6	R	R	T	T	G	0	C	T	T	C	T	C	RC	F	C	G	C	RC
G1Cm7	С	C	T	T	A	T	С	T	T	C	T	C	C	na	C	G	C	C Africa
G3Cm7	С	C	T	T	A	T	C	T	T	C	T	C	C	na	C	G	C	C Africa
G2Cm7	R	R	T	T	A	T	С	T	T	C	T	C	RC	F	C	G	C	RC Africa
G2Wm3	R	R	T	T	A	T	C	T	T	C	T	C	RC	F	C	G	C	RC Africa
G3Wm4	С	C	C	C	G	A	C	T	T	C	C	T	CR	na	T	A	G	CR Africa
G3Wm5	С	C	T	T	A	T	C	T	T	C	T	C	C	na	C	G	C	C Africa
G4Wm5	С	C	T	T	R.	W.	C	T	T	C	T	C	С	M	C	G	C	C Africa
G1Wm5	С	C	T	T	A	A	C	T	T	C	T	C	C	na	C	G	C	C Africa
G5Wm5	R	R	T	T	A	T	С	T	T	C	T	C	RC	F	C	G	С	RC Africa
G2Wm6	С	С	T	T	A	T	C	T	T	C	T	C	С	na	C	G	C	C Africa

G2Wm7	С	С	T	T	A	T	С	T	T	С	T	С	С	na	С	G	С	C Africa
G3Wm7	С	C	T	T	A	T	C	T	T	C	T	C	C	na	C	G	C	C Africa
G1Wm7	С	С	T	T	A	T	С	T	T	С	T	C	C	na	C	G	С	C Africa
G3Wm8	С	C	T	T	A	T	C	T	T	C	T	C	C	na	C	G	C	C Africa
G2Wm8	C	С	T	T	A	T	С	T	T	С	T	C	C	na	C	G	C	C Africa
G4Am1	C	C	T	T	A	0	C	T	T	C	T	C	C	na	C	G	C	C Africa
G3Am5	C	C	T	T	A	T	C	T	T	C	T	C	C	na	C	G	C	C Africa
G3Am6	R	R	T	T	A	T	C	T	T	C	T	C	RC	F	C	G	C	RC Africa
G3Am7	C	С	T	T	A	T	C	T	T	C	T	C	С	na	C	G	C	C Africa
G1Am8	C	C	T	T	A	0	C	T	T	C	T	C	C	na	C	G	C	C Africa
G2BAm1	C	С	T	T	R.	W.	C	T	T	C	T	C	С	M	C	G	C	С
G1BA2	C	C	T	T	A	0	C	T	T	C	T	C	C	na	C	G	C	C Africa
G1BAm3	R	R	T	T	A	T	C	T	T	C	T	C	RC	F	C	G	C	RC Africa
G1BAm4	C	C	T	T	A	T	C	T	T	C	T	C	C	na	C	G	C	C Africa
G3BAm4	C	C	T	T	A	T	T	A	T	C	T	C	C	na	C	G	C	C Africa
G1BAm5	R	R	T	T	A	T	C	T	T	C	T	C	RC	F				RC
G3BAm5	C	C	T	T	R.	W.	C	T	T	C	T	C	C	M	C	G	C	C Africa
G1Vm1	R	R	T	T	A	T	C	T	T	C	T	C	RC	F	C	G	C	RC Africa
G1Vm5	R	R	T	T	A	0	C	T	T	C	T	C	RC	F				RC
G2Vm7		C	T	T	A	T	C	T	T	C	T	C	C	na	C	G	C	C Africa
G1Vm6	С	C	T	T	A	T	С	Y.	T	С	T	C	C	M	C	G	C	C Africa
G2Vm8	С	С	T	T	R.	W.	С	T	T	С	T	С	C	M	C	G	С	C Africa

G2Vm9	С	С	T	T	A	T	С	T	T	С	T	С	С	na	С	G	С	C Africa
G1Em2	С	C	T	T	A	T	C	T	T	C	T	C	C	na	C	G	C	C Africa
G2E2	R	R	T	T	A	T	С	T	T	С	T	С	RC	F	С	G	C	RC Africa
G3Em5	R	R	T	T	A	T	C	T	T	C	T	C	RC	F	C	G	C	RC Africa
G4Em6	С	С	T	T	A	T	C	T	T	C	T	C	С	na	C	G	C	C Africa
G3Em8	С	C	T	T	R.	W.	C	T	T	C	T	C	C	M	C	G	C	C Africa
G2Em3	С	C	T	T	A	0	C	T	T	С	T	С	С	na	C	G	C	C Africa
G1Em4	С	C	T	T	A	T	C	T	T	C	T	C	C	na	C	G	C	C Africa
G1NM1	C	C	T	T	A	T	C	T	T	С	T	С	C	na	C	G	C	C Africa
G1NM2	С	C	T	T	G	A	C	T	T	C	T	C	C	na				C
G1NM3	С	C	T	T	A	T	C	T	T	C	T	C	C	na	C	G	C	C Africa
G3NM3	C	C	C	T	A	0	C	T	T	C	T	C	C	na	T	A	G	C Africa
G5N1	C	C	T	T	A	T	C	T	T	С	T	С	C	na	C	G	C	C Africa
G1N2	С	C	T	T	A	T	C	T	T	C	T	C	C	na				С
G2N2	С	C	T	T	A	0	C	T	T	C	T	C	С	na	C	G	C	C Africa
G4N2	С	C	Y.	Y.	R.	W.	C	T	T	C	T	C	CR	M	C	G	C	С
G2N3	С	C	T	T	A	T	C	T	T	С	T	С	С	na	C	G	C	C Africa
G1N4	С	C	C	C	A	T	T	C	T	C	T	C	CR	F	C	G	C	CR Africa
G2N4	С	C	C	C	G	A	C	T	T	С	T	С	CR	na				CR
G3N4	C	C	Y.	Y.	A	0	C	T	T	C	T	C	C	M	C	G	C	C Africa
G4N4	C	C	T	T	A	T	C	T	T	C	T	C	C	na	C	G	С	C Africa
G1Nm4	C	C	T	T	R.	W.	C	T	T	C	T	C	C	M	С	G	C	C Africa

G4Nm4	С	С	T	T	A	T	С	T	T	С	T	С	С	na	С	G	С	C Africa
G1Nm5	R	R	T	T	R.	W.	C	T	T	C	T	C	RC	M	C	G	C	RC Africa
G1Nm6	C	С	T	T	A	T	C	T	T	C	T	C	С	na	C	G	C	C Africa
G1Nm7	C	C	T	T	G	A	C	T	T	C	T	C	C	na	C	G	C	C Africa
G1Nm8	R	R	T	T	A	T	C	T	T	C	T	C	RC	F	C	G	C	RC Africa
G2Nm8	R	R	T	T	A	T	C	T	T	C	T	C	RC	F	C	G	C	RC Africa
G1UEM1	C	C	T	T	A	T	С	T	T	С	T	C	C	na	C	G	C	C Africa
G2UEM2	C	C	T	T	R.	W.	C	T	T	C	T	C	C	M	C	G	C	C Africa
G1UEm1	C	C	T	T	A	T	C	T	T	C	T	C	C	na	C	G	C	C Africa
G2UEm1	C	C	T	T	A	T	C	T	T	C	T	C	C	na	C	G	C	C Africa
G3UEm1	C	С	T	T	A	T	C	T	T	C	T	C	С	na	C	G	C	C Africa
G2UEm2	С	C	T	T	A	T	C	T	T	C	T	C	C	na	C	G	C	C Africa
G1UEm3	R	R	T	T	A	T	C	T	T	C	T	C	RC	F				RC
G2UEm3	C	C	T	T	G	A	C	T	T	C	T	C	C	na				С
G1UEm4	C	С	T	T	R.	W.	C	T	T	C	T	C	C	M				С
G2UEm5	C	C	T	T	A	T	C	T	T	C	T	C	C	na	C	G	C	C Africa
G3UEm5	C	С	T	T	A	T	C	T	T	C	T	C	C	na	C	G	C	C Africa
G1UWM1	C		T	T	A	T	C	T	T	C	T	C	C	na	C	G	C	C Africa
G2UWm1	C	C	T	T	A	T	С	T	T	С	T	C	C	na	C	G	C	C Africa
G3UWm1	С	C	T	T	R.	W.	C	T	T	C	T	C	C	M	C	G	C	C Africa
G1UW2	R	R	T	T	R.	W.	C	T	T	C	T	C	RC	M	C	G	C	RC Africa
G2UW2	С	C	T	T	A	T	С	T	T	С	T	С	C	na	C	G	С	C Africa

G3UW2	С	C	T	T	A	T	C	T	T	С	T	С	C	na	C	G	C	C Africa
G4UW2	С	C	T	T	R.	W.	C	T	T	C	T	C	C	M	C	G	C	C Africa
G5UW2	С	C	T	T	A	T	C	T	T	С	T	C	C	na	С	G	C	C Africa
G2UWm2	R	R	T	T	A	A	A	T	T	C	T	C	RC	M	C	G	C	RC Africa
G1UWm4	R	R	T	T	R.	W.	C	T	T	C	T	C	RC	M	C	G	C	RC Africa
G1UWm5	R	R	T	T	A	T	C	T	T	C	T	C	RC	F	C	G	C	RC Africa
G1UWm6	С	С	T	T	R.	W.	C	T	T	C	T	C	C	M	C	G	C	C Africa
G2UWm6	С	C	T	T	A	T	C	T	T	C	T	C	C	na	C	G	C	C Africa