UNIVERSITY OF CAPE COAST

BIOLOGY AND SUSCEPTIBILITY OF *Spodoptera frugiperda* (J.E. SMITH) (FALL ARMYWORM) TO SELECTED INSECTICIDES

PHYLLIS AKWAA-SEKYI

2021

UNIVERSITY OF CAPE COAST

BIOLOGY AND SUSCEPTIBILITY OF *Spodoptera frugiperda* (FALL ARMYWORM) TO SELECTED INSECTICIDES

BY

PHYLLIS AKWAA-SEKYI

Thesis submitted to the Department of Conservation Biology and Entomology of the School of Biological Sciences, College of Agriculture and Natural Sciences, University of Cape Coast in partial fulfilment of the requirements for the award of Master of Philosophy degree in Entomology

JULY 2021

DECLARATION

Candidate's Declaration

I hereby declare that this thesis is the result of my original research and that no part of it has been presented for another degree in this university or elsewhere.

Candidate's Signature: …………………………   Date …………………….....

Name: Phyllis Akwaa-Sekyi

Supervisor's Declaration

We hereby declare that the preparation and presentation of the thesis were supervised in accordance with the guidelines for supervision of thesis laid down by the University of Cape Coast.

Principal Supervisor's Signature: ………………...   Date …………………….....

Name: Prof. Benjamin A. Mensah

Co-Supervisor's Signature: ………………………...Date …………………….....

Name: Dr. John Abraham

ABSTRACT

Environmental factors and the use of insecticides form a major component in the management of insects of agricultural importance. The development of fall armyworm, *Spodoptera frugiperda*, an invasive insect pest in Ghana was studied. The efficacy of six selected insecticides against them was also determined. Larvae of fall armyworm were collected from the field at three locations in the Central Region of Ghana and reared at the Entomology laboratory of the University of Cape Coast. The duration of events during the life cycle, including egg hatching, larval moulting, pupation and adult lifespan were studied at two periods at different temperatures and humidity regimes. Five insecticides recommended by the Ministry of Food and Agriculture for the control of fall armyworm in Ghana were assayed for their efficacy against the eggs and second instar larvae of the fall armyworm in a completely randomized design. Aqueous neem extract was also assessed for its potency against the second instar larvae. Study results indicated that, rate of development was faster from February to March when higher range of temperatures were recorded and there were significant differences between the average duration of all the events of the life cycle studied. None of the commercial insecticides assessed prevented treated eggs from hatching. In the larval bioassay, Emamectin benzoate and *Bacillus thuringiensis* caused 100% mortality 24 hours after larvae fed on treated leaves. Indoxacarb+Acetamiprid caused 100% mortality 48 hours after application while Acetamiprid+Lambda cyhalothrin and Imidacloprid could not cause more than 85% mortality even at twice the recommended doses. Three concentrations of the aqueous neem extract caused 100% mortality by the 8th day after larvae were fed on leaves treated with these concentrations.

KEY WORDS

Spodoptera frugiperda

Development of *Spodoptera frugiperda*

Egg susceptibility

Larval susceptibility

Efficacy of synthetic insecticides

Aqueous neem extract

ACKNOWLEDGEMENTS

I am most grateful to the Almighty God for strength and grace to complete this work. I duly appreciate my family, the Akwaa-Sekyi Family, without whose financial support this work would not have come to fruition.

I am appreciative to my Principal Supervisor, Prof. Benjamin A. Mensah and my Co-Supervisor, Dr. John Abraham whose critical review, constructive criticisms and encouragement have made this thesis worth it. I acknowledge the effort of Dr. Rofela Combey in identification of some insect species that came up in the course of the data collection for this thesis. I am grateful to all the lecturers of the Department of Conservation Biology and Entomology for their criticisms and input that made this thesis better.

Special thanks go to my friends, Eugenia Amador, Godwin Deku, Justina Ekuwa Annan, Ebenezer Smith Forson, Barbara Adu-Boadu, Anita Edith Wilson, Vivian Frimpomaa Asamoah, Thomas Oppong, and Smyrna Ofori-Quaye for their encouragement and help during the challenging period of data collection and putting together this thesis. I say a very big thank you to my brother, Dr. Ellis Akwaa-Sekyi for his relentless efforts in ensuring that this thesis was completed.

Finally, I sincerely appreciate the efforts of Mr. George Ofori; the MOFA Director of Bibiani Anhwiaso Bekwai Municipal, Mr. Nyantakyi, Mr. Aidoo of PPRSD - Twifo Praso, Mr. Atta Mensah of Agricultural Extension Services; Cape Coast Metropolitan Assembly, Mr Mensah and Mr. Tahir of the University of Cape Coast Science Botanical Garden and all the farmers whose assistance made the data collection successful, I am most grateful.

DEDICATION

This work is dedicated to my brother, Mr. Solomon Akwaa-Sekyi.

TABLE OF CONTENTS

	Page
DECLARATION	ii
ABSTRACT	iii
KEY WORDS	iv
ACKNOWLEDGEMENTS	v
DEDICATION	vi
TABLE OF CONTENTS	vii
LIST OF TABLES	x
LIST OF FIGURES	xi
LIST OF ACRONYMS	xiii
CHAPTER ONE	1
INTRODUCTION	1
Background to the Study	1
Insecticides	6
Emamectin benzoate	6
Acetamiprid + Indoxacarb	7
Imidacloprid	7
Bacillus thuringiensis	8
Lambda-cyhalothrin + Acetamiprid	8
Aqueous Azadirachta indica (neem) extract	9
Statement of the Problem	10
Objectives of the Study	11
Hypotheses	11
Significance of the Study	11

Delimitations of the Study	13
Limitations of the Study	13
Organization of the Study	13
CHAPTER TWO	15
LITERATURE REVIEW	15
Biology of *Spodoptera frugiperda*	15
Taxonomy of *Spodoptera frugiperda*	15
Origin and distribution of *Spodoptera frugiperda*	15
Lifecycle of *Spodoptera frugiperda*	16
Behaviour and development of *Spodoptera frugiperda* on different host plants	20
Management Methods for *Spodoptera frugiperda*	22
Monitoring for Fall Armyworm Population	24
Biological Control of the Fall Armyworm	25
Cultural control of *Spodoptera frugiperda*	27
Chemical control of *Spodoptera frugiperda*	28
Synthetic insecticides	29
Botanical insecticides	31
CHAPTER THREE	34
MATERIALS AND METHODS	34
Maintenance of *Spodoptera frugiperda* Colony	34
Studying the growth and development of *Spodoptera frugiperda*	36
Laboratory Bioassays	37
Egg susceptibility test	38
Larval susceptibility test with synthetic insecticides	39

Larval susceptibility test with *Azadirachta indica* extract 40

Statistical Analyses 41

CHAPTER FOUR 42

RESULTS AND DISCUSSION 42

Development of *Spodoptera frugiperda*. 42

Morphometric and behavioural study of the life stages of *Spodoptera frugiperda* 44

Susceptibility of the life stages of *Spodopera frugiperda* to insecticides 50

Egg susceptibility 50

Larval Susceptibility 51

Aqueous Neem Extract Bioassay 53

Discussion 56

Development of the Fall Armyworm (*Spodoptera frugiperda*) 56

Susceptibility tests 60

Effect of aqueous neem extract on the larvae of *Spodoptera frugiperda* 67

CHAPTER FIVE 70

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS 70

Summary 70

Conclusions 72

Recommendations 72

REFERENCES 75

APPENDICES 91

LIST OF TABLES

Table		Page
1	Active ingredients in the Insecticides used for susceptibility tests against fall armyworm	38
2	Mean development period (days) ± S.E (range) of *Spodoptera frugiperda* studied at two periods with different temperature and relative humidity ranges	42
3	Mean ± S.E (range) periods for *Spodoptera frugiperda* larval instars studied at two periods with different temperature and relative humidity ranges	43
4	Lifespan (days) ± S.E (range) of male and female *Spodoptera frugiperda* moths studied at two periods with two different temperature and relative humidity ranges	44
5	Mean ± S.E (range) body lengths and head widths (mm) of the larval instars of *Spodoptera frugiperda*	46
6	Mean ± S.E (range) number of *Spodoptera frugiperda* larvae that emerged after eggs were sprayed with recommended doses of four insecticides	51
7	Percentage (%) Mean mortality ± S.E of second instar larvae of *Spodoptera frugiperda* exposed to twice the recommended dose of two of the five selected insecticides for 24-72 hours	53
8	% Mean mortalities caused by different concentrations of aqueous *Azadirachta indica* (Neem) extract on second instar larvae of *Spodoptera frugiperda*	55

LIST OF FIGURES

Figure		Page
1	Feeding damage caused by *Spodoptera frugiperda* to the leaves (A), stalk (B) and kernels (C) of maize plant	10
2	Sampling *Spodoptera frugiperda* from the field.	34
3	Larval cages containing maize leaves and larvae.	35
4	Larval cages being cleaned (A) and stocked with maize leaves (B).	36
5	Eggs of *Spodoptera frugiperda* covered with fibre (A), uncovered (B), laid on filter paper (C) or on frames of cage (D).	45
6	*Spodoptera frugiperda* larvae showing yellow stripes (A), inverted 'Y' (B) and four dark spots (C).	46
7	Freshly hatched neonates of *Spodoptera frugiperda* (A), neonates dispersing after hatching (B), leaf mining by neonates (C)	47
8	Head capsule of sixth instar larva of *Spodoptera frugiperda* showing well-developed mandibles.	47
9	Fourth instar larva of *Spodoptera frugiperda* feeding on another larva of the same species.	48
10	Larvae of *Spodoptera frugiperda* about to pupate.	48
11	Fresh pupa (A), pupa after tanning (B), pupa prior to eclosion (C).	49
12	*Apocephalus borealis* (A), pupa of *Spodoptera frugiperda* being consumed by larvae *Apocephalus borealis* (B).	50
13	Adult moths: Female (left), Male (right).	50

14 % Mean mortality of second instar larvae of *Spodoptera frugiperda* exposed to recommended doses of five insecticides at 24, 48 & 72 hours 52

15 Larvae unable to completely shed off their exuviae after ecdysis. 56

LIST OF ACRONYMS

CABI	Centre for Agriculture and Bioscience International
FAO	Food and Agriculture Organization
FAW	Fall armyworm
MoFA	Ministry of Food and Agriculture
PPRSD	Plant Protection and Regulatory Services Directorate

CHAPTER ONE

INTRODUCTION

The fall armyworm (FAW), *Spodoptera frugiperda* (Smith, 1797) (Lepidoptera: Noctuidae) is an exotic pest to the African continent. It was first reported in Ghana in April 2016 and by March, 2017, it had invaded all the 16 regions of Ghana, causing massive destruction to over 14,000 hectares of maize and sorghum farms worth $163 million all over the country (Godwin, Hevi & Day, 2017). The presence of this pest in Ghana poses a threat to food security and availability. Financial loss is incurred through reduced yield and attempts at managing the pest with chemicals. It is necessary to manage the pest to prevent further damage to several crops, considering the polyphagous nature of the FAW. There is inadequate knowledge about the growth and development of the pest with respect to the weather conditions in Ghana and insecticides to which the fall armyworm is susceptible. In order to implement successful management strategies, there is the need for the biology of the FAW to be studied in Ghana and insecticides screened to ascertain their efficacy for the management of the population of this notorious insect pest. This study will contribute to the knowledge needed to formulate strategies to combat this insect pest.

Background to the Study

The increase in human population in Ghana and the world at large makes food security an issue of importance. The quest to feed the populace has shifted the world's focus towards agriculture since it is the main activity that ensures constant supply of food as well as cause increase in economic growth (FAO 2015). In Ghana, this quest has led to the initiation of "Planting for Food and Jobs" in which the youth are brought on board to cultivate large hectares of farmlands to produce enough food to feed the people while providing an income for those involved in the cultivation. Among the main food crops cultivated in this programme, are maize, sorghum and rice which are staple food for most Ghanaians. According to the Food and Agriculture Organisation, these crops comprise about 60% of the total food calorie intake worldwide (FAO, 2017), which makes it obvious that investing in agriculture is a step in the right direction. That notwithstanding, the problem of pest outbreak is an inevitable occurrence in agriculture, and Ghana, Africa and the world at large have not been spared from this (Dent, 2000). These crops suffer major attacks from pre-harvest pests like the fall armyworm (*Spodoptera frugiperda*), the African armyworm (*Spodoptera exempta*), the maize stalk borer (*Busseola fusca*), pink borer (*Chilo partellus*) and post-harvest pests like the larger grain borer (*Prostephanus truncatus*) and the lesser grain borer (*Rhyzopertha dominica*) (Bosque-Pérez, 1995). These insect pests cause damage to host plants, usually at levels that reduce yield or in severe cases result in complete loss of yield (Peairs & Saunders, 1979). In order prevent such losses and improve yield, pest populations are managed so they do not reach economic injury level.

Africa was hit by the sudden appearance of the fall armyworm in January 2016 and by April 2016, Ghana had recorded cases of the pest's attack on maize plants (Godwin *et al*., 2017). The fall armyworm, *S. frugiperda*, is a pest that is native to the Americas (Capinera, 2002). It is the most important pest of maize in Mexico, Central America, South America and the Pacific Basin (Mitchell, Waddill, & Ashley, 1984; Ashley, Wiseman, Davis, &

Andrews, 1989). In Mexico, *S. frugiperda* is present in all of the regions where maize is grown, causing severe damage (Sifuentes, 1967; Blanco *et al*., 2014). Heavy outbreaks of this pest cost the American continent over $300 million annually, resulting from losses and the cost of control (Gross & Pair, 1986). Two strains have been identified according to their host preference: a rice-associated strain that feeds primarily on forage grasses and rice, and a corn-associated strain that feeds primarily on corn (Pashley, 1986). The fall armyworm had not previously been established outside the Americas but its two strains appeared in Africa and are rapidly spreading throughout the continent. Research to date suggests that both strains entered Africa as stowaways on commercial aircraft, either in cargo containers or airplane holds, before subsequent widespread dispersal by the wind (Goergen, Kumar, Sankung, Togola, & Tamo, 2016). Currently, it is present in all countries of sub-Saharan Africa except Lesotho. By the end of July 2018, *S. frugiperda* was detected in Yemen and in India: the first occurrence in Asia and as at January 2019, it was reported in Bangladesh, Sri Lanka and Thailand. In May 2019, Nepal confirmed its presence and as at June 2019, it had been reported in Myanmar, China, Indonesia, Laos, Malaysia and Vietnam and the Republic of Korea. Japan reported the presence of FAW in July, 2019 (FAO, 2019). The FAW now threatens Africa and Asia and is obviously established in Africa. This is a widespread invasion over a short period of time and calls for robust control measures to be implemented to battle this notorious pest as its presence poses a threat to food security and will also lead to financial loss to the continent in a pursuit to control it. This makes this research work imperative.

Spodoptera frugiperda has a wide range of host plants, about 80 species of plants, but has high preference for grasses (Barros, Torres, Ruberson, & Oliveira, 2010; Silva *et al.*, 2015). Feeding damage is also observed on other major agricultural crops such as cowpea, groundnut, potato, soybean, cotton and vegetables. Due to its polyphagous nature, it is expected that its accidental introduction in the African continent will constitute a lasting threat to several important crops. In Ghana, the main crop attacked is maize. The damage caused by this pest is enormous and its effect can be seen at the household and national levels and can also affect international trade in affected crops. At the household level, farmers may spend extra money and labour to control the pest; should their efforts fail it could result in diminished cultivation of these crops. At the international level, recent estimates show that in the absence of control measures, FAW causes annual loss of about 21-53% of the total maize production in twelve countries in sub-Saharan Africa (Day *et al.*, 2017). This percentage is estimated to be 8.3 to 20.6 million tonnes of maize, equivalent to US $2481-6187 million of total expected value of US$ 11,590.5 m per year (Day *et al.*, 2017). International trading will have its own share of the effects of the FAW invasion as countries that have not yet recorded the presence of the pest could be infested through importation of products from affected countries.

In view of this, the Ministry of Food and Agriculture (MoFA) in Ghana has beckoned research institutions, universities and other relevant stakeholders to contribute their quota to help control the populations of this devastating pest, making this research vital.

The outbreak of the FAW in Ghana was so sudden that the use of synthetic insecticides was the immediate remedy for the menace, while the ecology of the pest in the ecosystem is studied and biological and other control options are assessed. In other parts of the world where the pest migrated from, synthetic insecticides are used mainly as control because of the pest's ability to migrate long distances and feed on a broad host range (Straub & Hogan, 1974). Although synthetic insecticides can provide effective control of crop pests including *S. frugiperda* (Young, 1979), the pest has developed resistance to major classes of insecticides in several locations (Pitre, 1988; Yu, 1991; Berta *et al*., 2000). For instance, in Central, South and South-Eastern America, resistance to major classes of insecticides such as pyrethroids, carbamates and organophosphate has been reported (Day *et al*., 2017).

That is the case with places outside Africa and as a result, measures have to be put in place to delay the onset of insecticide resistance in the fall armyworm, in Ghana. There is the need to use insecticides with different modes of action alternatively and discriminately, as well as practise integrated pest management so that the onset of resistance in the pest population can be delayed. In view of this the Plant Protection and Regulatory Services Directorate (PPRSD) of MoFA has recommended eleven insecticides to be used by farmers in Ghana to control the populations of the fall armyworm. The susceptibility of the FAW to these insecticides has to be ascertained through research so that informed recommendations about these insecticides could be made to farmers and other stakeholders.

Since the pest is novel to the African continent and specifically to Ghana, little is known in literature concerning the biology and ecology of the pest and its susceptibility to insecticides in Ghana. This research work therefore focused on studying the biology of the FAW and evaluating the susceptibility of the eggs and second instar larvae to five synthetic insecticides (out of which four are among the eleven recommended insecticides by PPRSD), and one plant extract.

The active ingredients in the five synthetic insecticides are Emamectin Benzoate, *Bacillus thuringiensis* (*Bt*), Acetamiprid + Indoxacarb, Lambda-cyhalothrin + Acetamiprid, and Imidacloprid; the active ingredient of the plant extract is azadirachtin.

Insecticides

The selection of the various insecticides for this study was done based on the behaviour of the FAW. Insecticides that had contact and systemic toxicity as well as single and broad spectrum attack on targeted pests were selected. Early instar larvae of FAW are vulnerable and can easily be reached when insecticides that work via contact are sprayed while late instar larvae have the ability to hide in developing whorl of the leaves and so are usually shielded from the reach of insecticides; hence the selection of systemic insecticides.

Emamectin benzoate

Emamectin benzoate belongs to the class of insecticides called avermectins. It works on the nerves and muscles as glutamate-gated chloride channel allosteric modulator to cause paralysis of targeted insects (IRAC, 2017). It has a natural origin and was isolated from *Streptomyces avermitili*, a naturally occurring soil bacterium. This insecticide is non-systemic and works as a contact insecticide.

Acetamiprid + Indoxacarb

This insecticide is made up of a combination of two active ingredients from two classes of insecticides; a neonicotinoid (Acetamiprid) and a carbamate (Indoxacarb). Neonicotinoids act on nicotinic receptors of insects and are less toxic to mammals and other non-targeted organisms due to their specificity (Xu *et al*., 2010). They do not readily pass the blood-brain barrier, further reducing the potential for mammalian toxicity. When administered orally, they are rapidly absorbed, metabolized primarily in the liver and excreted primarily in urine. They do not accumulate in the body, and are neither carcinogenic, mutagenic, teratogenic nor a reproductive toxicant (Xu *et al*., 2010).

This insecticide is indicated to be effective against caterpillars of lepidopteran pests. It works as a systemic insecticide, with both Acetamiprid and Indoxacarb acting on the nerves of the target insect as a nicotinic acetylcholine receptor modulator and an acetylcholinesterase inhibitor respectively (IRAC, 2017).

Imidacloprid

Imidacloprid is a neonicotinoid, which is a class of neuro-active insecticides modelled after nicotine; and works as a systemic insecticide. Imidacloprid has a high margin of safety due to the high insecticidal specificity and low mammalian toxicity (Xu *et al*., 2010). Even though not one of the insecticides recommended by the PPRSD, it was selected to compare its efficacy with the other insecticides that have two active ingredients. Due to its low toxicity to mammals and high toxicity to insects, this insecticide was selected to ascertain its potency against the FAW so that if found to be effective, it could be added to what has been recommended already.

Bacillus thuringiensis

Bacillus thuringiensis (*Bt*) is a naturally-occurring, soil-borne bacterium that is used for insect control. It is a biopesticide made up of a spore that makes it persistent, and a protein crystal within the spore, which is toxic (Castro *et al*., 2019). The toxic protein differs depending on the subspecies of *Bt* used to produce the insecticide, yielding a variance of *Bt* toxic to different insect species and this makes it target-specific. *Bacillus thuringiensis* works as a microbial disruptor of the epithelial membrane of the midgut of targeted insects. When the bacterium is consumed by a particular insect, the toxic crystal is released to attack the insect's midgut epithelial cells which leads to disorganization of the cells, degeneration of microvilli and destruction of the peritrophic membrane, which protects the pest's stomach from its own digestive enzymes. The stomach is penetrated, and the insect dies by poisoning from the stomach contents and the spores themselves (Planet Natural Research Center, 2004; Castro *et al*., 2019).

Lambda-cyhalothrin + Acetamiprid

This insecticide is a combination of a pyrethroid (Lambda-cyhalothrin) and a neonicotinoid (Acetamiprid). The combination of the two active ingredients confers on the insecticide a strong knock-down effect against caterpillars. Lambda-cyhalothrin is a broad-spectrum insecticide that has a low potential to contaminate ground water due to its ability to bind to the soil as well as its poor solubility in water. It targets the nerves as a sodium channel modulator while Acetamiprid provides a systemic action to plants (Leistra *et al*., 2004; IRAC, 2017).

Aqueous Azadirachta indica (neem) extract

Neem, *Azadirachta indica,* is a plant widely known for its pesticidal ability. Substances with pesticidal properties are found in all parts of the neem tree, but the greatest concentrations are found in the seed. The leaves are also known to contain some of these chemicals with potency to kill pests. The active ingredient in neem is azadirachtin which consists of more than 25 different but closely related compounds. It works as a feeding deterrent by blocking the ability of the pest to swallow and at the same time interrupts the release of ecdysteroid hormones which prevents moulting; the pest eventually dies. Neem is also known to disrupt the development of eggs and pupae of insects it is used against. Adults are not killed by the growth-regulating properties of azadirachtin but mating and sexual communication may be disrupted, which results in reduced fecundity (National Research Council, 1992). Again, neem extract is not persistent in the environment but is easily degraded by ultraviolet light and rainfall. It has low toxicity to mammals and other non-targeted beneficial organisms as compared with other chemical pesticides (Stone, 1992; Quarles, 1994).

Neem plant was selected because it is readily available in Ghana and the aqueous extract can be easily prepared by farmers.

Finally, neem extract is compatible with Integrated Pest Management practices and remains a pesticide to which resistance development would be difficult if not completely impossible due to its diverse modes of action.

Statement of the Problem

In Ghana, the fall armyworm has invaded and destroyed vast areas of maize farms which poses a threat to food security and availability. It mainly attacks maize and feeds on the leaves, buds, whorls and ears (Figure 1).

Figure 1: Feeding damage caused by *Spodoptera frugiperda* to the leaves (A), stalk (B) and kernels (C) of maize plant

The Centre for Agriculture and Bioscience International (CABI) in a report commissioned by the Department for International Development, UK, have estimated that, the pest could affect up to 500,000 tonnes of maize and sorghum in Ghana, potentially costing the country up to $163 million in 2017 (Godwin *et al*., 2017). If these huge losses are not controlled, they can lead to food shortages and eventually famine, considering how Ghanaians rely on maize and rice for food.

There is inadequate literature on the growth and development of the FAW with respect to the weather conditions in Ghana. Therefore, there is the need to study the biology of *S. frugiperda* in Ghana in order to come up with effective control methods that could be included in integrated pest management programmes.

Moreover, the most effective insecticides against the FAW in Ghana are also not known. The extent of the problem has triggered indiscriminate use of insecticides by farmers that may lead to resistance development in the insects. Hence it was relevant to find out the efficacy of the insecticides recommended by the PPRSD of the Ministry of Food and Agriculture.

Objectives of the Study

The main goal of this research was to study the development of *Spodoptera frugiperda* and to ascertain the efficacy of selected insecticides on the life stages.

Specifically, the study sought to:

1. determine the duration of development of *S. frugiperda* at two periods with different temperature and relative humidity ranges.
2. assess the susceptibility of the eggs and second instar larvae of *S. frugiperda* to selected insecticides.

Hypotheses

1. Temperature and relative humidity do not affect the development of *Spodoptera frugiperda.*
2. The eggs and second instar larvae of *S. frugiperda* are not susceptible to the selected insecticides.

Significance of the Study

This study was imperative as its successful completion would make available reliable information about the growth and development of the fall armyworm with respect to the weather conditions in Ghana. In the study of a pest's biology, distribution and abundance of the pest species is greatly influenced by the relationship between temperature and the rate of development of the pest (Tobin, Nagarkatti & Saunders, 2003). The development of insects occurs within specific temperature and relative humidity ranges that affect the duration of the lifecycle, the rate of development and the survival of the insects (Howe, 1967). Temperatures fluctuate in natural environments and affect insect population dynamics differently from conditions where insects are only exposed to constant temperatures. Insects develop faster under fluctuating temperatures when the maximum and minimum temperatures are within their optimal range of development (Hagstrum & Hagstrum, 1970). In view of this, conducting this study under uncontrolled temperature would provide an outcome that would not be so far from a field outcome. Consequently, it was essential that the effects of temperature and humidity; on the development of *S. frugiperda* under the current changing climatic conditions were known so that predictions would be made and efficient management strategies deployed to manage the population of the pest.

Moreover, studying the biology of the pest would also make known any parasitoids and predators associated with the FAW as field sampling of the larvae was done. The parasitoids and predators ascertained could be conserved or augmented for biological control of the FAW.

Again, the efficacy of the insecticides recommended by the MoFA would be determined so that the use of the ineffective ones among the recommended insecticides would be stopped.

Finally, information about which stage of the lifecycle (egg or larva) of *S. frugiperda* is more susceptible to insecticides would be ascertained and targeted accordingly. Finding the efficacy of the insecticides on the eggs would determine whether or not the presence of eggs of FAW in an infested field could possibly cause a reappearance of the pest a few days after the field has been sprayed with insecticides.

Delimitations of the Study

The study covers extensively, the biology of the FAW under the following synopsis: duration for egg hatching, number of larval instars, duration for larval development, duration from pupal development to adult and adult male and female lifespan. Some selected insecticides have also been evaluated to ascertain the susceptibility of the egg and larvae of the FAW to these insecticides. However, aspects like the mating behaviour of the FAW adults, female oviposition preference on the host plant under consideration (maize plant) and quantity of egg masses laid by a single female in its lifetime could not be studied.

Limitations of the Study

The laboratory where the study was done lacks a humidifier and an insectary with controlled temperature and photoperiod, so the temperature, humidity and photoperiod could not be controlled and this affected the continual survival and maintenance of the subsequent generations of the FAW larvae in the laboratory. That notwithstanding, the quality of this research work was not compromised, an improvised insect-rearing area was prepared to raise the insect colony and the susceptibility tests were carried out successfully.

Organization of the Study

This study has been structured into five chapters with each chapter having specific sub-topics that are well discussed. Chapter one provides a general introduction to the research which includes: background to the study, statement of the problem, significance of the study, general and specific objectives, hypotheses, limitations and delimitations of the study. Chapter two consists of a review of related and relevant literature, considering the theoretical framework of the study. Hence sufficient literature regarding this research were sought for and carefully reviewed. Chapter three describes the study design and materials and methods employed for data collection. Chapter four highlights and discusses the results of the study in accordance with the objectives. Major findings of the study in relation to relevant literature that were reviewed in chapter two are also discussed. Finally, chapter five presents the summary, conclusions and recommendations made based on the findings of the study.

CHAPTER TWO

LITERATURE REVIEW

In this chapter, the works of other authors related to this study were reviewed. Literature concerning the biology of *Spodoptera frugiperda* and its susceptibility to the insecticides assessed in this study or other insecticides belonging to the same classes of insecticides as the ones assessed in this study was reviewed.

Biology of *Spodoptera frugiperda*

Taxonomy of *Spodoptera frugiperda*

The fall armyworm, *Spodoptera frugiperda*, belongs to: Kingdom Animalia; Subkingdom Bilateria; Infrakingdom Protostomia; Superphylum Ecdysozoa; Phylum Arthropoda; Subphylum Hexapoda; Class Insecta; Subclass Pterygota; Infraclass Neoptera; Superorder Holometabola; Order Lepidoptera; Superfamily Noctuoidea; Family Noctuidae; Subfamily Noctuinae; Tribe Prodeniini; Genus Spodoptera; Species *Spodoptera frugiperda* (Integrated Taxonomic Information System, 2020).

Origin and distribution of *Spodoptera frugiperda*

The fall armyworm, *S. frugiperda*, is a pest that is native to the Americas (Capinera, 2002). It is the most important maize pest in Mexico, Central America, South America and the Pacific Basin (Mitchell *et al*., 1984; Ashley *et al*., 1989). In Mexico, *S. frugiperda* is present in all of the regions where maize is grown, causing severe damage (Sifuentes, 1967; Blanco *et al*., 2014).

In January 2016, it was reported for the first time in Africa and research suggests that two strains (rice and corn strains) entered Africa as stowaways on commercial aircraft, either in cargo containers or airplane holds, before subsequent widespread dispersal by the wind (Goergen *et al.*, 2016). Currently, it is present in all countries of sub-Saharan Africa except Lesotho. In July 2018, the fall armyworm was detected in Yemen and in India: the first occurrence in Asia and as at January 2019, it was reported in Bangladesh, Sri Lanka and Thailand. Now, it is present in Nepal, Myanmar, China, Indonesia, Laos, Malaysia, Vietnam, Japan and the Republic of Korea (FAO, 2019). The FAW is now present in Africa and Asia.

Lifecycle of *Spodoptera frugiperda*

The fall armyworm, has a lifecycle consisting of egg, larva, pupa, and adult just like all other insects that undergo complete metamorphosis. Generally, temperature affects its development. The length of time for hatching of eggs to pupation varies based on temperature and other environmental conditions (Luginbill 1928; Hogg *et al.*, 1982). The thermal optimum of an insect is the temperature at which it develops, reproduces and survives. When temperatures are lower or higher than the optimum temperature of an insect species, it leads to a decrease in its rate of development (Begon, Townsend & Harper, 2006). Temperature influences egg hatching time, duration for larval moulting and the life-span of the adult male and female insects (Aguilon & Velasco, 2015). When the developmental rate of an insect is faster, it can be beneficial to the insect since it results in less time spent in susceptible stages during which it can be attacked by predators, parasitoids and entomopathogens (Du Plessis, Schlemmer & Van den Berg, 2020). There may be several generations of *S. frugiperda* occurring within a year and because it does not undergo diapause in its development, the appearance of dispersing adults determines the number of generations that may occur in a particular area (Luginbill 1928).

The adult is a strong flier, able to fly up to 100 km per night and disperse long distances annually during the summer months. In Minnesota and New York where fall armyworm moths do not appear from January to July in a year, there may be a single generation. In Kansas there may be one or two generations, there may be three generations in South Carolina but four in Louisiana. In coastal areas of north Florida, moths are abundant from April to December (Capinera, 2002).

In areas of the southern United States, six or more generations per year may occur in summer (Luginbill, 1928).

Completion of the lifecycle usually takes about four weeks in Southern cotton production regions, but can take as long as twelve weeks during periods of low temperatures in the spring and fall (Hardke, Lorenz III, & Leonard, 2015). Barfield, Mitchell, and Poeb (1978) reported that mean developmental periods for *S. frugiperda* from egg to adult ranged from 66.6 days (15.6°C) to 18.4 days (35.0°C) and Sparks (1979) reported a period of 30 days at 25^{o}C. In a recent laboratory work done by Du Plessis *et al*. (2020), the development of *S. frugiperda* from egg to adult was favourable within 18-32^{o}C; the development from egg to adult was observed to be 71.44 days at 18^{o}C and 20.27 days at 32^{o}C while Basuto *et al*. (2005), reported a period of 77.3 days at 18^{o}C and 21.0 days at 32^{o}C. In a study by Silva *et al.* (2017) in Cameroon, the number of days taken for *S. frugiperda* larvae to develop to adults was 21.41 days at temperature 25°C ± 2, 70 ± 10% relative humidity and photoperiod 14:10 (L:D).

The egg of the fall armyworm is dome-shaped; pointed at the apex with the base flattened. It measures about 0.4 mm in diameter and 0.3 mm in height (Capinera, 2002). Du Plessis *et al*. (2020) observed that at a lower temperature of 18^{o}C and a higher temperature of 32^{o}C, eggs hatched at a mean of 6.38 and 2.00 days, respectively. They opined that though eggs were viable at 18^{o}C through to 32^{o}C, the rate of egg development was faster and similar at 30 and 32^{o}C. However, Ali, Luttrell and Schneider (1990) reported a much broader favourable range of egg development at temperature range of 17-38^{o}C.

There are usually six larval instars in fall armyworm, with the head capsules for each moult measuring 0.34, 0.48, 0.81, 1.22 and 1.96 mm, respectively for the five moults (Sharnbasappa, Kalleshwaraswamy, Maruthi, & Pavithra, 2018). However, in a work published by Capinera, (2002), head capsule widths were found to be 0.35, 0.45, 0.75, 1.3, 2.0 and 2.6 mm for instars 1-6, respectively. Larvae attain lengths of about 1.7, 3.5, 6.4, 10.0, 17.2 and 34.2 mm, respectively, during these instars. The head capsule values obtained by the two authors mentioned above do not differ much and the difference could possibly be due to the variation in the quantity and the type of food the larvae were fed with. Again, Sharnbasappa *et al*. (2018) only measured the moulted head capsule hence had five values since there are five moults in a six-instar larval development. Capinera (2002) on the other hand probably measured the head capsules of the larvae while the larvae were in their respective larval stages but not the moulted head capsules thereby reporting six values for head capsule width.

Young larvae are greenish with a black head, which turns orange in the second instar. In the third instar, the dorsal surface of the body becomes brownish, and lateral white lines begin to form. 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. The dorsal part of the body bears dark spots and spines and the face of the mature larva is also marked with a white inverted "Y".

The average development time taken for a larva to go through the 6 instar stages was determined to be 3.3, 1.7, 1.5, 1.5, 2.0 and 3.7 days for instars 1 to 6, respectively, when larvae were reared at 25ºC (Pitre & Hogg, 1983). The larval stage lasts for 14 days in summer while it lasts for 30 days in winter (Luginbill, 1928). When larvae were reared at 26ºC, 75 - 80% relative humidity; larval period lasted for 15.9 days with L_1-L_6 stages taking 2.6, 2.2, 2.0, 2.0, 2.4 and 4.5 days, respectively (Sharnbasappa *et al*., 2018).

Pupation normally takes place in the soil at a depth of 2 to 8 cm. The larva constructs a loose cocoon, oval in shape and 20 to 30 mm in length. Pupal duration lasts about 8-9 days in summer but up to 20-30 days in winter in Florida (Pitre & Hogg 1983). Silva *et al.* (2017) in Cameroon, reported that pupation occurred in 8.5 days at temperature 25°C ± 2, 70 ± 10% relative humidity and photoperiod 14:10 (L:D). In India, Sharnbasappa *et al*. (2018) ascertained a pupal period of 10.5 days at 26 ºC and 75 - 80% relative humidity.

The adult moths have a wingspan of 32 to 40 mm, the hind wing being whitish with a narrow dark border. The forewing of both the male and the female is brown in colour but that of the male has white spots while the female lacks those spots. Adults are nocturnal and have a lifespan of about 10 days (Capinera, 2002). According to Sharnbasappa *et al*. (2018), adult males have a lifespan of 7 - 9 days while adult females have a lifespan of 9 - 12 days. Oviposition lasts for 2 - 3 days during which the adult female lays about 835 - 1169 eggs and the female also deposits a layer of grey-like scales in-between and over the egg mass, giving it a furry appearance (Sharnbasappa *et al*., 2018). Usually, the number of eggs per mass varies but is often 100 to 200, and total egg production per female is about 1500 with a maximum of over 2000 (Prasanna, Huesing, Eddy & Peschke, 2018). Though the number of eggs laid by the female moth as reported by Sharnbasappa *et al*. (2018) and Prasanna *et al*. (2018) differ, they both depict a high fecundity in this species.

Behaviour and development of *Spodoptera frugiperda* on different host plants

The fall armyworm is known to be polyphagous. It feeds on about 80 species of plants; among which are some plants of the Family Poaceae, Fabaceae, Solanaceae, Asteraceae, Rosaceae, Chenopodiaceae, Brassicaceae and Cyperaceae (Silva *et al*., 2015; Casmuz *et al*., 2017).

Two strains of the FAW have been identified; the corn strain (C-strain) and the rice strain (R-strain) based on the preferred host plant, however, each strain may infest other plants in addition to the preferred host plants (Nagoshi & Meagher, 2004).

The C-strain prefers corn and cotton as host plants while the R-strain prefers rice, Bermuda grass and other grasses of the Family Poaceae. The C-strain develops in large numbers compared with the R-strain and inter-strain breeding is reported. However, mating preference exists such that, R-strain females prefer C-strain males but C-strain females are not reproductively compatible with R-strain males (Whitford, Quisenberry, Riley & Lee, 1988; Quisenberry, 1991). The two strains can only be distinguished at the genetic level using molecular markers because they are morphologically identical. Identification of the two strains is important in order to deploy effective management practices since each strain differs in its development on host plants, resistance to insecticides, mating behaviour and susceptibility to plants with *Bacillus thuringiensis* proteins (Nagoshi & Meagher, 2004).

Spodoptera frugiperda is successful as a pest because the adult female oviposits usually directly on the bark of leaves and funnels of host plants, protecting the eggs from the reach of insecticides sprayed on the plants. Unlike most other *Spodoptera* species, the mandibles of caterpillars of the fall armyworm have comparatively stronger, serrated cutting edges, which make feeding easy. Older larvae are cannibalistic. Young larvae hide in the funnel during the day but emerge at night and early morning to feed on the leaves while older larvae stay inside the funnel and so are protected from traditional insecticides, pesticide applications and natural enemies. In older plants, the larger larvae can bore into the developing reproductive structures, such as maize cobs and cotton bolls, reducing quantity and quality of yield (Capinera, 2002; Pannuti *et al*., 2016).

The ability of the fall armyworm to infest a host plant depends on the developmental stage of the plant. For instance, its attack on maize usually occurs during the development of the vegetative whorls so the larvae can feed on the leaves of the plant but in cotton, the bolls, the blooms and the squares are the targeted structures. Older larvae feeding on cotton hide and feed in the bolls hence are protected from the action of insecticides (Barros *et al*., 2010). In maize, early instars feed by mining the green tissues of leaves while older larvae feed massively on the leaves, buds, developing whorls as well as on the ears. The presence of FAW infestation on maize plant is characterised by perforations in the leaves and the presence of frass on the leaves and stalk of the plants (Goergen *et al*., 2016).

Larvae are found to survive best, gain the most weight, pupate faster and adults emerge fastest on millet as compared to maize, cotton and soybean under field conditions. Thus, comparing the survival of FAW on these host plants, millet is the best and maize is next to millet for suitable larval survival. (Barros *et al*., 2010). There is low survival and delayed development of the larvae of FAW on cotton because cotton leaves contain several anti-herbivory secondary compounds such as gossypol, a sesquiterpene aldehyde that serves as a deterrent to feeding, delays larval development, and reduce larval weight gain and survival (Montandon, Stipanovic, Williams, Sterling & Vinson, 1987; Stipanovic, Lopez-Junior, Dowd, Puckhaber & Duke, 2006). Silva *et al.* (2017) reported in Brazil that, when FAW larvae were reared on soybean, cotton, maize, wheat and oat leaves, the larvae fed on the grasses (wheat, maize and oats), had the shortest larva-adult period and weighed heavier compared with the larval period and weights for larvae fed on cotton and soybean. They concluded that FAW larvae have high preference for grasses to other host plants.

Management Methods for *Spodoptera frugiperda*

Pest outbreaks could be as a result of the concentration and high yielding of a single plant species in a larger area, reduction of natural enemies of pests, importation and exportation of plant materials around the world and indiscriminate use of pesticides which could result in resistance development in a pest species.

Pest management practices have currently been channelled towards the concept of integrated pest management (IPM). IPM refers to the use of a combination of management practices that reduce the population of a pest species below the economic injury levels whilst ensuring the safety of the environment and other non-targeted animals. It involves the use of chemical, biological, cultural control and host plant resistance mechanisms in the management of a pest population (Smith, 1966; Thomas & Waage, 1996). According to Hugh (2015), IPM is dependent on the following steps:

1. prevention: this involves the steps taken to manage crop production such that pest problems may not arise. With this, crops are managed healthily to improve their tolerance to pests. Native and introduced natural enemies are also preserved to control the population of the pests naturally.
2. accurate identification, monitoring and knowledge of pests, pest damage and natural enemies: pests are identified accurately, the nature of the damage they cause is noted through monitoring and scouting for early detection of pest infestations so that efficient management strategies could be deployed.
3. applying Economic Thresholds: here, the damage caused by the pest is assessed and controlled so that economic injury level (damage level at which the loss caused by the pest equals the cost of control) is not reached; and

4. suppressing Pest Populations: this involves the management of the pest population using biological, cultural, chemical, behavioural or mechanical control methods.

Considering the polyphagous nature of the fall armyworm, its biology on the different host plants, distribution and pesticide resistance status, IPM has been the major management strategy used for its control in its native countries. Recent studies in Africa indicate that, the use of a single control method in the management of FAW larvae is not as effective in increasing maize yield as a combination of two or more methods of control (Kumela *et al*., 2019; Kassie, Wossen, De Groote, Tefera, Sevgan & Balew, 2020). Thus, a combination of two or more control methods that are compatible in the management of the fall armyworm works more efficiently than practising each control method in isolation (Teklewold, Kassie, Shiferaw & Kohlin, 2013; Day *et al*., 2017; Tambo & Mockshell 2018). For instance, in a study done in Ghana and Zambia, a combination of synthetic insecticides and handpicking of FAW eggs and larvae, proved to be successful and resulted in about 125% increase in maize yield compared to the use of the methods in isolation (Tambo *et al*., 2020).

Monitoring for Fall Armyworm Population

Populations of fall armyworm are monitored using coloured traps, black light and pheromone traps. These traps are mounted at canopy heights in the field to attract flying FAW moths (Meagher, 2001). Pheromone-based monitoring is one of the more effective means to detect the presence of pests in an area. It works efficiently via: mass trapping, ‘lure and kill’ and mating disruption. This method has been successfully used in the United States of

America to monitor male fall armyworm moths (Wyatt, 1997). Monitoring for the presence or absence of the moth when coupled with crop inspection for the presence of FAW eggs informs the decision whether or not to employ other management strategies of IPM for the control of FAW populations (Beuzelin *et al.*, 2014).

Biological Control of the Fall Armyworm

The reliance on parasitoids, predators and entomopathogens constitutes biological control of insect pests. Biological control involves the conservation, augmentation and introduction of the natural enemies of the pest to be controlled (Hugh, 2015). This method of control is considered safe for the environment and the host plant. There are 53 species of parasites from 43 genera and ten families that attack the fall armyworm (Ashley, 1979; Sparks, 1986). Current documentation shows the presence of 150 species of parasitoids present in the countries of origin of the pest; United States, Mexico, South and Central America. They attack the eggs and larvae of fall armyworm (Molina-Ochoa *et al.*, 2003).

In Africa where the pest is novel, there are a few documented natural enemies of the larvae and eggs of FAW. In Ethiopia, *Cotesia icipe* (Hymenoptera: Braconidae), *Palexorista zonata* (Diptera: Tachinidae) and *Charops ater* (Hymenoptera: Ichneumonidae) have been noticed. *C. icipe* caused 33.8, 45.3 and 33.8% parasitism in Hawassa, Jimma and Awash-Melkasa respectively, *P. zonata* caused 6.4 and 5.7% parasitism in Hawassa and Awash-Melkasa respectively and *C. ater* caused 4.6% parasitism in Jimma (Sisay, 2018). In Kenya, the parasitoids identified were the Tachinid fly, *Archytas marmoratus* causing 12.5% parasitism and *C. ater* with parasitism range from 6 - 12%. In Tanzania, *Coccygidium luteum* has been identified with parasitism range of 4 - 8.3% (Sisay *et al*., 2018). In Ghana and Benin, the parasitoids identified were *Telenomus remus*, *Chelonus bifoveolatus*, *C. luteum*, *C. icipe*, *Meteoridea cf. testacea*, *Charops* sp, *Drino quadrizonula* while *Trichogramma* sp and *Pristomerus pallidus* have been identified only in Benin. *Metopius discolor* has also been identified only in Ghana (Agboyi *et al*., 2020).

The commonly known predators of FAW are ground beetles (Coleoptera: Carabidae), *Labidura riparia* (Dermaptera: Labiduridae), *Podisus maculiventris* (Hemiptera: Pentatomidae) and *Orius insidiosus* (Hemiptera: Anthocoridae) (Capinera, 2001).

The control of FAW populations using entomopathogens works via utilisation of naturally occurring diseases, introduction and establishment of pathogens into the FAW population as natural regulatory agents and continual application of pathogens as microbial insecticides (Wayne *et al*., 1980). Fall armyworm larvae are susceptible to entomopathogens like viruses, bacteria (*Bacillus thuringiensis*), fungi (*Metarhizium anisopliae* and *Beauveria bassiana*), nematodes and protozoans which cause significant levels of mortality (Wayne *et al*., 1980; Molina-Ochoa *et al*., 2003).

In Mexico, Molina-Ochoa *et al*. (2003) recorded 3.5% FAW larval mortality due to naturally occurring entomopathogens and parasitic nematodes. Three species of entomopathogenic fungi representing two different classes, Hyphomycetes (*Nomuraea rileyi*, and *Hirsutella* sp.) and Zygomycetes (*Entomophthora* sp.) were retrieved from fall armyworm larvae.

Cultural control of *Spodoptera frugiperda*

Continuous cultivation of plants that serve as host for fall armyworm creates a suitable environment for its spread as gravid moths gain easy access to host plants which serve as oviposition sites. The practice of crop rotation, intercropping and the use of multiple varieties reduce the rate of oviposition by the female moth and thus reduce the level of infestation of the FAW (FAO, 2018).

Cultural control involves intentional manipulation of a cropping environment to avert or subdue pest development and damage. This includes trap cropping, crop rotation, growing resistant varieties, minimizing tillage to conserve soil-dwelling natural enemies and managing alternative hosts plants of the pest (Hugh, 2015). Fall armyworm infestation on maize has successfully been managed using cultural control.

The cultivation of resistant corn varieties with antibiosis and non-preference mechanisms has yielded much more produce than susceptible varieties at similar levels of FAW infestations (Wiseman & Davis, 1990).

The push-pull cultural control method which has been recently modified as 'climate-adapted push-pull' for control of cereal stem-borers in drier agro-ecologies, for the management of fall armyworm proved successful in Kenya, Uganda and Tanzania. The technology comprises intercropping maize with drought-tolerant green leaf, *Desmodium intortum* (Leguminosae) (push) and planting *Brachiaria* cv Mulato II (Poaceae) (pull) as a border crop around the intercrop. An assessment of this control practice revealed that the method was effective in controlling FAW population per plant by 82.7% and reduced plant damage by 86.7% compared with a maize monocrop plot. This resulted in higher yield in the climate-adapted push-pull plot compared with the maize monocrop plot (Midega, Pittchar, Pickett, Hailu & Khan, 2018).

Handpicking of egg masses and larvae as a cultural control method is also practised by 29.53% of farmers in Zambia and was ranked the second highest method of control used by most farmers in Zambia to control the fall armyworm population (Tambo *et al*., 2020).

Chemical control of *Spodoptera frugiperda*

The use of chemical insecticides dates back to the 1950s and organochlorines were the first to be widely used. From that time onwards, the misuse of insecticides together with its effects on the environment and the farmers that apply them, has led to the initiation of IPM. That notwithstanding, the use of insecticides remains the bedrock of pest management since they are usually effective at causing mortality in the targeted pest. Selecting the appropriate insecticide, applying it according to the dose and time between successive applications recommended by the manufacturer is important in determining the efficiency of a chemical insecticide (Dent, 2000). Again, efficacy of insecticides is largely dependent on the method and effectiveness of application of the insecticides. Young (1979) described factors that limit efficient use of insecticides as a control method to be; plant height and canopy density. According to him, large volumes of insecticides for adequate penetration into whorls and plant terminals should be used. He also opined that instead of using large volumes of water, baits; granular formulations of insecticides and conventional and ultra-low volume formulation aerial application should rather be used.

Out of the various strategies employed in IPM, many *S. frugiperda*-infested countries have used chemical insecticides as the main control strategy (Straub & Hogan, 1974; Bass, 1978; Young, 1979; Gutierrez-Moreno *et al*., 2019). In Africa, the use of chemical insecticides is the main method of control against the FAW populations in Ethiopia, Ghana, Kenya and Zambia (Kumela *et al*., 2019; Tambo *et al*., 2020).

Synthetic insecticides

Synthetic insecticides are deemed important options in the management of the fall armyworm in the Americas and remains the main method of control employed against the FAW (Gomez & Gomez, 1984; Yu, 1991; Gutierrez-Moreno *et al*., 2019). In Africa, the use of synthetic insecticides is the major method of control (Kumela *et al*., 2019; Tambo *et al*., 2020).

In Mexico, carbamates and pyrethroids which work as acetylcholinesterase inhibitors and sodium channel modulators respectively are among the oldest insecticides used to control the FAW (Gutierrez-Moreno *et al*., 2019). In southern United States, FAW populations on sweetcorn are managed using synthetic insecticides applied three to four times in a week. In Florida, synthetic insecticides are applied against FAW to protect both the vegetative and reproductive stages of corn (Capinera, 2001). In Africa, survey done by Kumela *et al*., (2019) showed that about 50% of farmers in Ethiopia and Kenya use synthetic insecticides for the control of the fall armyworm. In Ghana, Tambo *et al*., (2020) found out that 51.22% of farmers use synthetic insecticides while in Zambia, 48.54% of farmers use synthetic insecticides. In Cameroon, Fotso *et al*. (2019) ascertained that 41% of farmers use synthetic insecticides in the control of FAW larvae. The common major classes of synthetic insecticides recommended by the MoFA in Ghana are *Bacillus thuringiensis*; a bio-pesticide, pyrethroids, carbamates, avermectins and neonicotinoids. In some parts of the Americas, the pest has developed resistance to many of the major synthetic insecticides such as pyrethroids in Mexico and Puerto Rico (Gutierrez-Moreno *et al*., 2019) and pyrethroids, carbamates and organophosphates in South-eastern United States (Day *et al*., 2017). Due to that, new classes of insecticides like diamides and spinosyn are now commonly used (Hardke, Temple, Leonard & Jackson, 2011). However, synthetic insecticides remain the main method of control for this notorious pest in Mexico and Puerto Rico (Gutierrez-Moreno *et al*., 2019).

In Mexico, some of the potent synthetic insecticides used for the control of FAW in maize are Methyl-parathion, Chlorpyrifos, Methamidophos, and Phoxim (Malo, Bahena, Miranda & Valle-Mora, 2004). Others are Emamectin benzoate, Chlorantraniliprole, Spinetoram, Triflumuron and Flubendiamide (Gutierrez-Moreno *et al*., 2019).

Emamectin benzoate, an avermectin, is known to be a potent insecticide against lepidopterous pests both in laboratory bioassays and field trials, with LC_{90} of 0.002-0.89 µg/mL. It is also known to be less toxic to non-target arthropods and found to be superior to other insecticides like permethrin and *Bt*. in controlling lepidopterous pests (Leibee, Jansson, Nuessly & Taylor, 1995; Jansonn *et al*., 1997).

Lambda Cyhalothrin-based insecticides (pyrethroid) seem to have an erratic potency for control of FAW larvae. For instance, it has been reported as being potent for the control of FAW larvae in Ethiopia (Sisay, Tefera, Wakgari, Ayalew & Mendesil, 2019) and Santa Isabel, Puerto Rico (Belay, Huckaba & Foster, 2012) but the opposite is the case in Baton Rouge, Louisiana (Hardke *et al*., 2011), most states in Mexico, Puerto Rico (Gutierrez-Moreno *et al*., 2019) and South-Eastern United States, Central and South America (Pitre, 1986).

In a laboratory bioassay conducted in Santa Isabel, Puerto Rico, an Indoxacarb- and a Lambda-Cyhalothrin-based insecticides caused more than 80% FAW larval mortality each after 96 hours of application while Spinosad, Spinetoram and Acephate-based insecticides caused more than 80% mortality after 48 hours of application (Belay *et al*., 2012). It is important to note that, though the Indoxacarb and Lambda-Cyhalothrin-based insecticides caused high mortality in FAW, they achieved that after a relatively longer period of four days.

In Cameroon, Fotso *et al*. (2019) ascertained that 16%, 15% and 10% of farmers used Emamectin benzoate-, Cypermethrin- and Lambda cyhalothrin-based pesticides, respectively to control FAW larvae. Other chemicals with Imidacloprid, Acetamiprid and Chlorpyriphos-ethyl as active ingredients were used by some farmers. However, it was concluded that these chemicals used by farmers did not have drastic effects on the larvae of *S. frugiperda* in maize farms and so current efforts are focusing on identifying potential indigenous natural enemies and screening of less toxic insecticides and bio-pesticides as part of an integrated control of the pest.

Botanical insecticides

The issue of ecotoxicity and the development of resistance in insect pests to synthetic insecticides has paved way for the use of botanical insecticides. Botanical insecticides are easily degraded by sunlight and rainfall hence do not persist in the environment. They are usually non-toxic to natural enemies of targeted insect pests as well as other benign insects (Miresmailli & Isman, 2014; Stevenson, Isman & Belmain, 2017; Tembo *et al*., 2018).

The fall armyworm is an example of a pest that has been successfully controlled with plant extracts including *Azadirachta indica, Jatropha curcas, Nicotiana tabacum, Argemone ochroleuca, Cedrela salvadorensis, Cedrela dugesia* and *Schinnus molle* (Martinez *et al*., 2017; Sisay *et al*., 2019). These plants possess anti-feedants, repellents, juvenile hormone inhibitors, growth-disrupters, ecdysis disrupters and other chemicals that cause alterations in the morphology of mid-gut epithelium and peritrophic membrane which results in inefficient nutrient absorption by the FAW (Metcalf & Metcalf, 1992; Roel, Dourado, Matias, Porto, Bednaski & Costa, 2010; Mochiah *et al*., 2011). A notable example is *A. indica* (neem) extract (both seed and leaf extract) which is known to be effective in causing mortality in the larvae of *S. frugiperda*. It contains azadirachtin as the main active ingredient together with other compounds such as nimbin and salannin which are toxic to FAW larvae. Neem works as a feeding deterrent, growth and metamorphosis hormone interrupter and eventually result in mortality (Schmutterer, 1990; Mordue, & Nisbet, 2000; Simmonds, Jarvis, Johnson, Jones, Morgan, 2004; Isman, 2006; Silva *et al*., 2015).

Though found to be potent, botanical insecticides are used by just a handful of farmers for the control of fall armyworm larvae. In Zambia and Ghana, a survey done to analyse the control measures used by farmers showed that less than 1% of farmers relied on botanical insecticides as a control method against the FAW (Tambo *et al.*, 2020).

CHAPTER THREE

MATERIALS AND METHODS

This chapter describes the methods used in the research work to achieve the objectives of the study. It elaborates the steps taken to keep the colony of *Spodoptera frugiperda*, how the development of the insect was studied and how the laboratory bioassays were carried out to evaluate the efficacy of the various insecticides.

Maintenance of *Spodoptera frugiperda* Colony

Larvae of *S. frugiperda* were collected from infested maize farms (Figure 2) in Cape Coast, (Longitude 1.32^{o}W, Latitude 5.18^{o}N and Altitude 58 m) Dehia (Longitude 1.3^{o}W, Latitude 5.24^{o}N and Altitude 69.6 m) and Twifo Praso (Longitude 1.46^{o}W, Latitude 5.63^{o}N and Altitude 106 m) in the Central Region of Ghana.

Figure 2: Sampling *Spodoptera frugiperda* from the field

The larvae were conveyed to the Entomology laboratory of the University of Cape Coast and kept in ventilated plastic containers (15 × 9 ×5 cm) (Figure 3).

Figure 3: Larval cages containing maize leaves and larvae

Rearing of the larvae was done from September 2018 to August 2019. The larvae were reared under the prevailing ambient conditions of the period. Temperature was 25-34^{o}C, photoperiod; 12L: 12D and relative humidity; 44-80%. The larvae were fed with fresh maize leaves cut into pieces (length, 5 cm). Larval cages were cleaned and stocked with maize leaves every other day (Figure 4).

Figure 4: Larval cages being cleaned (A) and stocked with maize leaves (B)

Once larvae pupated, pupae were transferred into square-shaped cages (30 × 30 × 30 cm) with outlets sealed with nets, where eclosion would occur. Adults were fed with 10% sugar solution to provide them energy for mating followed by oviposition. Potted maize plants were kept inside the cages to serve as substrates on which females would lay eggs. Portions of the leaves where eggs had been laid were clipped off and transferred into new larval cages. When the eggs hatched, neonates were fed with fresh maize leaves and kept for subsequent generations. Some of the larvae raised from these generations were kept singly to study the development of FAW and the others were used as samples to carry out the insecticide susceptibility tests.

Studying the growth and development of *Spodoptera frugiperda*

The following aspects of the biology of the FAW were studied in the laboratory: duration for egg maturation and hatching, number of larval instars, duration for larval development, duration for pupal development and adult male and female lifespan. In order to study the duration for egg maturation and hatching, fifteen batches of eggs; each containing different number of eggs, were kept in separate cages. These eggs were observed and the number of days taken for them to mature and hatch noted. After hatching, fifteen neonates were kept singly in disposable cups, fed with maize leaves and the number of days taken for them to pupate noted. The width of the head capsules and the body length of each of the larval stages were measured during the period of larval development. Larval colouration was also observed for the occurrence of ecdysis. Once pupation occurred, the pupae were kept singly and observed till adults emerged. Adults were fed with 10% sugar solution and their lifespan noted.

The study was conducted under the natural temperature, humidity and photoperiod prevailing in the laboratory. This mimicked the conditions under which FAW develops naturally. Two groups of larvae were studied at different periods. The first group was studied from December 2018 to January 2019 (temperature: ca. 25 - 28^{o}C; relative humidity: ca. 75 - 80%; photoperiod: ca. 12L: 12D) and the second group was studied from late February to March 2019 (temperature: ca. 31 - 34^{o}C; relative humidity: ca. 44 - 55%; photoperiod: ca. 12L: 12D).

Laboratory Bioassays

Recommended doses of five selected insecticides - Emamectin Benzoate, *Bacillus thuringiensis* (Bt), Acetamiprid+Indoxacarb, Lambda-cyhalothrin+Acetamiprid, and Imidacloprid recorded in Table 1 were prepared and used for the susceptibility tests.

Table 1: Active ingredients in the Insecticides used for susceptibility tests against the eggs and second instar of the fall armyworm

Active ingredient	Mode of action[a]	Recommended dose (chemical/ water)	Dosage used
Emamectin benzoate	GLUCl[b] allosteric modulators	15 mL/15 L	1 mL/1 L
Acetamiprid + Indoxacarb	nAChR[c] modulators. AChE[d] inhibitors	70 mL/15 L	4.7 mL/1L
Acetamiprid + Lambda cyhalothrin	nAChR[c] modulators. sodium channel modulators	40 mL/15 mL	2.7 mL/1L
Imidacloprid	nAChR[c] modulators	30 mL/15 L	2 mL/1 L
Bacillus thuringiensis	microbial disruptors of insect midgut	50 g/15 L	3.3 g/1 L

[a]Mode of action and group numbers as given by Insecticide Resistance Action Committee (IRAC), [b]Glutamate-gated chloride channel, [c]Nicotinic acetylcholine receptor, [d]Acetylcholinesterase

Egg susceptibility test

Four insecticides, namely Imidacloprid, Acetamiprid+Lambda cyhalothrin, Acetamiprid+Indoxacarb and Emamectin Benzoate, with tap water as control, were evaluated on batches of eggs of the FAW. Each treatment was applied to one batch of eggs and replicated three times, in a completely randomized design. Thus, three batches of eggs were used for each insecticide and for the control. The number of eggs varied from one batch to the other.

The eggs used were 24 hour-old. The insecticides and water were sprayed upon the egg masses using a plastic mist spray bottle after which they were kept in separate cages and observed for hatching. Neonates that hatched from the treated eggs were not used for any of the experiments described in the subsequent pages of this chapter.

Larval susceptibility test with synthetic insecticides

A trial susceptibility test was done before the actual test was carried out. The insecticides recommended by MoFA came with concentrations that were higher than the manufacturer's recommended concentrations. In order to settle on which concentration to use in the actual experiment, a trial susceptibility test was done with four different concentrations (manufacturer's recommended concentration, concentration recommended by MoFA, half and twice the dose recommended by MoFA) of one insecticide - Emamectin Benzoate. The susceptibility at each concentration was tested with ten larvae per treatment. The treatment was replicated five times to bring the total number of larvae used to 50, at each concentration. Tap water was used for the control units. Ten larvae with four replications were used as control for all four concentrations, due to insufficient samples.

In the actual experiment, the bioassay was carried out using only the manufacturer's recommended dose for all five insecticides (Table 1), in a completely randomized design. For each insecticide, ten larvae were used with five replications; thus 50 larvae were used for each insecticide and 50 were used as the control. The laboratory bioassay method known as leaf dipping (Paramasivam and Selvi, 2017) was used to assess the susceptibility of *S. frugiperda* larvae to the recommended concentrations of each of the synthetic insecticides.

Three week-old maize leaves were collected, washed, wiped dry and cut into pieces, each measuring 5 cm long. The pieces of maize leaves were dipped separately in each test insecticide for ten seconds after which they were air-dried for ten minutes before being fed to the larvae. Due to the cannibalistic nature of third – sixth instar larvae of FAW second instar larvae (L2) of F_1 generation were used to prevent mortality due to cannibalism. The leaves for the control samples were also dipped in tap water for ten seconds, air-dried for ten minutes and kept in the larval cages and was later fed on by the larvae. Each cage containing ten second instar larvae was served with ten pieces of leaves and observed after 24, 48 and 72 hours for mortality.

A sequel susceptibility test was done with twice the recommended dose of Imidacloprid and Acetamiprid+Lambda cyhalothrin after it was ascertained that the recommended doses of both insecticides could not cause expected mortality in the second instar larvae of the FAW.

Larval susceptibility test with *Azadirachta indica* extract

Three concentrations of *Azadirachta indica* (neem) extract: 0.25 g/mL, 0.50 g/mL and 1 g/mL were prepared from 25 g of fresh neem leaves, 50 g and 100 g, respectively in 100 mL of water. Each mass of fresh leaves was blended in 100 mL of water, the mixture was filtered with a fine mesh and the aqueous extract used for the experiment.

For each of the three concentrations of the aqueous neem extract, ten larvae with five replications were used to test susceptibility. Due to insufficient samples, ten larvae with three replications were used as control units for the three concentrations of neem extract.

Three week-old maize leaves were collected, washed, wiped and cut to the length of 5 cm and used for the susceptibility test. The leaves were dipped for ten seconds separately in each mixture and air-dried for ten minutes. The treated leaves were served as food to the larvae and replaced with fresh leaves after 72 hours. Mortality in the larvae was observed and recorded from 24 to 192 hours (8 days) after treatment.

Statistical Analyses

Mean duration for the development of the fall armyworm during the two periods of rearing, was calculated (Microsoft Office Excel, Version 2013, Redmond, WA). Student's T-test (MS Excel) was used to determine statistically significant differences between the mean developmental periods for the life stages (egg hatching, larval duration, pupation, eclosion and adult lifespan) as observed for the two different periods.

Mean mortality caused by each of the five insecticides was calculated and subjected to one-way analysis of variance (ANOVA) to determine statistically significant differences between the means. This was followed by a post hoc test, using Tukey pairwise comparisons (MINITAB 17) to separate the percent mean mortalities caused by each of the insecticides.

Data obtained from the neem extract larval bioassay were subjected to probit analysis followed by regression analysis from which the LC_{50} and LC_{90} were computed. The probit analysis was done using the percentage mortality caused by each of the concentrations by the fifth day and the mortalities were corrected using Abbott's (1925) formula as follows:

Corrected Percent Mortality = $(P - P_0)/ (100 - P_0) \times 100$; where P is the percent mortality in the treated insects and P_0 is the percent mortality in the untreaed control.

CHAPTER FOUR

RESULTS AND DISCUSSION

Development of *Spodoptera frugiperda*.

The development period of *S. frugiperda* studied for December 2018–January 2019 (temperature: 25 - 28°C; relative humidity: 75 - 80%) was different from that for February 2019 - March 2019 (temperature: 31 - 34°C; relative humidity: 44 - 55%). Development was faster but adult male and female had shorter lifespans at the higher temperature of 31 - 34°C and relative humidity range of 44 - 55%. There were significant differences in duration of all life stage events including: duration for egg hatching, larval moulting (six larval instars; L_1-L_6), pupation and adult lifespan at the different temperature and relative humdity ranges (Tables 2, 3 and 4).

Table 2: Mean development period (days) ± S.E (range) of *Spodoptera frugiperda* studied at two periods with different temperature and relative humidity ranges

	Metamorphosis (no. of days)			
Temperature/ Relative Humidity	**Egg-1st instar larva**	**Larva-Pupa**	**Pupa-Adult**	**Total period (Egg-Adult)**
25 - 28°C/ 75 - 80%	2.47 ± 0.13 (2 - 3)	21.53 ± 0.38 (19 - 24)	9.33 ± 0.16 (8 - 10)	33.33 ± 0.35 (31 - 35)
31- 34°C/ 44 - 55%	2.00 ± 0.00 (2)	10.93 ± 0.23 (10 - 12)	8.13 ± 0.17 (7 - 9)	21.07 ± 0.18 (20 - 22)
T-test				
Sample size	15	15	15	15
p-value	0.002	$p < 0.001$	$p < 0.001$	$p < 0.001$

It is evident that, the number of days taken for the eggs to develop into adults were significantly shorter (20 - 22 days) during the second period (February – March), when the temperature was high (31 - 34°C) and relative humidity was low (44 - 55%) compared with the first period (December – January) when temperature was low (25 - 28°C) and relative humidity was high (75 - 80%) (31 - 35 days).

Table 3: Mean ± S.E (range) periods for *Spodoptera frugiperda* larval instars studied at two periods with different temperature and relative humidity ranges

	Larval Development (Days)		**t-test**
	Period 1	**Period 2**	
Instar (L)	25 - 28°C/ 75-80%	31 - 34°C/ 44 - 55%	p-value
L_1	2.6 ± 0.13 (2 - 3)	2.07 ± 0.07 (2 - 3)	0.001
L_2	2.73 ± 0.12 (2 - 3)	1.53 ± 0.13 (1 - 2)	< 0.001
L_3	2.87 ± 0.09 (2 - 3)	1.53 ± 0.13 (1 - 2)	< 0.001
L_4	3.4 ± 0.19 (2 - 4)	1.4 ± 0.13 (1 - 2)	< 0.001
L_5	4.13 ± 0.13 (3 - 5)	2.00 ± 0.00 (2)	< 0.001
L_6	2.5 ± 0.12 (5 - 6)	2.4 ± 0.13 (2 - 3)	< 0.001

Sample size (N) =15

Number of days spent by larvae in each instar stage was significantly shorter during the second period of the study (February - March) when the temperature was higher and the relative humidity was lower.

Table 4: Lifespan (days) ± S.E (range) of male and female *Spodoptera frugiperda* moths studied at two periods with two different temperature and relative humidity ranges

	Adult Lifespan (Days)	
Temperature/ Relative Humidity	**Male**	**Female**
25 - 28°C/ 75 - 80%	7.6 ± 0.19 (7 - 9)	10.33 ± 0.29 (9 - 12)
31-34°C/ 44-55%	5.7 ± 0.23 (5 - 7)	8.0 ± 0.22 (7 - 9)
T-test		
Sample size	15	15
p-value	$p < 0.001$	$p < 0.001$

Adult females had longer lifespans than the males during both periods of the study. However, the lifespans of both adults were significantly shorter during the second period, when temperature was relatively higher and relative humidity was lower. Thus, adults lived longer when the temperature was lower and relative humidity was higer.

Morphometric and behavioural study of the life stages of *Spodoptera frugiperda*

Eggs

In the laboratory, cream coloured eggs were laid in masses (Figure 5) mostly on the lower surface of leaves (Figure 5A & B) of the potted maize plants or on filter paper (Figure 5C) provided in cages. Eggs were laid occasionally on the frames of the cages (Figure 5D). The eggs were stuck to the substrate and the females laid silky fibre to cover them (Figure 5A) while others were uncovered (Figure 5B).

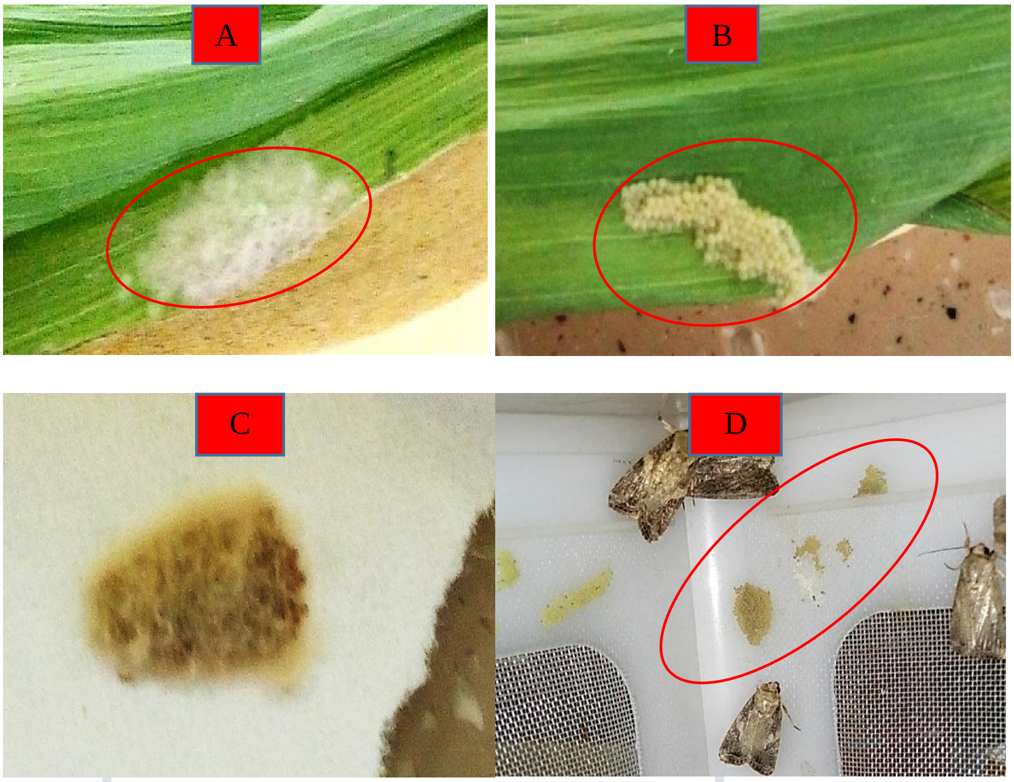

Figure 5: Eggs of *Spodoptera frugiperda* covered with fibre (A), uncovered (B), laid on filter paper (C) and on frames of cage (D)

Larvae

Spodoptera frugiperda larvae were observed to have some peculiar features that distinguished them from other lepidopteran larvae which include; one yellow stripe each on the dorsolateral parts of the body (Figure 6A), an inverted 'Y' mark on the head capsule (Figure 6B) and the presence of four dark spots on the dorsal part of the eighth abdominal segment (Figure 6C). These features were not obvious at the initial stages of the larval development (L_1-L_2), but were prominent in the late instars (L_3-L_6).

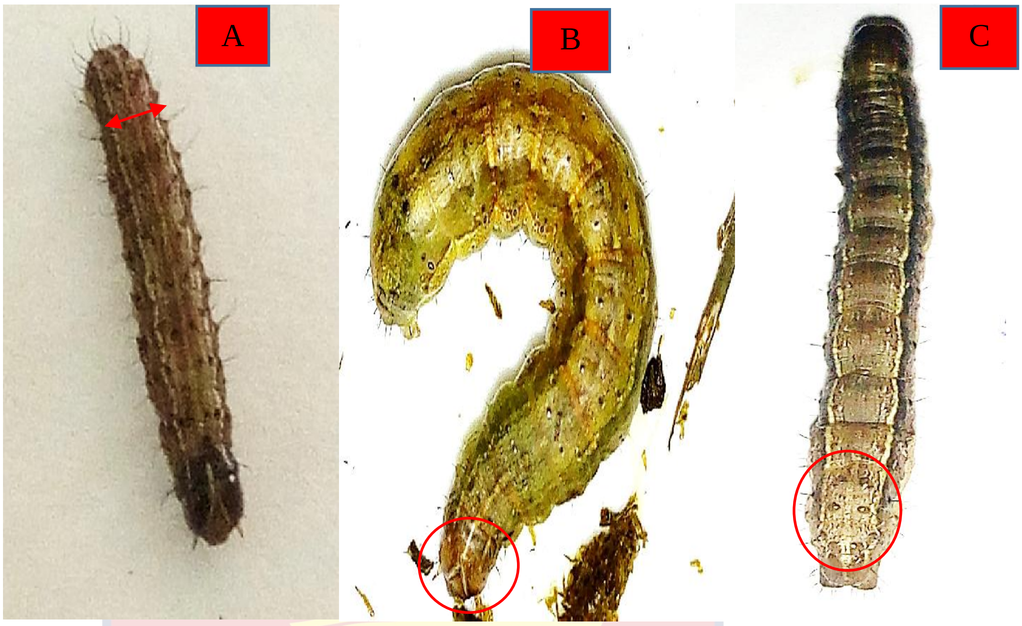

Figure 6: *Spodoptera frugiperda* larvae showing yellow stripes (A), inverted 'Y' (B) and four dark spots (C)

Generally, larval head width correlated positively with larval body length; increase in head width after moulting resulted in a corresponding increase in the body length (Table 5).

Table 5: Mean ± S.E (range) body lengths and head widths (mm) of the larval instars of *Spodoptera frugiperda*

Instar (L)	Body length (mm)	Head width (mm)
L_1	1.7 ± 0.02 (1.6 - 1.8)	0.31 ± 0.02 (0.2 - 0.4)
L_2	6.9 ± 0.25 (5.0 - 8.0)	0.81 ± 0.02 (0.7 - 0.9)
L_3	12.1 ± 0.21 (11 - 13)	1.17 ± 0.01 (1.1 - 1.2)
L_4	18.5 ± 0.25 (17 - 20)	1.77 ± 0.02 (1.6 - 1.9)
L_5	23.9 ± 0.22 (23 - 25)	2.34 ± 0.03 (2.1 - 2.5)
L_6	29.8 ± 0.36 (28 - 32)	3.0 ± 0.02 (2.9 - 3.1)

1st instar larvae had the least mean head and body size of 0.31 ± 0.02 mm and 1.7 ± 0.02 mm, respectively while 6th instar larvae had the greatest mean head and body size of 3.0 ± 0.02 mm and 29.8 ± 0.36 mm, respectively.

Neonates of *S. frugiperda* (Figure 7A) usually consumed the remains of the eggs from which they hatched (Figure 7B). Freshly emerged larvae dispersed in search of food in approximately an hour after hatching. However, dispersal was limited by the presence of food in the cages, leaving them clustered on the surfaces of the maize leaves (food) provided. In the laboratory, freshly-emerged instars fed by mining the green tissues of the leaves, leaving the epidermal layer (Figure 7C).

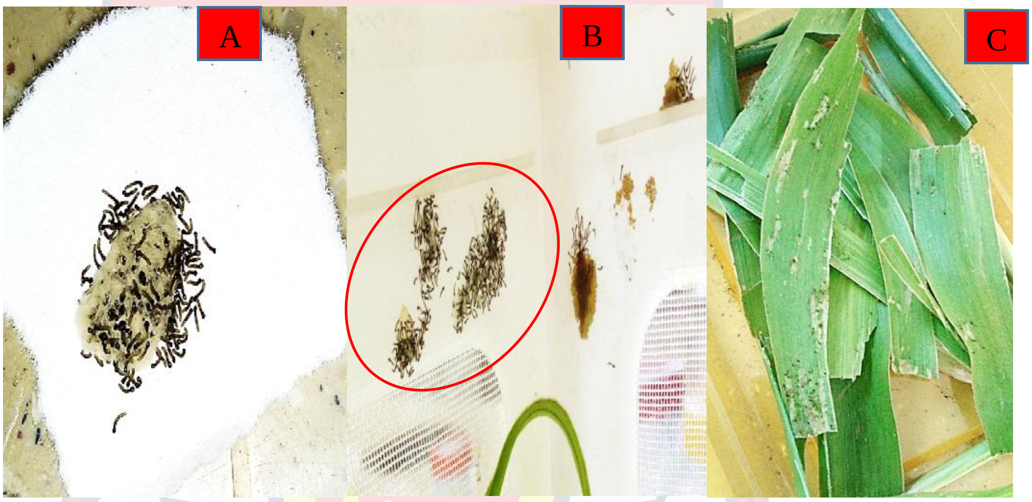

Figure 7: Freshly hatched larvae of *Spodoptera frugiperda* (A), neonates dispersing after hatching (B) and leaf mining by neonates (C).

The feeding behaviour of L2-L3 instars was characterised by perforations of the maize leaves while L_4-L_6 instars which had well developed mandibles (Figure 8), fed voraciously.

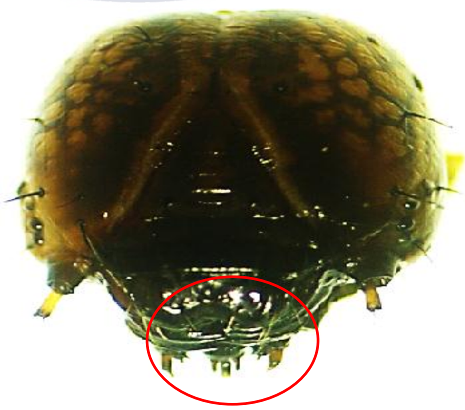

Figure 8: Head capsule of sixth instar larva of *Spodoptera frugiperda* showing well-developed mandibles.

Larval cannibalism (Figure 9), was observed both in the presence and absence of food as older instars fed on the early instars or on other larvae of the same stage. Prior to pupation, the larvae became shortened (Figure 10), sluggish and stopped feeding. It was observed that the larva would hide under frass and weave a silky fibre around itself.

Figure 9: Fourth instar larva of *Spodoptera frugiperda* feeding on another larva of the same species

Figure 10: Larvae of *Spodoptera frugiperda* about to pupate

Pupa

Fresh pupae were green in colour (Figure 11A) and very fragile. Tanning and hardening occurred within a period of 24 hours with a bright brown pupal case (Figure 11B). Prior to eclosion, the puparium became dark brown (Figure 11C) and then eclosion occurred in a day or two.

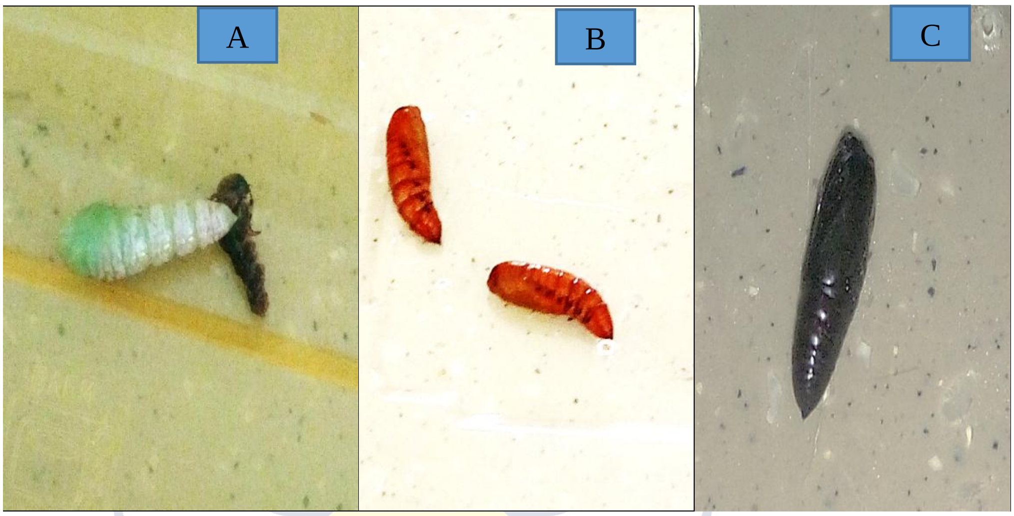

Figure 11: Fresh pupa (A), pupa after tanning (B), pupa prior to eclosion (C)

It was observed that pupae were susceptible to the larvae of the phorid fly *Apocephalus borealis*. Larvae of *A. borealis* consumed whole pupal content of *S. frugiperda*, leaving only the pupal case. They caused 14.3% mortality in the pupae of *S. frugiperda*. Dead larvae of *S. frugiperda* were also consumed by the larvae of *A. borealis* (Figure 12). *Apocephalus borealis* might have possibly been collected from the infested maize farms, together with the larvae of the FAW during the field collections.

Figure 12: *Apocephalus borealis* fly (A), pupa of *Spodoptera frugiperda* being consumed by larvae *Apocephalus borealis* (B)

Adult

Sexual dimorphism exists in the adults of *S. frugiperda* (Figure 13); the male has white patches in the forewing unlike the female that has only brown patches in the forewings. Both have white hind wings.

Figure 13: Adult moths: Female (left), Male (right)

Susceptibility of the life stages of *Spodopera frugiperda* to insecticides

Egg susceptibility

All the insecticides failed to prevent eggs from hatching, thus eggs were not susceptible to the insecticides. Apart from emamectin benzoate, all the other insecticides did not cause mortality in the first instar larvae after treated eggs hatched. Only neonates that emerged from the eggs treated with emamectin benzoate (135 ± 7.64) died on the same day of hatching (Table 6).

Table 6: Mean ± S.E (range) number of *Spodoptera frugiperda* larvae that emerged after eggs were sprayed with recommended doses of four insecticides

Insecticide	Larvae that emerged	Larvae that died
Imidacloprid	121.67 ± 4.41 (115 - 130)	0
Acetamiprid+Lambda cyhalothrin	144.33 ± 16.69 (113 - 170)	0
Acetamiprid+Indoxacarb	119.33 ± 8.09 (105 - 133)	0
Emamectin Benzoate	135 ± 7.64 (125 - 150)	135 ± 7.64 (125 - 150)
Control	148 ± 20.48 (110 - 180)	0

Larval Susceptibility

Generally, mean larval mortality in the control was less than 10%. Considering the insecticides at the recommended dose rates, Emamectin benzoate and *Bacillus thuringiensis* caused 100% mean mortality in less than 24 hours (Figure 14). Single factor analysis of variance (ANOVA), showed that there was a significant difference in the mortalities caused by the five insecticides together with the control, at the end of 24 hour trial ($F = 29.35$; d.f $= 5$; $P < 0.001$). Tukey's pairwise post-hoc test carried out proved that, there was significant difference between the percent mean mortality caused by Emamectin benzoate (100%) and *B. thuringiensis* (100%) and that caused by Acetamiprid+Indoxacarb (36%), Acetamiprid+Lambda cyhalothrin (24%) and Imidacloprid (12%), after 24 hours. After 48 hours of treatment, Acetamiprid+Indoxacarb caused 100% mean larval mortality and there was significant difference in this percent mean mortality and that caused by Acetamiprid+Lambda cyhalothrin (60%) and Imidacloprid (28%) (F = 91.68; d.f = 3, P < 0.001).

Acetamiprid+ Lambda cyhalothrin and Imidacloprid could not cause 75% mortality even after 72 hours (Figure 14). However, there was a significant difference in mortality caused by Imidacloprid (42%) and Acetameprid+Lambda cyhalothrin (74%) after 72 hours of treatment (t = 4.82; df = 4; P = 0.004).

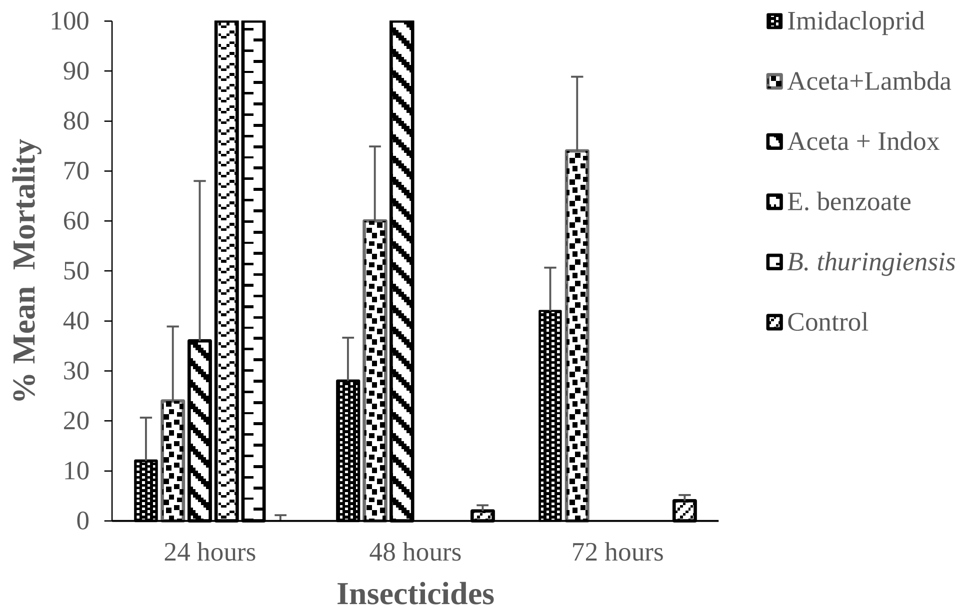

Figure 14: Percentage mean mortality of second instar larvae of *Spodoptera frugiperda* exposed to recommended doses of five insecticides at 24, 48 & 72 hours

From the graph, it is evident that percentage mean mortality caused by each of the five insecticides increased with time.

Considering Acetamiprid+Lambda cyhalothrin and Imidacloprid at twice the recommeded dosages, the two insecticides caused mean mortalities of 85% and 55%, respectively after 72 hours of treatment (Table 7). There were no significant differences in mortalities caused by Imidacloprid ($t = 1.29$; $df = 7$; $p = 0.12$,) and Acetamiprid+Lambda cyhalothrin ($t = -1.5$; $p = 0.09$; $df = 7$) at the recommended and twice the recommeded dosages after 72 hours of treatment.

Table 7: Percentage (%) Mean mortality ± S.E of second instar larvae of *Spodoptera frugiperda* exposed to twice the recommended dose of two of the five selected insecticides for 24-72 hours

	% Mean mortality		
Insecticides	**24 hours**	**48 hours**	**72 hours**
Imidacloprid	27.5± 8.54	30 ± 10.8	55 ± 9.57
Acetamiprid+Lambda cyhalothrin	67.5 ± 8.54	80 ± 8.16	85 ± 6.45
Control	0	5 ± 2.89	5 ± 2.89

Aqueous Neem Extract Bioassay

Larval mortality was assessed for eight days using three different concentrations of aqueous neem extract; 0.25 g/mL, 0.5 g/mL and 1 g/mL. All three concentrations caused 100% mortality by the eighth day. The highest concentration, 1.00 g/mL, caused 100% mortality six days after application while the other two concentrations caused 100% mortality eight days after application (Table 8).

The LC_{50} (lethal concentration 50%) and LC_{90} (lethal concentration 90%) ascertained from the probit analysis done using the percent mortalities caused by the fifth day, were 0.3 g/mL and 0.78 g/mL, respectively. Regression analysis performed on the data as indicated in the summary output of the regression stastistics (Appendix E) showed a significant difference in the mortalities caused by the fifth day by the three concentrations used (F =

13.56; df = 2; $P = 0.03$; $R^2 = 0.93$;). Incompletely-shed exuviae were found on the last two abdominal segments of dead larvae (Figure 15).

Table 8: % Mean mortalities caused by different concentrations of aqueous *Azadirachta indica* (Neem) extract on second instar larvae of *Spodoptera frugiperda*

Concentration (g/mL)	% Mean mortalities per day ± S.E							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
0.25 g/mL	0	0	16 ± 2.45	28 ± 3.74	46 ± 2.45	64 ± 2.45	84 ± 2.45	100 ± 0
0.50 g/mL	0	4 ± 2.45	28 ± 3.74	50 ± 3.16	66 ± 2.45	80 ± 3.16	90 ± 3.16	100 ± 0
1.00 g/mL	0	16 ± 5.1	48 ± 3.74	80 ± 3.16	96 ± 2.45	100 ± 0		
Control	0	0	0	3.3 ± 3.33	6.7 ± 3.33	6.7 ± 3.33	6.7 ± 3.33	6.7 ± 3.33

Figure 15: Larvae unable to completely shed off their exuviae after ecdysis.

Discussion

Development of the Fall Armyworm (*Spodoptera frugiperda*)

Development of *S. frugiperda* during the higher temperature regime of February to March 2019 was faster because temperature and humidity are important environmental conditions that influence growth and development of insects since insects are poikilothermic (Aguilon & Velasco, 2015). The length of time from hatching of eggs to pupation varies based on temperature and environmental conditions (Luginbill, 1928; Hogg *et al*., 1982). Temperature affects physiological processes such as metabolism, hormone and enzyme secretions which are cardinal for the rate of development of an organism (Neven, 2000). This speeds up the process of metamorphosis and moulting therefore the development of the fall armyworm from egg to adult in this study was faster during the higher temperature regime but slower when the temperature was relatively low. That notwithstanding, both temperature and relative humidity ranges were favourable for the survival of the fall armyworm. On the contrary, the lifespan of the adult male and female was shorter during the warmer period than when the temperature was relatively low. Insects expend more energy on warmer days hence rate of respiration, nervous activities, their risk to predation also increase which may consequently reduce their lifespan (Acar, Smith, Hansen & Booth, 2001; Hulbert, Pamplona, Buffenstein, & Buttemer, 2007) and that may be the reason the adult moths had shorter lifespan when temperature was slightly higher. It is known that the female lays about 1500-2000 eggs in its lifetime (Prasanna *et al*., 2018) hence the "shorter" lifespan during higher temperatures may not be a factor of advantage to farmers and other stakeholders, considering the number of eggs the female can lay.

The findings on the developmental periods of *S. frugiperda* from this study are similar to the work done earlier by Du Plessis *et al*. (2020) at constant temperatures in South Africa. The fixed temperature conditions used by these authors fell slightly within the range of temperatures at which the current study was conducted. Egg hatching period (2 days) obtained by these authors at 30 and 32°C corroborates that obtained in this study at 31 - 34^{o}C. Moreover, Du Plessis *et al*. (2020) reported similar larval development period (10 - 12 days) at 32^{o}C as was observed in the present study at 31 - 34^{o}C. However, the range ascertained from this study for larval development at 25 - 28^{o}C was higher (19 - 24 days) than what Du Plessis *et al*. (2020) reported for 26^{o}C (13 - 19 days). In their study, pupal development at 26^{o}C took 10 - 13 days which was longer than what was observed in this study (8 - 10 days). Pupal development at 32^{o}C which lasted 7 - 9 days conforms to findings in this study. The difference in the duration of larval and pupal development periods observed in the current study at 25 - 28^{o}C and the study by Du Plessis *et al*. (2020) in South Africa (26^{o}C) could be due to the differences in temperature, relative humidity and photoperiod under which both experiments were conducted. Du Plessis *et al*. (2020) worked at constant temperature (26 ± 1^{o}C), relative humidity (65 ± 5%) and a photoperiod of 14L: 10D; while in the current study none of these laboratory conditions were kept constant. The adult life span obtained in this study at 25 - 28^{o}C is consistent with what was reported by Sharnbasappa *et al*. (2018) at 26 ± 2^{o}C; 75 - 80% relative humidity and L12: D12 photoperiod.

Climatic and weather changes affect insect population dynamics, abundance and distribution (Ayres & Schneider, 2009). It can therefore be inferred from this study that relatively high temperatures which shortened the developmental period of *S. frugiperda* would increase its abundance and further facilitate its distribution in Ghana and Africa knowing that it does not undergo diapause in its development. A faster rate of development implies reduced number of days spent by the larvae; which is the most vulnerable stage to insecticides, predators and parasitoids. This offers an advantage to the FAW as it is likely to escape from predators, parasitoids and the action of insecticides. Based on the data obtained on the developmental periods of the fall armyworm, it could be inferred that in a year, *S. frugiperda* could have about eight to ten generations. Considering the weather pattern in Ghana, the presence of the host plants of the fall armyworm and the fact that it does not undergo diapause in its development, the adults have the ability to migrate to other regions where they would survive and perpetuate their generations. In countries where the FAW is native, the number of generations occurring within a year depends on temperature and the presence of migratory moths.

In Minnesota and New York, where fall armyworm moths do not appear from January to July, there may be a single generation. In Kansas, there may be one or two generations, while there may be three generations in South Carolina but four in Louisiana. In coastal areas of North Florida, moths are abundant from April to December (Capinera, 2002).

Usually during extremely low temperatures in fall and spring, the fall armyworm may complete its lifecycle in as long as twelve weeks but in summer, duration of its lifecycle is shorter so that, there could be about six generations (Luginbill, 1928; Hardke *et al.*, 2015). Deductions could be made from the above occurrences in the native countries of the FAW that, in Ghana, the FAW is likely to persist throughout the year. This is obviously due to the fact that Ghana is a tropical country that does not record extremely cold temperatures (below 19^{o}C) coupled with the polyphagous nature of the FAW, the presence of different host plants would enhance the continual existence of the generations of the fall armyworm.

This would be detrimental to the success of food production, especially grains in Ghana. Having ascertained that females live longer than males, together with the number of eggs a female can lay in a lifetime, the impact of the presence of FAW on food security and availability in Ghana and Africa can therefore, never be overemphasised.

Susceptibility tests

In this study, when the eggs of *Spodoptera frugiperda* were sprayed with selected insecticides to determine their efficacy on the stage of the lifecycle, the results obtained proved that the eggs were not susceptible to any of the insecticides since they all hatched 24 hours after treatment. This could be due to the fact that the chorion of the eggs coupled with the silky fibre covering the eggs served as a barrier to prevent the insecticides from penetrating the yet-to-hatch larvae. It also suggests that the insecticides are not formulated as ovicides hence their inability to function as such. However, all the neonates that emerged from the eggs treated with Emamectin benzoate died while larvae that emerged survived when eggs were treated with the other insecticides.

This could be due to the fact that Emamectin benzoate works as a contact insecticide (IRAC, 2017) coupled with a high residual potency of the active ingredient, which remained on the leaves till the eggs hatched in order to cause mortality in the neonates.

Lambda cyhalothrin+Acetamiprid and Imidacloprid, even at twice the recommended doses, could not cause more than 85% mortality in the second instar larvae and this could be attributed to tolerance of the larvae to these insecticides. Insecticide tolerance is the natural ability for an insect to withstand insecticide exposure and this has been identified in many insect species such as *Drosophila melanogaster*, houseflies and mosquitoes (Spiller, 1958). The continuous high tolerance levels of bed bugs to DDT has also been confirmed in Sri Lanka (Karunaratne, Hawkes, Perera, Ranson & Hemingway, 2007). The tolerance of the FAW larvae to the insecticide could be due to exposure of the larvae to sub-lethal or overdose concentrations of these insecticides by farmers in the fields where the larvae were sampled from.

Emamectin Benzoate and *Bacillus thuringiensis* (*Bt*) proved to be the most potent insecticides. Both recorded 100% mortalities 24 hours after treatment. Emamectin Benzoate works via contact as a Glutamate-Gated Chloride (GLUCl) channel allosteric modulator which works on the nerves of insects causing paralysis and death eventually (IRAC, 2017). This was observed in the larvae that were killed by feeding on leaves treated with Emamectin benzoate. Dead larvae were severely deformed and their bodies contorted from the action of the chemical.

Larvae that were exposed to *Bt*; a bio-pesticide that targets the midgut cells, became very soft after death and practically melted when touched with a brush. *Bt* works as a microbial disruptor of the midgut epithelium of targeted insects. When the bacterium is consumed by a particular insect, the toxic crystal is released to attack the insect's midgut epithelial cells which leads to disorganization of the cells, degeneration of microvilli and destruction of the peritrophic membrane, which protects the pest's stomach from its own digestive enzymes. The stomach is penetrated, and the insect dies by poisoning from the stomach contents and the spores themselves (Planet Natural Research Center, 2004; Castro *et al*., 2019).

Acetamiprid+Indoxacarb was also found to be very potent as it caused 100% mortality 48 hours after its application. Larvae that were exposed to this insecticide became sluggish, their rate of feeding was slowed down, hence became smaller compared with the control larvae, and eventually died.

Two insecticides caused the least mortality, with Acetamiprid+Lambda cyhalothrin causing 74% mortality, while Imidacloprid caused 42% mortality 72 hours after application. Larvae exposed to Imidacloprid, a neonicotinoid, which works systemically on the nerves of insects as a nicotinic acetylcholine (nACh) receptor modulator (IRAC, 2017) made the treated larvae cease feeding for about a day or two, became sluggish and smaller compared with the control larvae but after 24 - 48 hours the treated larvae that did not die began feeding again, while those that died were stiff when touched with a brush. This observation was not so different from what was observed in the larvae that were treated with Acetamiprid+Lambda cyhalothrin, which is made up of a neonicotinoid and a pyrethroid. Due to the relatively low percentage mortalities caused by the two insecticides, a sequel bioassay was done with twice the recommended doses to verify whether that could cause significant levels of mortality but the mortalities caused by twice the recommended doses were not significantly different from what was caused by the recommended doses.

The findings from this study about the potency of Emamectin benzoate agree with what was reported by Gutierrez-Moreno *et al*. (2019). In their study, Emamectin benzoate recorded the highest toxicity profile when a number of synthetic insecticides were screened against the larvae of fall armyworm in Mexico. Belay *et al*. (2012) reported that an Indoxacarb-based insecticide when used, was potent and caused more than 80% mortality which conforms to the finding from this study. Even though in the study by Belay *et al*. (2012), the Indoxacarb-based insecticide could not cause 100% mortality like it did in the current study, their conclusion that the insecticide is potent for the control of FAW larvae is proven in the current study.

The report by Fosto *et al*. (2019) about the inefficacy of Imidacloprid and Acetamiprid-based insecticides in the control of the larvae of FAW agrees with what was observed in the current study. However, Sisay *et al*. (2019) and Belay *et al*. (2012) reported that Lambda Cyhalothrin-based insecticides are potent for the control of FAW in Ethiopia and Santa Isabel; Puerto Rico, respectively. Meanwhile, that was not the case in the current study because the Lambda cyhalothrin-based insecticide used was second to the least potent insecticide. Hardke *et al*. (2011) ascertained that Lambda Cyhalothrin-based insecticides were not effective in the control of fall armyworm in Baton Rouge; Louisiana, which agrees with the finding from this current study.

The efficacy of an insecticide on a pest depends on a number of factors. For instance, the life stage of the target pest on which the insecticide works best, is cardinal (Nansen & Ridsdill-Smith, 2013). Thus, applying an insecticide on a pest at a stage where it is unaffected by the chemical renders the insecticide ineffective; as was the case of the egg susceptibility test carried out in this research. Inference could, therefore, be made from the outcome of the egg bioassay that when an infested farmland is sprayed with insecticides at a time when there are *S. frugiperda* eggs present, there may be an appearance of a subsequent larval population emerging from the eggs. This would likely result in the branding of the insecticides used for the treatment as not being effective which would further trigger the use of more chemicals or even overdose concentrations. It was obvious from this study that, the egg stage of the fall armyworm cannot be targeted for chemical control of the pest except where Emamectin benzoate is used.

Another factor that influences the toxicity of insecticides is the volume of the insecticide applied and the route through which the insecticide works; i.e. whether via contact or systemic (Nansen & Ridsdill-Smith, 2013). If a contact insecticide is used, the question of whether or not the targeted pest comes into contact with a lethal dose of the chemical at the time of application or at a later time matters. If a systemic insecticide is used, whether or not a lethal dose is translocated through the vascular tissues of the leaves to cause mortality of the pests that ingest the leaves also matter. It is in this regard that the mobility of the pest as well as the phenology of the host plant matters. In the case of the fall armyworm on a maize plant, because the first and second instars do not hide in the stalk of the maize plant but remain practically sedentary as they feed by mining the leaves and perforation respectively, it would be expedient to spray insecticides at the onset of infestation when the larvae are still young, less motile and less devastative.

The ineffectiveness of Imadacloprid and Acetamiprid+Lambda Cyhalothrin as observed in this study implies that farmers using these insecticides may be using overdoses in a quest to control the population of the fall armyworm. Meanwhile, analysis made on the results obtained from the bioassay proved that there were no significant differences between percentage mortalities caused by the recommended doses and twice the recommended doses. This shows clearly that using more of the chemicals than the quantity recommended by the manufacturer results in no substantial difference in the desired mortality but would only speed up the onset of resistance of the FAW population as well as pose harm to other benign and non-targeted animals; not forgetting the harm it poses on the health of the farmers using the chemicals. For some of the insecticides the MoFA of Ghana recommended doses that were much higher than the manufacturer's recommended doses, due to the level and extent of the infestations (Owusu, 2017). For instance, for Emamectin benzoate, MoFA recommended a dose of 75 mL/ 15 L of water when the manufacturer's dose is 15 mL/ 15 L of water. It is worth noting that the manufacturer's recommended concentration, yielded 100% mortality in this study after 24 hours of application. It is therefore instructive to stick to the manufacturer's dose. It is important for farmers to apply the insecticides correctly by ensuring that developing whorls and leaf sheaths of host plants are bathed with the insecticides. Extrapolating the findings of this particular analysis about the insignificant difference in mortality caused by recommended and twice the recommended dose, to other insecticides, it is apparent that there is no need for those higher doses to be used in a quest to control the population of the FAW. Excessive and indiscriminate use of these chemical insecticides would only worsen the plight of a nation. In fact, it is on record that FAW has developed resistance to some of the major classes of insecticides in its native countries. In Mexico and Puerto Rico, FAW has developed resistance to pyrethroids (Gutierrez-Moreno *et al*., 2019) and in South-Eastern United States, FAW has developed resistance to pyrethroids, carbamates and organophosphates (Day *et al*. 2017). The use of the correct doses in control programmes could rather delay if not completely prevent any potential development of resistance in the FAW population to these insecticides in Ghana. Lessons from regions where insect pests have developed resistance to prescribed insecticides should make farmers and other stakeholders use the insecticides prescribed by MoFA, according to the manufacturer's recommendation under field conditions.

Time is of essence as far as efficacy of chemical insecticides is concerned (Nansen & Ridsdill-Smith, 2013). Apart from Emamectin Benzoate and Bt that caused 100% mortality 24 hours after application, larvae exposed to the other insecticides had percentage mortalities increasing after every 24 hours until the third day. This means that each insecticide used has some level of residual potency. It is an indisputable fact that insecticides applied on the field degrade with time (Nansen & Ridsdill-Smith, 2013). However, they are able to achieve their maximum effect at certain points in time before the rate of mortality slows down again until no further mortality is caused. It is then necessary to allow some time to elapse after insecticide application for the results that would be achieved.

The fact that there were significant differences in the mortalities caused by the insecticides 24, 48 and 72 hours after treatment, proves that the percentage mortality caused depended on the type of insecticide used. Thus, the potency of the insecticides depends on the toxicity of the active ingredient, the mode of action and the residual potency of a particular insecticide (Singh, 2006; Nansen & Ridsdill-Smith, 2013). Therefore, findings from this study suggest that Emamectin benzoate, *Bacillus thuringiensis* and Acetamiprid+Indoxacarb are potent insecticides that can be used for the control of *Spodoptera frugiperda*.

Effect of aqueous neem extract on the larvae of *Spodoptera frugiperda*

The effect of the aqueous neem extract on the larvae of *S. frugiperda* was evident in the cessation of feeding and the inability of the larvae that moulted to completely shed off their exuviae. The remaining exuviae on the last two abdominal segments of the larvae is an indication of incomplete ecdysis, owing to the effect of the neem extract. Neem extract is noted for its effect on the process of ecdysis in insects (Nisbet, 2000).

The aqueous neem extract worked slowly, it took 8 days to achieve 100% mortality. However, it took 6 days for the highest concentration used, 1 g/mL, to achieve 100% mortality. The LC_{50} (based on percentage mean mortalities for the first five days) for the extracts was 0.3 g/mL and the LC_{90} was 0.78 g/mL. Such small concentrations indicate that the leaf extract is toxic enough to cause 50 and 90% mortalities. Ideally, 0.25 and 0.5 g/mL concentrations were not supposed to cause up to 100% mortality but both recorded 100% mortality 8 days after treatment. It is highly probable that, these levels of mortality were achieved because during the study, larvae fed on the treated leaves for the first two days but ceased feeding from the third day onward. Even though the treated leaves were replaced with fresh leaves after 72 hours, larvae could not feed. It is however not surprising that larvae stopped feeding on the neem-treated maize leaves because neem contains azadirachtin which is a known feeding deterrent (Martinez & Van Emden, 2001).

Nisbet (2000) described the effects of neem on insects as working as a deterrent to feeding, interfering mainly in the physiology of ecdysis and as a growth regulator which consequently results in the death of the insect.

Prates, Viana and Waquil (2003) also showed that neem extract works slowly when 1% neem extract caused low larval mortality in FAW for the first three days but mortality increased by the tenth day. Considering the duration taken for 100% mortality to be achieved, it can be predicted that neem extract requires a relatively longer period of time to work compared with most synthetic insecticides.

In a previous study, both neem seed and leaf extracts were assessed with a high concentration of 2% for both extracts but 100% mortality could not be achieved (Silva *et al.*, 2015). In that study, maize leaves were dipped in the extracts for 2 seconds but in the present study, leaves were dipped for 10 seconds. This could possibly account for the differences in mean mortalities obtained from the result of this study compared with the previous study done by Silva *et al*. (2015). Dipping leaves for two seconds may not be enough to cover the whole surfaces of the leaves with the insecticide and may result in certain portions of the leaves being devoid of the extract. Differences in the methods of preparation of the neem extract could also account for the different mean mortalities recorded by these researchers. In this study, fresh neem leaves were collected, weighed, blended and sieved before use. However, in the study by Silva *et al*. (2015), leaves were collected, dried and triturated and a stock solution prepared. Portions of the concentrated solution were diluted at different percentages, from 0.5 - 2% and used for the bioassays. It is apparent that, the method of preparation of the neem extracts might have rendered the active ingredients in the earlier study ineffective.

Mortality caused by the different concentrations of the aqueous neem extract together with the low LC_{50} and LC_{90} values, prove that this botanical insecticide is potent and could be considered for the control of the fall armyworm. Neem is not persistent in the environment but is easily degraded by ultraviolet light and rainfall and has low toxicity to mammals and other non-targeted beneficial organisms as compared with other chemical pesticides (Stone, 1992; Quarles, 1994). Considering the abundance and accessibility of neem in Ghana coupled with how easily the aqueous extract can be prepared, farmers should be encouraged to use it as one of their organic insecticides. A survey done by Tambo *et al*. (2020) in Ghana, showed that few (2.44%) farmers used neem-based products as control of the fall armyworm and found the method to be efficient. It has been ascertained from the current study that the aqueous neem extract is effective in controlling the larvae of the fall armyworm, therefore, it could be adopted and implemented by farmers in Ghana for the management of FAW.

Finally, neem is compatible with Integrated Pest Management practices and remains a pesticide to which resistance development would be difficult if not impossible due to its diverse modes of action. Therefore, this botanical insecticide should be considered as a traditional remedy in addition to the insecticides that have been recommended by the MoFA of Ghana.

CHAPTER FIVE

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

In this chapter, the summary, conclusions and recommendations of the study are presented. The main findings of the study are summarized, conclusions based on the findings are drawn and relevant recommendations based on the data obtained from the study are made.

Summary

The development of the fall armyworm (FAW), *Spodoptera frugiperda* was studied and the susceptibility of the eggs and second instar larvae to selected insecticides was determined. The duration of egg hatching, larval development, pupation, eclosion and adult lifespan studied at two different periods (December 2018 - January 2019 and February - March 2019) showed that, temperature and relative humidity affect the duration of the life stages of the fall armyworm. The duration for hatching of eggs, larval development, pupation, eclosion and adult lifespan was shorter during the February-March regime of the study, when the temperature was relatively high (31 - 34^{o}C) with a lower relative humidity (44 - 55%), than what was observed during the December-January regime when the temperature was relatively low (25 - 28^{o}C) with a higher relative humidity (75 - 80%). Though not a stated objective, the fall armyworm larvae sampled from one of the locations unknowingly came with a parasitoid (*Apocephalus borealis*) whose larvae caused 14.3% mortality in the pupae of the fall armyworm.

The outcome of the study on the duration of the development of FAW implies that relatively high temperatures which shortened the developmental period, would increase the abundance and further facilitate the spread of the

FAW in Ghana. The FAW is likely to persist throughout the year in Ghana, since it does not undergo diapause in its development coupled with the fact that Ghana is a tropical country which does not record extremely low temperatures (below 19^{o}C).

In order to ascertain the efficacy of four insecticides against the eggs of the fall armyworm, three egg masses for each insecticide were sprayed with recommended doses of the selected insecticides while the egg masses for the control were sprayed with water. In the assessment of the efficacy of five selected synthetic insecticides and one botanical insecticide (aqueous neem extract at three different concentrations) against the second instar larvae of the fall armyworm, the laboratory bioassay method known as “leaf dipping” was used. All the insecticides failed to prevent the treated eggs from hatching. This implies that the presence of eggs of the fall armyworm on an infested farm could result in the reappearance of the larvae after the farm has been sprayed, since the eggs are not susceptible to synthetic insecticides.

In the larval bioassay, Emamectin benzoate and *Bacillus thuringiensis* caused 100% mortality 24 hours after larvae fed on treated leaves while Acetamiprid+Indoxacarb caused 100% mortality after 48 hours. Acetamiprid+Lambda cyhalothrin and Imidacloprid could not cause high mortality at the recommended dose and when a sequel bioassay was done using twice the recommended dose, there was no significant difference in the mortality caused by the recommended and twice the recommended doses. The implication of this is that farmers using Acetamiprid+Lambda cyhalothrin and Imidacloprid might be applying overdose concentrations due to the ineffectiveness of these insecticides.

All the three concentrations of the aqueous neem extract used (0.25 g/mL, 0.5 g/mL and 1 g/mL) caused 100% mortality by the 8th day after larvae fed on treated leaves.

Conclusions

Temperature and relative humidity affect the development of *Spodoptera frugiperda*. A higher temperature with a lower relative humidity results in a shorter period for egg hatching, larval development, pupation, eclosion and adult life-span and vice versa.

The eggs of the fall armyworm are not susceptible to Emamectin benzoate, Acetamiprid+Indoxacarb, Acetamiprid+Lambda cyhalothrin and Imidacloprid. However, the second instar larvae of the fall armyworm were susceptible to the synthetic insecticides Emamectin benzoate, *Bacillus thuringiensis* and Acetamiprid+Indoxacarb and could be used for the management of fall armyworm.

The aqueous neem extract was found to be potent against the second instar larvae of fall armyworm and could be used as a traditional insecticide for the management of *Spodoptera frugiperda*.

Recommendations

I recommend that the data on the developmental periods ascertained from this study be used to predict the survival and the number of generations FAW can have within a year in Ghana; using these environmental conditions as baseline for the prediction.

Also, having ascertained that high temperature speeds up the completion of the lifecycle of the fall armyworm, intensive education should be given the public, on the need to minimize human activities that result in global warming and climate change. The populace must be taught to appreciate nature, use resources sustainably so that the stability of the ecosystem can be maintained.

Again, the susceptibility of the second instar larvae of fall armyworm to the selected insecticides suggests that management practices should be deployed early enough to target the early instars for effective control. In order to achieve this, farmers should be encouraged to do regular scouting for the eggs and signs of the presence of the larvae on their farms. Eggs could then be destroyed manually and potent insecticides applied against the larvae to prevent excessive damages to host plants. Farmers should be encouraged to adhere to the strict application of the recommended doses of synthetic insecticides to prevent any health problems they might be exposed to as well as to delay if not completely prevent the possible onset of insecticide resistance development in the fall armyworm. This would also prevent damages to benign and non-targeted animals and ensure the persistence of a nontoxic ecosystem. It would also minimize the cost farmers incur in controlling the pest population in case of infestations.

Moreover, more insecticides should be screened to ascertain their efficacy against the fall armyworm so that such insecticides could be recommended for the control of the pest in Ghana to prevent indiscriminate use of insecticides. Having found out that aqueous neem extract is effective against the second instar larvae of the fall armyworm, it could be used as an additional biopesticide to control FAW in order to minimise the use of synthetic insecticides which may have detrimental effects on the ecosystem.

Finally, geographical-based potential parasitoids and predators of the fall armyworm could be reared and augmented for biological control of the fall armyworm.

REFERENCES

Abbott, W. S. (1925). A method of computing the effectiveness of an insecticide. *Journal of economic Entomology*, *18*(2), 265-267.

Acar, E. B., Smith, B. N., Hansen, L. D., & Booth, G. M. (2001). Use of calorespirometry to determine effects of temperature on metabolic efficiency of an insect. *Environmental entomology*, *30*(5), 811-816.

Agboyi, L. K., Goergen, G., Beseh, P., Mensah, S. A., Clottey, V. A., Glikpo, R., Buddie, A., Cafà, G., Offord, L., Day, R., Rwomushana, I. & Kenis, M. (2020). Parasitoid complex of fall armyworm, *Spodoptera frugiperda*, in Ghana and Benin. *Insects*, *11*(2), 68.

Aguilon, D. J., & Velasco, L. R. (2015). Effects of larval rearing temperature and host plant condition on the development, survival, and coloration of African armyworm, *Spodoptera exempta* Walker (Lepidoptera: Noctuidae). *Journal of Environmental Science and Management*, *18*(1), 54-59.

Ali, A., Luttrell, R. G., & Schneider, J. C. (1990). Effects of temperature and larval diet on development of the fall armyworm (Lepidoptera: Noctuidae). *Annals of the Entomological Society of America*, *83*(4), 725-733.

Ashley, T. R. (1979). Classification and distribution of fall armyworm parasites. *Florida Entomologist*, 114-123.

Ashley, T. R., Wiseman, B. R., Davis, F. M., & Andrews, K. L. (1989). The fall armyworm: a bibliography. *Florida Entomologist*, 152-202.

Ayres, J. S., & Schneider, D. S. (2009). The role of anorexia in resistance and tolerance to infections in Drosophila. *PLoS Biology*, *7*(7), e1000150.

Barfield, C. S., Mitchell, E. R., & Poeb, S. L. (1978). A temperature-dependent model for fall armyworm development. *Annals of the Entomological Society of America*, *71*(1), 70-74.

Barros, E. M., Torres, J. B., Ruberson, J. R., & Oliveira, M. D. (2010). Development of Spodoptera frugiperda on different hosts and damage to reproductive structures in cotton. *Entomologia Experimentalis et Applicata*, *137*(3), 237-245.

Bass, M. H. (1978). Fall armyworm: evaluation of insecticides for control. *Leaflet 93*, 1-9.

Busato, G. R., Grützmacher, A. D., Garcia, M. S., Giolo, F. P., Zotti, M. J., Bandeira, J .D. M. (2005). Exigências térmicas e estimative do número de gerações dos biótipos “milho” e “arroz”de Spodoptera frugiperda. Pesq. Agropec. Bras. 40, 329–335.

Begon, M., Townsend, C. R., & Harper, J. L. (2006). Ecology: From Individuals to Ecosystems. Oxford, UK: Blackwell.

Belay, D. K., Huckaba, R. M., & Foster, J. E. (2012). Susceptibility of the fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae), at Santa Isabel, Puerto Rico, to different insecticides. *Florida Entomologist*, *95*(2), 476-478.

Berta, C. D., Virla, E., Colomo, M. V., & Valverde, L. (2000). Effect on the parasitoid Campoletis grioti of an insecticide used to control *Spodoptera frugiperda* and contributions to the bionomy of the parasitoid. *Integrated Pest Management (CATIE) (57)* 65-70.

Beuzelin, J., Brown, S., Davis, J., Foil, L. D., Hall, M., Huang, F., Kerns, D., Morgan, A., Pollet, D. & Reed, D. (2014). Cotton insect pest management, *Louisiana Insect Pest Management Guide*, 7-12.

Blanco, C. A., Pellegaud, J. G., Nava-Camberos, U., Lugo-Barrera, D., Vega-Aquino, P., Coello, J., Teran-Vargas, A. P. & Vargas-Camplis, J. (2014). Maize pests in Mexico and challenges for the adoption of integrated pest management programs. *Journal of Integrated Pest Management*, *5*(4), E1-E9.

Bosque-Pérez, N. A. (1995). Major insect pests of maize in Africa: biology and control. International Institute of Tropical Agriculture Research Guide 30. Training Programme, 2nd Edition, 9-16.

Busato, G. R., Grützmacher, A. D., Garcia, M. S., Giolo, F. P., Zotti, M. J., & Bandeira, J. D. M. (2005). Thermal requirements and estimate of the number of generations of biotypes "corn" and "rice" of *Spodoptera frugiperda*. *Pesquisa Agropecuária Brasileira*, *40* (4), 329-335.

Capinera, J. L. (2001). Handbook of vegetable pests 4th Edition. University of Florida. Retrieved from http://creatures.ifas.ufl.edu

Capinera, J. L. (2002). Fall armyworm, *Spodoptera frugiperda* (JE Smith) (Insecta: Lepidoptera: Noctuidae). *EDIS*, *2002*(7), 1-6.

Casmuz, A., Juarez, M. L., Socias, M. G., Murua, M. G., Prieto, S., Medina, S., Willink, E. & Gastaminza, G. (2017). Review of the host plants of fall armyworm, Spodoptera frugiperda (Lepidoptera: Noctuidae). *Revista de la Sociedad Entomológica Argentina*, *69*(3-4).

Castro, B. M. D. C., Martinez, L. C., Barbosa, S. G., Serrão, J. E., Wilcken, C. F., Soares, M. A., da Silva, A. A., de Carvalho, A.G & Zanuncio, J. C. (2019). Toxicity and cytopathology mediated by *Bacillus thuringiensis* in the midgut of *Anticarsia gemmatalis* (Lepidoptera: Noctuidae). *Scientific reports*, *9*(1), 1-10.

Day, R., Abrahams, P., Bateman, M., Beale, T., Clottey, V., Cock, M., Colmenarez, Y., Corniani, N., Early, R. Godwin, J. & Gomez, J. (2017). Fall armyworm: impacts and implications for Africa. *Outlooks on Pest Management*, *28*(5), 196-201.

Dent, D. (2000). *Insect Pest Management*. New York, USA: CABI publishing.

Du Plessis, H., Schlemmer, M. L., & Van den Berg, J., (2020). The effect of temperature on the development of *Spodoptera frugiperda* (Lepidoptera: Noctuidae). *Insects*, *11*(4), 228.

FAO (2015). World Food Programme; The State of Food Insecurity in the World 2014: Strengthening the Enabling Environment for Food Security and Nutrition, Rome: Food and Agriculture Organization International Fund for Agriculture Development.

FAO (2017). Staple foods: what do people eat? Corporate Document of Food and Agriculture Organization Repository. Retrieved from http://www.fao.org/docrep/u8480e/u8480e07.htm.

FAO (2018). Integrated management of the Fall Armyworm on maize a guide for Farmer Field Schools in Africa. Food and Agriculture Organization of the United Nations. Retrieved from http://www.fao.org/faostat/en/.

FAO (2019). "Global Platform: FAW Monitoring and Early Warning Systems. Food and Agriculture Organization of the United Nations.

Fotso Kuate, A., Hanna, R., Doumtsop Fotio, A. R., Abang, A. F., Nanga, S. N., Ngatat, S., Tindo, M., Masso, C., Ndemah, R., Suh, C. & Fiaboe, K. K. M. (2019). Spodoptera frugiperda Smith (Lepidoptera: Noctuidae) in Cameroon: Case study on its distribution, damage, pesticide use, genetic differentiation and host plants. *PloS one*, *14*(4), 0215749.

Godwin, J., Hevi, W., & Day, R. (2017). Fall armyworm response in Ghana: Stakeholders workshop. Accra-Ghana, Erata Hotel (May 2017).

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), 0165632.

Gomez, K. A., & Gomez, A. A. (1984). *Statistical Procedures For Agricultural Research*. John Wiley & Sons. Retrieved from: KA Gomez, AA Gomez - 1984 - books.google.com

Gross Jr, H. R., & Pair, S. D. (1986). The fall armyworm: status and expectations of biological control with parasitoids and predators. *Florida Entomologist*, *69*(3), 502-515.

Gutiérrez-Moreno, R., Mota-Sanchez, D., Blanco, C. A., Whalon, M. E., Terán-Santofimio, H., Rodriguez-Maciel, J. C., & DiFonzo, C. (2019). Field-evolved resistance of the fall armyworm (Lepidoptera: Noctuidae) to synthetic insecticides in Puerto Rico and Mexico. *Journal of Economic Entomology*, *112*(2), 792-802.

Hagstrum, D.W. & Hagstrum, W.R. (1970). A simple device for producing fluctuating temperatures, with an evaluation of the ecological significance of fluctuating temperatures. *Annals of Entomological Society of America*, *63*, 1385–1389

Hardke, J. T., Lorenz III, G. M., & Leonard, B. R. (2015). Fall armyworm (Lepidoptera: Noctuidae) ecology in southeastern cotton. *Journal of Integrated Pest Management*, *6*(1), 10.

Hardke, J. T., Temple, J. H., Leonard, B. R., & Jackson, R. E. (2011). Laboratory toxicity and field efficacy of selected insecticides against fall armyworm (Lepidoptera: Noctuidae). *Florida Entomologist*, *94*(2), 272-278.

Hogg, D. B., Pitre, H. N. & Anderson, R. E. (1982). Assessment of early-season phenology of the fall armyworm (Lepidoptera: Noctuidae) in Mississippi. Environmental Entomology 11, 705-710.

Howe, R. W., (1967). Temperature effects on embryonic development in insects. *Annual Review of Entomology*, *12*(1), 15-42.

Hugh, P., (2015). Field Crop and Forage Pests and their Natural Enemies in Western Canada: Identification and Management Field Guide. Available at www.publications.gc.ca. 9-10.

Hulbert, A. J., Pamplona, R., Buffenstein, R. & Buttemer, W. A. (2007). Life and Death: Metabolic Rate, Membrane Composition, and Life Span of Animals. *The American Physiological Society, Review 87*: 1175–1213, doi: 10.1152/physrev .00047.2006.

IRAC (2017). Mode of Action Classification Scheme. Version 8.3. Insecticide Resistance Action Committee, 5-10.

Integrated Taxonomic Information System (2020). Retrieved from http://www.itis.gov.

Isman, M. B. (2006). Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. *Annual Review Entomology*, *51*, 45-66.

Jansson, R. K., Peterson, R. F., Mookerjee, P. K., Halliday, W. R., Argentine, J. A., & Dybas, R. A. (1997). Development of a novel soluble granule formulation of emamectin benzoate for control of lepidopterous pests. *Florida Entomologist*, 425-443.

Karunaratne, S. H. P. P., Hawkes, N. J., Perera, M. D. B., Ranson, H., & Hemingway, J. (2007). Mutated sodium channel genes and elevated monooxygenases are found in pyrethroid resistant populations of Sri Lankan malaria vectors. *Pesticide Biochemistry and Physiology*, *88*(1), 108-113.

Kassie, M., Wossen, T., De Groote, H., Tefera, T., Sevgan, S., & Balew, S. (2020). Economic impacts of fall armyworm and its management strategies: evidence from southern Ethiopia. *European Review of Agricultural Economics*.

Kumela, T., Simiyu, J., Sisay, B., Likhayo, P., Mendesil, E., Gohole, L., & Tefera, T. (2019). Farmers' knowledge, perceptions, and management practices of the new invasive pest, fall armyworm (*Spodoptera frugiperda*) in Ethiopia and Kenya. *International Journal of Pest Management*, *65*(1), 1-9.

Leibee, G. L., Jansson, R. K., Nuessly, G., & Taylor, J. L. (1995). Efficacy of emamectin benzoate and *Bacillus thuringiensis* at controlling diamondback moth (Lepidoptera: Plutellidae) populations on cabbage in Florida. *Florida Entomologist*, 82-96.

Leistra, M., Zweers, A. J., Warinton, J. S., Crum, S. J., Hand, L. H., Beltman, W. H., & Maund, S. J. (2004). Fate of the insecticide lambda-cyhalothrin in ditch enclosures differing in vegetation density. *Pest Management Science: formerly Pesticide Science*, *60*(1), 75-84.

Luginbill, P. (1928). The fall armyworm. United States departament of agriculture, Washington, DC, USA. *Technical Bulletin*, *34*.

Malo, E. A., Bahena, F., Miranda, M. A., & Valle-Mora, J. (2004). Factors affecting the trapping of males of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) with pheromones in Mexico. *Florida Entomologist*, *87*(3), 288-293.

Martínez, A. M., Aguado-Pedraza, A. J., Viñuela, E., Rodríguez-Enríquez, C. L., Lobit, P., Gómez, B., & Pineda, S. (2017). Effects of ethanolic extracts of *Argemone ochroleuca* (Papaveraceae) on the food consumption and development of *Spodoptera frugiperda* (Lepidoptera: Noctuidae). *Florida Entomologist*, *100*(2), 339-345.

Martinez, S. S., & Van Emden, H. F. (2001). Growth disruption, abnormalities and mortality of *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) caused by azadirachtin. *Neotropical Entomology*, *30*(1), 113-125.

Meagher Jr, R. L. (2001). Collection of fall armyworm (Lepidoptera: Noctuidae) adults and nontarget Hymenoptera in different colored unitraps. *Florida Entomologist*, 77-82.

Metcalf, R.L. & Metcalf, E.R. (1992). Plant kairomones in insect ecology and control. 1st edition. Chapman and Hall Ltd, UK.

Midega, C. A., Pittchar, J. O., Pickett, J. A., Hailu, G. W., & Khan, Z. R. (2018). A climate-adapted push-pull system effectively controls fall armyworm, *Spodoptera frugiperda* (JE Smith), in maize in East Africa. *Crop Protection*, *105*, 10-15.

Miresmailli, S., & Isman, M. B. (2014). Botanical insecticides inspired by plant–herbivore chemical interactions. *Trends in Plant Science*, *19*(1), 29-35.

Mitchell, E. R., Waddill, V. H., & Ashley, T. R. (1984). Population dynamics of the fall armyworm (Lepidoptera: Noctuidae) and its larval parasites on whorl stage corn in pheromone-permeated field environments. *Environmental Entomology*, *13*(6), 1618-1623.

Mochiah, M. B., Banful, B., Fening, K. N., Amoabeng, B. W., Ekyem, S., Braimah, H., & Owusu-Akyaw, M. (2011). Botanicals for the management of insect pests in organic vegetable production. *Journal of Entomology and Nematology*, *3*(6), 85-97.

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. *Florida Entomologist*, *86*(3), 254-289.

Montandon, R., Stipanovic, R. D., Williams, H. J., Sterling, W. L., & Vinson, S. B. (1987). Nutritional indices and excretion of gossypol by *Alabama argillacea* (Hübner) and *Heliothis virescens* (F.) (Lepidoptera: Noctuidae) fed glanded and glandless cotyledonary cotton leaves. *Journal of Economic Entomology*, *80*(1), 32-36.

Nagoshi, R. N., & Meagher, R. L. (2004). Seasonal distribution of fall armyworm (Lepidoptera: Noctuidae) host strains in agricultural and turf grass habitats. *Environmental Entomology*, *33*(4), 881-889.

Nansen, C., & Ridsdill-Smith, T. J. (2013). The performance of insecticides–a critical review. *Insecticides. InTech Europe, Croatia*, 195-232.

National Research Council (US) Panel on Neem (1992). Neem: A Tree for Solving Global Problems. Washington (DC): National Academies Press (US); 5, Effects on Insects. Retrieved from: https://www.ncbi.nlm.nih.gov/books/ NBK234642/

Neven, L. G. (2000). Physiological responses of insects to heat. *Postharvest Biology and Technology*, *21*(1), 103-111.

Nisbet, A. J. (2000). Azadirachtin from the neem tree *Azadirachta indica*: its action against insects. *Anais da Sociedade Entomológica do Brasil*, *29*(4), 615-632.

Owusu, M.E. (2017). Highly effective pesticides to fight fall armyworm. Retrieved from www.ghananewsagency.org

Pannuti, L. E. R., Baldin, E. L. L., Hunt, T. E., & Paula-Moraes, S. V. (2016). On-plant larval movement and feeding behavior of fall armyworm (Lepidoptera: Noctuidae) on reproductive corn stages. *Environmental Entomology*, *45*(1), 192-200.

Paramasivam, M. & Selvi, C. (2017). Laboratory bioassay methods to assess the insecticide toxicity against insect pests-A review. *Journal of Entomology and Zoology Studies*, 5(3), 1441-1445.

Pashley, D. P. (1986). Host-associated genetic differentiation in fall armyworm (Lepidoptera: Noctuidae a sibling species complex? Annals of Entomological Society of America, 79: 898-904.

Peairs, F. B., & Saunders, J. L. (1979). Fall armyworm, *Spodoptera frugiperda* (JE Smith): a review. *Ceiba*.

Pitre, H. N. (1986). Chemical control of the fall armyworm (Lepidoptera: Noctuidae): an update. *Florida Entomologist*, 570-578.

Pitre, H. N. (1988). Relationship of fall armyworm (Lepidoptera: Noctuidae) from Florida, Honduras, Jamaica, and Mississippi: susceptibility to insecticides with reference to migration. *Florida Entomologist*, 56-61.

Pitre, H. N., & Hogg, D. B. (1983). Development of the fall armyworm on cotton, soybean and corn [*Spodoptera frugiperda*]. *Journal of the Georgia Entomological Society*.

Planet Natural Research Center (2004). Answers and Advice for Organic Gardeners-*Bacillus thuringiensis* products.

Prasanna, B. M., Huesing, J. E., Eddy, R., & Peschke, V. M. (2018). Fall armyworm in Africa: a guide for integrated pest management. Retrieved from: repository.cimmyt.org

Prates, H. T., Viana, P. A., & Waquil, J. M. (2003). Activity of neem tree (*Azadirachta indica*) sobre *Spodoptera frugiperda*. *Pesquisa Agropecuária Brasileira (Brazil)*.

Quarles, W. (1994). Neem tree pesticides protect ornamental plants. *The IPM Practitioner: The Newsletter of Integrated Pest Management (USA)*.

Quisenberry, S. S. (1991). Fall armyworm (Lepidoptera: Noctuidae) host strain reproductive compatibility. *Florida Entomologist*, 194-199.

Roel, A. R., Dourado, D. M., Matias, R., Porto, K. R., Bednaski, A. V., & Costa, R. B. D. (2010). The effect of sub-lethal doses of *Azadirachta indica* (Meliaceae) oil on the midgut of *Spodoptera frugiperda* (Lepidoptera, Noctuidae). *Revista Brasileira de Entomologia*, *54*(3), 505-510.

Schmutterer, H. (1990). Properties and potential of natural pesticides from the neem tree, *Azadirachta indica*. *Annual review of entomology*, *35*(1), 271-297.

Sharnbasappa, Kalleshwaraswamy, C.M., Maruthi, M.S. & Pavithra, H.B. (2018). Biology of invasive fall army worm *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) on maize. *Indian Journal of Entomology*, *80(3)*, 540-543.

Sifuentes, A. J.A. (1967). Oviposition of fall armyworm moths and damage to larvae in corn and sorghum seedlings in a greenhouse. *Technical Agriculture in Mexico*, *2* (7).

Silva, D. M. D., Bueno, A. D. F., Andrade, K., Stecca, C. D. S., Neves, P. M. O. J., & Oliveira, M. C. N. D. (2017). Biology and nutrition of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) fed on different food sources. *Scientia Agricola*, *74*(1), 18-31.

Silva, M. S., Forti Broglio, S. M., Prédes Trindade, R. C., Ferrreira, E. S., Gomes, I. B. & Micheletti, L.B. (2015). Toxicity and Application of Neem on Fall Armyworm. *Comunicata Scientiae 6*(3): 359-364

Simmonds, M. S., Jarvis, A. P., Johnson, S., Jones, G. R., & Morgan, E. D. (2004). Comparison of anti-feedant and insecticidal activity of nimbin and salannin photo-oxidation products with neem (*Azadirachta indica*) limonoids. *Pest Management Science: formerly Pesticide Science*, *60*(5), 459-464.

Singh, D. K. (2006). Toxicology of Insecticides. University of Delhi, Delhi-110007, India. 1-8.

Sisay, B. (2018). *Evaluation of Different Management options of Fall Armyworm, (JE Smith) (Lepidoptera: Noctuidae) and Assessment of its Parasitoids in Some Parts of Ethiopia*. Unpublished Doctoral dissertation, Haramaya University. Ethiopia

Sisay, B., Simiyu, J., Malusi, P., Likhayo, P., Mendesil, E., Elibariki, N., Wakgari, M., Ayalew, G. & Tefera, T. (2018). First report of the fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae), natural enemies from Africa. *Journal of Applied Entomology*, *142*(8), 800-804.

Sisay, B., Tefera, T., Wakgari, M., Ayalew, G., & Mendesil, E. (2019). The efficacy of selected synthetic insecticides and botanicals against fall armyworm, *Spodoptera frugiperda*, in maize. *Insects*, *10*(2), 45.

Smith, R. A. (1966). Principles, definitions and scope of integrated pest control. In *Proc FAO Symp Integr Contr* (1), 11-17.

Sparks, A. N. (1979). Fall Armyworm Symposium: A review of the biology of the fall armyworm. *Florida Entomologist*, 82-87.

Sparks, A. N. (1986). Fall armyworm (Lepidoptera: Noctuidae): potential for area-wide management. *Florida Entomologist*, 603-614.

Spiller, D. (1958). Variations in the Insecticide Tolerance of Insects. *Nature*, *182*(4643), 1177-1177.

Stevenson, P. C., Isman, M. B., & Belmain, S. R. (2017). Pesticidal plants in Africa: a global vision of new biological control products from local uses. *Industrial Crops and Products*, *110*, 2-9.

Stipanovic, R. D., Lopez, J. D., Dowd, M. K., Puckhaber, L. S., & Duke, S. E. (2006). Effect of racemic and (+)-and (−)-gossypol on the survival and development of *Helicoverpa zea* larvae. *Journal of Chemical Ecology*, *32*(5), 959-968.

Stone, R. (1992). A Biopesticidal Tree Begins to Blossom. *Science*. February 28, 1992.

Straub, R., & Hogan, H. (1974). Feasibility of fall armyworm, *Spodoptera frugiperda* (Smith) control on late-planted dent corn.

Tambo, J. A., & Mockshell, J. (2018). Differential impacts of conservation agriculture technology options on household income in Sub-Saharan Africa. *Ecological Economics*, *151*, 95-105.

Tambo, J. A., Day, R. K., Lamontagne-Godwin, J., Silvestri, S., Beseh, P. K., Oppong-Mensah, B., Phiri, N. A. & Matimelo, M. (2020). Tackling fall armyworm (*Spodoptera frugiperda*) outbreak in Africa: an analysis of farmers' control actions. *International Journal of Pest Management*, *66*(4), 298-310.

Teklewold, H., Kassie, M., Shiferaw, B., & Köhlin, G. (2013). Cropping system diversification, conservation tillage and modern seed adoption in Ethiopia: Impacts on household income, agrochemical use and demand for labor. *Ecological Economics*, *93*, 85-93.

Tembo, Y., Mkindi, A. G., Mkenda, P. A., Mpumi, N., Mwanauta, R., Stevenson, P. C., Ndakidemi, P. A. & Belmain, S. R. (2018). Pesticidal plant extracts improve yield and reduce insect pests on legume crops without harming beneficial arthropods. *Frontiers in Plant Science*, *9*, 1425.

Thomas, M., & Waage, J. (1996). *Integration of biological control and host-plant resistance breeding: a scientific and literature review*. CTA.

Tobin, P. C., Nagarkatti, S., & Saunders, M. C. (2003). Phenology of grape berry moth (Lepidoptera: Tortricidae) in cultivated grape at selected geographic locations. *Environmental Entomology*, *32*(2), 340-346.

Wayne A. Gardner & Fuxa. R.J., (1980). Pathogen for the suppression of fall armyworm. *Florida Entomological Society*, *63*(4): 439-447.

Whitford, F., Quisenberry, S. S., Riley, T. J., & Lee, J. W. (1988). Oviposition preference, mating compatibility, and development of two fall armyworm strains. *Florida Entomologist*, 234-243.

Wiseman, B. R., & Davis, F. M. (1990). Plant resistance to insects attacking corn and grain sorghum. *Florida Entomologist*, 446-458.

Wyatt, T. D. (1997). Putting pheromones to work: paths forward for direct control. *Insect Pheromone Research* (445-459).

Xu, T., Wei, K. Y., Wang, J., Ma, H. X., Li, J., Xu, Y. J., & Li, Q. X. (2010). Quantitative analysis of the neonicotinoid insecticides imidacloprid and thiamethoxam in fruit juices by enzyme-linked immunosorbent assays. *Journal of AOAC International*, *93*(1), 12-18.

Young, J. R. (1979). Fall armyworm: control with insecticides. *Florida Entomologist*, 130-133.

Yu, S. J. (1991). Insecticide resistance in the fall armyworm, *Spodoptera frugiperda* (JE Smith). *Pesticide Biochemistry and Physiology*, *39*(1), 84-91.

APPENDICES

APPENDIX A: Egg hatching period for 25 - 28 and 31 - 34^{o}C

t-Test: Paired Two Sample for Means

	Variable 1	*Variable 2*
Mean	2.466667	2
Variance	0.266667	0
Observations	15	15
Pearson Correlation	#DIV/0!	
Hypothesized Mean Difference	0	
df	14	
t Stat	3.5	
P(T<= t) one-tail	0.001768	
t Critical one-tail	1.76131	
P(T<= t) two-tail	0.003535	
t Critical two-tail	2.144787	

APPENDIX B: Larval Development for 25-28 and 31-34°C

t-Test: Paired Two Sample for Means

	Variable 1	*Variable 2*
Mean	21.53333	10.93333
Variance	2.12381	0.780952
Observations	15	15
Pearson Correlation	-0.08135	
Hypothesized Mean Difference	0	
df	14	
t Stat	23.26335	
P(T< = t) one-tail	6.87E-13	
t Critical one-tail	1.76131	
P(T< = t) two-tail	1.37E-12	
t Critical two-tail	2.144787	

APPENDIX C: Pupa - Adult Development

t-Test: Paired Two Sample for Means

	Variable 1	*Variable 2*
Mean	9.333333	8.133333333
Variance	0.380952	0.40952381
Observations	15	15
Pearson Correlation	0.241121	
Hypothesized Mean Difference	0	
df	14	
t Stat	6	
P(T< = t) one-tail	1.63E-05	
t Critical one-tail	1.76131	
P(T< = t) two-tail	3.25E-05	
t Critical two-tail	2.144787	

APPENDIX D: Overall Development (Egg-Adult)

t-Test: Paired Two Sample for Means

	Variable 1	*Variable 2*
Mean	33.33333	21.06667
Variance	1.809524	0.495238
Observations	15	15
Pearson Correlation	-0.32697	
Hypothesized Mean Difference	0	
df	14	
t Stat	27.78418	
P(T< = t) one-tail	6.01E-14	
t Critical one-tail	1.76131	
P(T< = t) two-tail	1.2E-13	
t Critical two-tail	2.144787	

APPENDIX E: Summary Output of the Regression Statistics of Mortality Caused by Neem Extract

Regression Statistics	
Multiple R	0.965061243
R Square	0.931343202
Adjusted R Square	0.862686405
Standard Error	0.355176013
Observations	3

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	1.71125	1.71125	13.5652	0.168780202
Residual	1	0.12615	0.12615		
Total	2	1.8374			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	6.605	0.324229857	20.37135033	0.0312257	2.485269059	10.724731	2.48526906	10.7247309
X Variable 1	3.072783488	0.834293495	3.683096545	0.1687802	-7.52792047	13.673487	-7.5279205	13.6734874

RESIDUAL OUTPUT

Observation	*Predicted Y*	*Residuals*
1	4.755	0.145
2	5.68	-0.29
3	6.605	0.145