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FUNCTIONING OF A COMMUNITY OF LEPIDOPTERAN MAIZE STEMBORERS AND ASSOCIATED PARASITIDS FOLLOWING THE FALL ARMYWORM INVASION IN KENYA

Bonoukpé Mawuko

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**FUNCTIONING OF A COMMUNITY OF LEPIDOPTERAN MAIZE
STEMBORERS AND ASSOCIATED PARASITIDS FOLLOWING
THE FALL ARMYWORM INVASION IN KENYA**

BONOUKPOÈ MAWUKO SOKAME

A80/52426/2017

**A THESIS SUBMITTED IN FULFILMENT OF THE
REQUIREMENTS FOR THE AWARD OF THE DEGREE OF
DOCTOR OF PHILOSOPHY IN CROP PROTECTION**

DEPARTMENT OF PLANT SCIENCE AND CROP PROTECTION

FACULTY OF AGRICULTURE

UNIVERSITY OF NAIROBI

Soutenance le 26/11/2020

2020

DECLARATION

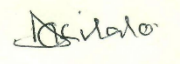
I, Bonoukpoè Mawuko Sokame, duly declare that this thesis is my original work and has not been presented for a degree or any award in any other University.

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and associated parasitoids following the Fall Armyworm
invasion in Kenya

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DEDICATION

To my late elder sister Mrs Houchi Sokame for your constant sacrifices and supports during your life. This PhD thesis is the fruit of your dreams for me which has been materialized today. May the earth be light to you, and may your soul be accepted in the crown of God.

To my beloved mother Mrs Sénadé Sossa-Sokame and my late father Mr Edou Sokame who took me to school and encouraged me to acquire knowledge in the field of agriculture. Thanks for your sacrifices.

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LISTE OF ABBREVIATIONS AND ACRONYMS

°C	Degree Celsius
ANOVA	Analysis of Variance
ARCU	Animal Rearing Containment Unit
Bf	<i>Busseola fusca</i>
BCED-Unit	Behavioural and Chemical Ecology Department Unit
CAVS	College of Agriculture and Veterinary Sciences
CCA	Canonical Correspondence Analysis
Cp	<i>Chilo partellus</i>
FAW	Fall ArmyWorm
GC/MS	Gas Chromatograph/ Mass Spectrometry
GLM	Generalized Linear Model
HIPVs	Herbivore-Induced Plant Volatiles
<i>icipe</i>	International Centre of Insect Physiology and Ecology
IPM	Integrated Pest Management
IRD	Institut de Recherche pour le Developpement
L:D	Light-Darkness photoperiod proportion
LR	Likelywood Ratio
NMDS	Non-metric Multi-Dimensional Scaling
NSBB	Noctuid Stem Borer Biodiversity
OR	Odd Ratio
PCA	Principal Component Analysis
PVC	PolyVinyl Chloride
RGR	Relative Growth Rate
Sc	<i>Sesamia calamistis</i>
SNK	Student-Newman and Keuls
SPME	Solid Phase MicroExtraction
VOCs	Volatile Organic Compounds

ABSTRACT

Lepidopteran stemborers are still among the most important pests that are reported to cause maize yield losses in sub-Saharan Africa. In Kenya, there are three main stemborer species, *Busseola fusca*, *Sesamia calamistis* and *Chilo partellus* that seriously limit potential maize yield. The recent invasion of the fall armyworm (FAW), *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in the maize growing region of the country has further complicated the management of stemborer pests in maize fields. For proper management of maize pests, the knowledge of the behavioural and chemical ecology, eco-environmental factors and interaction among various pests species in the field is essential. Therefore, this study aimed to identify the main reservoir source of maize stemborers and associated parasitoids that give rise to new populations in succeeding cropping season and evaluate the chemical basis of the female moth oviposition site preference. Furthermore, the communal larval interactions among the stemborer species and FAW larvae and the factors such as larval dispersal potential, environment temperature, larval density and duration of the interactions as well as the susceptibility of FAW larvae to the stemborer associated parasitoids were also studied. The occurrence of maize stemborers and associated parasitoids was investigated in maize stem residues and wild grasses during the non-cropping seasons as potential carry-over populations to subsequent early-season maize plants. Chemical mechanism of oviposition choice was conducted in a community of noctuids, *B. fusca* and *S. calamistis* and crambid *C. partellus* between un-infested, conspecific and interspecific larvae-infested maize plants. The impact of FAW introduction in maize stemborers and associated parasitoids communities was evaluated in maize fields. The performance studies were also carried out on the associated larval parasitoids of maize stemborers, namely *Cotesia flavipes*, *C. sesamiae* Inland, *C. sesamiae* Coastal and *C. typhiae* (Hymenoptera: Braconidae) in laboratory conditions with FAW larvae as host. Larval dispersal either through ballooning or crawling was compared between FAW and individual stemborer species. The study of factors that influence the interactions of stemborers larval communities with the FAW with respect to temperatures (15°C, 20°C, 25°C and 30°C), larval densities (4 larvae, 8 larvae and 12 larvae), and the durations of the interactions (5 days, 10 days, 15 days and 20 days) was conducted using restricted artificial stems in the laboratory. The sources of infestation study showed that

the carry-over of these stemborer species and their associated parasitoids during the non-cropping season was significantly ($P < 0.05$) ensured by the maize residues left in the fields from the previous harvests. The chemical basis of female moth oviposition site preference data highlighted that either conspecific or heterospecific larvae-infested maize plants elicited specific volatile signatures that attract female moths for oviposition. Following the introduction of FAW in maize fields, the study showed that single-species infestation significantly ($P < 0.05$) decreased in maize field upon communal stemborer and fall armyworm larval interactions. It was also recorded that multi-species significantly ($P < 0.05$) increased at field level with the introduction of FAW while the overall lepidopteran infestation incidences and larval densities significantly ($P < 0.05$) increased leading to the accelerated increase of yield losses in the fields. All the tested associated parasitoids inserted their ovipositor into the FAW larvae but without depositing eggs in them except *C. typhiae*. However, they induced significant ($P < 0.05$) non-reproductive mortality of FAW larvae. The FAW neonate larvae had a significant ($P < 0.05$) greater potential of dispersion than of the stemborer species counterparts. Temperature was an important factor that significantly ($P < 0.05$) influenced the intra- and interspecific interactions, both on survival and relative growth rates (RGR) between larvae of the three stemborer species and the FAW larvae using the same resource. However, in interspecific interactions, the competition was significantly ($P < 0.05$) less pronounced between the FAW and the stemborers compared to that among the stemborer species across the temperature tested. The results also showed that negative density-dependence survival and RGR significantly ($P < 0.05$) affected both survival and RGR. The time partitioning of the resource use significantly ($P < 0.05$) influenced the coexistence of these competing insect species. The carry-over niches findings give rise to new habitat management considerations in IPM strategies. Attractant volatiles identified generate applications in the development of a multi-species lure targeting female moths. FAW constitutes an additional production constraint of cereal crops that can co-exist with stemborer species along different temperature gradients. In addition, the nonreproductive mortality induced by common stemborer specific parasitoids can be explored further as part of FAW biological control contribution.

CHAPTER ONE

INTRODUCTION

1.1. Background

African traditional crops (Poaceae) including sorghum (*Sorghum* spp.), pearl millet (*Pennisetum glaucum* (L.) R. Br.), finger millet (*Eleusine coracana* (L.) Gaertn.) and African rice (*Oryza glaberrima* Steud.) are among the most cultivated cereal crops in Africa. However, maize (*Zea mays* L.) cultivation has exceeded the production of the traditional cereals, since its introduction into Africa during the 16th century (Macauley & Ramadjita, 2015). Today, maize is the main staple food crop grown in diverse African agro-ecological zones and farming systems (Macauley & Ramadjita, 2015). It is estimated that approximately 208 million people in the African continent depend on maize as a staple (Macauley & Ramadjita, 2015). For example, in Kenya, where maize is considered a staple crop, the national food security for the country has often been pegged on the availability of adequate supplies to meet the over-increasing domestic demand (FAOSTAT, 2017). In 2014, the total area under maize cultivation in Kenya was estimated at 2.1 million hectares yielding a total of 3.6 million tonnes (FAOSTAT, 2017). Most of the maize production is by small scale farmers reported to account for over 70% of the total production (De Groote, 2002).

Many abiotic and biotic constraints limit maize production in East Africa. Among the biotic constraints, the insect pests, especially lepidopteran stemborers and the recent invasion of fall armyworm are considered the most crucial pests of maize in the region. These insect pests are reported to severely limit the potentially attainable maize yields as a result of

continuous infestation of the crop throughout its growth stages, from seedling to maturity (Oben *et al.*, 2015). Estimates of crop loss due to stemborers are reported to vary widely ranging between 30 to 100 % depending on the regions and the agro-ecological zones (Kfir *et al.*, 2002). In Kenya, the annual yield loss due to stemborers infestation has been estimated to cost Kshs 7.2 billion (US\$ 90 million) (EPZA [2005] cited by Midingoyi *et al.* [2016]).

The most economically important maize lepidopteran stemborers in Africa belong to the family of the Pyralidae, Noctuidae and Crambidae. The noctuids *Busseola fusca* (Fuller), *Sesamia calamistis* Hampson and the crambid *Chilo partellus* (Swinhoe) are considered the most damaging. Cumulatively, these pests are reported to cause losses ranging from 10 to 70 % of the expected yields (Kfir *et al.*, 2002; Sétamou *et al.*, 2000; Polaszek, 1998). In Kenya, these three major stemborer species co-infest maize fields especially in the mid-altitudes with variation in species dominance depending on location and season (Ong'amo *et al.*, 2006a; Guofa *et al.*, 2001). Several methods have been used for their management including chemical control, biological control, host plant resistance, crop rotation, cultural practices and integrated pest management (IPM). In biological control program of these stemborers, the most common parasitoids used in order of increasing efficiency are the larval parasitoids including *Cotesia flavipes* Cameron and *Cotesia sesamiae* (Cameron) (Hymenoptera: Braconidae), the pupal parasitoids are *Xanthopimpla stemmator* (Hymenoptera: Ichneumonidae) *Pediobius furvus* Gahan (Hymenoptera: Eulophidae) and the tachinid *Siphona* sp. respectively (Mailafiya *et al.*, 2009). The larval parasitoids *C. flavipes* and *C. sesamiae* are the most efficient in the control of lepidopteran stemborers in Kenya (Kfir *et al.*, 2002; Overholt *et al.*, 1997).

Maize stemborers have been the major pests of maize in Africa for a long time, until recently when the invasion of fall armyworms *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera, Noctuidae) was reported West and Central Africa causing extensive damage to the maize crop (Cock *et al.*, 2017; Goergen *et al.*, 2016). Since then, this pest has spread to most Sub-Saharan African Countries. Substantial economic impacts of fall armyworm on agricultural productivity have been reported across Africa (CAB International, 2017). For example, preliminary assessments on the economic impact of this pest on maize yield losses in 12 major maize producing countries of Africa has been estimated to range between 8.3 to 20.6 M metric tonnes annually (CAB International, 2017; Day *et al.*, 2017). Globally and outside Africa, the fall armyworm is reported to be a major insect pest of maize in Brazil and is estimated to cause up to 34% reduction in grain yield in the country (Lima *et al.*, 2010). In Kenya, the pest was initially reported in the western part of the country in 2017 and was confirmed to have spread throughout the country by the early cropping season in 2018 (Sisay *et al.*, 2018).

Maize lepidopteran pests, stemborers (Le Ru *et al.*, 2006a & 2006b) or fall armyworm (Goergen *et al.*, 2016) cause widespread destruction. It is imperative to determine the mechanisms that drive the ecology of the major groups of destructive maize insects and evaluate whether the invasive fall armyworm can be controlled by biological control options using the stemborer associated parasitoids. In maize fields, the maize stemborer pests and the invasive fall armyworm are reported to exist as communities or species with differential interactions (Hailu *et al.*, 2018). It has already been demonstrated that within insect communities both direct and indirect interactions occur, which lead to either positive, negative or neutral outcomes within and between different species. These usually

affect the structure and functions of insect communities on the plants (Kaplan & Eubanks, 2005; Wootton & Emmerson, 2005). These outcomes dependent on conspecific or heterospecific interactions, the larval dispersal potential of the species involved in the interactions, the environment temperature, larval density and the duration of the competition among the species (Ntiri *et al.*, 2016; Berger, 1992). Generally, competitive interactions among insect species often result in negative outcomes, while facilitative interactions result in positive outcomes on species involved (Denno & Kaplan, 2007; Denno *et al.*, 1995).

1.2. Statement of the problem

Globally, the demand for maize as a food crop is on the increase in most developing countries. It has been estimated that by the year 2025, maize is projected to become the most widely produced crop (Rosegrant *et al.*, 2008). In Kenya, although about 98 % of Kenya's 3.5 million small-scale farmers grow maize (Kariuki, 2015), the maize food security situation is still of a great challenge (Kariuki, 2015). This has partly been attributed to constant losses in maize yields over the years largely due to the lepidopteran stemborers (Midingoyi *et al.*, 2016; Oben *et al.*, 2015; Sétamou *et al.*, 2000) and recently by fall armyworm field crop infestations (Hailu *et al.*, 2018).

In order to limit insect pest infestation levels, various management strategies including chemical, biological, cultural control strategies as well as use of semiochemicals among others have been developed (Azerefegne & GebreAmlak, 1994). However, a number of these pest control approaches have not been widely adopted due to several socio-economic and biological challenges (Van den Berg, 1997). In particular, the use of chemical

pesticides to control stemborer and fall armyworm pests is largely ineffective. This has been partly associated with the physical protection provided to the immature stemborer pests by the host crop stem and the resistance of fall armyworm towards a number of commercial and conventional pesticides. Therefore, proper management of these lepidopteran and the recent invasive fall armyworm calls for an integrated and sustainable pest management approaches.

From one maize cropping season to another, hibernating stemborers are reported to take refuge in other and adjacent habitats that later give rise to infestations in the new crop (Matama-Kauma *et al.*, 2008; Le Ru *et al.*, 2006a & 2006b; Khan *et al.*, 1997) while no information regarding the associated parasitoids is available for better habitat management for conservative biological control design. Furthermore, induction of volatile emissions by stemborer infested host plants has recently been a subject of several studies aimed on the understanding of how host plant preferences are achieved among a community of stemborer species (Calatayud *et al.*, 2008a; Glas *et al.*, 2007; Van Den Berg, 2006; van den Berg *et al.*, 2006). However, the information on how these infochemicals drive the conspecific and interspecific interactions in female moth oviposition site selection remains understudied for the development of a multi-species lure purpose. In addition, the invasion of stemborer infested maize fields by the fall armyworm might induce a disequilibrium in maize stemborers communities population depending on the factors such as the larval dispersal potential, the environment temperature, larval density and the duration of the interaction as well as parasitism by stemborer associated parasitoids.

1.3. Justification and significance of the study

Lepidopteran stemborers seriously limit the potential maize yields by infesting the crop throughout its growth stages (Van den Berg, 2010). In Kenya, maize yield losses due to lepidopteran stemborers attack have been estimated to be 12.9 %, amounting to 0.39 million tonnes of the country's total annual maize production (De Groot, 2002).

Several methods that include chemical control, biological control, host plant resistance, crop rotation and cultural practices (Azerefege & GebreAmlak, 1994) have been used for the management of lepidopteran stemborers in maize fields across Africa. Moreover, an Integrated Pest Management (IPM) system combining control components such as chemical, host plant resistance, biological and cultural practices have also been developed to better manage these pests (Akinsola, 1990). Although all these control methods play an important role in the management of stemborers, most of them have some inherent limitations that impact negatively on resource-poor farming communities where these methods are commonly practiced. Cost of inputs, insect resistance, inefficiency in the reduction of the pests population below economic damage levels have been demonstrated to be the major constraints of the methods (Kfir, 1994; 1990; Dent, 1991). Furthermore, the recent outbreak of the fall armyworm, *Spodoptera frugiperda* (J. E. Smith) in East Africa, especially in maize fields, has further complicated the already complex management strategies for the stemborer pests. For proper management of maize pests, the knowledge of the behavioural and chemical ecology, eco-environmental factors and interaction among various pests species in the field is essential.

The identification of the sources of stemborer infestations and associated parasitoids have the advantage of providing data that is essential for habitat management in the

agroecosystem. The information concerning the main reservoir source of maize stemborers and associated parasitoids that give rise to new populations as well as indicators of the intensity and species dominance in the succeeding farming season is necessary for the development of an integrated pest management strategy for lepidopteran maize stemborers. In a semiochemical-based stemborer management approaches, lepidopteran sex pheromones and the push-pull system have already been successfully applied (Khan *et al.*, 2000). Moreover, some studies have demonstrated that, following infestation of their host plants, stemborers, induce the production of plant volatiles (VOCs). These plant volatiles may be in the form of new compounds unrelated to those released by the healthy intact plant (Bruce *et al.*, 2005; Dicke & van Loon, 2000) and have been associated with female attraction to host plant for oviposition (Bruce *et al.*, 2005; Dicke & van Loon, 2000). For instance, Ntiri *et al.*, (2018) demonstrated that *B. fusca*, *C. partellus* and *S. calamistis* females exhibited a significant preference for infested over un-infested maize plants for oviposition. Although knowledge of the chemical compounds in host preference by the female stemborers can open new avenues for the development of specific chemical attractants to trap female stemborer moths in the field, there is scarce information on the specific compounds or blends that signal oviposition suitability of the infested host plants to these female moths. Furthermore, no studies have investigated the behaviour or the fate of stemborers community and associated parasitoids when their niche is invaded by other insect species as has recently occurred following the invasion by fall armyworm. Information on the behaviour of the stemborers species and associated parasitoids in the presence of other species is thus key when successful management strategies are considered.

Hence this study entailed the identification of the main reservoir source of maize stemborers and associated parasitoids that give rise to new populations in succeeding cropping season. It also involved the evaluation of the chemical basis for the female moth oviposition site preference on un-infested, conspecific and interspecific larvae-infested maize plants. This was to determine how plants info-chemicals drive the conspecific and heterospecific interactions of stemborer pests in female oviposition site choice. In addition, the work also attempted to understand the larval interactions between the stemborer communities and the fall armyworm to interrogate the importance of this relationship for pest management in maize fields. This work focused on the study of three stemborer species, *B. fusca*, *S. calamistis* and *C. partellus* as the most economically important stemborers species which attack maize fields in Kenya (Kipkoech *et al.* 2006; Kfir *et al.* 2002), fall armyworm, the new invasive species and the associated parasitoids *C. flavipes* and *C. sesamiae*, the most efficient parasitoids for biological control of lepidopteran stemborers (Kfir *et al.*, 2002; Overholt *et al.*, 1997).

1.4. Objectives

1.4.1 General objective

The study analysed the functioning of a community of lepidopteran maize pests and associated parasitoids for sustainable production of maize.

1.4.2 Specific objectives

The specific objectives of this study included:

- (i). To identify refugia of lepidopteran maize stemborers and associated parasitoids during the non-cropping season;

- (ii). To determine the role of plant volatiles in the attraction of conspecific and heterospecific lepidopteran maize stemborers adults for oviposition;
- (iii). To determine the impact of fall armyworm invasion on population of maize stemborers larval communities and their associated parasitoids in maize field;
- (iv). To assess the performance of *Cotesia* spp. associated with maize stemborers on fall armyworm in terms of acceptability and suitability;
- (v). To determine maize stemborers and the fall armyworm larval dispersal capacity within and between maize plants;
- (vi). To evaluate the effect of larval density and duration of interactions on the competition outcomes between communities of lepidopteran maize stemborers and the fall armyworm;
- (vii). To evaluate the effect of temperature on larval interactions in a mixed community of lepidopteran maize stemborers and the fall armyworm.

1.5. Hypotheses

The following hypotheses drove the study:

- (i). Previous on-farm maize harvest residues serve as refugia for hibernating stemborers and associated parasitoids and are key indicators of the intensity of stemborer's species dominance in the succeeding cropping season;
- (ii). Conspecific or interspecific-larvae-infested maize plants emit specific chemical signatures attractive to conspecific and heterospecific lepidopteran maize stemborers adults for oviposition;

- (iii). The invasive fall armyworm (*Spodoptera frugiperda*) competes for limited resources with stemborer species and associated parasitoids in maize fields;
- (iv). *Cotesia* spp. associated with maize stemborers are efficient for biological control of fall armyworm;
- (v). A similar dispersal potential for the redistribution of larval populations within or between plants exists between the maize stemborers and the fall armyworm .
- (vi). Interaction outcomes between communities of lepidopteran maize stemborers and fall armyworm larvae depend on temperature, larval density, and duration of the interactions.

CHAPTER TWO

LITERATURE REVIEW

2.1 Global distribution and importance of maize crop

Maize (*Zea mays* L.) is a cereal crop grown worldwide in a wide range of climatic conditions; from latitude N 56° to S 40°, below the sea level to the Caspian plains about 3000 m in the Andes forest and in the Arid regions (Russell & Hallauer, 1980). Maize is the most important cereal crop grown under both irrigated and rainfed agricultural systems across the tropics (Hussan *et al.*, 2003). Maize is considered an important source of food and nutritional security in most of the developing world. In Africa, maize is an important staple crop and base of food for approximately 1.2 billion people in sub-Saharan Africa (SSA) (IITA, 2009), which is reported to represent more than 40% of Africa total cereal production (CRDI, 2003). This is because of its ease cultivation and ready adaptability to different agro-ecological climates. Additionally, maize forms a basis of a variety of foodstuff and has good storage characteristics (Shiferaw *et al.*, 2011).

Production of maize varies by region with yearly worldwide yields estimated at 1.014 billion metric tonnes (De Groote *et al.*, 2013). The United States of America (USA) and China are the first and second largest maize producers with an estimated 40% and 20% respectively of total world production (FAOSTAT, 2017). Other large-scale maize producers include Brazil, Mexico, Argentina, India and France (FAOSTAT, 2017). Africa produces approximately 7 % of the total world maize production (FAOSTAT, 2017). Most of it, almost two-thirds of all maize produced in Africa comes from eastern and southern Africa (FAOSTAT, 2017). In these regions, most of the maize is produced by smallholders

accounting for over 70 % of the total production and above 80 % of the total maize growing area (De Groote, 2002).

In Eastern Africa, maize accounts for almost half of the calories and protein consumed in the region (Macauley & Ramadjita, 2015). For example, according to some previous studies, approximately 400 grammes of maize are consumed daily per person in Tanzania with an average national consumption estimated to be over three million metric tonnes per year (Ranum *et al.* 2014). In Kenya, maize is the staple food crop, with reports indicating that each household in Nairobi, the capital city of Kenya consumes a monthly average of 5.67 kgs per adult of maize products (Muyanga *et al.*, 2005).

2.2 Constraints facing maize production in Africa

Despite the importance of maize as a food crop for human consumption and the efforts made to expand production by increasing tillage acreage, maize production levels have remained low in Africa. This phenomenon has been mainly occasioned by increased population growth and low maize yields which has often led to serious food insecurity and poverty among the African populace (Ngoko *et al.*, 2002). In Africa, maize yields are estimated to be 2 tonnes per hectare (FAOSTAT, 2017). For example, maize yield in Kenya is reported to be approximately 1.7 tonnes per hectare, which is comparatively lower than the average yields of 6 tonnes per hectare reported in developed countries (FAOSTAT, 2014).

The low maize yields are attributed to many factors such as soil infertility, poor managerial skills, high costs of farming inputs, environmental stresses, pests and diseases (Ngoko *et*

al., 2002). Among these factors, pests and diseases remain the most severe cause of losses in maize yields, which often range from between 20 - 99 % (Cardwell *et al.*, 1997). Moreover, insect pests especially the lepidopteran and coleopteran are among the most important pests and often considered the principal cause of both maize field and grain losses in Africa (Gwinner *et al.*, 1996). Lepidopteran stemborer species are responsible of yield losses in the range of 34 - 46 % under field conditions (De Groote, 2002; Kfir *et al.*, 2002) while Coleopteran species have been reported to cause upto 40% losses of stored grains (Midega *et al.*, 2016).

In Africa, the most frequent insect pests of maize are the cereal stemborers such as *Busseola fusca* Fuller (Lepidoptera: Noctuidae), *Sesamia calamistis* Hampson (Lepidoptera: Noctuidae), *Chilo partellus* Swinhoe (Lepidoptera: Crambidae), *Chilo orichalcociliellus* Strand (Lepidoptera: Crambidae), *Eldana saccharina* Walker (Lepidoptera: Pyralidae) (Schulthess *et al.*, 1997) and the cob miners like *Mussidia nigrivenella* Ragonot (Lepidoptera: Pyralidae) (Agboka *et al.*, 2009). However, their pest status varies significantly according to the agroecological zones and country (Schulthess *et al.*, 1997). Stored products pests associated with maize grains include grain borer, *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae), sawtoothed grain beetle, *Oryzaephilus surinamensis* (L) (Coleoptera: Silvanidae), and maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) (Midega *et al.*, 2016). Besides, the fall armyworm *Spodoptera frugiperda* (J. E. Smith) has been widely demonstrated as one of the most damaging crop pests in the Americas (Faretto *et al.*, 2017; Lima *et al.*, 2010). Previous studies on the feeding behaviour of this insect pest indicates that larval feeding

on foliage, ear tips and whorl of maize leads up to 34% reduction in grain yield with annual losses estimated at \$300 to \$500 million in the United States (Lima *et al.*, 2010).

2.3 Major maize pests of Africa

About twenty moth species in the families of Crambidae, Pyralidae and Noctuidae, constitute the most important cereal pests found in many parts of Africa (Polaszek, 1998). The larvae of these moths bore into the stems of the host plants, often killing the plant hence their name stemborer. These stemborer pests have important regional differences in the inhabiting ecozones (Kipkoech *et al.*, 2006). In East Africa, *B. fusca*, *C. partellus*, *C. orichalcociliellus*, *E. saccharina*, and *S. calamistis* are reported as the important and widely distributed stemborers of maize and sorghum (Kfir *et al.*, 2002). In Kenya, the most economical important stemborers species that attack maize are, the noctuids *B. fusca* and *S. calamistis* and the crambids *C. partellus* (Kipkoech *et al.*, 2006; Kfir *et al.*, 2002). Recently, fall Armyworm *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera, Noctuidae), a new invasive pest species in maize farming fields in Africa was reported (Goergen *et al.*, 2016)

2.3.1 Bioecology and economic importance of the noctuids *Busseola fusca* and *Sesamia calamistis* and the crambid *Chilo partellus*

Most of lepidopteran stemborer pest species are holometabolous insects exhibiting complete metamorphosis life cycle including eggs, larvae, pupae and adults (Figure 2.1).

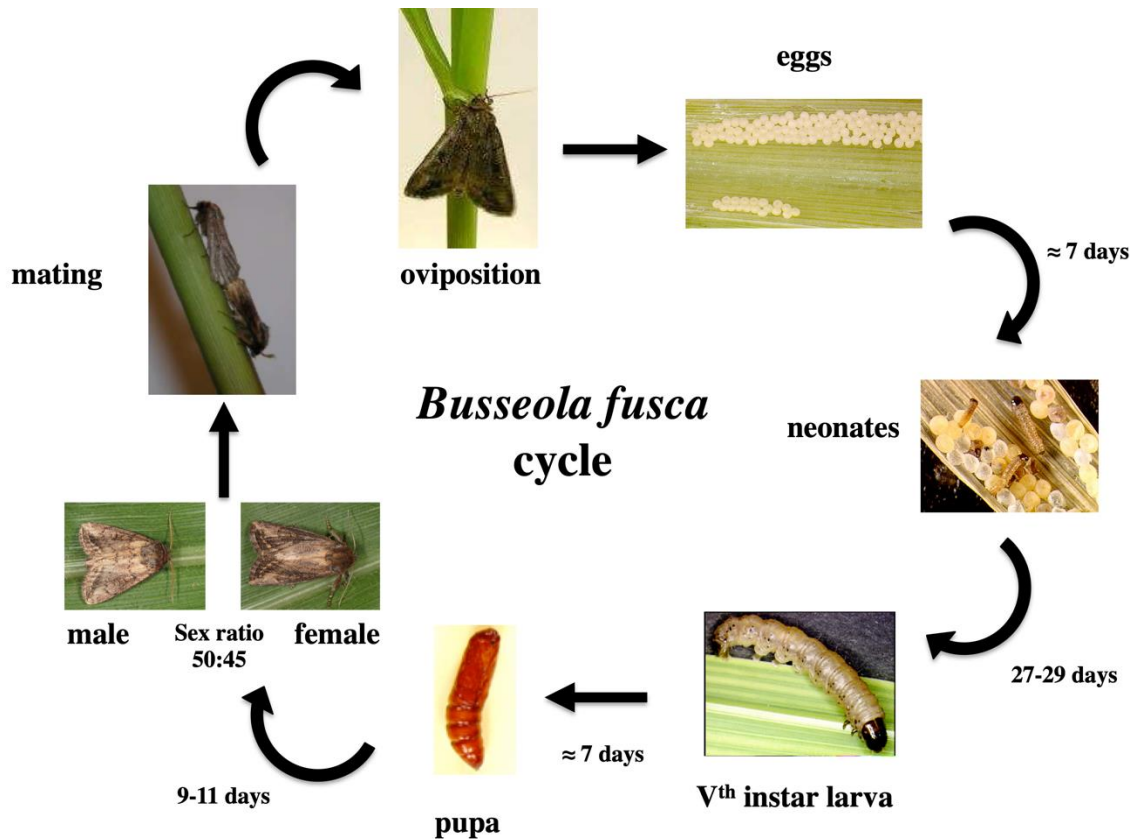


Figure 2. 1: Typical biological life cycle of maize stemborers species (Source: Calatayud *et al.*, 2014a).

Busseola fusca (Fuller) (Lepidoptera: Noctuidae) takes about 60 days to develop from egg to the adult stage though this duration varies greatly depending on climatic conditions which include variation in humidity, temperature and atmospheric pressure (Calatayud *et al.*, 2014a). Maize yield losses due to *B. fusca* damage have been estimated to vary between 20 to 80 % depending on the extend of infestation (Haile & Hofsvang, 2002). Losses due to *B. fusca* damage have a strong economic impact in developing countries, especially where agriculture plays a major economic role (Haile & Hofsvang, 2002).

Sesamia calamistis Hampson (Lepidoptera: Noctuidae), the African pink stemborer complete its life cycle within 44 -56 days (Khadioli *et al.*, 2014a). The attack of *S.*

calamistis on plants induces killing of the plant growing points often known as dead-heart which results in lodging due to weakened stems, leaf senescence and reduced translocation (Bosque-Perez & Mareck, 1990). Tesfaye & Solomon (2007) reported that the yield losses caused by *S. calamistis* in maize crop infestation varies up to 54 %. In addition, *S. calamistis* infestation increases the incidence and severity of stem rots (Bosque-Pérez & Mareck, 1991).

Chilo partellus (Swinhoe) (Lepidoptera: Crambidae) in, the spotted stemborer, an indigenous traditional Asian species (Bleszynski, 1970) invaded Africa in 1930 when it was first reported in Malawi (Sylvain *et al.*, 2015). The complete life cycle of *C. partellus* range between 38-55 days. *Chilo partellus* has been estimated to cause the greatest loss to maize production, in the order of 24.7%, 20.7% and 10% in the Kenyan dry transitional, lowland tropics and mid-altitude respectively (Ong'amo *et al.*, 2006a). Damage of this pest to maize and sorghum often exceeds 50-70% of the total yield loss (Kfir *et al.*, 2002).

Ecological differences influence the geographic population distributions of the three main species. *Busseola fusca* is abundant especially in the high altitude (about 1500 to 2000 meters) (Ndemah *et al.*, 2001) while *C. partellus* is mainly present in the dry lowland tropics and in the moist and dry mid-altitudes (Ong'amo *et al.*, 2006a). *Sesamia calamistis* is widespread species in Africa mostly in all altitudes (Kfir *et al.*, 2002). These stemborers species are poly/oligophagous attacking both cultivated and wild plants (Le Ru *et al.*, 2006a & 2006b). For example, all these pests have been found on cultivated plants such as rice, maize, sorghum, millet and sugar cane. Similarly, wild Poaceae plants including different species of *Cyperus* are recorded as potential host plants. (Calatayud *et al.*, 2014a; Otieno *et al.*, 2008; Le Ru *et al.*, 2006a & 2006b).

2.3.2 Bioecology and economic importance of fall Armyworm *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera, Noctuidae)

The fall armyworm, *Spodoptera frugiperda* (J. E. Smith) is a polyphagous pest indigenous throughout the American continent (Todd & Poole, 1980). The pest has for long been regularly intercepted in intercontinental trade (CAB International, 2017) but not been previously reported outside the American continent. The pest was reported in some parts of the African continent from 2016 (Cock *et al.*, 2017; Goergen *et al.*, 2016) and has since then spread throughout the subtropical and tropical regions of the continent (Day *et al.*, 2017). The life cycle of fall armyworm is presented in Figure 2.2.

In pest endemic areas, the infestations occur throughout the year since the pest does not have the ability to diapause (Prasanna *et al.*, 2018). In non-endemic areas, migratory fall armyworm arrives when environmental conditions allow and may have a few as one generation before they become extinct (Prasanna *et al.*, 2018). The larvae of the moth feed on leaves, stems and reproductive parts of over 80 different crop species. While fall armyworm has a preference for maize, it can also attack many other major cultivated crops, such as sorghum, rice, cabbage, sugarcane, groundnut, beet, onion, soybean, cotton, millets, potato, tomato and pasture grasses (Prasanna *et al.*, 2018). The moth has both a migratory habit and a more localised dispersal habit. In migratory habit, the moth can migrate up to 100 Km per night or more (Prasanna *et al.*, 2018). The voracious feeding and long-distance flight behaviours of fall armyworm indicate a significant menace to African agriculture with the potential for quick spread throughout the continent (Nagoshi *et al.*, 2017). The fall armyworm economic impacts on agricultural productivity across Africa are substantial. In the absence of appropriate control methods, it is estimated that the potential

of fall armyworm to cause maize yield losses might range from 8.3 to 20.6 M metric tonnes annually in just 12 sampled Africa's maize producing countries (CAB International, 2017; Day *et al.*, 2017).

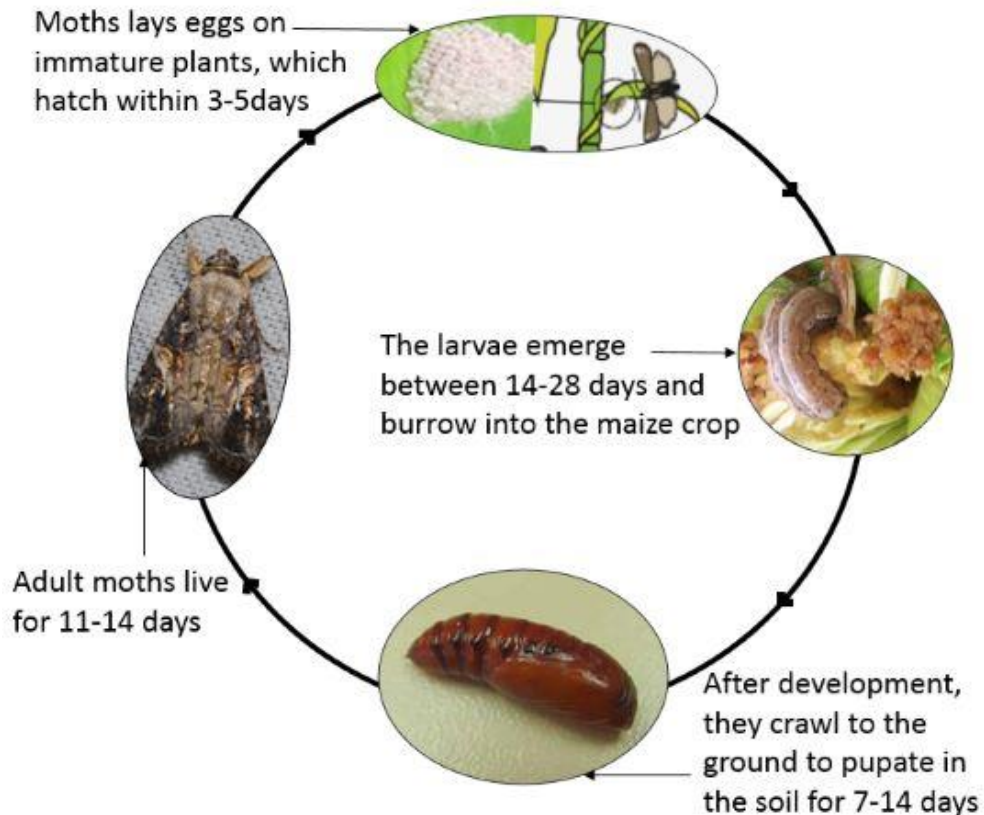


Figure 2. 2: The Fall armyworm life cycle including egg, six growth stages of caterpillar development (instars), pupa and moth. Source IMWIC & CABI, modified by Sokame.

2.3.3 Distribution and occurrence of fall Armyworm in Africa

Native to the American continent, the fall armyworm was first reported in the Africa in the early months of 2016 (Goergen *et al.*, 2016). Subsequent investigations have revealed the presence of this pest in nearly all parts of the sub-Saharan Africa, where it is reported to

cause widespread damage in the maize fields (Goergen *et al.*, 2016). Infact, over 30 African countries including the island countries of Cape Verde, Madagascar, São Tomé and Príncipe, and Seychelles have reported the presence of the pest within their borders (Prasanna *et al.*, 2018). Some studies have suggested that the fall armyworm haplotype currently spreading in Africa is similar to that initially reported in south Florida (USA) and the Caribbean (Prasanna *et al.*, 2018; Nagoshi *et al.*, 2017). The polyphagous nature of this pest and the sub-Saharan Africa favourable ecological conditions suggest that fall armyworm is likely to get established as an endemic, multigenerational pest (Prasanna *et al.*, 2018).

2.4 Pest management practices for lepidopteran maize stemborers and fall armyworm in maize fields

Several methods have been used for the management of lepidopteran stemborers and fall armyworm in maize fields. The most commonly used management options include chemical, biological, host plant resistance and cultural control methods such as Crop rotation, Intercropping, Manipulation of planting dates(Hailu *et al.*, 2018; Azerefegne & GebreAmlak, 1994).

2.4.1 Chemical methods for the control of stemborer and fall armyworm pests

In Eastern Africa, most varieties of maize and sorghum grown are susceptible to stemborer and fall armyworm infestations which often led to increased yield losses (Hruska & Gould, 1997). Chemical pesticides are the most commonly used method for the control of a variety of insect pests in maize fields (Azerefegne & GebreAmlak, 1994). Pitre (1986) reported that chemical combinations of pyrethroids and chlorpyrifos even at rates lower than

recommended results in effective control fall armyworm invasion in maize fields. However, it has been reported that some strains of fall armyworm confer some resistance to some of the commonly used insecticides (Yu *et al.*, 2003). In a study carried out in Florida, USA, resistance of *S. frugiperda* to pyrethroids was reported to range from 2- to 216-fold, 12- to 271-fold for the organo-phosphorates and 14- to > 192-fold for carbamate based insecticides (Yu *et al.*, 2003). In addition, the distribution of different immature development stages, location in the plant and mixed populations of stemborer species were commonly reported in the maize fields spread with agrochemicals (Van Rensburg & Van den Berg, 1992) indicating that effective chemical control of stemborers is difficult to attain in the field (Van den Berg, 1997). Van den Berg (1997) recommended an economic threshold level (ETL) for control of *B. fusca* and *C. partellus* on maize and sorghum plants to ensure cost-effective chemical control measures. This ETL control measure is reached when 10% of the total field plants exhibit infestations by the pests as indicated by leaf whorl damage. For maize fields manifesting clear signs of stem borers attack, a cost-effective chemical method of control has been the application of insecticide granules in plant whorls as soon as damage is observed. Van den Berg & Van Rensburg (1993) applied a persistent, granular insecticide (beta-cyfluthrin) in the plant whorls and observed a reduction in the larval number of 78.2% and a 25.2% decrease in maize yield loss. However, although granular insecticide application is effective and economical, it is less effective in large scale farming system when crop fields are large due to limited practicability (Van den Berg, 1997). It has been suggested that in some cases the pest status of *C. partellus* sometimes increase in the presence of some chemicals. In addition, chemical control is not always effective and sometimes too expensive, especially for small-scale subsistence African farmers (Kfir, 1990). Due to the high costs of insecticides (Pathak,

1985), subsistence farmers cannot afford to buy chemicals (Pathak, 1991). Despite the popularity of insecticides, there is inadequate information among farmers on application methods, time of application and efficacy of available insecticides for an effective control (Ebenebe, 1998). Therefore, chemical control is not generally a viable option for use by resource-poor farmers to control stemborers and fall armyworm especially in resource poor African farming systems.

2.4.2 Host plant resistance mechanism for the control of stemborers and fall armyworm in the field

Resistance of host plant to pest infestation is the heritable qualities possessed by a plant that allows the host to tolerate, avoid or recover from the attacks of insect pests under conditions that should cause greater damage to other plants that belong to the same species (Kumar, 1984). Together with the desirous characteristics of the capacity to be effective when alone or as a component of an overall pest management strategy, host plant resistance is considered most beneficial to the farmer (Pathak, 1990). The use of insect-resistant cultivar has been demonstrated to be the cheapest, and the safest mode of pest control means even under low farm input and poor management conditions (Pathak, 1985). In addition, similar reports have been made for fall armyworm on different maize cultivars with varying degree of resistance being reported (Rojas *et al.*, 2018). For example, different cases of transgenes-based fall armyworm resistance in maize have been reported (e.g. Chandrasena *et al.*, 2018; Horikoshi *et al.*, 2016). Furthermore, the development of resistance crop variety has been demonstrated to be much less costly than the development of a new pesticide (Kumar, 1984). Although a number of crop resistance varieties seems to be available at no extra cost to the farmer and requires little management skills, the

technique is faced by incomplete resistance as well as resistance breakdown by insect pests (Dent, 1991).

2.4.3 Biological control options for stemborers and fall armyworm in the field

Biological control, the oldest form of managing insect pests is defined as the pest management tactic in which the manipulation of natural enemies leads to the reduction of a pest population. Different from natural control, biological control or bio-control is a pest management option that heavily relies on natural pest enemies for control (Hegge, 2007). In biological control strategies, a natural enemy feeds or preys on the host to extend its own population at the expense of the pest population. Natural enemies are living organisms, which kill or weaken insects to reduce their population numbers (Hegge, 2007; Orr & Suh, 1998). Insects' natural enemies are diverse and include insects themselves, other invertebrates, vertebrates, nematodes and microorganisms. These natural enemies have been effectively divided into parasites, parasitoids, predators or pathogens (Pedigo, 1996). In Kenya, the koinobiont (parasitoids that allow host to continue to grow in size after parasitism) larval endoparasitoid (feeding from inside the host larva) *Cotesia flavipes* Cameron (Hymenoptera: Braconidae) was released in 1993 by *icipe* in the coastal region to control *C. partellus* an invasive exotic stemborer of maize and sorghum in Eastern and Southern African lowlands (Overholt *et al.*, 1997; 1994a). Due to its successful history in its Asian aboriginal home (Overholt *et al.*, 1994a), *Cotesia flavipes* was chosen as the best candidate to complement the activity of *Cotesia sesamiae* which was initially associated with the indigenous borer species such as the noctuids *S. calamistis* and *B. fusca* (Zhou *et al.*, 2001; Overholt *et al.*, 1994a;). Following its release, the parasitoid successfully established in the coastal region and later spread to other areas of the country within four

years following its release. The percentage parasitism of this parasitoid was subsequently established between 1995 and 2004, while the average annual parasitism due to the parasitoid increased linearly with time following its introduction (Omweya *et al.*, 2006; Zhou *et al.*, 2001). At the coast, *C. flavipes* reduced the *C. partellus* population densities by 57% while at the same time maize yields increased by 10-15% (Zhou *et al.*, 2001). Following its success in Kenya and western Tanzania (Omweya *et al.*, 1997), this pest control option was adopted by eleven other countries of the Eastern and Southern Africa but became established in 10 of these countries (Omweya *et al.*, 2006). However, as demonstrated by Jiang *et al.*, (2006) parasitism by this parasitoid is still on the increase, indicating that the pest-parasitoid system has not yet equilibrated. Parasitoids such as *Cotesia icipe* Fernandez-Triana & Fiaboe (Hymenoptera: Braconidae) against *S. frugiperda* has been recently shown to give promising results in the laboratory. Its effectiveness in the maize fields is yet to be demonstrated (Sisay *et al.*, 2018).

2.4.4 Crop rotation strategies for the management of stemborers and fall armyworm

Stemborers and fall armyworm damage may considerably be reduced by following the principle of crop rotation, in which a pest non-host plant is cropped after a host crop. It has been demonstrated that in such practices, a new insect pest generation that might have pupated in the vicinity of the host crop become exposed to a different non-host crop in a subsequent season leading to its death (Dent, 1991).

2.4.5 Intercropping to control stemborers and fall armyworm field infestations

Most studies on field infestation by pests conclude that intercropping reduces stemborers and fall armyworm infestation. In particular, studies at *icipe*, Kenya, have focused both on the practice of intercropping itself and on how the developed systems can be adapted for use by small-scale farmers (Hailu *et al.*, 2018). These studies have mainly focused on intercropping with non-host plants especially cowpeas, beans and cassava, which have shown a considerable reduction in the incidence of most insect pest on the host species (maize and sorghum usually intercropped with cowpea). Ampong-Nyarko *et al.* (1994) reported that 30% of *C. partellus* oviposition in a maize/sorghum/cowpea intercropping system was on cowpea. Besides, it has been reported that maize/cassava intercropping practices in Nigeria reduced larval numbers of *E. saccharina*, *B. fusca* and *S. calamistis* by approximately 50% compared to monocropped maize fields (Kaufmann, 1983). Similar results were recently reported in maize fields intercropped with either beans, soybeans or groundnuts targeting the management of fall armyworm infestation compared to a maize monocrop (Hailu *et al.*, 2018).

2.4.6 Manipulation of planting dates as stemborers and fall armyworm control strategy

Manipulation of planting date as one of the cultural practices is an effective measure to escape serious stemborers or fall armyworm attack and should not merely be seen as purely of agronomic concern. In order to efficiently utilize planting date as a means of escaping stemborers and fall armyworm damage, information on the local seasonal patterns of the pest life cycle is important. Therefore, the coincidence of the most susceptible stage of crop growth with periods of peak moth activity can be disoriented by manipulating the planting

dates. However, any effect on planting dates might impact on stemborers and fall armyworm incidence is dependent on local or regional conditions.

Other cultural practices such as planting density, physical control and removal of infested plants, volunteer plants, fertilizer use, choice of variety and water management practices have also been used in the management of stemborers and fall armyworm infestations on the farm (Prasanna *et al.*, 2018; Van den Berg, 1994). However, all these cultural practices have been demonstrated to be inefficient in the reduction of pest populations of both species below economic damage levels in maize fields (Kfir, 1994).

2.4.7 Semiochemicals for the control of lepidopteran stemborers and fall armyworm infestations in maize fields

Although chemical control, host plant resistance, biological control and cultural practices play an important role in the control of lepidopteran pests in maize fields, some of these methods have unique limitations that impact negatively on resource-poor farming communities. These have thus led to the continued search of other pest control mechanisms that involve use of semiochemicals.

A Semiochemical is a chemical that conveys a signal from one organism to another and modifies the behaviour of the recipient organism. Semiochemicals that have been most successfully used in lepidopteran pests control include lepidopterous sex pheromones and the push-pull system which is also important in habitat management (Hailu *et al.*, 2018; Khan *et al.*, 2000). Many commercially developed systems in existence exploit lepidopterous sex pheromones, either as monitoring systems or as a slow-release formulations to disrupt normal mating location (Félix *et al.*, 2009; Meagher & Mitchell, 2001). Currently, the chemical blend consisting of (Z)-11-tetradecen-1-yl acetate (62%),

(E)-11-tetradecen-1-yl acetate (15%), (Z)-9-tetradecen-1-yl acetate (13%) and (Z)-11-hexadecen-1-yl acetate (10%) is recommended for monitoring the flight phenology and for timing control measures for optimal efficacy of *B. fusca* (Félix *et al.*, 2009). Meagher & Mitchell (2001) have developed commercially produced pheromone lures to capture fall armyworm male moths in maize and peanut fields.

The use of semiochemicals in habitat management system referred to as "push-pull" has also been developed to repel the pests from maize plants and subsequently lure them to more attractive barrier around the maize crop (Khan *et al.*, 2000; 1997). The two most important trap crops that have since been used are *Pennisetum purpureum* and *Sorghum vulgare sudanense*, both fodder crops of economic importance.

In the push-pull strategy, grasses which emit chemicals, such as hexanal, (*E*)-2-hexenal, (*Z*)-3-hexen-1-ol and (*E*)-3-hexen-1-yl acetate are planted to surround the maize fields so as to attract the intrusive adult moths (Khan *et al.*, 2010). Therefore, the insects become more attracted to grasses, where they lay their eggs instead of landing onto the maize crops. The neonate larvae subsequently hatched are not able to develop on the alternative grass hosts, leading to their fatalities. Two non-host plants repulsive to stemborers, namely *Melinis minutiflora* and *Desmodium uncinatum* have been extensively used in the 'push-pull' system (Khan *et al.*, 2000; 1997). These non-hosts plants are intercropped with maize crops and produce volatile compounds which repel (push) gravid female stemborers from the field. The active compounds found not in trap plants but specifically in *Melinis minutiflora* and *Desmodium uncinatum* are comprised of (*E*)-ocimene, (*E*)-4,8-dimethyl-1,3,7-nonatriene, β -caryophyllene, α -terpinolene, humulene, and α -cedrene (Khan *et al.*, 2010). However, the effectiveness of these attractive and repulsive plants towards

lepidopteran stemborers is still not fully convincing (Calatayud P.-A., Le Ru, B., Sétamou M. & Schulthess F., pers. Comm.). Moreover,, it has been recently observed that the use of *Desmodium uncinatum* might also be effective in the control the fall armyworm infestation especially in the early and the tasseling stages of maize growth phases (Hailu *et al.*, 2018).

This habitat management system has also been used to promote the successful establishment of natural enemies generally employed in biological control strategies (Landis *et al.*, 2000). For instance, *Melinis minutiflora* when planted as an intercrop with maize is reported to attract parasitoids (Khan *et al.*,2000; 1997) though this has not yet been fully demonstrated (Sétamou M. & Schuthess F., pers. Comm.). If demonstrated, the system might favour the survival, fecundity, longevity, and behaviour of natural enemies to increase their effectiveness (Landis *et al.*, 2000). The natural enemies attack the hosts in the field in order to reduce their populations to below economic injury levels.

2.4.8 Integrated pest management (IPM) strategies for the management of stemborers and fall armyworm infestations in maize fields

Integrated pest management system utilises all available and suitable techniques and methods in a compatible manner at many possible extend to reduce pest populations and maintain them at levels below those causing economic injury (Kumar, 1984). The system combines control components such as chemical, host plant resistance, biological and cultural practices (Akinsola, 1990). For instance, the IPM tactics for lepidopteran maize pest controls have been recently developed using a semiochemical-based approach. An IPM program can thus be employed to suppress stemborer and fall armyworm populations in maize-based production systems (Prasanna *et al.*, 2018; Van den Berg, 1997). Therefore,

the understanding of the driven mechanisms of the mode of functioning of the interacting stemborer and fall armyworm communities could lead to the improvement of the ongoing IPM strategies for their control. In the fields, they occur together, constituting biological communities and have ecological interactions.

2.5 Carry-over of maize lepidopteran communities and associated parasitoids during the non-cropping seasons

Wild vegetation surrounding small-scale cereal fields can often serve as alternative host plants for lepidopteran pests. These alternative hosts are considered to serve as refugia of lepidopteran pests during the off-cropping season and are hence important sources of infestation during the succeeding cropping season (Khan *et al.*, 1997). However, some studies have demonstrated that the refugia role of the wild grasses for maize lepidopteran pests during the off-cropping is limited (Matama-Kauma *et al.*, 2008; Ndemah *et al.*, 2007). Studies conducted in Eastern and Southern parts of Africa have demonstrated that previous reports of the presence of maize stemborers on wild grasses might have accrued from possible misidentification of the pest during the surveys (Le Ru *et al.*, 2006a).

For instance, the densities of stemborers on wild grasses generally ranges from 0.001 to 0.03/tiller which are largely less than the densities found on cultivated plants (Matama-Kauma *et al.*, 2008; Ndemah *et al.*, 2007; Le Ru *et al.*, 2006a). A study conducted in Western Kenya assessing the potential refugia role of wild grasses for *B. fusca* demonstrated that the proportion of wild host-plant was below 10% (Kanya *et al.*, 2004). Moreover, in the moist-transitional and lowland tropical zones of Kenya specifically in Kakamega and Muhaka, Otieno *et al.* (2006) showed that the host range of *C. partellus* and *B. fusca* was limited in both abundance and number, suggesting that the study of Kanya *et*

al. (2004) may have overestimated the species populations. Based on these findings, it may be argued that maize residues are probably the main refugia of these stemborers species especially during the non-cropping seasons. Several studies have also reported that in their larval stage, stemborer species enter into diapause in maize residues left in the field after following previous harvest (Polaszek, & Khan, 1998) from where they infest young crops after planting during the succeeding cropping season (Schulthess *et al.*, 1997). For the carry-over of associated parasitoids during the non-cropping season, there might exist synchronisation between development in diapausing larva and its parasitoid to allow the carrying over of the parasitoids to the next cropping season. For example, Chinwada and Overholt (2001) reported that *Sturmiopsis parasitica* (Curran) (Diptera: Tachinidae) overwinters by parasitising larvae of *B. fusca* which later go into diapause. Soon after the onset of rains, diapause of the larvae is terminated allowing the parasitoids to develop to pupae and adults (Scheltes, 1978).

2.6 Ecological and biological interactions among insect communities

Biological community refers to all types of populations living and interacting within a particular geographic area. On the other hand, an ecosystem constitutes both living organisms in a community and their non-living or abiotic environment (Duyck *et al.*, 2006). Interactions between insect phytophagous species are usually reviewed in terms of competition and facilitation (Speight *et al.*, 2008; Denno *et al.*, 1995). Competition interactions are structured as: Intra- and interspecific competitive interactions, direct and indirect competitive interactions, resource and apparent competitive interactions, and interference and exploitative interactions.

2.7 Host plant selection for oviposition by phytophagous gravid female insects

During host plant selection for oviposition, the insect encounters two obstacles. The first one is the ability to locate its host at a distance within heterogeneous habitat patches. The second is the need to confirm the suitability (through quality assessment) of available host plants after identifying a suitable habitat patch (Knolhoff & Heckel, 2014). Successful egg deposition then depends on a sequence of behavioural events related to the presence of one or more host plant species. Generally, six different main steps events that lead gravid females to host plant selection can be distinguished (Schoonhoven *et al.*, 2005): (i) the insect has no physical contact with the plant and flies around randomly; (ii) it detects visual and/or olfactory signals of the plant; (iii) it responds to these signals by approaching the plant; (iv) it finds a suitable plant, lands and makes contact with it; (v) it examines the surface of the plant (palpation of leaves surface for example); (vi) the plant is accepted if the insect lays eggs on it. If the insect leaves immediately, the plant is rejected (Figure 2.6).

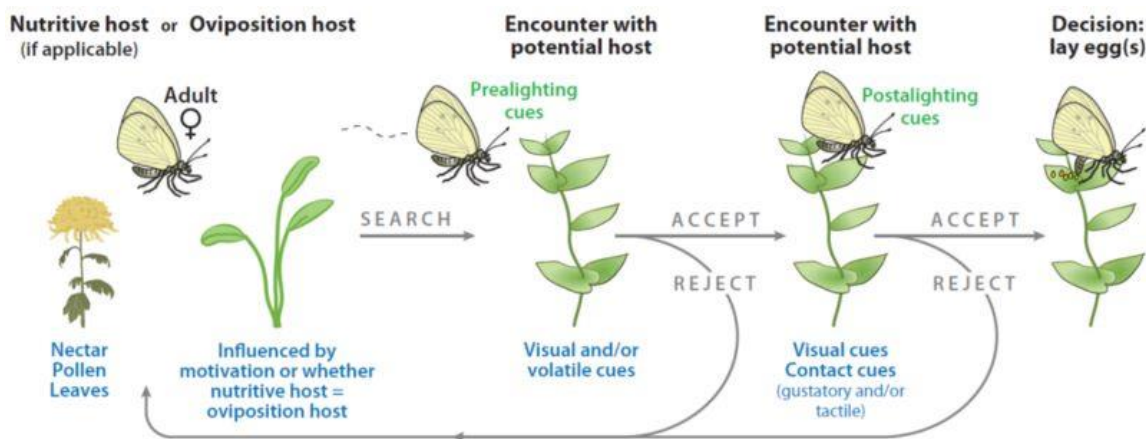


Figure 2. 3: Events leading to female moth oviposition choice. These sequence of events assumes that females are freely mobile and have the ability to choose a host plant. The sequence of events is probabilistic, and females may opt-out of the sequence at any step. Experience may influence future choices such that the first completion of the sequence may

shorten the time period required to make a subsequent decision. Source: Knolhoff & Heckel, 2014.

Moreover, insects display different behavioural responses to plant that affect their oviposition site choice and egg-laying decision. This has well been illustrated by oviposition and egg-laying behaviour of two cruciferous pests, cabbageworm *Pieris brassicae* L. (Lepidoptera: Pieridae) and cabbage maggot *Delia radicum* L. (Diptera: Anthomyiidae), (Mitchell, 1977; Chun & Schoonhoven, 1973). *Pieris brassicae* females are attracted to green surfaces (Mitchell, 1977) and land on any host plants where they briefly drum the leaf's upper surface with anterior tarsi, and immediately fly away after recording the non-host plant. However, on an encounter with a crucifer plant, drumming with anterior tarsi occurs at high speed followed by egg-laying on the underside of the leaf. In this case, oviposition is not controlled by volatile compounds but by the chemical stimuli of contact chemicals perceived at the tarsal level (Chun & Schoonhoven, 1973). However, ovipositing *D. radicum* females perceive crucifer plants volatile kairomonal odours at approximately 5 to 10 m away from the host which stimulate the activity of insects that fly, and then rapidly approach the host plants (Finch & Skinner, 1982). About 25 cm, the choice of the landing site is entirely visual (Prokopy *et al.*, 1983). After landing, the plant recognition is confirmed by the perception of glucosinolates at tarsal contact receptors of the flies that do not exist in males. Then the females, stimulated by the contact kairomones, move on the leaves and then descend on the ground at the base of the stem to lay the eggs (Nair & McEwen, 1976).

In addition, some plants attract and retain the females repetitively, while others are regularly avoided. *Cactoblasty cactorum* Berg. (Lepidoptera, Phycitidae) selects the greenest and largest cactus plants with good photosynthetic activity. Similarly, *Pieris rapae*

L. (Lepidoptera: Pieridae) prefers to oviposit on plant rich in water, while the American, corn borer *Ostrinia nubilalis* Hübn. (Lepidoptera: Pyralidae) first chooses the cultivars richest in sugars (Derridj & Fiala, 1983).

Furthermore, the preference of good hosts over poor hosts and poor hosts over non-hosts depends on the host–plant abundance (Cunningham *et al.*, 2001; Jaenike, 1978), its age (Durbey & Sarup 1984), adult feeding sites (Scheirs *et al.*, 2004), insect learning (Cunningham & West, 2008), larval movement (Cunningham *et al.*, 2001;) and predator avoidance (Ballabeni *et al.*, 2001).

In addition, various plant factors including physical factors (toughness, size, foliar shape and surface texture) and chemical factors have been demonstrated to significantly influence host selection in phytophagous insect (Calatayud *et al.*, 2008a).

2.8 Role of phytochemicals in host selection by phytophagous insect

Plant volatiles organic compound (VOCs) and contact chemical (non-volatiles) cues are chemical factors that strongly influence the final decision by phytophagous insects to accept or reject a host plant (Haribal & Feeny, 1998). Both volatile and contact semiochemicals have been demonstrated to influence host selection in phytophagous insects.

2.8.1 Volatile compounds in host selection by phytophagous insects

Insects are able to move and orient themselves to their host plants through the windward transport of volatile organic compounds (VOCs) emitted by the host plants. VOCs are examined by the perception by the insect at a distance; through the distance over which odour stimuli are first perceived and induce a response (Prokopy & Roitberg, 1989) which

can probably depend on nature of odours stimuli and senses of insect involved. It was previously demonstrated that VOCs are emitted in the pockets form and distributed in very short period of time. The insect thus perceives a series of "odour pockets", separated by odour deficient periods (Murlis, 1986). The insect moves in the opposite direction of the wind using its antennae-directional eyelashes sensitive to the direction of the wind (Bernays & Chapman, 1994; Visser & Taanman, 1987). If the force of the wind is too strong, the insect losses its markers and turn back to capture the plant odour signals again. In various phytophagous insects, volatile kairomones are reported to be involved in the discovery of the short- and medium-distance spawning sites (Kamm & Buttery, 1983) as well as intervene in the stimulation of spawning (Harris & Miller, 1983). Indeed, VOCs emitted by host-plant provide sufficient information for phytophagous insects to distinguish between preferred and unpreferred plants during suitable host plants selection. Several studies have investigated the effect of many plant VOCs on many phytophagous insects and found that some compounds are attractive and increase trap catches while others stimulate oviposition (e.g. Dethier & Schoonhoven, 1969). Thus, attractive compounds allow insects to orient themselves towards the source of the odour signal while the repellent compounds orient them in the opposite direction.

However, most of these compounds emitted by the plant are due to phytophagous insects attack that alters host plant physiology (Dicke & Baldwin, 2010). Then, the damage plant starts to produce secondary metabolites either in response to developing resistance against next attack by repellent compounds for herbivores insects (Erb *et al.*, 2012; Dicke & Baldwin, 2010), to attract the natural enemies of the herbivore or warn nearby plants (Rodriguez-Soana & Frost, 2010;).

2.8.2 Non-volatile compounds in host selection by phytophagous insects

The plant cuticle is the last stage in which the insect is in contact with stimuli that allow it to accept or refuse the plant for oviposition or feeding (Schoonhoven *et al.*, 2005). It is composed of soluble lipids or waxes and a cutin matrix which is an insoluble lipid-polymer. Among the waxes, epicuticular and intra-cuticular waxes are distinguished based on their biosynthetic pathways, cuticular location, chemical composition as well as their structural organization (Schoonhoven *et al.*, 2005). Epicuticular waxes are those which constitute the most superficial part of the cuticles and which are in contact with the insect when it lands on the plant. It is an amorphous film from which emerge crystalline structures consisting exclusively of aliphatic compounds: alkanes, alcohols, aldehydes, fatty acids and alkyl acetates (Schoonhoven *et al.*, 2005). This composition differentiates them from intra-cuticular waxes, which contain a great majority of triterpenoids and some aliphatic compounds. There are also plant surface metabolites that are derived from either primary or secondary metabolism (Schoonhoven *et al.*, 2005). These metabolites can be implicated in the recognition mechanisms of specific proportions on the surface of the plant. For example, sugar alcohols and the soluble sugars participate in recognition and acceptance mechanisms of the plant by a number of insects for oviposition purposes (see, for example, Calatayud *et al.* [2013] for review).

2.9 Interactions between invasive and native communities of insect pest species

Invasive insect pest species termed exotic, introduced, non-indigenous or non-native species, refers to the insect pest that is introduced by humans intentionally or otherwise through a human agency or accidentally from one region to another. The biological process

of invasion of these species can be divided into four steps (i) introduction, (ii) establishment, (iii) spread and (iv) naturalisation (Sharma *et al.*, 2018). Invasive insect pest species have the potential to rapidly established and spread in a new area and cause major crop loss that can adversely affect food security. Such introduced insects are characterized by being hardy, long-lived, voracious, aggressively pervasive, very resilient, rapid growth, generalised diet, ability to move long distances and rapid spread (Richardson *et al.*, 2000). The organisms that arrive and establish themselves in a new range of hosts, either for intentional or unintentional introductions, are positioned to have adverse effects on the surrounding fauna and also results in the extinction of other species (Pimentel *et al.*, 2000). They may affect native species populations and communities by competing for the same resource (Reitz & Trumble, 2002a). For example, the Asian adelgid, *Pineus boemeri* Annand, has been shown to be competitively superior and to displace a native congener, *P. coloradensis* (Gilette) in red pine (*Pinus resinosa* Aiton) plantation in Eastern USA, possibly through the reduction of host plant quality and forcing *P. coloradensis* to less suitable sites (McClure, 1989). *Icerya purchasi* Maskell (Hemiptera: Coccinellidae) has also been found to cause local extinction of host-specific lepidopteran (Roque-Albelo, 2003). Fabre *et al.*, (2004) demonstrated a form of resource competition between native and exotic seed chalcids, *Megastigmus* spp. and displacement of the native species. Similarly, the African stemborer *Busseola fusca* (Fuller) seems to have been displaced from sorghum fields by the Asian invasive stemborer *Chilo partellus* (Swinhoe) (Kfir, 1997) possibly due to deterrence of the native species by the invasiveness or due to differences in host plant phenology. An invasive herbivores species may also displace other indigenous species *via* behavioural interference. This has been demonstrated in asymmetric mating interactions that has driven widespread invasion and displacement in the whitefly *Bemisia tabaci* (Liu

et al., 2007). Alien species could also impact native species and community through other more complex mechanisms such as apparent competition, disease transmission, pollination disruption, among others.

Biological control may provide an opportunity to confirm competitive displacement. For example, the displacement of native Lepidoptera by the exotic noctuid moth *Penicillaria jocosatrix* Guenee in Guam was confirmed by a successful biological control program against *P. jocosatrix* which allowed the native species to recover (Kenis *et al.*, 2008).

Invasive herbivores species do not only affect closely related species. In agro-ecosystems, they can transform the structure and species composition of agroecosystem by excluding or repressing native species, whether directly by out-competing them for resources or indirectly by modifying the way nutrient are cycled through the system (Sharma *et al.*, 2018). Invasive insects can decrease native species' rates of growth, development, survival, reproduction, and movement (Caswell, 2000). Once they are established, they become invasive and with staggering economic and environmental costs (Pimentel *et al.*, 2000). One of the environmental costs is the irretrievable loss of native species.

2.10 Distribution of internal and external feeder larvae on maize plants

In the case of the internal feeder on maize plants, lepidopteran stemborer moths lay eggs on the lower leaf and/or on the inside bract depending on the species. After hatching, the larvae migrate severally, during the phases of their life cycle (Berger, 1992). The newly hatched larvae move from the egg batch to the first feeding site in the plant whorl. During this period of between 20 min to 20 hours, some larvae may balloon on a silken thread away from the plant (Bernays & Bernays, 1982). The larvae start feeding within the funnel

leaves, usually gregariously once the larvae have entered the whorl (Alghali & Saxena, 1988). Although some larvae stay inside the plant whorl until they migrate down into the stem, some leave the whorl and balloon away from the plant or crawl onto another when the leaf contact allows. The larvae leave the whorl as first and second instars (Berger, 1992; Bernays, & Simpson, 1982) While the third instar larvae or older larvae leave the plant and crawl on the ground or on the leaves to other plants. The older larvae from third instar change feeding site from whorl to stem (Leuschner, 1990). The larvae move outside the stem often within the leaf sheaths and bore into internodes where they start tunnelling until pupation.

The fall armyworm larvae are a folivory or an external feeder insect. The fall armyworm moths usually lay eggs on the underside of the leaves on maize plants. After the young caterpillar hatch, they feed superficially on the undersides of the leaves that results in semitransparent patches on the leaves called “windows”. Young caterpillars can spin silken threads which catch the wind and transport the caterpillars to a new plant. On young plants, the larvae of one to third instars usually move into the whorl leaf, where they preferentially feed on leaves and around the cob silks in older plants. By the fourth to sixth instars, the larvae reach the protective region of the whorl, where they cause the most damage resulting in ragged holes in the leaves of the young plants. However, in older plants with already developed cobs, these larvae prefer to eat cob through the protective leaf bracts into the side of the cob, where they begin to feed on the developing kernels (CAB International, 2017). Therefore, either in internal or external feeder case, the stemborer and fall armyworm could physically interact at least from larvae hatched to third instar

development, since they are still share the same parts of maize plants. Therefore, direct and indirect interactions could occur in their communities in maize fields.

The present study entailed the identification of the main reservoir source of maize stemborers and associated parasitoids that give rise to new populations in succeeding cropping season. The study also involved the evaluation of the chemical basis for the female moth oviposition site preference on un-infested and conspecific and interspecific larvae-infested maize plants. In addition, the work also involved the study of the existing larval interactions between the stemborer communities and the fall armyworm. This work focused on the study of three stemborer species including *B. fusca*, *S. calamistis* and *C. partellus* as important stemborers species which attack maize fields in Kenya (Kfir *et al.* 2002; Kipkoech *et al.* 2006), fall armyworm, the new invasive species and the associated parasitoids *C. flavipes* and *C. sesamiae*, the most efficient parasitoids important in the biological control of lepidopteran stemborers (Overholt *et al.*, 1997; Kfir *et al.*, 2002).

CHAPTER THREE

CARRY-OVER NICHES FOR LEPIDOPTERAN MAIZE

STEMBORERS AND ASSOCIATED PARASITIDS DURING THE

NON-CROPPING SEASON

Abstract

Sources of stemborer field infestation are the key elements to be considered in the development of habitat management techniques for the control of maize stemborers. Though several wild plants, grasses mostly, have been identified that serve as hosts for stemborers and their parasitoids during the off-season when maize is absent in the field, their role as a reservoir is still controversial, particularly in agro-ecosystems with reduced wild habitat. In this study the occurrence of different maize stemborers and associated parasitoids in maize stem residues and wild grasses was evaluated during the non-cropping seasons. Surveys were conducted in the central region of Kenya during long and short dry seasons. Similar studies were also carried out during the two rainy seasons in maize fields in an early maize growing and late whorl stages during the years of 2017 and 2018. Wild habitat had a significant ($p < 0.05$) higher pest and parasitoid species diversity than maize residues habitat. In the contrary, maize residues had a significant ($p < 0.05$) higher abundance of maize stemborer species, *Busseola fusca*, *Sesamia calamistis*, and *Chilo partellus* (290 and 38 in maize residues and wild habitat, respectively) and associated parasitoids (*Cotesia flavipes* and *Cotesia sesamiae*). The main source of both stemborers and associated parasitoids that are carried over to the maize plants during the subsequent cropping season in this study area. Therefore, systematic destruction of maize

residues would not help the biological control of lepidopteran stemborers mostly in areas with reduced wild habitat.

Keywords: Wild plants; maize residues; habitat and pest management; biological control

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3.1 Introduction

Though more than 300 stemborer species infest wild plants, only 21 stemborer species attack cereal crops, mainly maize (*Zea mays* L.), sorghum (*Sorghum bicolor* L.), and millet (*Pennisetum glaucum* (L.) R. Br.), in various parts of Africa (Ong'amo *et al.*, 2018; Moolman *et al.*, 2014; Ong'amo *et al.*, 2014; Le Ru *et al.*, 2006b, 2006a). A few, the noctuids *Busseola fusca* Fuller and *Sesamia calamistis* Hampson, the crambid *Chilo partellus* Swinhoe, and the pyralid *Eldana saccharina* (Walker), are cereal stemborers of major economic importance (Kfir *et al.*, 2002). Yield losses due to stemborer attack vary regionally but generally range from 10% to 80% depending on infestation levels by the pest species and the crop growth stage (De Groote *et al.*, 2002).

Stemborers of the lepidopteran that attack maize are poly/oligo-phagous and feed on other cultivated and wild plants (Mailafiya 2009; Polaszek & Khan, 1998; Gebre-Amlak, 1988). In sub-Saharan Africa, cereal crops are mainly grown in small fields surrounded by land that is occupied by wild host plants of lepidopteran stemborers. Some poly/oligo-phagous stemborers that are found on wild plants, such as *Chilo orichalcociliellus* Strand and *Pirateolea piscator* (Fletcher), are occasionally found on cultivated cereal crops (Mailafiya *et al.*, 2009; Polaszek & Khan, 1998). However, they are more frequently found on

cultivated crops; for example, *Busseola segeta* (Bowden) infests 13–61% of maize fields in Western Kenya (Calatayud *et al.*, 2014b).

In Kenya, the larvae of *B. fusca*, *S. calamistis*, and *C. partellus* contribute up to 82% of the total maize yield losses (Kfir *et al.*, 2002; Chinwada & Overholt, 2001). In the context of a biological control program, the most commonly used parasitoids are the larval parasitoids, including *Cotesia flavipes* Cameron and *Cotesia sesamiae* (Cameron) (Hymenoptera: Braconidae), followed by the pupal parasitoids *Xanthopimpla stemmator* Thunberg (Hymenoptera: Ichneumonidae) and *Pediobius furvus* Gahan (Hymenoptera: Eulophidae), and then the tachinid *Siphona* sp. (Midingoyi *et al.*, 2016; Mailafiya *et al.*, 2009). During cropping seasons, *B. fusca*, *S. calamistis*, and *C. partellus* and their associated parasitoids *C. flavipes* and *C. sesamiae* are reported to be more abundant in maize fields as compared to wild plants (Ong'amo *et al.*, 2013; 2006b; Mailafiya *et al.*, 2011); and perennation occurs mainly in cultivated habitats (Mailafiya *et al.*, 2011). This supposes that the parasitoids follow their lepidopteran maize stemborer hosts during non-cropping seasons, either in larvae feeding on wild plants surrounding maize fields (Haile & Hofsvang, 2001; Polaszek, & Khan, 1998) or in diapausing larvae in maize residues left in the maize field after harvest (Polaszek, & Khan, 1998; Kfir, 1991; Kfir *et al.*, 1989). However, since the abundance of both lepidopteran stemborers and their parasitoids is much lower in wild plants compared to maize plants (Mailafiya *et al.*, 2011; Ong'amo *et al.*, 2006b), the role of wild plants as a reservoir for cereal stemborers and their parasitoids is still controversial, particularly in agro-ecosystems with reduced surrounding wild habitat. The objective of the present study was to identify refugia of lepidopteran maize stemborers and associated parasitoids during the non-cropping season.

3.2 Materials and Methods

3.2.1 Study locations and sampling design

Surveys on maize residues and alternative wild plants were conducted in two different maize growing localities (Makutano and Murang'a) situated in the central region of Kenya. These two localities are dominated with small cultivated maize fields but with few stemborers wild plants habitats as compared to other agroecological areas such as Kitale, Mtito Andei, Kakamega, and Muhaka (Mailafiya, 2009), where many wild plants surround the maize fields (Appendice 1) (Calatayud, P-A. Pers. Obs.). Makutano (0°43'37'' S, 37°16'22'' E; 1150–1250 m above sea level (a.s.l.)) and Murang'a (0°55'23'' S, 37°09'00'' E; 1267–1500 m a.s.l) have a bimodal rainfall distribution, two cropping seasons (from April to June and from October to December), a mean annual precipitation of 981 mm and 1195 mm, and temperature ranges of 12.05–26.04 °C and 11.01–22.7 °C, respectively. Different regions of these study areas are dominated by different stemborer pest species (Sokame *et al.*, 2019a; Ntiri, 2015). For instance, in the Makutano area, *C. partellus* and *S. calamistis* co-infest maize fields (Sokame *et al.*, 2019a) while *B. fusca* and *S. calamistis* co-exist (Sokame *et al.*, 2019a) in Murang'a area. These areas were, therefore, considered for the study, to include these three major stemborer species, the most common and economically important pests for maize in Kenya.

The surveys were done for two years (2017 and 2018). Three farmers' fields were randomly selected in each locality. Similarly to Mailafiya (2009), maize residues in each field and adjacent wild habitat were sampled two to three times during each non-cropping season of each year.

One hundred maize plants were sampled two times in maize fields during each subsequent cropping season of each year. A total of 10 surveys (six times in 2017 and four times in 2018) were conducted in each selected farmer's field in maize residues and adjacent wild plants during the non-cropping seasons and eight surveys (four times in 2017 and four times in 2018) were conducted in each farmer's field in maize plants during the cropping seasons of the two years of the survey (Table 3.1).

Table 3. 1: Number of surveys in each study habitat and releases of the parasitoid in maize fields at Makutano and Murang'a. The parasitoid releases were done through release of adults, cocoon masses, or parasitized larvae.

Sampling /Release Sites	Number of Surveys in Each Habitat			Parasitoid species released	No. adults released	No. Cocoon Masses released	No. parasitized larvae released
	Maize Residues	Wild Plants	Maize Plants				
2017							
Makutano							
Field 1	6	6	4		500	20	8
Field 2	6	6	4	<i>C. flavipes</i>	400	20	28
Field 3	6	6	4		400	-	10
Total	18	18	12	<i>C. flavipes</i>	1300	40	46
Murang'a							
Field 1	6	6	4		300	100	50
Field 2	6	6	4	<i>C. sesamiae</i>	300	30	14
Field 3	6	6	4		400	3	-
Total	18	18	12	<i>C. sesamiae</i>	1000	133	64
2018							
Makutano							
Field 1	4	4	4		400	40	-
Field 2	4	4	4	<i>C. flavipes</i>	200	20	-
Field 3	4	4	4		200	20	-
TOTAL	12	12	12	<i>C. flavipes</i>	800	80	-
Murang'a							
Field 1	4	4	4		100	20	-
Field 2	4	4	4	<i>C. sesamiae</i>	100	10	-
Field 3	4	4	4		50	5	-
Total	12	12	12	<i>C. sesamiae</i>	250	35	-

∴ No release of that parasitoid stage in that field has been done

3.2.2 Sampling for the diversity and abundance of lepidopteran stemborers and associated larval and pupal parasitoids in maize residues during the non-copping seasons

The optimal number of maize residues sampled in each field during each survey was determined using the equation described by Zar (1999):

$$n = \frac{1}{4Dd^2} \left(Z_{\alpha/2}^2 \right) \quad (1)$$

where $Z_{\alpha/2}^2$ is the standard normal deviation (1.96), d is the permitted error (0.1) resulting in a uniform number of maize residues in all farms, and D is the design effect (1).

$$n = \frac{1}{4 \times 1 \times 0.1^2} (1.96^2) = 96.04 \approx 100 \quad (2)$$

Hence, 100 maize residues were randomly sampled in each field during each survey. Each maize residue sampled was inspected for stemborer infestations and subsequently dissected for the recovery of stemborer larvae or pupae (Plate 3.1). The recovered stemborer larvae and pupae were then counted, placed in glass vials (8.5 × 2.7 cm), and reared in the laboratory for the confirmation of species identification at the adult stage or for parasitoid recovery.



Plate 3. 1: Maze residue stems selection (A), maize residue habitat (B), dissecting of residue stem (C) and larvae recovery (D&E)

3.2.3 Recovery and identification of stemborers and their associated parasitoids

The collected stemborer larvae were reared on an artificial diet developed by Onyango and Ochieng'-Odero (1994) in cylindrical glass vials (8.5×2.5 cm) plugged with cotton wool and kept under ambient conditions in the laboratory (25 ± 1 °C; $67 \pm 4\%$ relative humidity) until pupation or cocoon formation in case of parasitism. Pupae were then taken out of the artificial diet/maize stems and sexed by examining their external morphology of the ventral surface of the eighth and ninth abdominal segments using a Wild dissecting microscope according to the method described by Underwood (1994). Then, they were kept in separate plastic containers (16×10 cm) closed with perforated plastic lids until adult emergence for stemborer species identification. Parasitoid cocoons and puparia collected from stemborer larvae/pupae were separately kept in glass vials (2.5 cm in diameter and 7.5 cm in height) until adult emergence and conserved in 70% ethanol for species identification

(Polaszek & Kimani, 1990; Kenis *et al.*, 2008;) in collaboration with the biosystematics unit of the International Centre of Insect Physiology and Ecology (ICIPE).

3.2.4 Sampling for the diversity and abundance of lepidopteran stemborers and associated larval and pupal parasitoids in wild plants during the non-copping seasons

Stemborer diversities and densities on wild plants (Plate 3.2) were determined using 50–100 plants/tillers that were randomly sampled for each plant species in each field depending on the availability of plant species.



Plate 3. 2: A photograph showing wild plant habitat in the areas studied

Each selected plant/tiller was then dissected in the field for recovery of the larvae and/or pupae and subsequently transported to the laboratory for both stemborer and parasitoid (in case of parasitism) species identification using the above-described protocol in section 3.2.2.

3.2.5 Sampling for the diversity and abundance of lepidopteran stemborers and associated larval and pupal parasitoids in maize plants during the cropping seasons

The stemborer present in the farmers' maize fields that ranged approximately 0.5–1.5 ha in size were sampled in each locality. The sampling followed the procedure described by Overholt *et al.*, (1994a). Briefly, each maize field (Plate 3.3), was sampled at both early and late whorl stages that is when plants were 4 and 8 weeks old, respectively (plant stages that are susceptible to stemborer attack). The field was initially divided into four quadrants, and 25 plants were randomly selected from each quadrant in a zig-zag pattern, to give a total of 100 plants per field, according to the equation described by Zar (1999). Plants with symptoms of stemborer damage, such as scarified leaves (window panes and pin holes), frass, dry leaves and shoots (dead hearts), or bored (entrance or exit) hole, were uprooted from the field and dissected for recovery of stemborer larvae and/or pupae from the stems or whorls. All collected stemborer larvae and pupae were counted and then brought into the laboratory for both stemborer and parasitoid species identification using the above-described protocol in section 3.2.2.



Plate 3. 3: Maize plants in cultivated habitat

3.2.6 Release of *Cotesia flavipes* and *Cotesia sesamiae* in the studied farmer's fields

The purpose of this experiment was to identify the refugia habitat (maize residues or wild plants) of parasitoids associated with maize stemborers during the non-cropping season. For that, the larval parasitoids *C. flavipes* and *C. sesamiae* were first released into the farmer's fields to "boost" their abundance in the studied fields. For each parasitoid species, adults, cocoon masses, and parasitized larvae were released (Table 3.1). These different stages were released to enhance the propensity of parasitoid establishment, as previously carried out by ICIPE in a biological control program using *C. flavipes* towards *C. partellus* in East and Southern Africa (Omwega *et al.*, 2006). The parasitoids were released from the center of each cultivated maize field to allow the parasitoids to locate their host.

Cocoon masses of *C. flavipes* and *C. sesamiae* were obtained from the Animal Rearing and Containment Unit (ARCU) at ICIPE Duduville, Nairobi, Kenya. When parasitoids were ready to emerge from cocoons, they were placed in a large sleeve cage (35 cm³) until emergence. After emergence, a 20% honey/distilled water solution offered on cotton wool

in a Petri dish was introduced into the cage to provide food for the parasitoid. The cage was then placed under incandescent light for ca. 24 h to stimulate mating. The parasitoid's hosts, the larvae of *C. partellus* for *C. flavipes* and larvae of *S. calamistis* for *C. sesamiae*, were also obtained from colonies reared at ARCU. These hosts were reared on an artificial diet until their fourth instar stage when they were finally removed from the artificial diet and transferred to maize stems, where they were allowed to feed for 24 h. The larvae were then exposed to the parasitoids using the hand stinging method (Overholt *et al.*, 1994b). After oviposition, the larva was immediately removed from the cage to avoid superparasitism. The parasitized larvae were placed into small vials (7.5 × 2.5 cm) containing artificial diet and incubated at 25 °C until cocoon formation 10–15 days after exposure. The cocoon masses were removed from the artificial diet, placed in a clean vial, and held until they darkened (15–20 days after exposure). At emergence, the females were allowed to mate for 24 h, after which they were ready for release. Prior to parasitoid release in the study areas, the farmer's fields were initially surveyed to confirm the presence of the parasitoids in the area.

Since *C. flavipes* was used in a classical biological control program against *C. partellus* (Midingoyi *et al.*, 2016; Overholt *et al.*, 1997) and *C. sesamiae* was found to efficiently parasitize *S. calamistis* and *B. fusca* larvae (Kfir *et al.*, 2002), *C. flavipes* was released in the Makutano area where *C. partellus* and *S. calamistis* co-infest maize fields (Sokame *et al.*, 2019a), whereas *C. sesamiae* was released in the Murang'a area where *B. fusca* and *S. calamistis* co-exist (Sokame *et al.*, 2019a). Field release of the parasitoids were done at the maize whorl stage, which is the favored stage for oviposition by stemborers. Adults and

cocoon parasitoid masses were released in the whorl of infested maize plants, while parasitized larvae were placed inside the leaf sheaths. Parasitoid field release was carried out in December 2017 and in June 2018 in three maize fields per locality (Table 3.1) early in the morning or late in the afternoon to allow the parasitoids to become acclimatized and locate refuges before the temperature became too high.

Thereafter, the parasitism rates after the parasitoid releases in stemborers found in either maize residues or wild plants during the non-cropping seasons and the parasitism rates in stemborers found in maize plants during the subsequent cropping seasons were estimated using the species identification protocol (Kenis *et al.*, 2008; Polaszek & Kimani, 1990) and the collected parasitized stemborer larvae.

3.2.7 Data Analysis

All analyses were carried out in the R software version 3.5.1 (R Core Team, Vienna, Austria) (R Core Team, 2018). The BioFTF R package (Di Battista *et al.*, 2017) was used to compare the diversity of maize stemborer communities and their associated parasitoids in maize residues and wild plants considering both the richness and the evenness. It exploits the β diversity profile model (Equation):

$$\Delta\beta = \sum_{i=1}^s \frac{(1 - p_i^\beta)}{\beta} p_i ; \beta \geq -1$$

For Equation (3), $\beta = -1$ generates the richness index, $\lim\beta \rightarrow 0$ represents the Shannon diversity index, and $\beta = 1$ returns the Simpson Index.

The number of larvae and pupae from the 100 sampled stems/tillers/plants were compared between habitats (maize residues and wild plants) using a generalized linear model with a negative binomial error distribution (GLM.nb) due to the nature of the count data of this parameter. Significant differences were separated by Tukey’s multiple comparisons tests performed using the R package “lsmeans” (Lenth, 2016). From the GLM results, the Odds Ratio (O.R.) with a 95% confidence level interval (O.R. (95% CI)) was calculated.

The stemborer and associated parasitoid species composition in either maize residues or wild plants and maize plants in cultivated field habitats were pairwise compared by calculating the Morista–Horn index (C_{mH}) (Magurran, 1988):

$$C_{mH} = \frac{2 \times \sum (n_{ia} \times n_{ib})}{(d_a + d_b) N_a \times N_b}$$

where N_a and N_b are the total number of individuals in maize residues and maize plants in cultivated fields or in wild plants and maize plants in cultivated fields, respectively; and n_{ia} and n_{ib} are the numbers of individuals of a given species i in maize residues and maize plants in cultivated fields or in wild plants and maize plants in cultivated fields, respectively:

$$d_a = \frac{\sum n_{ia}^2}{N_a^2}$$

and

$$d_b = \frac{\sum n_{ib}^2}{N_b^2}$$

High values of C_{mH} indicate increasing similarity between the two habitats, with a maximum of 1.

Principal component analysis (PCA) using two R packages called “FactoMineR” and “Factoextra” (Kassambara, 2017) was performed to establish whether the abundance of stemborer species in maize plants in the cultivated fields correlated to those in the maize residues or wild plants during the non-cropping season. A correlogram (R package “corrplot”) was also constructed and performed a correlation test using the Pearson method to elucidate correlations between the abundance of each species in maize plants of cultivated fields and those in either maize residues or wild plants. Furthermore, the proportions of females and males of each species (*B. fusca*, *S. calamistis*, and *C. partellus*) in maize residues and maize plants in cultivated fields were evaluated and compared using the two-tailed Fisher’s exact probability 2×2 test.

Parasitoids recovered in maize residues or wild plants during non-cropping seasons and in maize plants in cultivated fields during the subsequent cropping seasons in Makutano and Murang’a before (pre-release) and after or during *Cotesia flavipes* and *Cotesia sesamiae* release (post and during release) were expressed as mean (\pm standard error (SE)) of parasitized hosts recorded per field. The parasitism rate for each parasitoid for the *B. fusca*, *S. calamistis*, and *C. partellus* species was quantified as the proportion of parasitized larvae among the total number of the given species in each habitat (maize residues, wild plants, and maize plants) while parasitism rates between habitats were compared using a proportion test.

3.3 Results

3.3.1 Diversity and abundance of lepidopteran stemborers and associated parasitoids in maize residues and wild plants during the non-cropping seasons

A total of 785 stemborers were collected, of which 653 were obtained from maize residues and 132 from wild plants (Table 3.2). Among them were five Noctuidae, two Crambidae, and one Pyralidae (Table 3.2). Only three or four wild grass species were reported to surround the maize fields in Murang'a and Makutano, respectively, during field surveys (Table 3.2).

Table 3. 2: Stemborer species composition and total abundance in maize residues and wild plants during the non-cropping seasons in two different localities in the central region of Kenya in 2017 and 2018.

Habitats	Total Number	Stemborer Species Composition			
		Bf	Sc	Cp	Os
Makutano					
Maize residues	290	-	202	88	-
Wild plants	99	-	20	15	61
<i>Sorghum arundinaceum</i> *	25	-	4	8	9 Csp, 4 Mni
<i>Pennisetum purpureum</i> *	39	-	3	7	29 Csp
<i>Megathyrus maximum</i> *	22	-	7	-	15 Mn
<i>Cyperus</i> sp.†	13	-	6	-	7 Mn
Murang'a					
Maize residues	363	226	137	-	-
Wild plants	33	2	11	-	20
<i>Pennisetum purpureum</i> *	14		9	-	5 Sn
<i>Megathyrus maximum</i> *	6	2	1	-	3 Mn
<i>Cynodon dactylon</i> *	13	-	1	-	7 Ssp, 5 Mn

Os, Other species. Noctuidae (Bf, *Busseola fusca*; Mn, *Manga nubifera*; Sc, *Sesamia calamistis*; Sn, *Sciomesa nyei*; Ssp, *Sciomesa* sp.)/Crambidae (Cp, *Chilo partellus*; Csp, *Chilo* sp.)/Pyralidae (Mni, *Mussidia nigrivenella*). Plant family: * Poaceae, † Cyperaceae. -: species was absent.

In each locality, the wild plant habitat had a significant higher population of stemborer species diversity than the maize residue habitat (Table 3.3). The wild plants had a figure of 5 indicating a high species richness compared to the maize residues that had 2 implying less richness or diversity both in Makutano and in Murang'a. The same trend was showed

by Shannon and Simpson indices. No differences in species richness both on wild plants and maize residues were observed between sites.

Table 3. 3: The stemborer species diversity ranking in maize residue and wild plant habitats during the non-cropping seasons in two different localities in the central region of Kenya.

Habitats	Makutano			Murang'a		
	Richness	Shannon	Simpson	Richness	Shannon	Simpson
Maize residues	2	0.627768	0.4227348	2	0.6760856	0.4699436
Wild plants	5	1.503721	0.7378839	5	1.5600483	0.7584940

A high number indicates great diversity in the habitat.

However, wild plants had a lower stemborer abundance than maize residues, from which only *B. fusca*, *S. calamistis*, and *C. partellus* were obtained (Figure 3.1; GLM.nb results: for Makutano (O.R. = 0.09 (0.06–0.14), $p < 0.00010$) and for Murang'a (O.R. = 0.08 (0.05–0.11), $p < 0.0001$)).

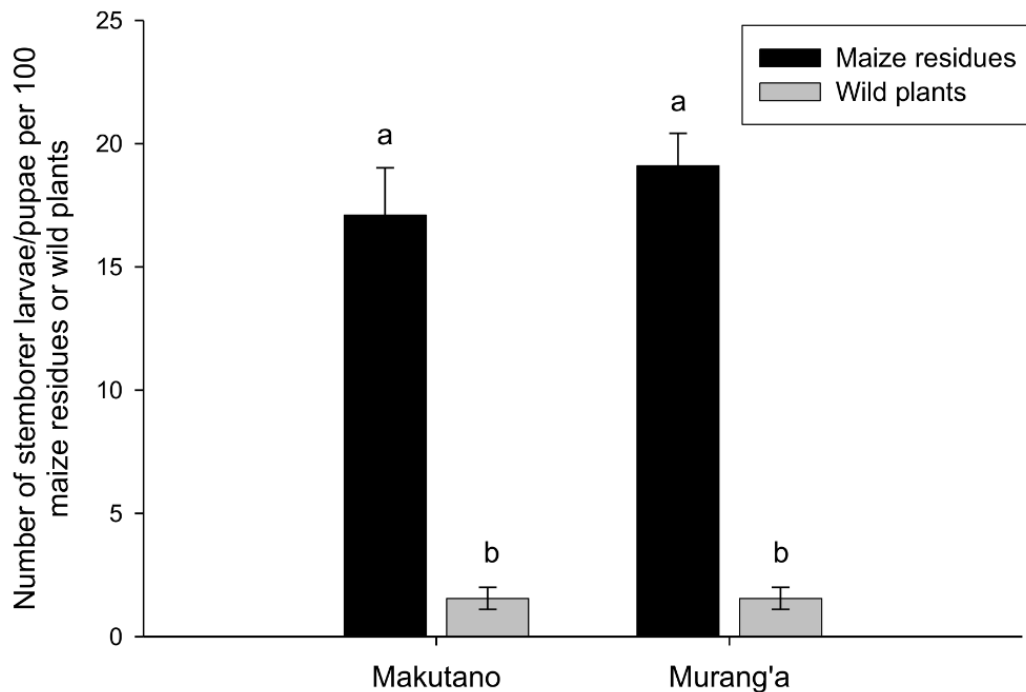


Figure 3. 1: Number of *Busseola fusca*, *Sesamia calamistis*, and *Chilo partellus* stemborers found in maize residues and wild plants per 100 maize residues or wild plants sampled in two different localities in the central region of Kenya (Makutano and Murang'a) during the non-cropping seasons in 2017 and 2018. Bars with the same letters are not significantly different following Tukey's multiple comparison tests.

During the two years of surveys, the parasitoid species that were obtained were from the families of Braconidae, Ichneumonidae, Eulophidae, and Tachinidae (Table 3.4). Among them, the braconids *C. flavipes* and *C. sesamiae* were the most abundant and widespread species after release; and were mainly obtained from *B. fusca*, *S. calamistis*, and *C. partellus* from the maize residues.

Table 3. 4: Parasitoid composition and abundance from stemborer species in maize residues and wild plants during the non-cropping seasons in two different localities in the central region of Kenya in 2017 and 2018 (i.e., before and after/during parasitoid release).

Parasitoid Species	Stem	Stem	Wild Plants	Total Number of Parasitized Hosts	Makutano		Murang'a	
	Borer Species	Borer Stages			Maize Residues	Wild Plants	Maize Residues	Wild Plants
Hymenoptera: Braconidae								
<i>Cotesia flavipes</i>	Cp, Sc	larva	Sa	9	8	1	-	-
<i>Cotesia sesamiae</i>	Bf, Sc	larva	-	12	-	-	12	-
Hymenoptera: Ichneumonidae								
<i>Syzectus sp.</i>	-	pupa	Sa, Pm	3	-	3	-	-
Hymenoptera: Eulophidae								
<i>Pediobius furvus</i>	Cp, Sc	pupa	Sa	4	1	1	-	2
Diptera: Tachinidae								
<i>Siphona (Meigen) sp.</i>	Bf, Mn	larva	Pm	3	-	-	-	3

Stemborer species: Bf, *Busseola fusca*; Sc, *Sesamia calamistis*; Cp, *Chilo partellus*; Mn, *Manga nubifera*. Plant species: Sa, *Sorghum arundinaceum*; Pm, *Panicum maximum*. -: absent.

Similar to the stemborer species, the wild plant habitat had a higher parasitoid species diversity than the maize residue habitat (Table 3.5). Maize residues from both sites had less richness of the parasitoid species compared to wild plants.

Table 3. 5: Stemborer-associated parasitoid species diversity ranking in maize residue and wild plant habitats during the non-cropping seasons in two different localities in the central region of Kenya.

Habitats	Makutano			Murang'a		
	Richness	Shannon	Simpson	Richness	Shannon	Simpson
Maize residues	2	0.3638339	0.1975309	1	0.2852830	0.1420118
Wild plants	3	0.9824805	0.5600000	2	0.6861506	0.4800000

A high number indicates great biodiversity in the habitat.

3.3.2 Relationships of abundance of maize stemborer species and their respective parasitoids in maize residues or wild plants during the non-cropping seasons and in maize fields during subsequent cropping seasons

A total of 1200 stemborer larvae and pupae consisting of *B. fusca*, *S. calamistis*, and *C. partellus* were collected in maize plants in the two localities during subsequent cropping seasons (Table 3.6). The average number per sample was 11.87 ± 6.51 and 9.00 ± 4.51 for *C. partellus* and *S. calamistis*, respectively, with an overall average of 10.72 ± 3.94 in Makutano. The average number per sample was 20.95 ± 7.76 and 7.58 ± 2.98 for *B. fusca* and *S. calamistis*, respectively, with an overall average of 14.21 ± 4.51 in Murang'a.

Table 3. 6: Total number of larvae/pupae of lepidopteran maize stemborer species (with the relative proportion (%) in parenthesis) collected from maize plants in cultivated fields during the subsequent cropping seasons in two different localities in the central region of Kenya in 2017 and 2018.

Localities	Total number	Stemborer Species Composition		
		<i>Busseola. fusca</i>	<i>Sesamia calamistis</i>	<i>Chilo partellus</i>
Makutano	515	-	230 (55.83)	285 (100)
Murang'a	685	503 (100)	182 (44.17)	-
Total number	1200	503 (100)	412 (100)	285 (100)

-: species was absent.

In each locality, the Morista–Horn indices for both stemborers and associated parasitoids species were higher between maize plants versus maize residues in comparisons between maize plants versus wild plants (Table 3.7).

Table 3. 7: The Morista–Horn similarity index between maize plants versus maize residues and between maize plants versus wild plants in the carry-over of lepidopteran maize stemborers and associated larval/pupal parasitoid species.

Localities	Morista–Horn Index (<i>CmH</i>)			
	Lepidopteran Maize Stemborer Species		Associated Larval/Pupal Parasitoid Species	
	Maize Plants vs. Maize Residues	Maize Plants vs. Wild Plants	Maize Plants vs. Maize Residues	Maize Plants vs. Wild Plants
Makutano	0.96	0.37	0.99	0.30
Murang'a	0.97	0.31	0.99	0.02

A value close to 1 indicates a greater similarity between the two habitats and vice versa.

The Principal Component Analysis (PCA), which was performed to correlate the abundance of stemborer species between habitats (maize residues, wild plants, and maize plants), revealed that stemborer species abundance in cultivated maize fields was highly

correlated to abundance in maize residues, but not to stemborer abundance in wild grasses (Figure 3.2).

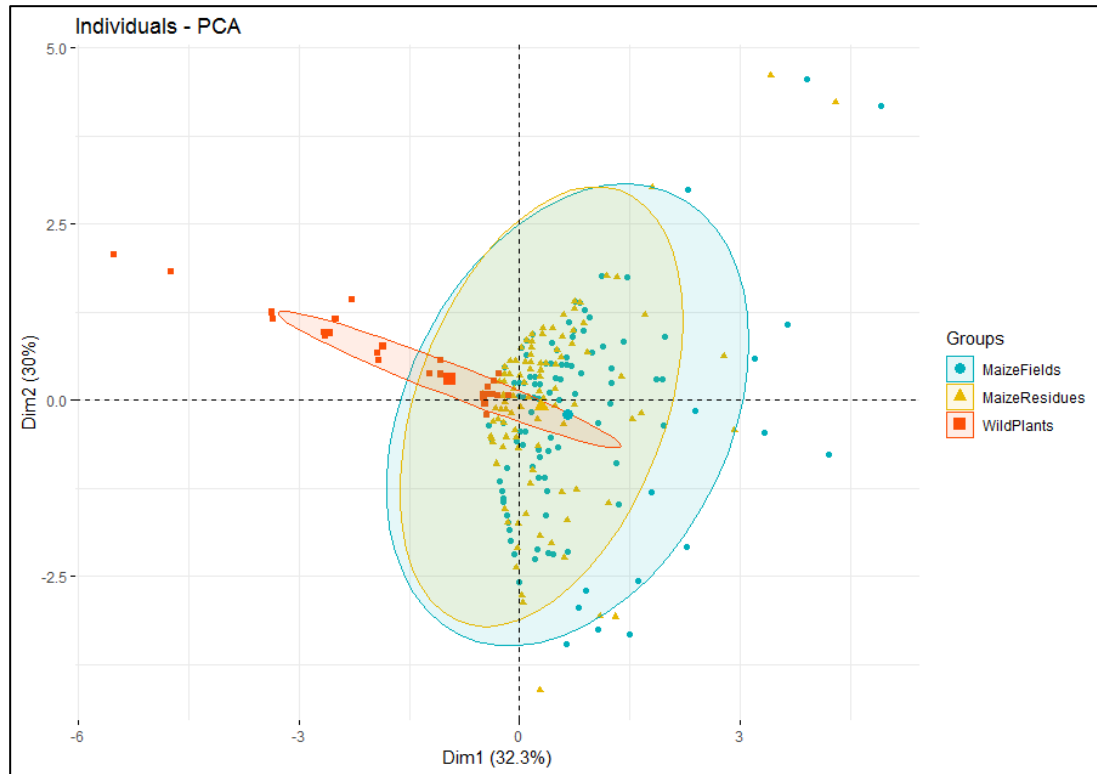


Figure 3. 2: The principal component biplot showing the relationship between abundance of stemborer species in maize plants in the cultivated fields during the cropping season and in maize residues or wild plants during the non-cropping season.

The correlations between the abundance of each stemborer species in maize plants in the cultivated fields and those in either maize residues or wild plants are illustrated in the correlogram shown in Figure 3.3. The abundance of *Busseola fusca*, *Sesamia salamistis*, and *Chilo partellus* in maize plants of cultivated fields were significantly and positively correlated with their carry-over abundances in maize residues during non-cropping seasons ($r = 0.84$, $t = 17.00$, $df = 118$, $p < 0.0001$; $r = 79$, $t = 14.06$, $df = 118$, $p < 0.0001$; $r = 0.96$, $t = 28.54$, $df = 118$, $p < 0.0001$, respectively), while no relationship was

evidenced between their abundances in wild plants ($r = 0.12$, $t = 1.34$, $df = 118$, $p = 0.18$; $r = 13$, $t = 1.45$, $df = 118$, $p = 0.14$; $r = 0.06$, $t = 0.68$, $df = 118$, $p = 0.49$, respectively).

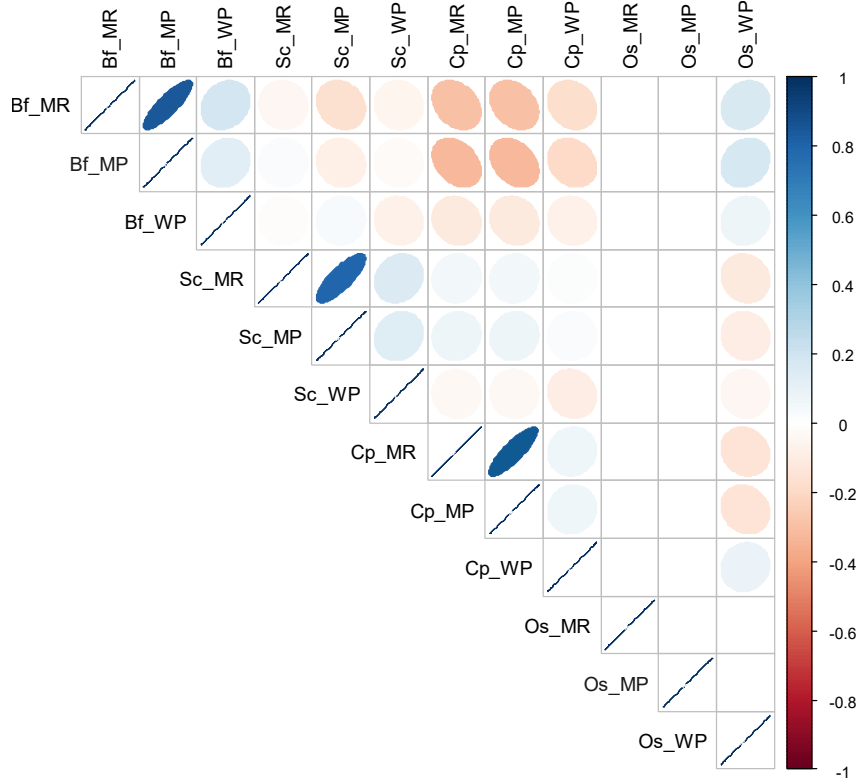


Figure 3. 3: A correlogram highlighting the direction and intensity of the correlation between the abundance of each stemborer species in maize plants in the cultivated fields during the cropping season and those in either maize residues or wild plants during the non-cropping season. The blue color denotes a positive correlation and the red color a negative correlation. Higher intensity of the color indicates a strong correlation. Bf_MR, *B. fusca* in maize residues; Bf_MP, *B. fusca* in maize plants; Bf_WP, *B. fusca* in wild plants; Sc_MR, *S. calamistis* in maize residues; Sc_MP, *S. calamistis* in maize plants; Sc_WP, *S. calamistis* in wild plants; Cp_MR, *C. partellus* in maize residues; Cp_MP, *C. partellus* in maize plants; Cp_WP, *C. partellus* in wild plants; Os_MR, other species in maize residues; Os_MP, other species in maize plants; Os_WP, other species in wild plants. For other species (Os), see Appendix 1.

Furthermore, for each species, the percentage of females to males was significantly higher in the maize residue habitat than in maize plants of the cultivated habitat (Figure 3.4; *B.*

fusca: $p = 0.0002$; *S. calamistis*: $p = 0.001$; and *C. partellus*: $p = 0.005$; two-tailed Fisher's exact test).

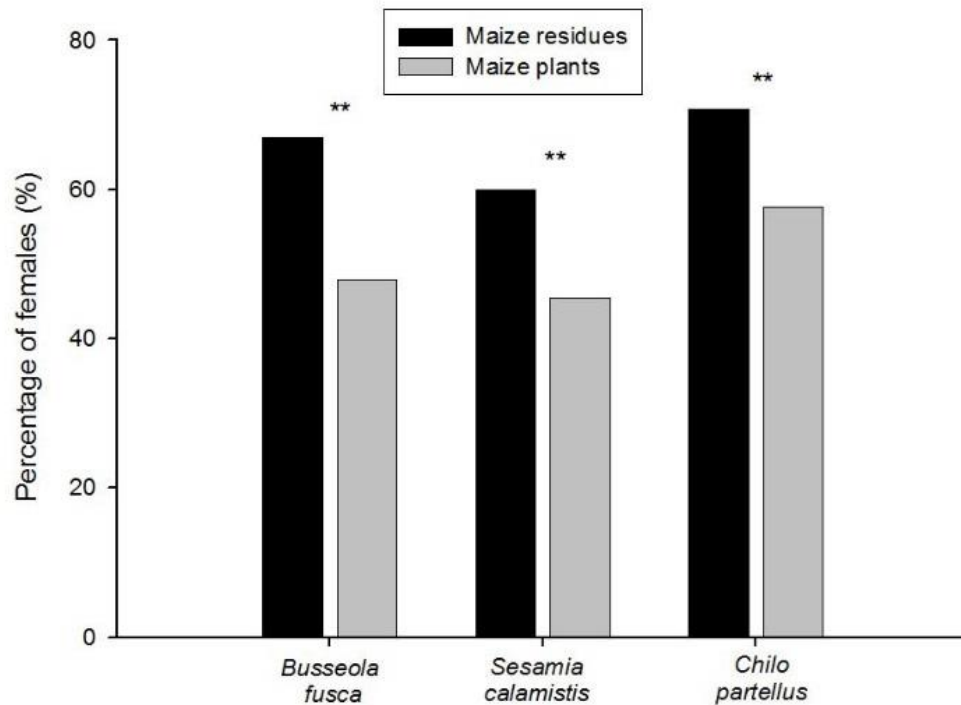


Figure 3. 4: Percentages of females of *Busseola fusca*, *Sesamia calamistis*, and *Chilo partellus* found in the maize residue habitat during the non-cropping seasons and on maize plants in the cultivated habitat during the subsequent cropping seasons. The proportion of males and females was set at 100% to calculate the percentage of females. The proportions of males and females were compared between habitats for each species using a two-tailed Fisher's exact probability 2×2 test (**: $p < 0.01$).

Before the release of parasitoids into the field, *C. flavipes* or *C. sesamiae* had not been recovered from either maize plants, maize residues, or wild plants in the study areas of Makutano and Murang'a. Three parasitoid species (*Syzectus* sp., *Pediobius furvus*, and *Siphona* sp.) were mainly recorded, on wild plants, of which only *Pediobius furvus* was present in both maize residues and maize fields in Makutano (Figure 3.5). After and during the releases, *C. flavipes* and *C. sesamiae* were recovered mostly from maize plants

during subsequent cropping seasons and maize residues during non-cropping seasons respectively (Figure 3.5).

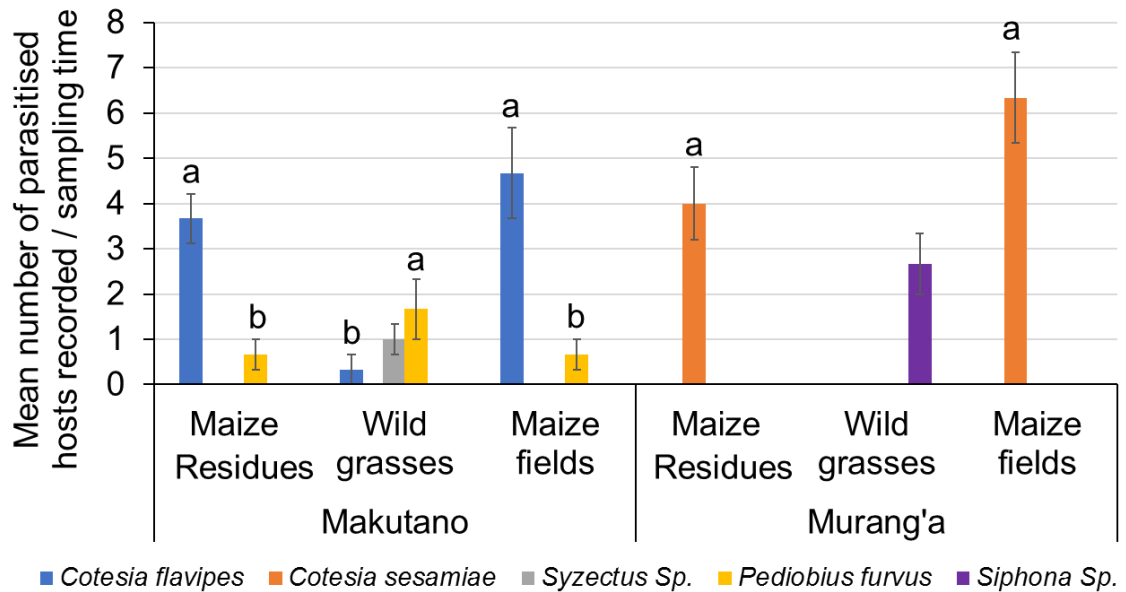


Figure 3. 5: The mean number (\pm SE) of parasitoid species recovered per each field in maize residues or wild plants during the non-cropping seasons as well as in maize plants from the cultivated fields during the subsequent cropping seasons in Makutano and Murang'a before (pre-release) and during or after (post and during release) *Cotesia flavipes* and *Cotesia sesamiae* releases.

The parasitism rates of *C. flavipes* and *C. sesamiae* obtained in the respective host species did not differ between habitats. As summarized in Table 3.8, there was no difference in parasitism rate between the different habitats. Overall, the parasitism levels were low, and generally nil in wild habitats.

Table 3. 8: The parasitism rate of *Cotesia flavipes* and *Cotesia sesamia* during and after the release according to the stemborer species and refugia habitat.

Parasitoids	Stemborer Species	Parasitism Rates (%)			Proportion Test		
		Maize Plants	Maize Residues	Wild Plants	χ^2	df	p
<i>Cotesia flavipes</i> (In Makutano)	<i>Sesamia calamistis</i>	3.48 a	5.68 a	5.00 a	0.8	2	0.7
	<i>Chilo partellus</i>	2.11 a	1.49 a	0.00 a	0.5	2	0.8
<i>Cotesia sesamia</i> (In Murang'a)	<i>Sesamia calamistis</i>	2.39 a	3.10 a	0.00 a	0.4	2	0.8
	<i>Busseola fusca</i>	3.85 a	3.65 a	0.00 a	0.4	2	0.8

The means in the same row followed by the same letters did not differ significantly

3.4 Discussion

This study highlights for the first time a broader host range of these insect species in wild habitats compared to maize residue habitats, although higher diversities of stemborers and parasitoids in wild habitats compared to cultivated habitats has already been well-reported in literature (Ong'amo *et al.*, 2018; 2013; 2006a; 2006b; Mailafiya *et al.*, 2011; 2009 Matama-Kauma *et al.*, 2008). This variation in distribution in insect diversity between wild and cultivated habitats might be a consequence of anthropogenic changes in the ecology of the availability of food resources, constraints of natural enemies, and the evolution of competitive interactions (Stewart *et al.*, 2015;). Diniz *et al.* (2010), who studied species richness of flower-head insects (Tephritidae: Diptera) in natural and cultivated habitats, have reported that anthropogenic alterations in the landscape determine the impoverishment of insect diversity in cultivated habitats. For stemborers'

parasitoids, Mailafiya *et al.* (2011) showed that parasitoid diversity was lower in locations where maize cultivation was practiced on a large commercial scale and where intense grazing activities persist across seasons. Nevertheless, a higher insect diversity does not mean a higher insect abundance. In fact, the abundance of stemborers (i.e., *B. fusca*, *S. calamistis*, and *C. partellus*) which are key maize plants pests and their associated parasitoids (i.e., *C. flavipes* and *C. sesamiae*), were found to be higher in the maize residue habitat as compared to the wild plant habitats. It was previously reported that both stemborers and their associated parasitoids are generally less abundant in wild habitats than in maize plants in cultivated habitats (Ong'amo, *et al.*, 2013; 2006a; Ndemah *et al.*, 2007). Although natural habitats surrounding cereal crops serve as refugia for sustaining the diversity of both stemborers and parasitoids from adjacent cereal fields (Mailafiya *et al.*, 2009), the abundance of stemborers and associated parasitoids is very low in the wild compared to cultivated fields (Diniz *et al.*, 2010; Ong'amo, *et al.*, 2006a). A possible explanation is the low abundance of wild plant species surrounding the studied fields. In fact, when analyzing the data obtained by Mailafiya (2009) in other agro-ecological zones in Kenya, the correlation between the abundance of stemborers in the maize fields and in maize residues was greater for maize fields surrounded by a low diversity of wild plants (Kitale and Mtito Andei) than for those surrounded by a high diversity of wild plants (Kakamega and Muhaka) (see Appendices 1, 2, 3 & 4).

In addition, these findings indicate that the existing correlation between stemborer species abundance in maize plants in cultivated fields with those in maize residues depend not only on the abundance of wild plant species in the agro-ecosystem but also on the

abundance of wild plants that are suitable hosts to maize stemborers. For instance, *Megathyrus maximus* and *Sorghum arundinaceum* (wild sorghum), surrounding maize fields suitable hosts to maize stemborers in wild habitat (Le Ru *et al.*, 2006a, 2006b). Another possible explanation is the generally higher survival and growth rates of the stemborers, and, thus, their associated parasitoids, on cultivated plants compared to wild plants (Juma *et al.*, 2013; 2015; Ndemah *et al.*, 2007).

In this study, the highest Morista–Horn similarity indices of both maize stemborers and their associated parasitoids obtained in maize plants from the cultivated habitat with maize residues compared to the wild habitats indicate that maize residues may constitute an important refugia source not only of the maize stemborers but also of their associated parasitoids. These results agree with those of Mailafiya (2009) on analyzing the data on stemborer and parasitoid recoveries between wild and maize residue habitats during non-cropping seasons from Kitale, Mtito Andei, Kakamega and Muhaka (see Appendix 5). It has also been reported that *S. calamistis* populations inhabiting wild habitats genetically differ from those living in cultivated habitats (Ong’amo *et al.*, 2008), which compromises wild habitats as a refugia source of that species coming from maize plants in cultivated habitats. Although it was shown that *B. fusca* infestation might originate from specimens that come from far away from the maize fields, probably from quite a distance (Ndjomatchoua *et al.*, 2016), based on the insect abundance relationships between maize residues and maize plants, it is not possible to preclude maize residues from being the main reservoir of maize stemborers, particularly when wild plants surrounding maize fields are scarce. It is reported that *B. fusca*, for example, survives the dry season as larvae

diapausing into maize residues left in the field after harvest (Polaszek & Khan, 1998; Kfir, 1991; Kfir *et al.*, 1989; Unnithan & Reddy, 1989; Gebre-Amlak, 1988). The high positive correlation on the abundance of stemborers in the maize fields and in maize residues obtained in this study reinforces the fact that maize residues might serve as the main reservoir of maize stemborers in the non-cropping seasons and are likely, the main source of the carry-over of the maize pest to the next cropping season.

In addition, each stemborer species recovered from maize residues gave rise to a significantly greater percentage of females compared to stemborers recovered in maize plants. This is in accordance with Gebre-Amlak (1989), who reported that the first generation of *B. fusca* coming from diapause larvae found earlier in the cropping season gave more females than males compared to subsequent generations. These seasonally dependent sex ratio variations might be due to either climatic and environmental factors or intrinsic factors of the insect to ensure the perennity of its species by a female-biased sex ratio distortion when the conditions became unfavourable. Kageyama *et al.*, (1998), studying the occurrence of feminizing bacteria in an insect by a female-biased sex ratio in *Ostrinia furnacalis* (Lepidoptera: Crambidae), the Asian corn borer, concluded that, in the sex determination systems in lepidopteran insects, chromosomal males are feminized by a cytoplasmic agent, most probably parasitic bacteria, according to the conditions. This phenomenon has also been confirmed in other insect species (Gempe & Beye, 2010; Hediger *et al.*, 2010;) and may need exploration in lepidopteran stemborers in relation to their habitats.

The absence of both *C. flavipes* and *C. sesamiae* in the field before any release could be because of: (i) the frequent use of pesticides; (ii) the systematic use of maize residues for animal feed during dry seasons; or (iii) the climate change adaptation of both parasitoids and hosts to dry seasons, which have been particularly long over the last five years (Calatayud P-A., Pes. Obs.). In contrast, during and after parasitoid releases, although the parasitism rates were low, the recovery of *C. flavipes* and *C. sesamiae* obtained in maize fields during the subsequent cropping seasons indicate a possible establishment of the parasitoids released in these areas. The low parasitism normally occurs after the first parasitoid release (Omweaga *et al.*, 2006) and according to the season, the year, and the locality (Jiang *et al.*, 2013; Overholt *et al.*, 1994a). The successful establishment of *C. flavipes* after release has already been observed in different countries, including coastal Kenya (Midingoyi *et al.*, 2016; Otieno *et al.*, 2008; Zhou *et al.*, 2001).

Similar to maize stemborers, *C. flavipes* and *C. sesamiae* were mostly recovered during and after the releases in maize residues during the non-cropping seasons. This confirms that the residues as being the main reservoir of maize stemborer parasitoids during the non-cropping periods. This aspect is important to consider in the context of biological control. In fact, it has been recommended that the maize residues (observed to be an important reservoir of maize stemborers) be burned (Kfir, 1991; Kfir *et al.*, 1989; Gebre-Amlak, 1988) in order to diminish the risk of maize infestation by stemborers for the subsequent cropping seasons (Moolman *et al.*, 2014). This management measure should not be adopted in areas where *C. flavipes* and *C. sesamiae* have been released or where the wild habitat has been drastically reduced. In this contexts, maize residues might ensure

the existence and/or survival of the parasitoids during dry seasons. Considering that: (i) *C. flavipes* and *C. sesamiae* were found to be rare or absent in all habitats prior to release; (ii) parasitism by *C. flavipes* is generally low or absent in the years after their release for biological control (Overholt, *et al.*, 1994a); and (iii) maize residues are also the main reservoir of parasitoids during dry periods (Mailafiya, 2009) (see Appendices 1 & 3), maize residues may also represent the main sources of stemborer parasitoids during non-cropping seasons. This suggests that maintaining maize residues in the field will promote parasitism of stemborers. However, to determine whether a buildup of the parasitoid population might occur over time, a further study need to be conducted on the comparable effect of infestation and parasitism over a longer time period to confirm the influence of maize residues on both infestation and the presence of parasitoids. In addition, some wild plants, such as wild sorghum *S. arundinaceum*, support a high survivorship of parasitized stemborers and, therefore, a relatively high performance of their larval parasitoids (Mailafiya *et al.*, 2011). The maintenance of these wild plants is vital for the survival and, thus, the perenity of *C. flavipes* and *C. sesamiae* in the field.

In conclusion, this study demonstrates the importance of maize residue habitats as an important reservoir source of maize stemborers and their associated parasitoids that ensures the continuation of the maize stemborer parasitoids in the field during dry periods. Therefore, systematic destruction of maize residues would not help the biological control of lepidopteran stemborers mostly in areas with reduced wild habitat.

CHAPTER FOUR:

**CATERPILLAR-INDUCED PLANT VOLATILES ATTRACT
CONSPECIFIC AND HETEROSPECIFIC ADULTS FOR
OVIPOSITION WITHIN A COMMUNITY OF LEPIDOPTERAN
STEMBORERS ON MAIZE PLANT**

Abstract

Olfactory cues may influence host plant preferences for oviposition of female moths within a community of stemborers that utilise the same resource. The objective of the study was to evaluate plant preferences for oviposition of gravid females of noctuid stemborers, *Busseola fusca* and *Sesamia calamistis*, and the crambid *Chilo partellus* for uninfested maize plants and plants infested by conspecific or heterospecific larvae. The involvement of volatile organic compounds (VOCs) emitted by uninfested and maize plants infested by conspecific or heterospecific larvae on moth orientation was studied in Y-tube olfactometer assays and in the field. All gravid female moths significantly ($P < 0.05$) preferred VOCs emitted by plants infested by conspecific or heterospecific larvae over those from uninfested plants and the female moths did not systematically prefer VOCs emitted by plants infested by conspecifics. Field trials confirmed these results. Chemical analysis by coupled gas chromatography/mass spectrometry showed that VOCs emitted by larvae-infested plants, regardless of the stemborer species, were compositionally richer than those released by uninfested plants, but their emission intensity varied with species involved in the infestation. *Busseola fusca* larvae induced a

compositionally richer VOCs profile than *S. calamistis* and *C. partellus* larvae. Eight plant volatile candidate attractants were associated with larvae-infested plants. These results open new avenues to develop specific VOC attractants to trap female stemborer moths in the field.

Key words: Lepidoptera stemborers, maize plant, volatiles, olfaction, interactions

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4.1 Introduction

Direct and indirect interactions among insect species maintain the structure and function of ecological communities (Wootton & Emmerson, 2005). Often, these interactions occur through the same resources utilisation by phytophagous insects. Its outcome can be negative (e.g. competition), positive (e.g. facilitation) or neutral (Speight *et al.*, 2008; Kaplan & Denno, 2007). These interactions can be directly intraspecific or interspecific (Memmott *et al.*, 2007) or indirectly through the mediation of the same host plant (Kaplan & Denno, 2007). The interactions among species can influence the oviposition preferences of gravid females utilising the same host plant in a community (Craig *et al.*, 2000). Thus, competition for or facilitation in the use of the same resource can influence the final choice by the female for oviposition. For example, prior larval feeding on a plant induces female oviposition (Facknath, 2012; Viswanathan *et al.*, 2005; Groot *et al.*, 2003; Craig *et al.*, 2000), while in some cases may deter subsequent oviposition on the same plant (Fatouros *et al.*, 2012; De Moraes *et al.*, 2001). Constitutive and inductive plant

volatile chemicals have been identified to play an important role in host plant selection by phytophagous insects for food or oviposition (Dicke & Van Loon, 2000). The infochemicals from insect infesting stages (eggs or larvae) from some insect conspecifics have been shown to influence gravid female oviposition choice, which serves to adjust population sizes to available resources with, in general, a preference for uninfested plants (Rothschild & Schoonhoven, 1977).

The lepidopteran species *Busseola fusca* (Fuller) (Noctuidae), *Sesamia calamistis* Hampson (Noctuidae) and *Chilo partellus* (Swinhoe) (Crambidae) are among the main pests of cereals in sub-Saharan Africa (Kfir *et al.*, 2002). These insect species are referred to as stemborers due to their cryptic habit of their larval stages to feed in plant stems. In East and Southern Africa, where they co-exist, these pests occur as communities of single or mixed species infesting not only cereal crops in the fields (Krüger *et al.*, 2008; Ong'amo *et al.*, 2006a & 2006b) but also wild graminaceous plants (Moolman *et al.*, 2014 ; Le Ru *et al.*, 2006b). The composition of these stemborer communities varies with locality, altitude and season. For example, in Kenya, *B. fusca* is the dominant species in the highlands, while *C. partellus* dominates in the lowlands. *Sesamia calamistis* is present at all altitudes in low numbers. In the mid-altitude regions, the stemborers occur as a mixed community of the three species, but with variation in species dominance with respect to the locality, season and year (Ong'amo *et al.*, 2006a & 2006b; Guofa *et al.*, 2001). In parts of Southern Africa *C. partellus* occurs in mixed populations with *B. fusca* in both the highland (Ebenebe *et al.*, 1999; Van Rensburg & Van den Berg, 1992) and lowland regions (Krüger *et al.*, 2008) but the dominance of a species has been reported to

vary with environment. Under laboratory studies, both intra- and interspecific competitions were observed between the three species with stronger interspecific competition for food resource utilisation recorded between *B. fusca*, *S. calamistis*, the two noctuids and the crambid *C. partellus* than between the two noctuids, *B. fusca* and *S. calamistis* (Ntiri *et al.*, 2016). Additionally, ovipositing females from these pest species prefer maize plant previously infested by larvae over uninfested plants suggesting the existence of a strong relation between larval feeding and female oviposition choice (Ntiri *et al.*, 2018). Hence maize plants infested by conspecifics or heterospecifics may produce or elicit chemical signatures rendering the plants more attractive to female moths.

Olfaction plays a major role in insect-plant interactions and this has been extensively investigated (e.g. Giunti *et al.*, 2016). Nevertheless, the impact of olfactory cues shaping the relationships among insects from the same guild competing for shared resources, mostly in hetero-specific interaction for oviposition site, is poorly understood. For instance, signals left by closely related species, as oviposition and host-marking pheromones, may be exploited by herbivores to detect competitor activity (Ukeh *et al.*, 2012). In this scenario, the prompt recognition of unsuitable or harmful habitats by host-seeking insects is critical for their survival and fitness. As such, semiochemicals emitted by infested plants could be exploited by host seeking insects to avoid competition. On the other hand, for some insect species, such cues rather elicit responses which indicate attractiveness (e.g. Horgan, 2012; Agrawal & Sherriffs, 2001).

This study focused on the olfactory responses of the gravid females of *B. fusca*, *S. calamistis* and *C. partellus* to maize plant odours infested by conspecific and

heterospecific larvae, compared to the uninfested plants. Initially, the attractiveness of each species to uninfested and maize plants infested by either *B. fusca*, *S. calamistis* or *C. partellus* larvae was evaluated. Secondly, the preference of odours emitted by plants infested by either *B. fusca*, *S. calamistis* or *C. partellus* larvae were compared. These results were finally linked to the levels of volatile organic compounds (VOCs) produced by both uninfested and larvae-infested maize plants captured by both dynamic headspace and Solid Phase MicroExtraction (SPME) techniques and analysed through gas chromatography/mass spectrometry (GC/MS).

4.2 Materials and methods

4.2.1 Plants and Insects

Maize plants of hybrid H513 (Simlaw, Kenya Seed Company, Nairobi, Kenya) were grown in plastic pots (12 cm in height x 13 cm in diameter) in a greenhouse at the Duduville campus of the International Centre of Insect Physiology and Ecology, (*icipe*) Nairobi, Kenya. Mean temperatures were approximately 31/17°C (day/night) with a L12:D12 photoperiod. Plants were used in experiments at the age of between 4-6 weeks old, i.e. about 60 - 75 cm tall.

Because wild insects are more responsive to plant odours compared to laboratory-reared insects, as shown for *B. fusca* (Calatayud *et al.*, 2008b), only field-collected insects of *B. fusca* (Bf), *S. calamistis* (Sc) and *C. partellus* (Cp) were used in the Y-tube experiments. For each species, fourth to fifth instar larvae were collected from maize fields. They were then reared until pupation on the artificial diet of Onyango & Ochieng'-Odero (1994) for *B. fusca* and *S. calamistis*, and on the artificial diet of Ochieng *et al.* (1985) for *C.*

partellus. Pupae were sexed according to the method described by Underwood (1994) and males and females separately kept in plastic containers (21 x 15 x 8 cm) until adult emergence. A cotton pad moistened with water was placed inside the container to maintain relative humidity at >80%. The insects were kept in a rearing room at a temperature of $25 \pm 0.05^\circ\text{C}$, RH of $58.5 \pm 0.4\%$, and a photoperiod of L12:D12.

Emerged adult males and females of each species were put together in a mating cage (40×40×63 cm), at the onset of the scotophase. The mating status was checked at an hourly intervals until the end of the scotophase. Pairs of mating moths were collected in plastic jars (8 cm high × 5 cm in diameter). The gravid females were used in experiments on the following night. After each experiment, all females used were dissected to check for the presence of spermatophores in the *bursa copulatrix*, which in Lepidoptera indicates successful mating (Lum, 1979). Only females bearing spermatophores were considered in the results. For plant infestations, larvae of *B. fusca*, *C. partellus* and *S. calamistis* were obtained from colonies reared at the Animal Rearing and Containment Unit (ARCU) at *icipe*, Nairobi, Kenya. All colonies were rejuvenated twice a year with field-collected larvae.

4.2.2 Plant infestations

For each stemborer species, single maize plants were manually infested by putting them in the whorl with 12 (for Y-tube experiments) or 5 (for field experiments) third and fourth instar larvae, the larval stages reported to co-occur with adult moths in the field (Le Ru B. & Calatayud P.-A., Pers. Observ.). These larval stage and infestation level were chosen to ensure a high degree of feeding damage within 24 hours prior to the start of the

experiments. Because of the long duration of the field experiments, the number of larvae used for infestation had to be lower to ensure the survival of the maize plants.

4.2.3 Olfactometer bioassays

This experiment was carried out in a Y-tube olfactometer (Plate 4.1), which is more useful for evaluating differences in attraction to odours in moths than wind tunnel (Calatayud *et al.*, 2014c). Similar to Petit *et al.* (2018), the olfactometer had the dimensions of 18cm of stem length, 34 cm of each arm length and 4 cm of diameter.

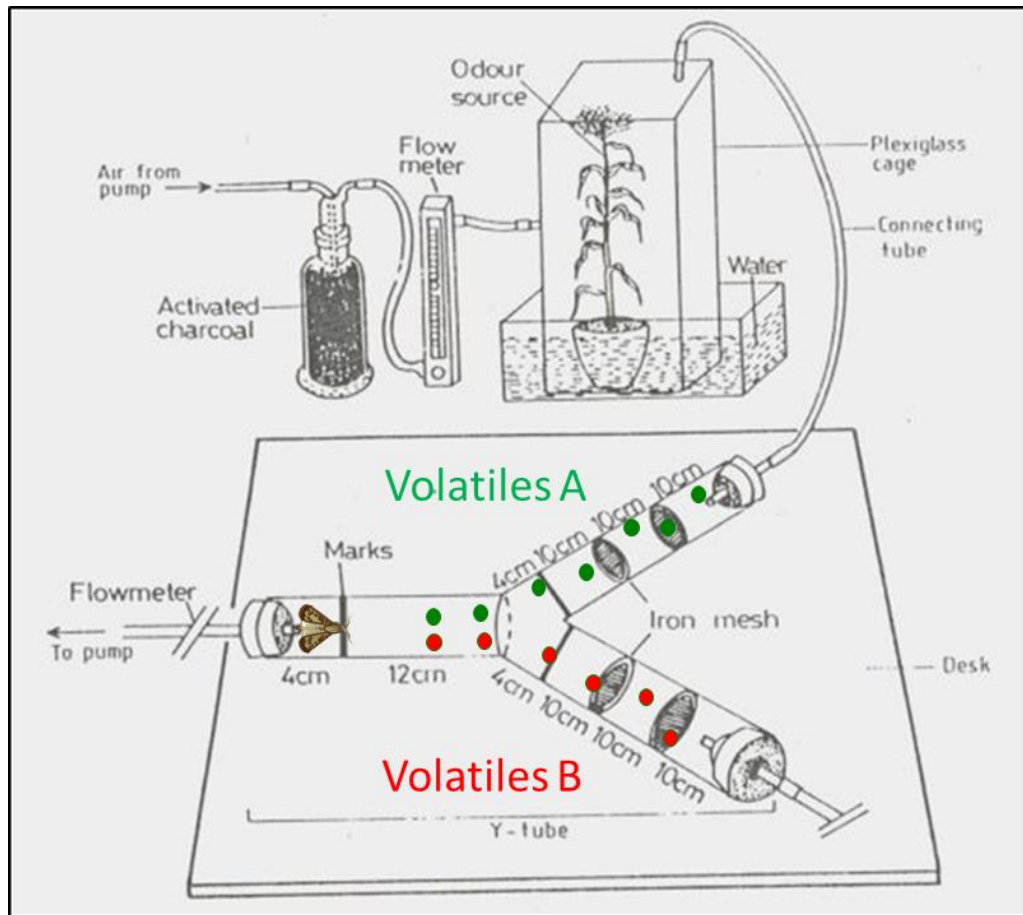


Plate 4. 1: Y-tube olfactometer of Ngi-Song et al. (1996)

Observations were performed 0–4 hours after the onset of the scotophase for females corresponding to the period of oviposition for the species under study (Calatayud *et al.*, 2007). The pot and soil of each potted plant (1 plant per pot) were wrapped with aluminium foil to prevent the introduction of foreign volatiles from the plastic pot and soil into the Y-tube. Each plant was introduced into a Perspex chamber measuring 30 x 30 x 120 cm large enough to contain the whole potted plant. The closed ends of each chamber were connected with Teflon tubing to either of the two arms of the Y-tube. Clean air was drawn into the system over the sample through the arms of the olfactometer. The airflow was set at 15 cm s⁻¹ per arm and measured by flow meters connected between the chambers and the activated charcoal. The air was left to flow through the olfactometer setup for approximately 30 min prior to each test for the system to reach equilibrium in the two chambers and the Y-tube. The Y-tube experiments were carried out at 25 ± 2°C and 50–60% RH. To avoid visual cues, all the experiments were carried out in a dark room illuminated with red fluorescent tubes (20 W). For each stemborer species, gravid females were released individually into the base of the Y-tube placed horizontally on the table and allowed to choose either of the two arms. The duration of a single evaluation progressed for a maximum of 10 minutes. For each species, the following choice combinations were done: uninfested maize *vs.* empty chamber; uninfested maize *vs.* maize infested by either *B. fusca*, *S. calamistis* larvae or by *C. partellus* larvae. To assess interactions among insects, the following choice combinations were offered to each species: maize infested by *B. fusca* larvae *vs.* maize infested by *S. calamistis* larvae; maize infested by *B. fusca* larvae maize *vs.* maize infested by *C. partellus* larvae; maize infested

by *S. calamistis* larvae maize vs. maize infested by *C. partellus* larvae. For each test, a choice was recorded when the insect passed 5 cm from the intersection into one arm and remained motionless there for more than 20s. Those that made no choice were also recorded. After every five insects, odour source connections to the chambers were reversed to minimise any location bias and the chambers were cleaned thoroughly with distilled water. In each case, the number of gravid females ranging from 20 to 37 were tested (n=20-37). For each conditioning procedure, the percentage of insects that made a distinct choice was calculated.

4.2.4 Field trials

Field trials were carried out evaluate if the caterpillar-induced volatiles attract con- and hetero-specific wild moths for oviposition under field conditions in Makutano (S 0°43.616, E 37°16.373) where *C. partellus* and *S. calamistis* are the most abundant species and in Murang'a (S 0°55.387, E 37°09.004), where *B. fusca* and *S. calamistis* co-infest maize fields, (Ntiri, 2015). The Makutano and Murang'a areas in central region of Kenya are intensively maize cultivated regions situated between 1150 to 1500 metres asl, respectively. Annual mean rainfall is 981 mm and 1195 mm while mean annual temperatures are 21.2 °C and 20 °C, respectively and the mean annual relative humidity range from 50 to 72% Six farmer fields (3 in Murang'a and 3 in Makutano) were selected for the experiment, and in each field, maize plants were grown in pots (12 cm in height x 13 cm in diameter) inside a cage (2× 2× 2 m) covered with a net to avoid natural infestations. The plants used in the experiments were between 4-6 weeks old, i.e. about 60-75 cm tall.

For each species, five potted maize plants were each infested with 5 individuals of 3rd instar larvae. Each potted plant was protected with a small cage (90 cm in height x 33 cm in diameter) equipped with a one-way drawstring mesh cloth bag to limit the larval escape to the plant for 24 hours prior to their exposure to the field to guarantee sufficient feeding damage. Each field consisted of treatments replicated five times (i.e. for each species five potted plants plus five uninfested plants as control). The treatments were distributed along a straight line in each field in a random pattern. Then, each mesh cloth bag over each plant was removed, and after one week, egg-laying from wild moths as well as the remaining larvae (for the infested plant) were checked on each potted plant, and the number of egg batches, as well as the numbers of eggs per batch, were recorded. Thereafter, oviposited eggs collected were transferred in the laboratory for hatching to confirm species identity. In parallel, three pheromone traps (Pherobank BV, Wageningen), one for each of the three species (*B. fusca*, *S. calamistis* and *C. partellus*) were also placed in nearby fields at a height of 1.5 m from the ground to monitor the male moth flight activity period of each stemborer species in the trial fields. The experiment was conducted from April to July 2017 corresponding the long rainy season (i.e. maize cultivation). A total of 16 replicates were conducted at Makutano and 13 replicates at Murang'a during the period.

4.2.5 Collection of VOCs by dynamic headspace

VOCs emitted from un-infested maize plants and plants infested with 5 and 12 individual larvae were collected by using a dynamic headspace sampling system as described by Fombong *et al.* (2016). Two blanks (odours collected from empty oven bags) were collected to verify the absence of background VOCs. The soil in the pot was covered with

aluminium foil to avoid odour emitting from the soil. The plant was covered with oven bags (520×580 mm) and equipped with a valve by which charcoal-purified air entered the system at 0.5 L/min. Volatiles chemicals were collected from the plants by passing the outlet air through a Super-Q filter (50 mg adsorbent) at a rate of 2 L/min. Before use, the Super-Q filter was cleaned using hexane, dried and placed in aluminium foil to avoid any contamination. After each collection, the volatiles were eluted from the traps with 150 μL of hexane and concentrated to 40 μL under a stream of nitrogen to enable detection of compounds that could have been present in very trace amounts when carrying out GC-MS analysis. To the 40 μL concentrated sample, 10 μL of the internal standard (heptadecane) concentrated at 4 $\text{ng}\cdot\mu\text{L}^{-1}$ were added and immediately injected into a gas chromatograph (GC) for analysis or stored at -80°C before analyses. VOCs were collected for 12 h (from 6:00 p.m. to 6:00 a.m.). Five plant headspace replicates were carried out in each case.

4.2.6 Collection of VOCs by Solid Phase MicroExtraction (SPME)

VOCs emitted from uninfested, and infested maize plants with 12 larvae were collected by placing a solid phase microextraction (SPME) fiber in a 20 x 20 x 120cm glass chamber with these plants. The open end of the cylinder was capped using aluminium foil. The cylinder cap was fortified using Parafilm to make it airtight. The SPME septum-piercing needle was driven through a self-sealing, gas-tight septum (sandwiched between the foil cap) into the plant headspace. Before use, SPME fibers (DVB/CARBOXEN/PDMS 50/30 1 m, Supelco) were cleaned by heating in a gas chromatograph injector at 250°C for 20 min. About 195 μg of heptadecane was injected into the cylinder as internal standards and

left to equilibrate for 10 min. Extraction of VOCs was carried out for a period of two hours per sample, and then the fibre was retracted from the headspace and immediately introduced into a gas chromatograph (GC) injector port for analysis or stored at -80°C before analyses. Four plant headspace replicates were carried out for each case.

4.2.7 Analyses of VOCs

After volatile collection, the eluates were analysed using coupled gas chromatography-mass spectrometry (GC-MS) (Plate 4.2) on an Agilent Technologies 7890B GC linked to a 5977 MS, equipped with a non-polar HP-5 MS ultra-inert column (30 m × 0.25 mm i.d., 0.25 µm) (J&W, Folsom, CA, USA). The temperature program was 5 min at 35 °C, then 10°C/min to 280 °C. A 1 µl aliquot of each volatile extract was analysed in the splitless mode using helium as a carrier gas at a flow rate of 1.2 ml/min. Spectra were recorded at 70 eV in the electron impact (EI) ionization mode.



Plate 4. 2: Coupled gas chromatography-mass spectrometry (GC-MS)

Similar to Leppik & Frérot (2014), compounds were identified by comparison of mass spectral data with library data: (Adams terpenoid/natural product library, 1995), (National Institutes of Standards and Technology, 2008) and ChemStation data system (G1701EA, version E.02.00). Furthermore, structure assignments of a fraction of each compound were confirmed based on co-injection with commercially authentic standards. These compounds included: Anisole (purity $\geq 98\%$), β -Bisabolene (purity $>85\%$), Butyl butanoate (purity 98%), (*E*)- Caryophyllene (purity $\geq 80\%$), α -Cedrene (purity $\geq 95\%$), (*E*)- β -Farnesene (purity $\geq 90\%$), (*Z*)-3-Hexenyl acetate (purity $\geq 98\%$), (*E*)-3-Hexenyl acetate (purity $\geq 98\%$), Indole (purity $\geq 99\%$), Linalool (purity 96%), (*R*)-(+)-Limonene

(purity \geq 95%), Methyl salicylate (purity \geq 99%), Myrcene (purity 98%), n-Nonanal (purity 95%), (S)-(-) α -Pinene (purity 99%), Sabinene (purity 99%), γ -Terpinene (purity 97%), Thymol (purity 99%) and α -Zingiberene (purity \geq 99%). A part of α -Zingiberene purchased from Santa Cruz Biotechnology, all the standards compounds were from Sigma-Aldrich Chemical Company.

The VOC peak area information was extracted from the raw GC/MS data and transformed into nanograms (ng) using internal standard peak area. The relative amount of each compound was calculated by dividing the overall weight of the compound (ng) by the sum of the detected compounds from the same analysis and expressed as percentages and calculated as the mean \pm standard error.

4.2.8 Statistical analyses

All analyses were carried out in R version 3.4.1 (R Core Team, 2017). The number of responding gravid female obtained from the dual choice olfactometer assays were recorded as the number of gravid females that responded to the different treatments and expressed as percent response $[(n/N) \times 100]$; N corresponds to the total number of responding gravid females, while n is the number of gravid females corresponding to a given treatment. The proportions of the females that made a choice from the dual choice olfactometer assays were analyzed using Chi-square goodness of fit performed at 5% significance level. From the field trials, the proportions of males captured using pheromone lures were compared using the Tukey Kramer's test. The total number of egg batches oviposited by wild moths recovered from uninfested potted plants was compared to the total number of egg batches recovered from infested plants using the Fisher's exact

test. Prior to the statistical comparisons of VOCs between plants status, all data were checked for normality and homogeneity of variance using Shapiro–Wilk and Bartlett tests, respectively. A non-parametric Kruskal-Wallis test was initially used to show differences of VOCs emitted between uninfested and infested plants and between infested plants. Dunn’s test (a non-parametric post-hoc test for unpaired data) was used to discriminate the means. Principal Component Analysis (PCA) was then performed on relative amount values of each VOCs using the R package, Factoextra. This allowed the assessment of the proportions of the VOCs distributed between uninfested and infested plants, and between stemborer species among infested plants. Mann–Whitney U Test was used to determine the significant differences of VOCs emitted by either 5 or 12 larvae that fed on a given plant.

4.3 Results

4.3.1 Olfactometer bioassays

In the Y-tube olfactometer, 78 to 95% of the females made a choice in the dual-choice bioassays (Figure 4.1 A&B&C). Regardless of the species, all adult females stemborer species used significantly oriented towards maize plants volatiles compared to blanks (Figure 4.1A), and had a significant ($P < 0.05$) preference for plants infested by the same or another species over uninfested plants (Figure 4.1B). By contrast, female orientation in interspecific dual choices depended on the species (Figure 4.1C).

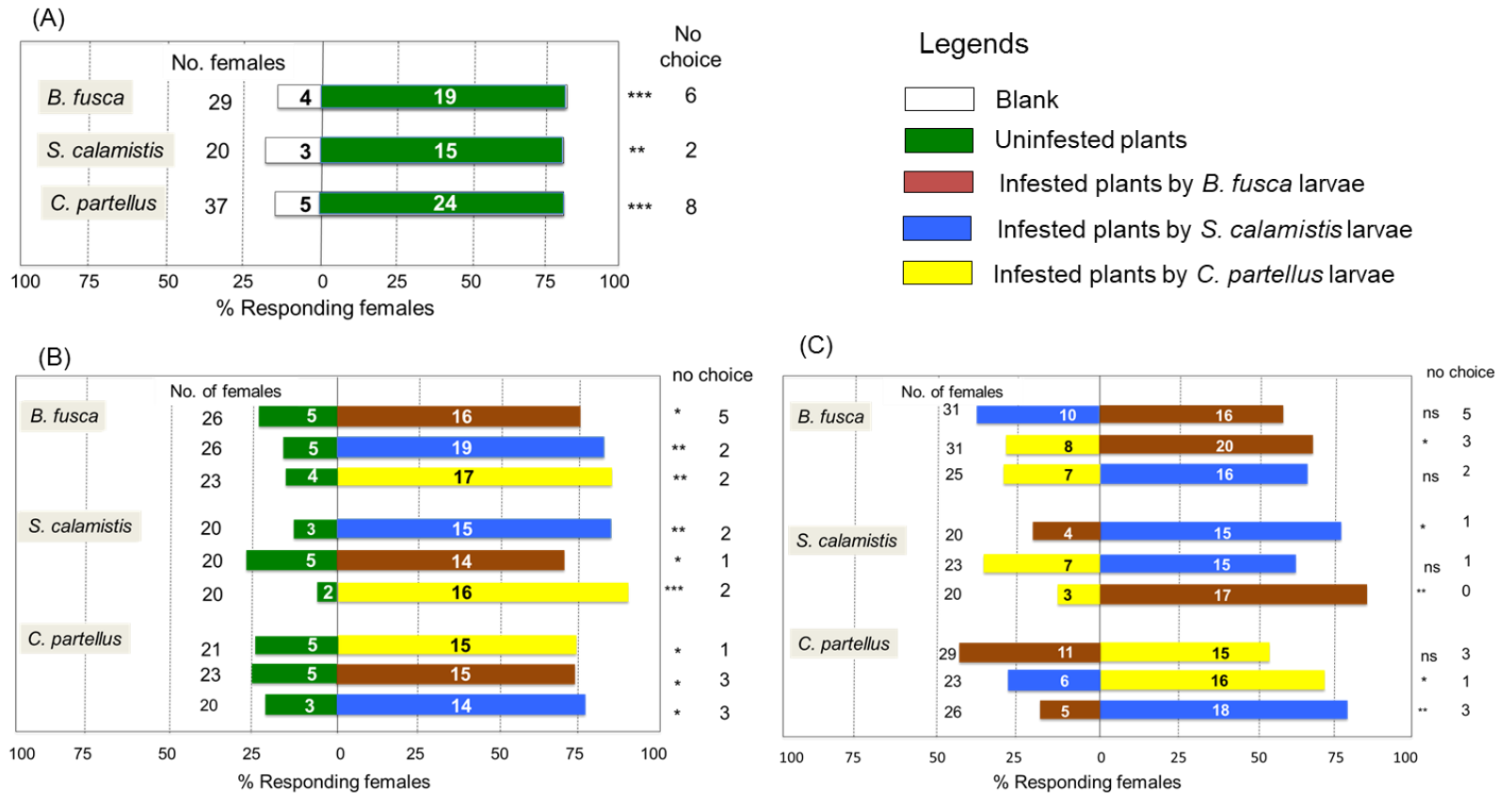


Figure 4. 1: Response of *Busseola fusca*, *Sesamia calamistis* and *Chilo partellus* females to uninfested maize plant and blank (A), uninfested and infested maize plants (B) and to maize plants infested by different stemborer species (C) (dual choice situation) (A), in a Y-tube olfactometer. On each bar, the number of females choosing the odours was given as well as the number of females making no choice (n ranging from 20 to 37). The number of females making a choice was set to 100% to calculate the percentage of responding females. * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.0001$ according to Chi-square goodness of fit performed at 5% significance level.

Busseola fusca did not discriminate among the odours emitted by maize plants infested by the noctuids (comparison *B. fusca* vs *S. calamistis* infested plants: $\chi^2 = 1.384$, $df = 1$, $P > 0.05$) but preferred odours emitted by maize plants infested with their conspecifics over maize plants infested by *C. partellus* (comparisons of *C. partellus* vs *B. fusca* infested plants: $\chi^2 = 5.143$, $df = 1$, $P = 0.023$). *Sesamia calamistis* oriented preferably towards maize plants infested with their conspecifics in a choice involving only noctuids (comparisons of *B. fusca* vs *S. calamistis* infested plants: $\chi^2 = 6.368$, $df = 1$, $P = 0.011$) but preferred plants infested by *B. fusca* over plants infested by *C. partellus* (comparisons of *C. partellus* vs *B. fusca* infested plants: $\chi^2 = 9.80$, $df = 1$, $P = 0.001$). *Chilo partellus* did not discriminate between plants infested by *B. fusca* and plants infested by their conspecifics (comparisons of *B. fusca* vs *C. partellus* infested plants: $\chi^2 = 0.615$, $df = 1$, $P > 0.05$) but it preferred conspecifics over *S. calamistis* (comparisons of *S. calamistis* vs *C. partellus* infested plants: $\chi^2 = 4.545$, $df = 1$, $P = 0.033$) and plants infested by *S. calamistis* over *B. fusca* (comparisons of *B. fusca* vs *S. calamistis* infested plants: $\chi^2 = 7.347$, $df = 1$, $P = 0.006$).

4.3.2 Field trials

In each field, ~ 2 to 3 larvae of each stemborer species remained per infested potted plant. The number of male moths captured inside pheromone traps differed significantly ($P < 0.05$) between species and site (Table 4.1).

Table 4. 1: Number of egg batch laid by females and number of male stemborer captured in traps baited with different stemborer pheromone lures from the natural population in two Kenyan localities (Makutano and Murang'a)

	Makutano				Murang'a			
	Control plants	Plants infested by <i>Busseola fusca</i>	Plants infested by <i>Sesamia calamistis</i>	Plants infested by <i>Chilo partellus</i>	Control plants	Plants infested by <i>Busseola fusca</i>	Plants infested by <i>Sesamia calamistis</i>	Plants infested by <i>Chilo partellus</i>
Wild moth species involved in egg laying	Total number of egg batches recorded on each maize plant							
<i>Busseola fusca</i>	0	0	0	0	3	1	2	3
<i>Sesamia calamistis</i>	2	1	3	2	2	3	2	3
<i>Chilo partellus</i>	2	4	4	3	0	0	0	0
	Uninfested plants		Infested plants		Uninfested plants			Infested plants
Total number of egg batches	4 a		17 b		5 a			14 b
Moths flight monitoring								
Pheromone lures	Lure of <i>Busseola fusca</i>	Lure of <i>Sesamia calamistis</i>	Lure of <i>Chilo partellus</i>	Lure of <i>Busseola fusca</i>	Lure of <i>Sesamia calamistis</i>	Lure of <i>Chilo partellus</i>		
Total males captured	0 a	68 b	5 a	593 a	87 b	0 c		

For each locality, the number of males captured followed by different letters were significantly different at the 5% level according to Tukey–Kramer test (proportion comparisons) and the total number of egg batches from uninfested and infested plants followed by different letters were significantly different at the 5% level according to Fisher's exact test. Number of egg batch laid by females (N=16 in Makutano and 13 in Murang'a), male captures in both localities (N=22).

Busseola fusca and *C. partellus* were both absent in Makutano and Murang'a, respectively. Therefore, no *B. fusca* eggs were found on potted maize plants in Makutano, and no *C. partellus* eggs were found in Murang'a study site. Although few *C. partellus* males were trapped by pheromone lures compared to *S. calamistis* in Makutano, both *S. calamistis* and *C. partellus* eggs were also found on the potted maize. In Murang'a, significantly ($P < 0.05$) higher numbers of *B. fusca* males were trapped than *S. calamistis* males and eggs of both species were also found on the potted maize plants. In total, 21 and 19 egg batches of wild moths were collected in Makutano and in Murang'a, respectively. For each locality, significantly ($P < 0.05$) more egg batches were collected on infested than uninfested plants (Table 4.1).

4.3.3 Identification of VOCs emitted by the uninfested and infested maize plants

From mass spectral data comparisons between GC/MS compounds with library data and from co-injection procedure with commercially authentic standards, a total of 42 compounds were tentatively identified and found to be emitted from both uninfested and infested maize plants. These compounds consisted of sesquiterpenes, monoterpenoids, green leaf volatiles, cyclic hydrocarbons and alkaloids. A total of 34 compounds were identified using the dynamic collection system, whereas 24 compounds were identified with the SPME collection system (Figure 4.2; Appendix 6). While twenty two (22) VOCs were produced by all maize plants tested, only twenty (20) VOCs were emitted by infested plants irrespective of the stemborer species involved (Figure 4.2).

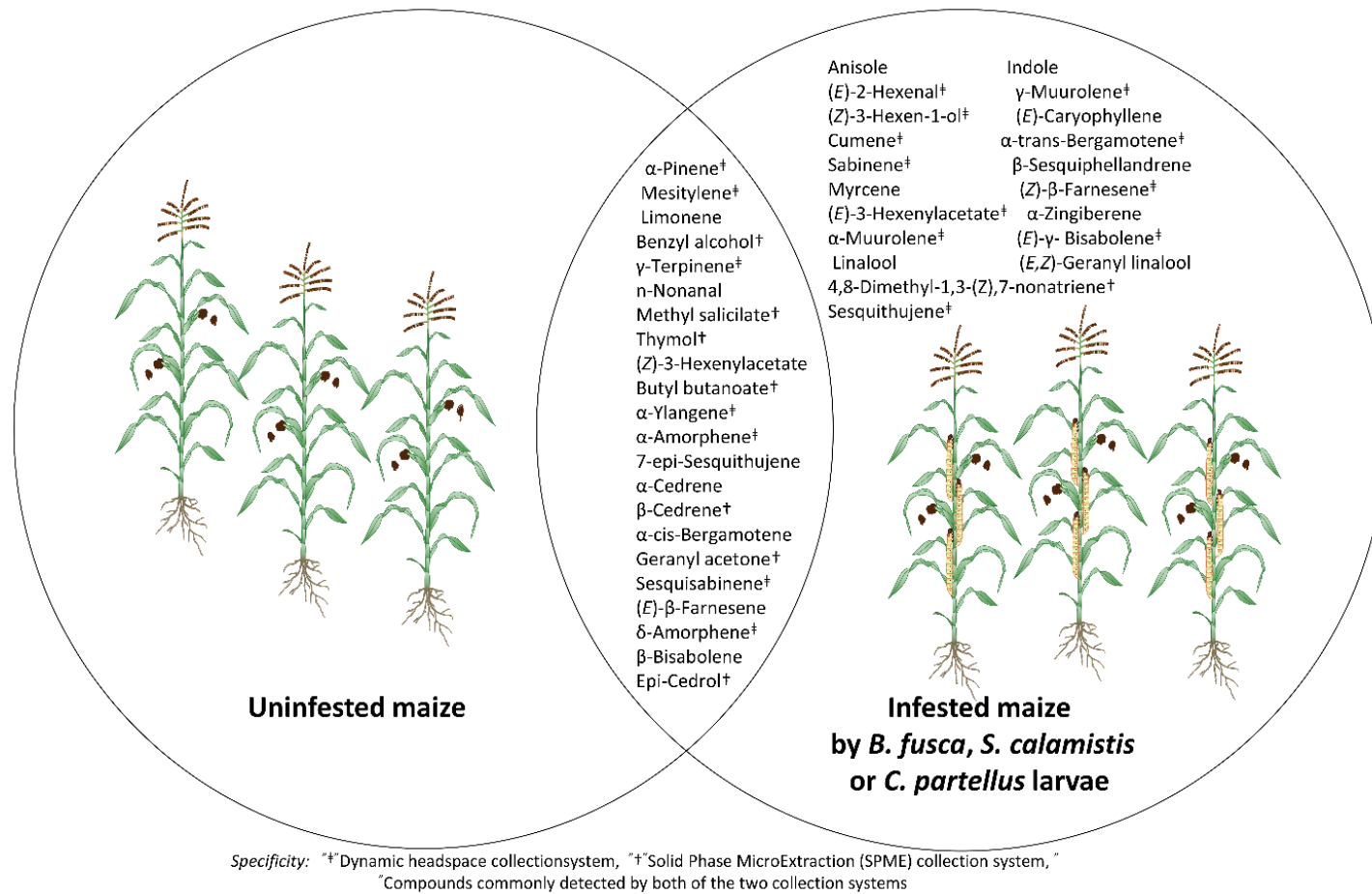


Figure 4. 2: Volatile organic compounds (VOCs) detected from uninfested and *Busseola fusca*, *Sesamia calamistis* and *Chilo partellus* larvae infested maize plant. None of the VOCs was detected exclusively in uninfested plants; 22 VOCs were commonly produced by both uninfested and infested plants while 20 VOCs were only detected from infested plants. 18 compounds were exclusively detected by dynamic system “‡”, while 8 were exclusively detected by SPME system “[‡]” and 16 compounds were common to both two collection systems

The mean ratios of some VOCs varied significantly between uninfested and infested plants irrespective of the stemborer species. Both collection systems produced higher amounts of α -pinene, n-nonanal, 7-epi-sesquithujene and butyl butanoate from uninfested than infested plants (Figure 3.3; Appendices 6 & 7), while greater amounts of (*E*)-2-hexenal, 4,8-Dimethyl-1,3-(*Z*),7-nonatriene, (*E*)-caryophyllene were emitted by infested plants irrespective of the stemborer species (Appendices 6 & 7).

However, there were some differences in the compound profiles emitted by insect species infesting the maize plants (Figure 4.3). With both collection systems, *B. fusca* larvae induced the compositionally richest VOCs profile, followed by both *S. calamistis* and *C. partellus* larvae. (*E*)- β -Farnesene, linalool, myrcene and β -bisabolene were characteristic VOCs emitted by maize plants infested by *B. fusca* only, whereas α -zingiberene, sesquisabinene, indole and α -cis-bergamotene were characteristic VOCs that were emitted by maize plants infested by both *C. partellus* and *S. calamistis* (Figure 4.3; Appendices 6 & 7).

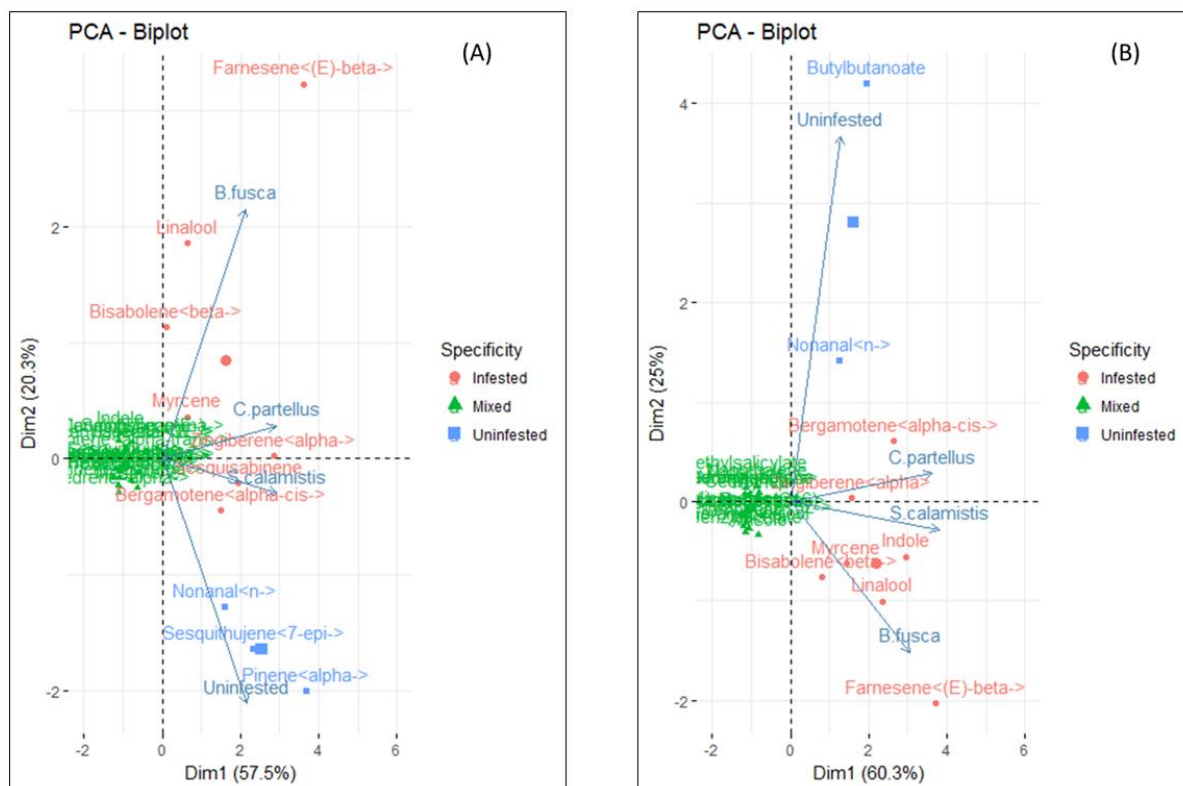


Figure 4. 3: Principal Component Analysis (PCA) of VOCs profiles from stemborer’s differentially infested maize plants. PCA score plot, highlighting cluster of volatiles attributable to different infestation status (ellipses = 95% of confidence) and PCA loading plot, showing variables correlations with the first and second principal component were together bi-plotted with Factoextra R Package. Biplot (A) from Dynamic headspace collection system and biplot (B) from Solid-Phase MicroExtraction (SPME) collection system.

4.3.4 VOCs produced with increase in larval density

Irrespective of the stemborer species most of the VOCs detected from plants infested with 5 larvae were also detected in plants infested with 12 larvae (Appendix 8). Among the 34 VOCs identified using the dynamic collection system, all were found with both larval densities and nine varied significantly with larval density (Appendix 8). Among them, linalool, indole, α -cedrene, sesquisabinene, (*E*)- β -farnesene and α -zingiberene increased while α -pinene, n-nonanal and 7-epi-sesquithujene decreased with decrease in larval density irrespective of the stemborer species.

4.4 Discussion

In the Y-tube olfactometer bioassays gravid females of the three stemborer species oriented preferably towards odours emitted by maize plants infested by both con- and hetero-specific larvae over odours emitted by uninfested plants. Field trials confirmed these results. These findings, though contrasting with the fundamental concept of competition, has been shown for several other lepidopteran phytophagous insects (Facknath, 2012; Viswanathan *et al.*, 2005;). Enhancements of oviposition by *C. partellus* on maize plants infested with conspecific larvae has already been reported by Kumar (1986). In addition, under interspecific choices, although the results are not conclusive enough to warrant a general interpretation, the Y-tube experiments showed that the female moths did not systematically preferably orient towards the plant infested by conspecific larvae. The findings of this study thus suggest an adaptive behaviour that may help ovipositing stemborer females to identify plants suitable for the survival of their offsprings.

Herbivores feeding often modifies the volatile profiles emitted by plants (El-Sayed *et al.*, 2016; Dicke & Van Loon, 2000). The common volatiles identified in the present study were previously reported by Konstantopoulou *et al.* (2004) and Khan *et al.* (2000), from uninfested maize plant and by Peñaflor *et al.* (2011), and Gouinguéné *et al.* (2001) from infested plants. However, in the present study, the volatile profile partly depended on the collection method. In the dynamic system more compositionally rich VOCs were collected than using the SPME system. Similarly, Elmore *et al.* (1997) reported that the dynamic headspace method extracted a greater number of volatile compounds from the same samples than did the SPME. This can be explained by several factors in SPME collection system such as the need to be in the linear range of detection and the competition effects

on the fiber between volatiles which can cause biases in the quantitative and qualitative determination of compounds (Roberts *et al.*, 2000).

Both methods however, showed a higher number of volatiles emitted by infested maize plants than uninfested ones irrespective of the stemborer species involved, a phenomenon already reported by several studies (Dicke & Van Loon, 2000; Ngi-Song *et al.*, 2000; Pare & Tumlinson, 1999). Some of these caterpillar-induced volatiles, which can either be produced *de novo* by the plant, as an indirect defence, as well as directly excreted by the larvae inside the plants, have also been reported in other studies (e.g. Pinto-Zevallos *et al.*, 2016; De Moraes *et al.*, 2001). The results of this study indicate that all the odour sources of infested plants were innately attractive to the female of the species tested. Giunti *et al.* (2018), and Kumar (1986) also demonstrated that infested plants attracted and increased the fitness of other conspecific species.

However, there were quantitative variations in the VOCs emitted by maize plants irrespective of the stemborer species involved in the infestation. The two collection systems revealed that the VOCs profiles emitted by plants infested by *B. fusca* were very distinct and compositionally richer in VOCs profile to those emitted by plants infested by both *S. calamistis* and *C. partellus*. Two elicitors in the oral excretion of larvae, volicitin and β -glucosidase, have been identified as being responsible for the induction of specific volatiles from plants they attack, which are different from intact or mechanically damaged plants (Gouinguéné *et al.*, 2001; Dicke & Van Loon, 2000). Thus, the type of elicitors specific to the different stemborer larval species may be responsible for these variations in HIPVs by the different stemborer species. This has been adequately reported in other herbivore species (Dicke, 2000).

In addition, the intensity of caterpillar-induced VOCs depended on the larval density. Skoczek *et al.* (2017) have shown, is directly influenced by the extent of plant feeding damage or the amount of plant tissue consumed. Similarly, Gouinguéné *et al.* (2003) found a correlation between the intensity of the HIPVs emission and the number of *Spodoptera littoralis* (Boisduval, 1833) (Lepidoptera, Noctuidae) larvae feeding on a plant, and with the amount of damage inflicted. In the present study, the VOCs which decreased with larval density were those characteristics of uninfested plants while the VOCs, which increased with larval density were those characteristics to those of the infested plants. However, all VOCs characteristics to infested plants were found in both larval densities. In conclusion, results from this study indicate that VOCs influence the interactions between members of the same guild and thus may play an attraction role in the coexistence among maize stemborers. Eight candidate attractants were associated with larvae-infested plants that attracted conspecific and heterospecific females. Thus, these results open new avenues to develop attractants specific to trap female stemborer moths in the field.

CHAPTER FIVE

INFLUENCE OF FALL ARMYWORM, *SPODOPTERA* *FRUGIPERDA* (LEPIDOPTERA: NOCTUIDAE), ON MAIZE STEMBORERS AND THEIR ASSOCIATED PARASITIDS COMPOSITION IN MAIZE FIELDS IN KENYA

Abstract

Interactions between pest communities influence the composition of pests complex that attack crops. The noctuid stemborers, *Busseola fusca* (Fuller) and *Sesamia calamistis* Hampson, the crambid, *Chilo partellus* (Swinhoe) as well as invasive leaf feeding fall armyworm (FAW), *Spodoptera frugiperda* J.E. Smith (Lepidoptera: Noctuidae) are currently major cereal pests in sub-Saharan Africa. It is not known how the new invasive species affects the relative importance, the distribution and structure of individual stemborers and associated parasitoids. Thus, pest and parasitoid surveys were conducted in maize fields at pre-tasseling, reproductive and senescence development stages after FAW had invaded Kenya in 2018 that were compared to ongoing survey in 2017 when there was no FAW. The surveys were conducted at two localities where the three stemborers used to co-occur on maize and at the same maize fields before and after FAW had invaded Kenya. The results indicated that the introduction of FAW had changed the equilibrium of maize stemborer communities and showed to be able to co-habit with stemborer species as additional pest in maize fields across the cropping seasons in the different localities. The interactions between FAW and stemborer larvae occurred mostly

from the reproductive to senescence stages of maize plants. However, no parasitoids associated with stemborers were recovered from FAW larvae and parasitoids' composition of stemborers did not differ between before and after the introduction of FAW.

Keywords: Community ecology, pest interactions, maize stemborers species, pest invasion, parasitoids.

This chapter is submitted in: *Environmental Entomology*.

5.1 Introduction

The stemborers, *Busseola fusca* (Fuller) and *Sesamia calamistis* Hampson (Lepidoptera: Noctuidae), and the invasive *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae), are pests of maize and sorghum in East and Southern Africa (Kfir *et al.*, 2002). Estimated crop losses due to these devastating pests vary with agro-ecological zones (Kfir *et al.*, 2002). The three stemborers frequently occur as single or mixed species communities (Krüger *et al.*, 2008; Ong'amo *et al.*, 2006a; van den Berg *et al.*, 1991) whose structure varies with locality, altitude, and season. *Busseola fusca* is generally the dominant species in the highlands, while *C. partellus* dominates in the lowlands (Calatayud *et al.*, 2016; Mwalusepo *et al.* 2015); and *S. calamistis* occurs at all altitudes (Ntiri *et al.*, 2019). On maize in the mid-altitudinal regions, these stemborer species often occur as a mixed community of the three species (Ong'amo *et al.*, 2006a; b; Guofa *et al.* 2001). As they share the same resource (i.e. maize stems), the likelihood of competition is high (Ntiri *et al.*, 2017). Both intra- and interspecific competitions were observed between *B. fusca*, *S. calamistis* and *C. partellus* with stronger interspecific competition recorded between the noctuids and the crambid than between the two noctuids (Ntiri *et al.*, 2016).

Several studies have documented parasitoids associated with the three stemborers in the different agroecological zones (Mailafiya *et al.*, 2011; 2009). In cultivated habitat in Kenya, the most common parasitoids of all three species are the larval parasitoids *Cotesia flavipes* Cameron and *Cotesia sesamiae* (Cameron) (Hymenoptera: Braconidae) followed by the pupal parasitoids *Xanthopimpla stemmator* (Hymenoptera: Ichneumonidae) and *Pediobius furvus* Gahan (Hymenoptera: Eulophidae), and the tachinid *Siphona* sp. (Mailafiya *et al.*, 2011; 2009). Among these, the larval parasitoids *C. flavipes*, which was introduced from Asia for classical biological control of *C. partellus* (Overholt *et al.*, 1997; 1994b), and *C. sesamiae* are the most common parasitoids of stemborers infesting maize in East and Southern Africa (Kfir *et al.*, 2002).

They were obtained from all three borer species in both cultivated and wild habitats (Sokame *et al.*, 2019a; Mailafiya *et al.*, 2011; 2009). This community of stemborers and parasitoids might be disturbed by the recent introduction of the fall armyworm (FAW), *Spodoptera frugiperda* J.E. Smith (Lepidoptera: Noctuidae) from America into sub-Saharan Africa, where it has invaded most countries causing severe damage in maize fields (Rwomushana *et al.*, 2018; Goergen *et al.*, 2016). In Kenya, this pest was first reported in the western region in 2017, and by the early cropping season in 2018, it has been confirmed throughout the country regions (Sisay *et al.*; 2018). The stemborer larvae feed on young leaves until the third instar, when they mainly feed on young leaves and from the third instar mainly feed into maize stems whereas FAW larvae feeds only on leaves during their whole development, essentially the central leaves in plant whorl (Van den Berg, 1997; Morrill & Greene, 1973). In addition, in maize fields at tasseling stage, the FAW larvae could be found feeding on the tassels and subsequently on the ear, silk, cob, and even in borer's holes. (CAB International, 2017; Morrill & Greene, 1973).

Therefore, FAW and stemborer larvae may interact by sharing the same niche at young developmental stages and even when the stemborer larvae migrate from the leaves to stems. The present study aims at evaluating how FAW affects the composition of stemborer community and associated parasitoids in the mid-altitudes of Kenya where the three stembores co-occur (Ong'amo *et al.*, 2006a, b; Guofa *et al.*; 2001) using the same fields surveyed before and after the introduction of FAW. In specific, the objectives of this study were to (i) describe the magnitude of single- and multi-species composition of stemborer communities and their associated parasitoids at maize plant and field levels before and after the introduction of FAW; (ii) assess the differences in stemborers density and associated parasitoid, and parasitism rates in maize fields following the fall armyworm introduction; and (iii) determine maize plant stages at which interactions are likely to occur.

5.2 Material and methods

5.2.1 Study localities and maize plants

The surveys on the composition of stemborer community and their associated parasitoids before and at the beginning of the introduction of FAW were done in maize fields of two localities with intense maize cultivation in central Kenya, where *B. fusca*, *S. calamistis* and *C. partellus* were known to occur together on maize before the introduction of FAW (Ntiri *et al.*, 2019; Sokame *et al.*, 2019a; Ndjomatchoua *et al.*, 2016; Ong'amo *et al.*, 2006a, b; Guofa *et al.*, 2001). All surveys were done during the cropping seasons at Makutano (S 0°43.616, E 37°16.373, 1150m asl), where *C. partellus* and *S. calamistis* co-infest maize and Murang'a (S 0°55.387, E 37°09.004, 1500m asl), where *B. fusca* and *S. calamistis* are present (Sokame *et al.*, 2019a, b). Mean annual rainfall are 981 mm and

1195 mm and mean annual temperatures are 21.2 °C and 20°C in Makutano and Murang`a, respectively. Mean annual relative humidity ranges from 50 to 72% in both sites. Both locations are characterized by a bimodal rainfall distribution with two cropping seasons, April to June and October to December with a dry season in between.

The three (3)-month maize variety Duma 43 variety (Simlaw, Kenya Seed Company, Nairobi, Kenya), which is commonly used by farmers in the study locations, was provided to selected farmers in each locality to minimize the effect of plant variety on infestation.

5.2.2 Sampling design and data collections

The surveys were conducted in the maize fields during maize cropping seasons of 2017 and 2018. FAW did not occur in the study area until the early cropping season of 2018, allowing the assessment of pest and parasitoid composition before and after the introduction of the invasive FAW. In each locality, three 0.5-1.5 ha large maize fields were selected and surveyed before and after FAW had invaded Kenya. During the long and short rainy seasons of each year, each field in each locality was sampled twice at three different plant growth stages (pre-tasseling, reproductive and senescence stages) to identify the phenological stages where interactions between maize stemborers and FAW are likely to occur. Six surveys were undertaken in each maize field during each cropping season. At the pre-tasseling stage, the “W” scouting pattern was used for sampling (Prasanna *et al.*, 2018), whereas at reproductive and senescence stages the “Ladder” scouting pattern was used (Prasanna *et al.*, 2018). The optimal number of maize plants sampled in each field during each survey was determined using the equation of Zar (1999):

$$n = \frac{1}{4Dd^2} \left(Z_{\alpha/2}^2 \right)$$

Where $Z_{\alpha/2}$ is the standard normal deviation (1.96), d is the permitted error (0.1) resulting in a uniform number of maize residues in all farms, and D is the design effect (1).

$$n = \frac{1}{4 \times 1 \times 0.1^2} (1.96^2) = 96.04 \approx 100$$

Hence, a total of one hundred maize plants were systematically inspected in each field during each survey. Plants with damage symptoms were uprooted and dissected for recovery of larvae and pupae from the stems, whorls, tassels and ears. The larvae and pupae collected were counted according to species and then placed individually in glass vials (8.5×2.5cm) on artificial diet and brought into the laboratory for rearing until adult stage to confirm the species identification or recovery of parasitoids in case of parasitism. Species richness, incidence and density, proportion of single and multi-species infestation as well as parasitism rate for each species were computed.

5.2.3 Recovery and identification of stemborers and their associated parasitoids from maize plant materials

Collected stemborer larvae were reared on artificial diet developed by Onyango & Ochieng'-Odero (1994) in cylindrical glass vials (8.5×2.5cm) plugged with cotton wool and kept under ambient conditions in the laboratory ($25 \pm 1^\circ\text{C}$; $67 \pm 4\%$ relative humidity) until pupation or parasitoids' emergence in case of parasitism. The pupae were kept in separate plastic containers (16 cm × 10 cm) closed with perforated plastic lids until adult emergence for stemborer species identification. In case of parasitism, the emerged parasitoids kept in glass vials (2.5 cm of diameter and 7.5 cm of higher) were conserved in 70% ethanol for species identification (Kenis *et al.*, 2019; Polaszek & Kimani, 1990) in collaboration with the Biosystematics Unit of *icipe*.

5.2.4 Data analysis

A Canonical multivariate Correspondence Analysis (CCA) (Zuur *et al.*, 2007) was performed to determine the effect of temperature and rainfall on the composition of maize pest communities before and after the introduction of FAW. The annual rainfall and temperature for the sampling localities were extracted from the CHIRPS dataset (Hijmans *et al.*, 2005) (<http://chg.geog.ucsb.edu/data/chirps/index.html#pub>). In addition, Non-metric Multi-Dimensional Scaling (NMDS) was performed to compare pest compositions before and after the introduction of FAW using MetaNMDS of vegan R package (Legendre & Legendre, 1998). The species communities of these two periods were compared with Permutational Multivariate Analysis of Variance Using Distance Matrices (PMAVUDM) of adonis R function. To check whether multivariate dispersion assumption is homogenic, dissimilarity indices of species community of each period was computed using vegdist R function and implemented multivariate homogeneity of groups dispersions (variances) using betadisper R function. Similarity/dissimilarity percentage (SIMPER) analysis was performed with simper R function to determine the relative contribution of different species to the dissimilarity species community compositions between before and after introduction of FAW.

The relative abundance of a species was expressed as the number of individuals of a given species divided by the total number of collected insects multiplied by 100. Incidence was computed as the number of plants hosting a given species divided by the total number of sampled plants and multiplied by 100. Density of infestation was calculated as a mean number of insect of a given species per 100 maize plants sampled. Pest incidence and density across seasons and phenological plant growth stages were compared using the

generalised linear model with binomial errors distribution. Parasitisms were assessed as the percentage of the number of parasitoids of a given species that emerged divided by the sum of host insects and/or parasitoids that emerged. The parasitism and the proportions of single and multi-species infestations between before and after the introduction of FAW periods were compared using a proportion Z-test. All analyses were performed with R software version 3.5.1 (R Core Team, 2018).

5.3 Results

5.3.1 Effect of temperature and rainfall on the composition of maize pest communities prior and after the introduction of FAW

The maize pest community is referred to both *S. calamistis* + *C. partellus* + FAW in Makutano and *B. fusca* + *S. calamistis* + FAW in Murang'a. A total of 3,238 larvae and pupae of FAW and stemborers were collected (Table 1). In both 2017 and 2018, at Makutano where temperature was high, *C. partellus* and *S. calamistis* co-infested maize fields, while in Murang'a, where temperature was low, *B. fusca* and *S. calamistis* co-occurred.

Table 5. 1: Total number of larvae/pupae of lepidopteran maize stemborer species and FAW (with the relative proportion [%] in parenthesis) collected from maize plants in cultivated fields during cropping seasons of 2017 and 2018

Maize pest composition	Makutano		Murang'a		Total number
	2017	2018	2017	2018	
<i>Busseola fusca</i>	-	-	592 (63.25)	341 (45.47)	933 (28.81)
<i>Sesamia calamistis</i>	309 (39.06)	194 (25.49)	344 (36.75)	151 (20.13)	998 (30.82)
<i>Chilo partellus</i>	482 (60.94)	389 (51.12)	-	-	871 (26.90)
Fall armyworm	-	178 (23.39)	-	258 (34.40)	436 (13.47)
Total number	791 (24.43)	761 (23.50)	936 (28.91)	750 (23.16)	3,238

- : species was absent in that area

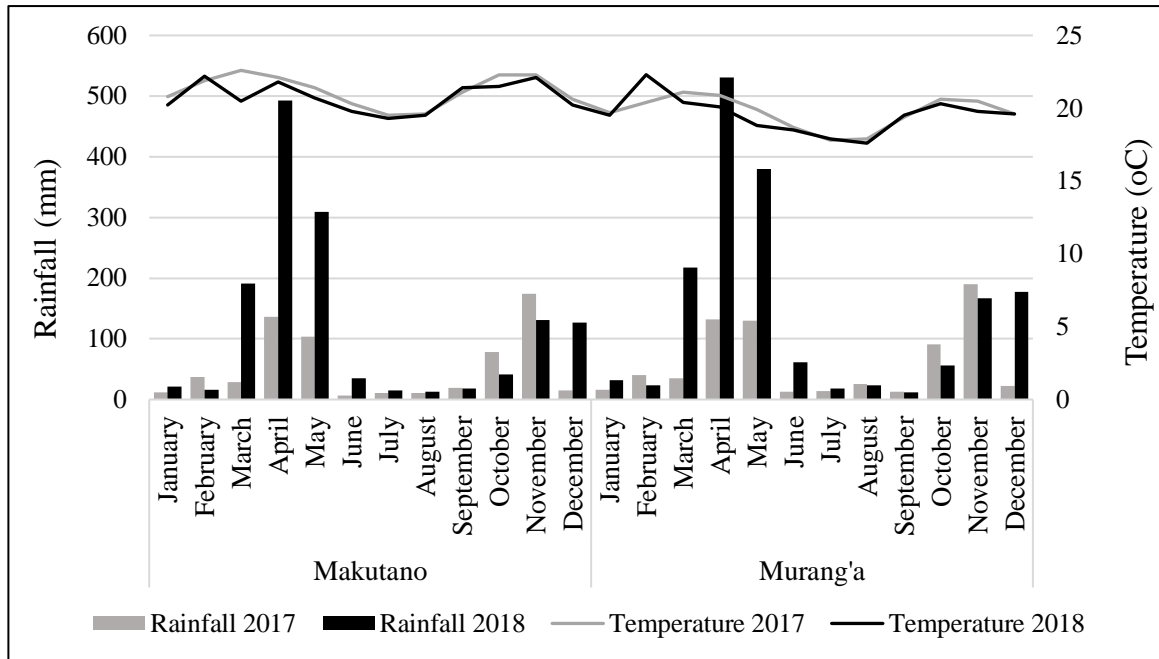


Figure 5. 1: Recorded temperature and rainfall in Makutano and Murang’a during the years of 2017 and 2018 extracted from the CHIRPS dataset (Hijmans *et al.*, 2005) (<http://chg.geog.ucsb.edu/data/chirps/index.html#pub>).

Chilo partellus was the most abundant species in Makutano while *B. fusca* dominated in Murang’a (Table 5.1). In 2018, FAW was recorded at both Makutano and Murang’a areas among the stemborer communities. During the long rainy season of 2018, rainfall was higher than in 2017 (Figure 5.1). The canonical correspondence analysis (Figure 5.2) showed that annual temperature and rainfall together explained only 11% of the variation in maize pests compositions before and after the introduction of FAW. This is represented by the constrained component of the model output (Table 5.2 A). The eigenvalues of each axis of the bi-plot and the proportion explained by these are provided in Table 5.2B. Neither single- nor multi-species compositions showed any relationship with neither temperature nor rainfall after the canonical multivariate correspondence analysis (Figure 5.2). Overall, both temperature and rainfall did not have a significant effect on the

composition of maize pest communities before and after the introduction of FAW periods (Table 5.2 C).

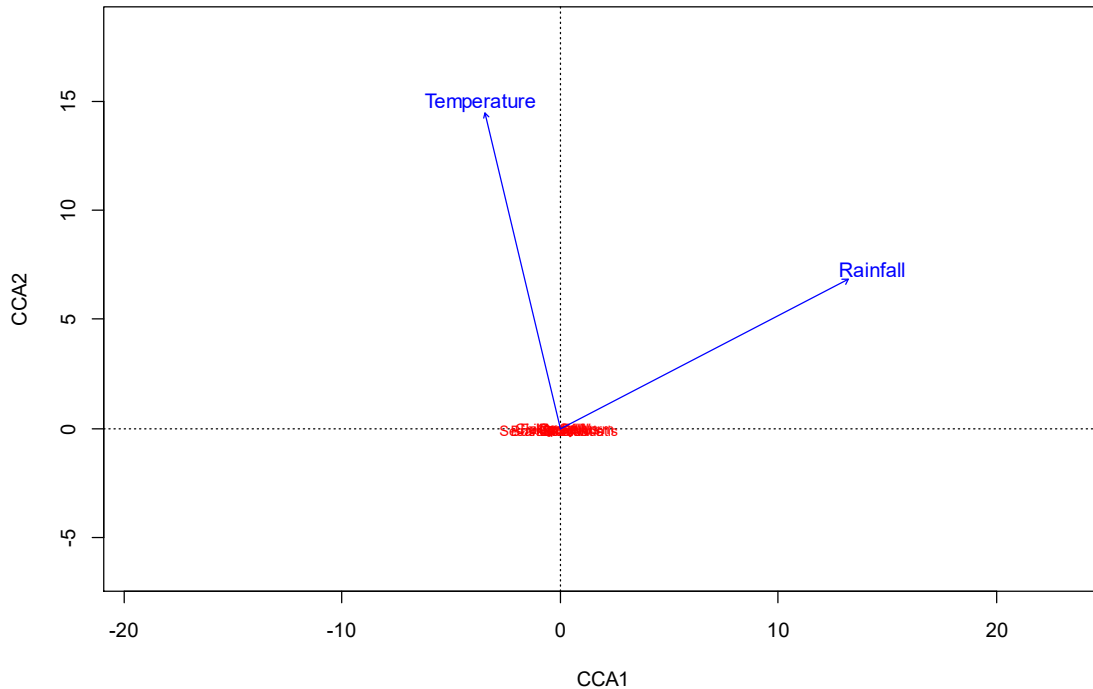


Figure 5. 2: Bi-plot showing the relationship between average annual temperature and rainfall and the occurrence of single- and multi-species communities of *Busseola fusca* (Bf), *Sesamia calamistis* (Sc), *Chilo partellus* (Cp), and fall armyworm (FAW).

Table 5. 2: Results of Canonical Correspondence Analysis (CCA) of the influence of temperature and rainfall on maize pest communities prior and after the introduction of fall armyworm

A)	Partitioning of scaled Chi-square	Inertia	Proportion
	Total	0.32377	1
	Constrained	0.03561	0.11
	Unconstrained	0.28816	0.89
B)	Importance of components	CCA1	CCA2
	Eigenvalue	0.0355	0.000107
	Proportion explained	0.9970	0.003006
	Cumulative proportion	0.9970	1
C)	Constrained	<i>F</i>	<i>P</i> -value
	Average annual temperature	0.4124	0.52
	Average annual rainfall	0.9178	0.56

(A) The proportion of variation in maize pests community composition explained by the axes of biplot (constrained) and the unexplained or residuals of regression (unconstrained); (B) Eigenvalues of each axes of the bi-plot, showing their contributions to the explained variations in the compositional maize pests data; and (C) results from the forward selection method to test the significance of the influence of average annual temperature and average annual rainfall on the composition and distribution of the different maize pests communities. Number of permutations = 9,999.

5.3.2 Effect of fall armyworm introduction on the composition of maize pest community in maize fields

The maize pest community before and after the introduction of FAW formed two distinct clusters (Figure 5.3). Composition of these communities significantly differed between the two periods (Figure 5.3, PMAVUDM results: $P = 0.001$, $R = 0.88$, Number of permutations = 999). Similarity/dissimilarity percentage (SIMPER) analysis indicated that the communities before and after the invasion of FAW were 77.6% dissimilar, and *B. fusca* alone contributed 30.2% of the dissimilarity followed by *S. calamistis*, FAW and *C. partellus* (Table 5.3). The dissimilarity indices of maize pest community composition of each time period showed that the multivariate dispersion of the communities' composition was statistically homogeneous ($F = 1.03$; $P = 0.31$) indicating that the variables were well represented by the two extracted coordinates of Non-metric Multi-Dimensional Scaling (NMDS) (Figure 5.3).

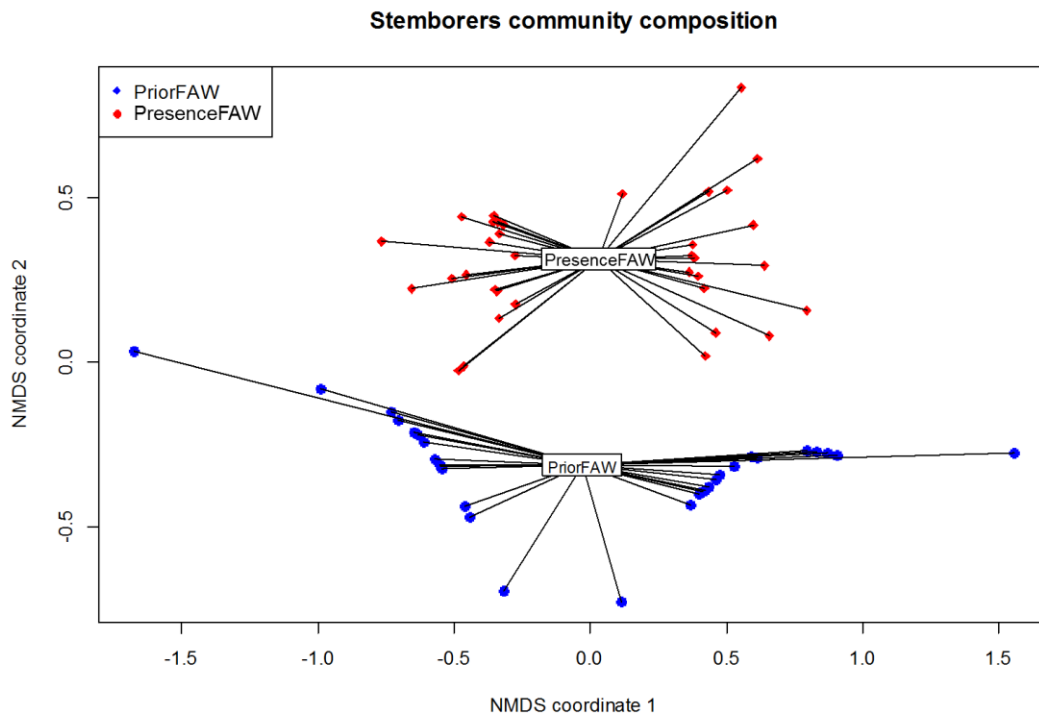


Figure 5. 3: Non-metric Multi-Dimensional Scaling (NMDS) ordination of stemborer community composition prior and after the introduction of FAW in maize fields (ordination based on Bray-Curtis dissimilarity matrix).

Table 5. 3: Disimilarity percentage (SIMPER) analysis results

Species	Average dissimilarity	Contributions %	Cumulative %	Mean abundance Prior FAW	Mean abundance during presence of FAW
<i>Busseola fusca</i>	19.21	30.23	30.23	16.4	9.47
<i>Sesamia calamistis</i>	17.55	27.62	57.85	18.1	9.58
Fall armyworm	14.21	22.37	80.21	0	12.1
<i>Chilo partellus</i>	12.57	19.79	100	13.4	10.8

At field level, both single- and multi-species stemborer infestations of maize plants were recorded, but at varying proportions before and after FAW introduction in each study locality (Figure 54A). The proportions of multi-species infestation significantly increased with the presence of FAW while those of single-species significantly decreased (Figure 5.4A; $\chi^2 = 4.28$, $df = 1$, $P = 0.03$ in Makutano and $\chi^2 = 8.50$, $df = 1$, $P = 0.003$ in Murang'a).

However, at plant level, neither the proportion of the single-species infestations nor the multi-species abundances significantly ($\chi^2 = 0.77$, $df = 1$, $P = 0.37$ in Makutano and $\chi^2 = 0.19$, $df = 1$, $P = 0.65$ in Murang'a) varied with the introduction of FAW (Figure 5.4B).

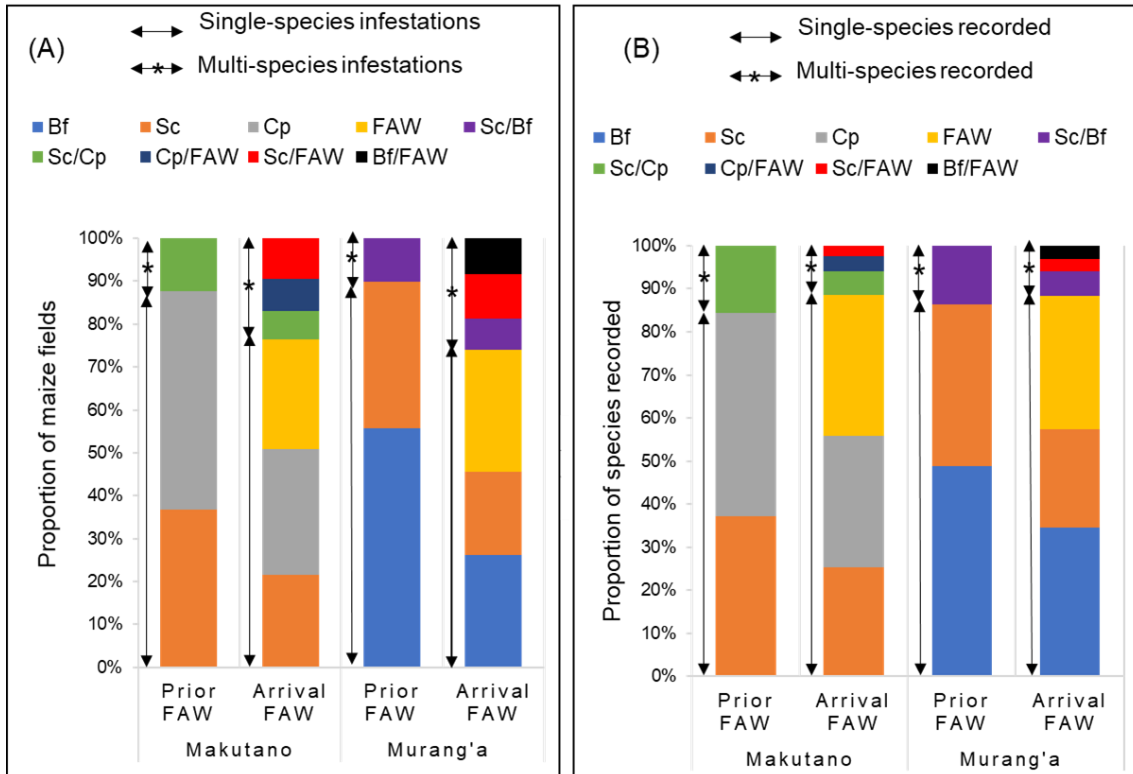


Figure 5. 4: Proportion of single- and multi-species infestations at the field level (A) and at the plant level (B) of each stemborer species after the introduction of FAW at Makutano and Murang'a areas. Bf: *Busseola fusca*, Sc: *Sesamia calamistis*, Cp: *Chilo partellus* and FAW: fall armyworm

5.3.3 Effect of fall armyworm introduction on infestation levels of maize pest community relative to rainy seasons

In both Makutano and Murang'a, the proportion of maize plants infested increased significantly ($P < 0.001$) after the introduction of fall armyworm in maize fields, regardless of the rain seasons (Figure 5.5).

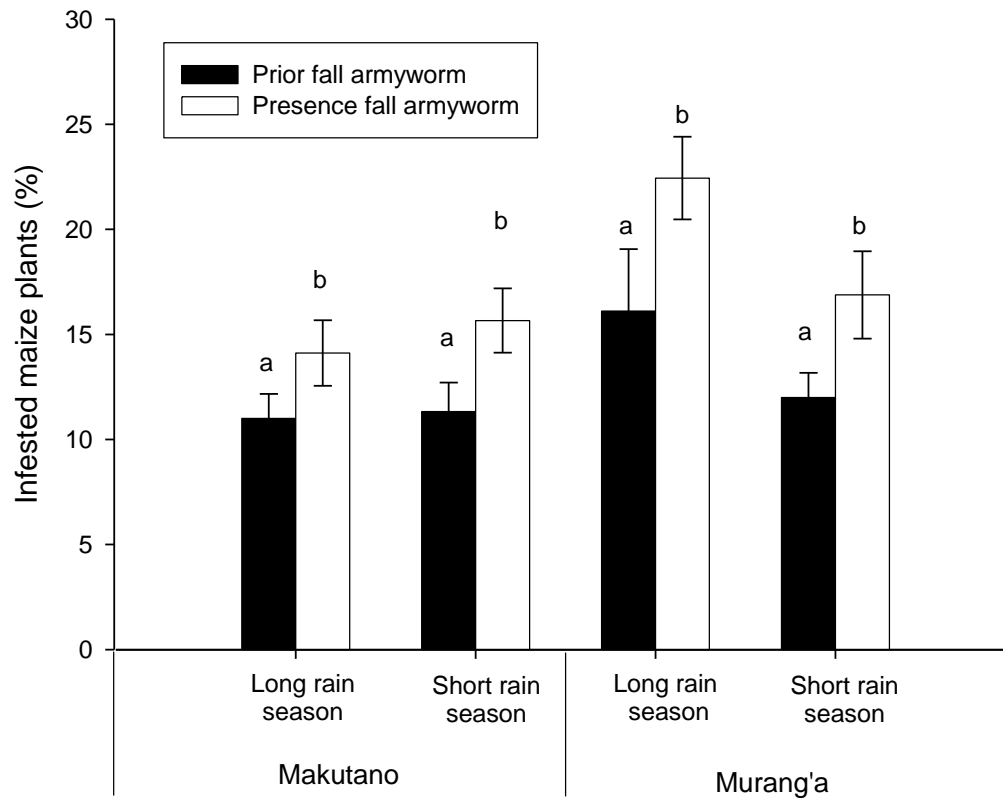


Figure 5. 5: Incidence of maize pest infestation in the fields before and after the introduction of fall armyworm relative to rainy seasons in Makutano and Murang'a. Non-significant differences between the infestation before and after the introduction of fall armyworm are shown by identical letters determined using Tukey's multiple comparisons tests with the R package "lsmeans", following generalized linear model (GLM) with binomial error distribution.

5.3.4 Effect of fall armyworm introduction on single and multi-species infestation by maize pest community relative to rainy seasons

Following the introduction of FAW, multi-species infestations of stemborers and FAW were more common and statistically higher than multi-species infestations involving stemborers only in both rainy periods (Fig. 6; $\chi^2 = 10.44$, $df = 1$, $P = 0.001$ (long rainy

season) and $\chi^2 = 18.86$, $df = 1$, $P < 0.0001$ (short rainy season) in Makutano; $\chi^2 = 2.42$, $df = 1$, $P = 0.04$ (long rainy season) and $\chi^2 = 8.94$, $df = 1$, $P < 0.002$ (short rainy season) in Murang'a). The Multi species infestation involving stemborers species seemed to be declined with FAW presence. *Sesamia calamistis/C. partellus* (Sc/Cp) multi-species infestation recorded during long rain season at Makutano was absent during short rainy season when FAW was present and *B. fusca*/FAW multi-species infestation was recorded only during short rainy season (Figure 5.6).

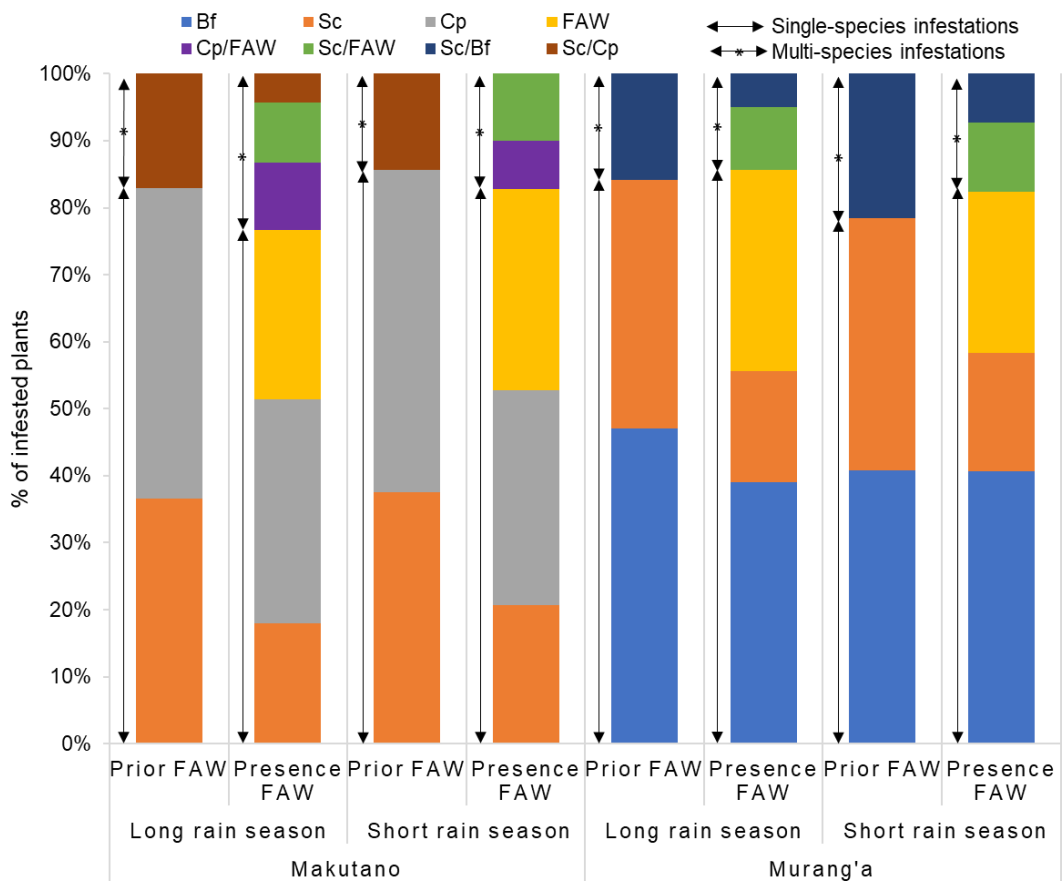


Figure 5. 6: Proportion of single- and multi-species maize pest infestations during long and short rainy seasons prior and after fall armyworm introduction.

5.3.5 Effect of fall armyworm introduction on the density of infestation by maize pest community relative to rainy seasons

At Makutano where *C. partellus* and *S. calamistis* were present, the abundance of *C. partellus* larvae decreased significantly in the presence of FAW during the long rainy season, while it was the reverse for *S. calamistis* during the short rainy season (Table 5.4). However, in the community with *B. fusca* and *S. calamistis* at Murang'a, the larval abundance of each species decreased significantly when FAW was present during both the long and short rainy seasons (Table 5.4).

Table 5. 4: Total number of *Busseola fusca*, *Sesamia calamistis*, *Chilo partellus* and *Spodoptera frugiperda* larvae per 100 maize plants sampled in each maize plot prior and after fall armyworm “invasion” relative to the rainy seasons in Makutano and Murang'a

Seasons	Species	Prior FAW	Presence of FAW	LR	Z-value	$P(> z)$
Makutano						
Long rain season	<i>Chilo partellus</i>	27.44 ± 2.25 b	16.00 ± 2.61 a	7.096	-2.666	0.007
	<i>Sesamia calamistis</i>	17.22 ± 2.76 a	12.66 ± 2.52 a	1.766	-1.329	0.184
	<i>Spodoptera frugiperda</i>	0.00 ± 0.00 a	8.55 ± 0.29 b	106.74	0.001	<0.0001
Short rain season	<i>Chilo partellus</i>	26.11 ± 5.14 a	27.22 ± 7.22 a	0.022	0.150	0.881
	<i>Sesamia calamistis</i>	17.11 ± 3.38 b	8.88 ± 1.96 a	5.650	-2.381	0.0173
	<i>Spodoptera frugiperda</i>	0.00 ± 0.00 a	11.22 ± 0.79 b	140.01	0.001	<0.0001
Murang'a						
Long rain season	<i>Busseola fusca</i>	34.77 ± 2.69 b	22.88 ± 2.52 a	21.542	-1.598	0.011
	<i>Sesamia calamistis</i>	19.55 ± 1.35 b	9.44 ± 1.87 a	17.939	-4.189	<0.0001
	<i>Spodoptera frugiperda</i>	0.00 ± 0.00 a	15.77 ± 4.21 b	79.39	0.002	<0.0001
Short rain season	<i>Busseola fusca</i>	31.00 ± 8.67 b	15.00 ± 4.06 a	3.967	-2.008	0.044
	<i>Sesamia calamistis</i>	18.66 ± 1.58 b	7.33 ± 1.58 a	28.687	-5.230	<0.0001
	<i>Spodoptera frugiperda</i>	0.00 ± 0.00 a	12.88 ± 2.4 b	91.598	0.002	<0.0001

LR = Likelihood Ratio. Non-significant differences of the density of each species between the two periods (prior FAW & arrival FAW) are shown by identical letters determined using Tukey's multiple comparisons tests with the R package “lsmeans”, following generalized linear model (GLM) with negative binomial error distribution.

5.3.6 Effect of fall armyworm introduction on infestation incidence of maize pest community infestation relative to maize plant phenological stages

During the pre-tasseling stage, incidences of infestation were significantly ($P < 0.001$) higher in 2018 than 2017 in both locations (Figure 5.7). However, from maize reproductive to senescence stages, the levels of infestation did not differ significantly ($P < 0.001$) before and after the presence of FAW (Figure 5.7).

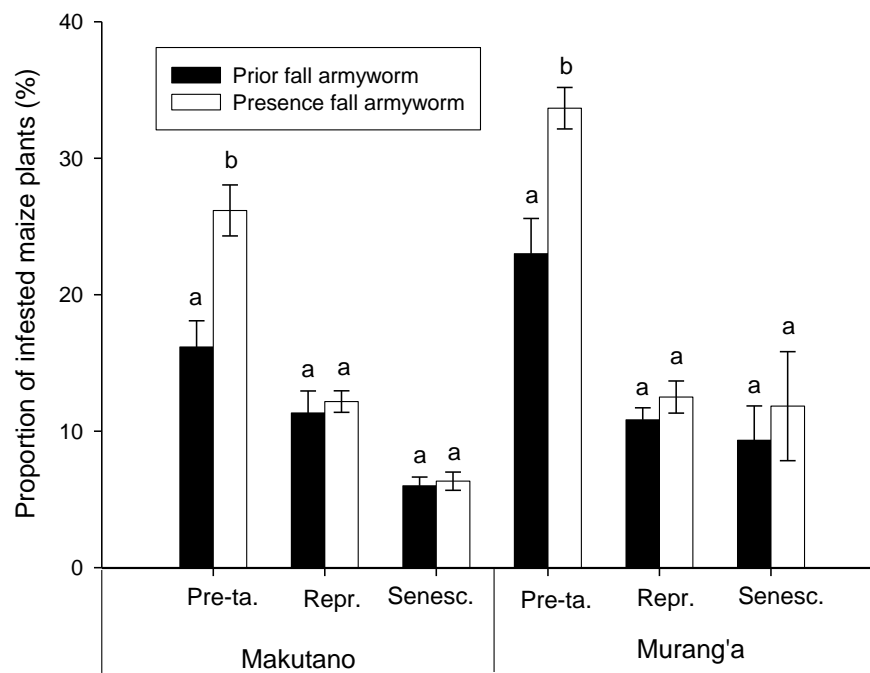


Figure 5. 7: Incidence of maize pest infestation in maize fields prior and after the introduction of fall armyworm relative to different developmental stages of maize plants in Makutano and Murang’a. Non-significant differences between the infestation prior and after the introduction of fall armyworm are shown by identical letters determined using Tukey’s multiple comparisons tests with the R package “lsmeans”, following generalized linear model (GLM) with binomial error distribution. Pre-ta : Pre-tasseling ; Repr. : Reproductive ; Senesc. : Senescent.

5.3.7 Effect of fall armyworm introduction on single and multi-species infestation by maize pest community relative to maize plant phenological stages

The proportion of FAW single-species infestation was significantly ($\chi^2 = 39.45$, $df = 2$, $P < 0.0001$ in Makutano and $\chi^2 = 42.37$, $df = 2$, $P < 0.0001$ in Murang'a) high during pre-tasseling as compared to the other developmental stages and it was almost absent on senescent maize (Fig. 5.8). The proportions of FAW/stemborer multi-species infestations were not affected by the maize plant phenology (Fig. 8; $\chi^2 = 2.35$, $df = 2$, $P = 0.30$ in Makutano and $\chi^2 = 3.65$, $df = 2$, $P = 0.16$ in Murang'a).

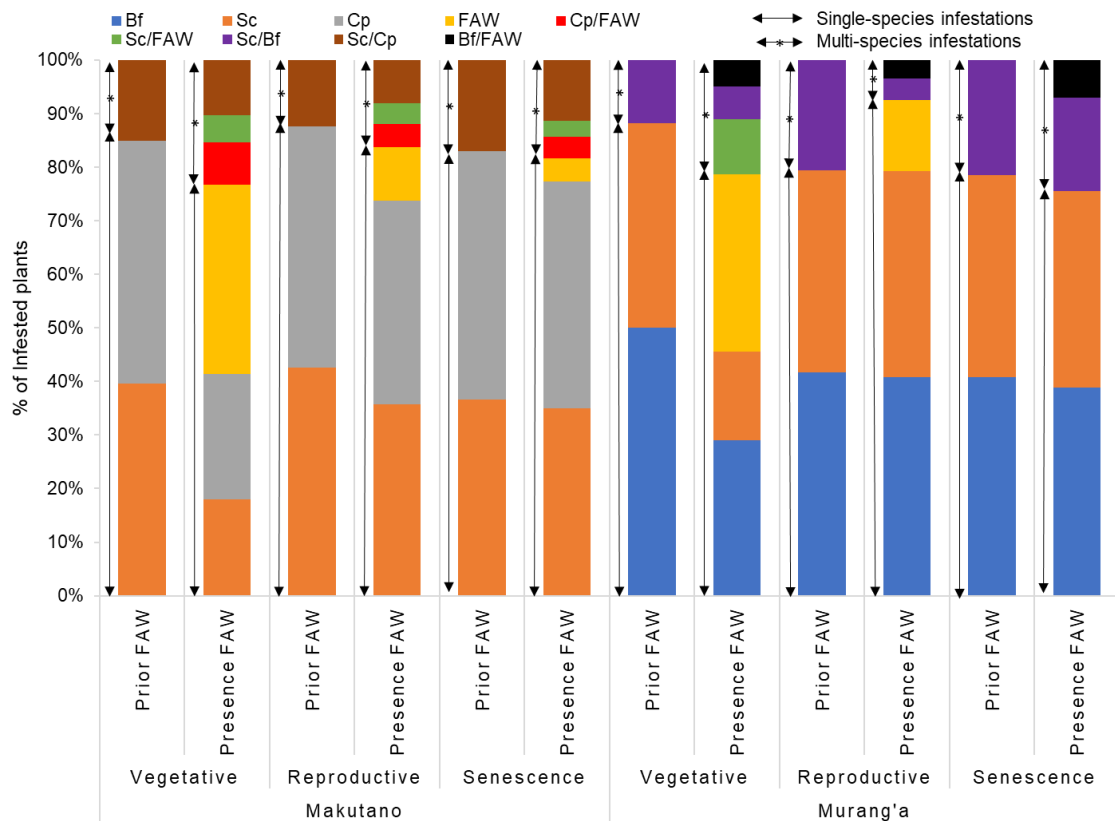


Figure 5. 8: Proportion of single- and multi-species maize pest infestations at different maize phenological stages prior and after the introduction of fall armyworm.

5.3.8 Effect of fall armyworm introduction on the abundance of maize pest community relative to maize plant phenological stages

The abundance of larvae of each species of stemborers larvae before and after the introduction of FAW varied with the developmental stages of maize. During pre-tasseling stage, there was no significant difference between the total number of larvae of each stemborer before and after the introduction of FAW (Table 5.5). However, at both maize reproductive and senescent stages, the total number of each stemborer larvae decreased significantly when FAW was present (Table 5.5).

Table 5. 5: Total number of *Busseola fusca*, *Sesamia calamistis*, *Chilo partellus* and *Spodoptera frugiperda* larvae per 100 maize plants sampled in each maize plot prior and after the introduction of fall armyworm relative to maize plant developmental stages in Makutano and Murang'a.

Developmental stages of maize plants	Species	Prior FAW	Presence of FAW	Likelihood Ratio	Z-value	$P(> z)$
Makutano						
Pre-tasseling	<i>Chilo partellus</i>	35.33 ± 4.80 a	36.17 ± 9.06 a	0.007	0.089	0.934
	<i>Sesamia calamistis</i>	22.00 ± 3.50 a	17.00 ± 3.15 a	1.2114	1.107	0.271
	<i>Spodoptera frugiperda</i>	0.00 ± 0.00 a	9.83 ± 1.30 b	81.782	0.001	<0.0001
Reproduction	<i>Chilo partellus</i>	27.67 ± 7.13 b	15 ± 3.15 a	5.0781	-2.258	0.023
	<i>Sesamia calamistis</i>	20.33 ± 3.73 b	9.50 ± 1.72 a	9.1964	-3.020	0.002
	<i>Spodoptera frugiperda</i>	0.00 ± 0.00 a	10.67 ± 0.88 b	88.717	0.001	<0.0001
Senscence	<i>Chilo partellus</i>	17.33 ± 0.76 a	13.67 ± 2.23 a	2.608	-1.609	0.108
	<i>Sesamia calamistis</i>	9.17 ± 0.30 b	5.83 ± 0.87 a	4.4817	-2.090	0.036
	<i>Spodoptera frugiperda</i>	0.00 ± 0.00 a	9.17 ± 0.31 b	76.243	0.000	<0.0001
Murang'a						
Pre-tasseling	<i>Busseola fusca</i>	50.00 ± 11.18 a	41.67 ± 7.13 a	1.3149	-1.151	0.250
	<i>Sesamia calamistis</i>	20.00 ± 5.10 b	11.67 ± 4.47 a	1.4646	-1.215	0.224
	<i>Spodoptera frugiperda</i>	0.00 ± 0.00 a	24.17 ± 4.91 b	95.677	0.002	<0.0001
Reproduction	<i>Busseola fusca</i>	29.17 ± 5.95 b	17.33 ± 3.39 a	4.6175	-2.151	0.031
	<i>Sesamia calamistis</i>	19.33 ± 2.47 b	8.33 ± 2.07 a	13.62	-3.646	0.0002
	<i>Spodoptera frugiperda</i>	0.00 ± 0.00 a	11.17 ± 1.56 b	90.319	0.001	<0.0001
Senscence	<i>Busseola fusca</i>	19.50 ± 1.67 b	7.83 ± 1.51 a	30.855	-5.281	<0.0001
	<i>Sesamia calamistis</i>	18.00 ± 0.82 b	5.16 ± 0.65 a	45.158	-6.126	<0.0001
	<i>Spodoptera frugiperda</i>	0.00 ± 0.00 a	7.67 ± 0.95 b	63.766	0.001	<0.0001

Non-significant differences of the density of each species between the two periods (prior FAW & arrival FAW) are shown by identical letters determined using Tukey's multiple comparisons tests with the R package "lsmeans", following generalized linear model (GLM) with negative binomial error distribution

5.3.9 Parasitoids species and their levels of stemborers parasitism in the field

The parasitoids recovered on stemborers were represented by the families of Braconidae and Eulophidae. *Cotesia flavipes* was recovered from *C. partellus* and *S. calamistis* at Makutano, while *C. sesamiae* was obtained from *S. calamistis* and *B. fusca* larvae at Murang’a. The eulophid, *Pediobius furvus* was found on *S. calamistis* and *C. partellus* pupae at Makutano (Table 5.6). Parasitism for each parasitoid and stemborer species did not differ between before and after the introduction of FAW. Overall, parasitism levels were low, and no parasitoid species was recovered on FAW larvae (Table 5.6).

Table 5. 6: Parasitoids parasitism rates on stemborers and fall armyworm in Makutano and Murang’a areas.

Localities	Parasitoids species	Host species	Parasitism rate (%)		Proportion Test		
			Prior FAW (2017)	Presence of FAW (2018)	χ^2	df	p
Makutano		<i>Sesamia calamistis</i>	1.99 a	5.94 a	0.155	1	0.69
	<i>Cotesia flavipes</i>	<i>Chilo partellus</i>	1.24 a	4.10 a	0.093	1	0.75
		<i>Spodoptera frugiperda</i>	0.00	0.00	-	-	-
	<i>Pediobius furvus</i>	<i>Sesamia calamistis</i>	2.34 a	2.59 a	0.458	1	0.49
		<i>Chilo partellus</i>	1.95 a	2.08 a	0.323	1	0.56
		<i>Spodoptera frugiperda</i>	0.00	0.00	-	-	-
Murang’a		<i>Sesamia calamistis</i>	2.37 a	5.18 a	0.009	1	0.92
	<i>Cotesia sesamiae</i>	<i>Busseola fusca</i>	1.87 a	4.98 a	0.012	1	0.91
		<i>Spodoptera frugiperda</i>	0.00	0.00	-	-	-

Non-significant different means (\pm SE) are shown by identical letters within rows

5.4 Discussion

The main maize pest community prior to the FAW introduction was composed of *C. partellus* and *S. calamistis* in Makutano, and *B. fusca* and *S. calamistis* in Murang’a (Ntiri *et al.*, 2019; Sokame *et al.*, 2019b). The difference in distribution in these two localities can largely be ascribed to altitudes and the related environment. Whereas *C. partellus* dominates Makutano (at lower altitude), *B. fusca* is the predominant species in Murang’a

(at higher altitude) (Ntiri *et al.*, 2019; Sokame *et al.*, 2019a, b). In 2018, the invasive maize pest, FAW, was recorded in both localities, indicating the capacity of FAW to occupy a wider range of altitudes and environmental conditions than *B. fusca* and *C. partellus*. The capacity of an invasive species to occupy a wide range of environment and thus to interact significantly with native species has been well documented (Kolar & Lodge, 2001). In the present study, both temperature and rainfall did not affect the composition and abundance of maize pest communities before and after the introduction of FAW periods.

However, as expressed by a dissimilarity of stemborer abundance before and after the presence of FAW; the FAW had perturbed the equilibrium of stemborers communities, modifying their species structure and composition by reducing the abundance of each native species. This could be due to the ability of FAW to probably out-compete some of the stemborers for use of maize resources (Sokame *et al.*, 2020a). In association with *Helicoverpa zea* (Boddle) and *Helicoverpa armigera* (Hubner), FAW had a competitive advantage over *Helicoverpa* spp. (Bentivenha *et al.*, 2017). Partial displacement of native stemborers by the introduction of *C. partellus* into Africa has been also reported (Mutamiswa *et al.*, 2017). Following the introduction of FAW, maize fields in each locality exhibited higher infestation levels of maize than before the introduction of FAW, as with an additional pest overall pest abundance was higher, increasing the levels of infestation (Dangles *et al.*, 2009; Van den Berg *et al.*, 1991).

However, although the presence of FAW increased multi-species infestation in the fields, it did not increase the proportion of multi-species larvae recorded at plant level. Such interactions of lepidopteran stemborers with FAW sharing the same maize resource could be competitive as previously demonstrated among lepidopteran stemborer species in

laboratory and greenhouse (Ntiri *et al.*, 2017, 2016) and fields experiments (Ntiri *et al.*, 2019). This present study showed that with the presence of FAW, some multi-species recorded during long rain season were absent in short season and the species dominance in the community also varied with rainy seasons. Such rotation in dominance of species with seasons seems a characteristic of multi-species communities as similar observations have also been reported for a community of stemborers in Eastern Uganda (Matama-Kauma *et al.*, 2008), Cameroon (Ndemah *et al.*, 2001) and South Africa (Van Rensburg & Van den Berg, 1992), and might depend on the species composition. For instance, in the community of *B. fusca* and *S. calamistis*, our study showed that the larval density of each species exhibited the same decrease trend following the invasion of FAW either in long or short raining seasons, whereas in *C. partellus* and *S. calamistis* community, the larval density decreased only for *C. partellus*. This trend on the multi-stemborer species infestation decrease with FAW prevalence could be due to difference in interspecific interactions among FAW and individual stemborer species as influenced by temperature (Sokame *et al.*, 2020a).

On the other hand, Hernandez-Mendoza *et al.* (2008) showed that the spatial distribution of the FAW larvae is random, and natural infestation is strongly associated with the maize phenological stages. Our study confirmed that the phenological stage of host is a crucial component of FAW infestation in maize field. According to the plant phenological stages, the level of infestation in maize fields only increased during pre-tasseling following the introduction of FAW. Besides, there was no significant effect on stemborer larval densities. However, from maize reproductive to senescence stages, the larval densities of stemborer species decreased in the fields whereas the level of infestation was not changed. Pre-

tasseling stage of maize plant seemed to be the most suitable host stage for FAW. This might allow the FAW larvae as foliar feeder to avoid interspecific competition with stemborers, explaining the significant increased level of infestation observed in the fields when FAW was present. Wyckhuys & O'Neil (2006) reported that FAW had an infestation peak during the whorl stage of maize. After tasseling stage, the feeding site of FAW larvae, which is essentially the central whorl (Morrill & Greene, 1973) becomes reduced resulting in a decline in FAW infestation level. In addition, after tasseling stage, the FAW larvae can be found everywhere on the plant (tassel, silk, cob, borer's holes etc.) (CAB International, 2017; Morrill & Greene, 1973), increasing a likelihood of contact interaction between FAW and stemborer larvae.

In addition, parasitoids' composition of stemborers did not differ between before and after the introduction of FAW. Overall, the parasitism levels found on lepidopteran stemborers were low and no parasitism was observed on FAW larvae indicating that FAW larvae are not suitable for the parasitoid species associated to lepidopteran stemborers. More studies need to be conducted to confirm the suitability of *Cotesia* parasitoids to parasitize FAW larvae in the study sites, since from other locations, *Cotesia* species such as *Cotesia icipe* Fernandez-Triana & Fiaboe (Hymenoptera: Braconidae) has been reported to be efficient on FAW larvae (Sisay *et al.*, 2018).

In conclusion, this study indicates that FAW had changed the equilibrium of maize stemborer communities and showed to be able to co-habit with stemborer species as additional pest in maize fields across the cropping seasons in the different study sites. The interactions between FAW and stemborer larvae occurred mostly from the reproductive to senescence stages of maize.

CHAPTER SIX

PARASITISM OF FALL ARMYWORM *SPODOPTERA* *FRUGIPERDA* (LEPIDOPTERA: NOCTUIDAE) BY *COTESIA* (HYMENOPTERA: BRACONIDAE) SPECIES ASSOCIATED WITH LEPIDOPTERAN STEM BORERS IN KENYA

Abstract

Exotic invasive insect herbivores have the potential to interfere with existing herbivores-natural enemies' interactions in new environments. Understanding the acceptability and suitability of a new invasive pest such as Fall Armyworm (FAW), *Spodoptera frugiperda* J.E. Smith (Lepidoptera: Noctuidae) to the existing parasitoids-maize stem borers interactions is the first step in elucidating the impact that this exotic pest can have on the existing natural enemies used in biological control of maize stem borers in Kenya. The most commonly used larval parasitoids to biocontrol maize stem borer communities in Kenya are *Cotesia flavipes* Cameron (Hymenoptera: Braconidae) and two populations of the native *Cotesia sesamiae* Cameron (Hymenoptera: Braconidae). The acceptability and host preference were carried out in Patri dishes and the parasitized larvae were followed up for their development until they formed cocoon, died or formed pupae. The odour preference was studied in Y-tube olfactometer assays. The results showed that all these parasitoids species have significantly ($P < 0.05$) attacked FAW larvae but none yielded offspring though they induced high nonreproductive host mortality when compared to natural mortality. Furthermore, the parasitoid that inserted their ovipositor into FAW larvae exhibited similar

acceptance between larvae of FAW and their respective stemborer hosts under dual-choice bioassays. In olfactometric bioassays, the parasitoids were more attracted ($P < 0.05$) to plants infested by FAW than uninfested plants and even a marked preference to the odours of plants infested by FAW over that of plants infested by their natural host counterparts. This study illustrates that exotic pest, such as FAW, can impact existing stemborer-parasitoid' interactions associated with *Zea mays*, even if it cannot be used as host by parasitoids associated with these stemborers. Although additional studies are needed, FAW might therefore have a negative impact on stemborer biological control already established before its invasion.

Keywords: *Busseola fusca*, *Chilo partellus*, *Sesamia calamistis*, *Sesamia nonagrioides*, biological control, *Cotesia flavipes*, *Cotesia sesamiae*, *Cotesia typhae*, nonreproductive host mortality

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6.1 Introduction

Generally, exotic invasive insect herbivores have the potential to interfere significantly with existing herbivores-natural enemies' interactions in the invasion zones (Desurmont *et al.*, 2014; Chabaane, *et al.*, 2015; Martorana *et al.*, 2017). The exotic insect herbivores may interfere with the foraging behaviour of natural enemies. Few studies have explored the impact of exotic invasive insect herbivores on existing herbivores-natural enemies' interactions. Most of the study focussed on the use of exotic insect herbivores by natural enemies, in classical biological program and the consequences of exotic natural enemies

for native parasitoid-host interactions (Chabaane *et al.*, 2015). However, the consequences of exotic insect herbivores on existing herbivores-natural enemies' interactions even if they cannot be used as hosts by natural enemies already present in the new environment is often less investigated.

In Sub-Saharan Africa, an alien invasive destructive pest on maize ((Hruska & Gould 1997), Fall Armyworm (FAW) *Spodoptera frugiperda* J.E. Smith (Lepidoptera: Noctuidae), originated in Americas started to invade Africa in 2016 (Goergen *et al.*, 2016) to spread in maize fields all over sub-Saharan Africa causing, for example, up to 11.5 and 30% yield losses in Ethiopia and Kenya, respectively (Kassie *et al.*, 2020; De Groote *et al.*, 2020). Before the introduction of FAW in Kenya, an effective biological control program for the maize stemborer communities with larval parasitoids was implemented (Midingoyi *et al.*, 2016). The most commonly used larval parasitoids are *Cotesia flavipes* Cameron (Hymenoptera: Braconidae), used in a classical biological control program against the invasive *Chilo partellus* Swinhoe (Lepidoptera: Crambidae) (Overholt *et al.*, 1994a), and two populations of the native *Cotesia sesamiae* Cameron (Hymenoptera: Braconidae), *Cs-Coast* and *Cs-Inland*, controlling respectively *Sesamia calamistis* Hampson (Lepidoptera: Noctuidae) and *B. fusca* (Kfir *et al.*, 2002). In addition, *C. typhae* Fernández-Triana (Hymenoptera: Braconidae), a recently described species, was found to be associated with *Sesamia nonagrioides* (Lefebvre) (Lepidoptera: Noctuidae) in Kenya (Kaiser *et al.*, 2017). The invasion of FAW, is likely to interfere with preexisting trophic interaction between maize-stemborer-natural enemies in Africa as showed elsewhere by Desurmont *et al.* (2014) and Chabaane *et al.* (2015). In maize fields, second larval instar of FAW are commonly found in communities of mixed species with stemborer species in the whorl of

the plants. Also, it is frequent to find older FAW larvae in the bored holes and tunnels from stemborers as well FAW larva feeding on corn with stemborer larvae (Sokame B.M., Personal Observations). This imply that FAW and the native African stemborers are sharing the same ecological niche. Such interference of FAW with existing trophic interactions, could potentially impact the biological control efforts of both FAW and maize stemborer. However, little is known about such interactions of FAW with lepidopteran stemborer communities in maize fields and their natural enemies. Such information will help to understand how the parasitoid associated with maize stemborer might evolve with the invader, *S. frugiperda*, in Kenya. The objective of this study was to assess the performance of *Cotesia* spp. associated with maize stemborers on fall armyworm and the implication in stemborer biological control already established before its invasion.

6.2 Material and methods

6.2.1 *Spodoptera frugiperda* colony

Laboratory rearing was carried out at $25 \pm 2^{\circ}\text{C}$; 50-70% RH and a photoperiod of 12:12 (L:D) h. Larvae of *Spodoptera frugiperda* were collected in 2018 from infested maize fields of Kitale (34.777E, 1.203N) and Bungoma (34.557E, 0.655N) in western region of Kenya and of Makutano (37.273E, 0.727S) and Murang'a (37.150E, 0.923S) in central region and reared prior to experiments, at least for two generations by the Animal Rearing and Containment Unit (ARCU) at *icipe*, Nairobi, Kenya. Adult moths (three females and two males moths per cage) were placed in cages (40×40×45 cm) constructed of Perspex with netting materials fitted on the back side and sleeve on the front side. They were fed with 1:9 honey:water solution soaked in cotton and hung on the well of the cage. Moths were provided small potted maize plants for oviposition. Potted plants were removed after

one-night exposure to the moths and the leaves with eggs transferred into the plastic boxes (15 × 7 × 5 cm) lined with a paper towel to absorb excess moisture until eggs' hatching. After eggs hatching, larvae were reared on detached maize fresh leaves in the plastic boxes until pupation. The detached maize leaves were changed every two days during the rearing process. Pupae were incubated under similar rearing conditions until adult emergence.

6.2.2 *Cotesia flavipes*, *C. sesamiae* Inland, *C. sesamiae* Coast and *C. typhae* colonies

Females of *C. flavipes*, an inland and coastal population of *C. sesamiae* (hereafter named Cs-Inland and Cs-Coast, respectively), as well as of *C. typhae*, came from laboratory-reared colonies established at *icip*e, Nairobi, Kenya. *Cotesia flavipes* was initiated with parasitoids collected in 2005 from *C. partellus* collected from maize fields in Mombasa, coastal Kenya (39.668E, 4.07S); while the indigenous parasitoids, *C. sesamiae* Inland and *C. sesamiae* Coastal were originated from maize fields infested by *B. fusca* larvae in Kitale in 2006 (Western region of Kenya: 34.818E, 1.195N), and from maize fields infested by *S. calamistis* in Mombasa in 2007 (Coastal region of Kenya: 39.667E, 4.05S), respectively; and *C. typhae*, from *Typha* sp. plants infested by *S. nonagrioides* larvae in Kobodo in 2013 (near Lake Victoria: 34.412E, 0.679S). Each parasitoid's colony was rejuvenated twice a year with field-collected parasitoids to maintain genetic diversity.

For each colony, the cocoons were kept until emergence. After emergence, adult parasitoids were fed on a 20% honey/water solution and placed under artificial light for 8 h to mate. In all experiments, 1-day-old naïve (i.e., without oviposition experience), mated females were used. Similar to Overholt *et al.* (1994a), experimental conditions were at 25 ± 2°C, at 50–80% relative humidity (RH) and with a 12:12 h (L:D) photoperiod.

Their respective hosts, *C. partellus* and *S. calamistis* were initially collected from maize fields in coastal regions of Kenya, and *B. fusca* from maize fields in Western Kenya (Kitale), while *S. nonagrioides* were initially collected from *Typha domingensis* in Makindu, Kenya. The larvae of *C. partellus* were continuously reared at *icipi* on artificial diets of Ochieng *et al.* (1985), whereas the larvae of the other species were fed on the artificial diet of Onyango & Ochieng'-Odero (1994). Twice a year, all host's colonies were rejuvenated by field-collected stemborer larvae.

6.2.3 Acceptability and suitability of *Spodoptera frugiperda* larvae by *Cotesia flavipes*, *C. sesamiae* Inland, *C. sesamiae* Coast and *C. typhae*

The experiments were conducted at $25 \pm 1^\circ\text{C}$, $50 \pm 6\%$ RH and a photoperiod of 12:12 (L:D) h. A single larva was placed under transparent glass tube ($t = 1.5$ cm; $d = 3$ cm), together with a wasp (Plate 6.1) as previously reported by Obonyo *et al.* (2010).

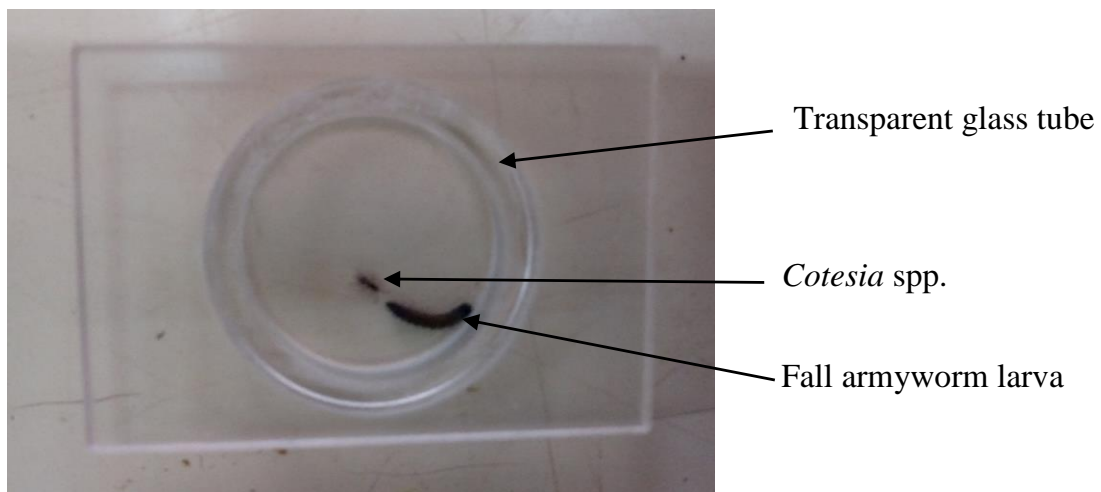


Plate 6. 1: Test of acceptance of fall armyworm larvae by *Cotesia* spp.

Naive, 1-day-old mated female parasitoid was randomly selected and introduced into an inverted transparent glass tube placed on a flat surface and allowed to climb to the top (i.e. the bottom of the glass tube placed). Once the parasitoid had reached the top, the Petri dish

was placed over a FAW larva. The behaviour of either the wasp or FAW larva was observed until the parasitoid stung the larva or for a maximum of 5 minutes. The number of dead parasitoids was recorded in case the defence behaviour of host-parasitoid led to its death. Second, third and fourth larval instars were tested with each parasitoid species. These instars were chosen because they generally constitute the most suitable stages as host for larval parasitoids (Godfray 1994).

Each wasp and larva were used only once. For each larval instar tested, there was a total of 60 FAW larvae exposed to each parasitoid species in 3 replicates of 20 larvae tested individually. After each replicate, the glass tube was cleaned in distilled water and air-dried. For each FAW larva, the searching and stinging times of each parasitoid species were recorded.

After exposure to the parasitoid, the FAW larvae were maintained individually in glass vials, and provided with fresh maize leaves. They were monitored daily until cocoon formation, pupation, death, or adult of host/parasitoid emergence. The control consisted of individually kept 60 FAW larvae that were not exposed to parasitoids in glass vials in 3 replicates of 20 larvae under the same rearing conditions to assess natural mortality of the host.

In nature, adult *C. flavipes* and *C. sesamiae* are generally active throughout the day but mostly around 12:00h, when light intensity and temperatures are high (Obonyo et al. 2010). Therefore, all experiments were conducted between 10:00 and 14:00h, in arenas kept at $25 \pm 2^\circ\text{C}$ and illuminated from above using a white 60W light bulb (Obonyo et al. 2010).

6.2.4 Parasitized host dissection and possible encapsulation ability of the host

To confirm eggs deposition of the parasitoids or to evaluate possible encapsulation of the eggs by FAW larvae, each larva tested was exposed to a gravid female of each parasitoid species using the hang stinging method (Overholt et al. 1994a). Parasitized larvae were placed individually into glass vials on fresh maize leaves maintained under laboratory conditions for 12 to 24 hours post-parasitism before dissection as previously reported by Buchori et al. (2009). *Chilo partellus* larvae parasitized by *C. flavipes* and *S. calamistis* parasitized by each population of *C. sesamiae* were used as positive controls for observations on egg deposition (Sallam et al. 1999) while *B. fusca* larvae parasitized by either *C. flavipes* or *Cs-Coast* were used as positive controls for determining encapsulated egg observations (Gitau et al. 2006). Thereafter, parasitized hosts were dissected *in vivo* suspended in a drop of Ringer's saline on a concave glass slide under a binocular microscope (Olympus UK Ltd) for counts of the number of normal and encapsulated eggs per host larva. For each examination, 5 replicates of 6 parasitized larvae per instar and parasitoid species were used.

6.2.5 Dual choice bioassay of *Cotesia* spp. between fall armyworm and maize stemborer species in Petri dish arena and in Y-tube olfactometer

Based on the outcome of the FAW larvae acceptability experiments (see results), only the parasitoid species which found to be more effective to attack the FAW larvae, namely *C. flavipes* and *Cs-Inland*, were used in both two-choice bioassays and Y-tube olfactometer tests.

This experiment is carried using the same procedure described above, except that the parasitoid was offered two larvae instead of one (FAW vs *C. partellus* for *C. flavipes* and

FAW *vs.* *S. calamistis* for *Cs*-Inland). The behaviour of each female wasp was observed until it either stung the preferred larva or for maximum of 5 minutes if the wasp did not sting any of the larvae. Second and third instars of hosts were tested with each parasitoid species. For each larval instar tested, 3 replicates of 20 choices were made. After each replicate, the glass tube was cleaned in distilled water and air-dried.

In Y-tube olfactometer choice experiments, maize plants of hybrid H513 (Simlaw, Kenya Seed Company, Nairobi, Kenya) grown in plastic pots (12 cm in height x 13 cm in diameter) were used when they were between 4-6 weeks old. Maize plants were manually infested with five third instar larvae of either FAW or a given stemborer species. These larval stages were chosen to ensure a high degree of feeding damage within 24 hours before their use. Each assay lasted for a maximum of 10 min. The following combinations were used for each female parasitoid: uninfested maize *vs.* empty chamber, uninfested maize *vs.* FAW infested maize. Additionally, for *C. flavipes* the treatments were uninfested maize *vs.* *C. partellus* infested maize; and FAW infested maize *vs.* *C. partellus* infested maize, while for *Cs*-Inland they were uninfested maize *vs.* *S. calamistis* infested maize; and FAW infested maize *vs.* *S. calamistis* infested maize.

For each parasitoid, gravid females were released individually into the base of the Y-tube and allowed to choose either of the two arms. The Y-tube had the dimensions of 18 cm of stem length, 34 cm of each arm length and 4 cm of diameter. For each test, a choice was recorded when the insect passed 5 cm from the intersection into one arm and remained motionless there for more than 20s. Those that made no choice were also recorded. After every five insects, odour source connections to the chambers were reversed to minimise any locational bias and the chambers were cleaned thoroughly with tap water. For each

conditioning procedure, the percentage of parasitoid that made a distinct choice was calculated.

6.2.6 Data analysis

The percentage of accepted hosts was expressed as the number of larvae stung divided by the total number of larvae exposed while successful parasitism was calculated as the number of parasitoid cocoon masses divided by the sum of host pupae and parasitoid cocoon masses. Larval and pupal nonreproductive mortality (i.e. larval or pupal mortality induced by parasitism not resulting in development of parasitoid offspring (Abram et al. 2019), as well as larval and pupal mortality in the control) were computed using Abbott formula (Abbott 1925).

The proportion of hosts stung, and the proportion of parasitoids killed by the host were analyzed using generalized linear model (GLM) with binomial error distribution (link = logit), due to the binary nature of these parameters (accept host *vs* does not accept, be killed *vs* is not killed). Means were separated with Tukey's multiple comparison test, performed in the R package 'lsmeans'. The searching and oviposition times of parasitoids and the actual nonreproduction host mortality were checked for normality and homogeneity of variance using Shapiro–Wilk and Bartlett tests, respectively and analysed using Kruskal-Wallis nonparametric procedure with `dunn.test` R package. The significance of larval and pupal nonreproductive mortality was assessed by comparing larval and pupal natural mortality rate in control with mortality rate in the presence of the parasitoid using analyse of variance (ANOVA) or Kruskal-Wallis nonparametric procedure depending on whether the data are normally distributed and homogeneous or not after Shapiro–Wilk and Bartlett tests, respectively. The number of eggs laid per female parasitoid and the number of eggs

encapsulated were compared between larval stages by ANOVA after been tested for normality and homogeneity of variance using Shapiro–Wilk and Bartlett tests, respectively. Means were separated with Student-Newman-Keuls (SNK) method at 5% significance level. Results of the olfactometer assays and the response in the dual bioassay were analyzed by Chi square at 5% significance level. All analyses were performed with R software version 3.5.1 (R Core Team 2018).

6.3 Results

6.3.1 Acceptability of fall armyworm larvae by *Cotesia flavipes* *C. sesamiae* Inland, *C. sesamiae* Coast and *C. typhae*

Upon release into the experimental arena, for the parasitoids species that initiated an active search behaviour followed by a stinging attempts using their ovipositors towards the FAW larvae, some of them were killed by FAW larvae. *C. flavipes* and *Cs* Coast mortality significantly increased with host larvae stages (Table 6.1). No mortality was observed for *C. typhae* since this parasitoid species never attempted to sting the larva for parasitism.

Table 6. 1: Percentage (mean \pm SE, n=3) of parasitoid killed by fall armyworm larvae.

Parasitoid species	FAW larval stages			LR	df	P
	L2	L3	L4			
<i>Cotesia flavipes</i>	0.00 \pm 0.00aA	0.00 \pm 0.00aA	5.00 \pm 2.83bA	6.69	2	0.03
<i>Cotesia sesamiae</i> Inland	0.00 \pm 0.00aA	3.33 \pm 2.33aA	6.67 \pm 3.24aA	5.68	2	0.05
<i>Cotesia sesamiae</i> Coast	3.33 \pm 2.33aA	10.00 \pm 3.90abB	23.33 \pm 5.50bB	11.93	2	0.002
<i>Cotesia typhae</i>	— ^a	—	—			
LR	4.41	9.87	11.27			
df	2	2	2			
P	0.10	0.007	0.003			

—^a No *Cotesia typhae* has been killed. Non-significant different means (\pm SE) are shown by identical letters within each row (i.e. larval stages comparisons) in lower case and within columns in upper case (i.e. parasitoid species comparisons), determined using lsmeans R package, following generalized linear model (GLM) with binomial error distribution.

For the parasitoid species that attacked FAW larvae, the attack occurred on 16.67 to 71.67% of FAW larvae exposed with an ovipositor insertion time ranging from 4.24 to 19.97 seconds, (Table 1). For *C. flavipes* and *Cs*-Inland, the percentage of FAW larvae attacked did not vary between larval stages (Table 1). By contrast, for *Cs*-Coast, percentage of FAW larvae attacked, varied with host age, being significantly higher for L2 than the other stages. Moreover, the times taken by the females' parasitoid to search the host and to insert their ovipositors tended to increase with FAW larval stage (Table 1). In overall, *C. flavipes* and *Cs*-Inland were found to be more effective than *Cs*-Coast to attack the FAW larvae (Table 6.2).

Table 6. 2: Percentage (mean \pm SE, n=3) of stung larvae of fall armyworm and searching and stinging times (mean \pm SE, n=3) by parasitoids.

Parasitoid species	FAW stages	% of larvae stung	Searching time (in seconds)	Stinging time (in seconds)
<i>Cotesia flavipes</i>	L2	71.67 \pm 5.85b	47.74 \pm 8.03a	4.30 \pm 0.37a
	L3	70.00 \pm 5.96b	34.48 \pm 6.26a	4.63 \pm 0.20a
	L4	66.67 \pm 6.13b	54.78 \pm 9.06b	6.86 \pm 0.95b
<i>Cotesia sesamiae</i> (Inland)	L2	71.66 \pm 5.86b	40.76 \pm 7.61a	4.24 \pm 0.22a
	L3	68.33 \pm 6.05b	54.83 \pm 10.19b	5.18 \pm 0.39a
	L4	63.33 \pm 6.27b	59.23 \pm 10.82b	19.97 \pm 2.65c
<i>Cotesia sesamiae</i> (Coast)	L2	65.00 \pm 6.20b	33.57 \pm 5.52a	6.00 \pm 1.10b
	L3	28.33 \pm 5.86a	77.40 \pm 23.36bc	11.90 \pm 2.40c
	L4	16.67 \pm 4.85a	119.64 \pm 22.24c	16.47 \pm 3.36c
<i>Cotesia typhae</i>	L2	– ^b		
	L3	–		
	L4	–		
LR/ χ^2		85.032	27.476	81.767
df		8	8	8
P		< 0.0001	< 0.0001	< 0.0001

–^b 0% of attacked larvae has been observed. LR = Likelihood Ratio. Non-significant different means (\pm SE) are shown by identical letters within each column (i.e. both parasitoid species and FAW stages comparisons), determined using lsmeans R package, following generalized linear model (GLM) with binomial error distribution (for proportion of stung larvae of FAW) or with Kruskal-Wallis nonparametric procedure using dunn.test R package (searching and stinging times) at $P < 0.05$.

6.3.2 Host suitability of fall armyworm for *Cotesia flavipes* *C. sesamiae* Inland, *C. sesamiae* Coast and *C. typhiae*

None of the larval stages of FAW yielded cocoons, irrespective of parasitoids species (Table 6.3). For all FAW larval stages, larval nonreproductive mortality was significantly higher with *C. flavipes* (23 to 36%) and *Cs*-Inland (22 to 37%) than with *Cs*-Coast (8 to 29%) (Tables 6.3 & 6.4).

Table 6. 3: Successful parasitism and nonreproductive mortality induced after stinging by parasitoids of fall armyworm larvae at different development stages (% , mean \pm SE, n=3).

Parameters	FAW stages	<i>Cotesia flavipes</i>	<i>Cotesia sesamiae</i> (Inland)	<i>Cotesia sesamiae</i> (Coast)
Successful parasitism (%)	L2	– ^c	–	–
	L3	–	–	–
	L4	–	–	–
FAW larval nonreproductive mortality (%)	L2	36.98 \pm 0.96aB	37.96 \pm 2.54aB	29.05 \pm 3.81aA
	L3	23.00 \pm 3.33abA	34.12 \pm 0.79bB	16.98 \pm 1.65aA
	L4	25.09 \pm 2.87bA	22.59 \pm 2.59bA	8.33 \pm 2.65aA
df		2	2	2
χ^2		5.80	6.21	4.39
<i>P</i>		0.04	0.04	0.11
FAW pupal nonreproductive mortality (%)	L2	11.49 \pm 1.51aA	14.39 \pm 1.89aA	14.05 \pm 0.84aA
	L3	15.05 \pm 2.14aA	11.20 \pm 0.72aA	20.55 \pm 2.42aA
	L4	10.06 \pm 0.58aA	8.33 \pm 2.16aA	8.33 \pm 4.16aA
df		2	2	2
χ^2		3.77	2.98	2.87
<i>P</i>		0.15	0.22	0.24

–^c No No Successful parasitism (i.e. cocoon mass production) has been observed. Non-significant different means (\pm SE) are shown by identical letters within each row (i.e. parasitoid species comparisons) in lower case (for larval, L2: $\chi^2 = 2.89$, $df = 2$, $P = 0.24$; L3: $\chi^2 = 6.54$, $df = 2$, $P = 0.04$; L4: $\chi^2 = 8.25$, $df = 2$, $P = 0.02$ and for pupal, L2: $\chi^2 = 2.15$, $df = 2$, $P = 0.34$; L3: $\chi^2 = 5.08$, $df = 2$, $P = 0.08$; L4: $\chi^2 = 0.34$, $df = 2$, $P = 0.83$) and within columns in upper case, determined using dunn.test function, following Kruskal-Wallis at $P < 0.05$.

Non-reproductive mortality of FAW larvae tended to decrease with larval stage but the differences were not always significant. Except for *Cs*-Coast and at L3 stage for *C. flavipes*, FAW larval mortality was significantly higher for larvae subjected to the parasitoid for all larval stage compared to their respective control (Table 6.4). For pupal mortality, no

significant difference was recorded neither between parasitoid species nor within host larval stages (Table 2). Comparing pupal nonreproductive mortality for each parasitoid vs its control at each host instar, larvae subjected to the parasitoid resulted in significant pupal nonreproductive mortalities in all host larval stages tested except L4 stage with *Cs-Coast* and *Cs-Inland*

Table 6. 4: Comparison between mortality (% , mean \pm SE, n=3) of fall armyworm larvae at different development stages after stinging or not (=control) by the parasitoids.

Parameters	FAW stages	Mortality after parasitoid stinging by	Natural mortality (control)	F/χ^2	df	P
<i>Cotesia flavipes</i>						
Larval mortality	L2	36.98 \pm 0.96b	16.66 \pm 4.40a	3.85	1	0.04
	L3	23.00 \pm 3.33a	15.00 \pm 2.88a	3.29	1	0.14
	L4	25.09 \pm 2.87b	13.33 \pm 1.66a	12.5	1	0.02
Pupal mortality	L2	11.49 \pm 1.51b	6.03 \pm 0.32a	112.7	1	0.004
	L3	15.31 \pm 2.14b	5.89 \pm 0.20a	3.85	1	0.04
	L4	10.06 \pm 0.58b	3.92 \pm 1.96a	9.03	1	0.03
<i>Cotesia sesamiae</i> (Inland)						
Larval mortality	L2	37.96 \pm 2.54b	16.66 \pm 4.40a	15.5	1	0.01
	L3	34.12 \pm 0.79b	15.00 \pm 2.88a	40.82	1	0.003
	L4	22.59 \pm 2.59b	13.33 \pm 1.66a	9.02	1	0.03
Pupal mortality	L2	14.39 \pm 1.89b	6.03 \pm 0.32a	3.97	1	0.04
	L3	11.20 \pm 0.72b	5.89 \pm 0.20a	50.02	1	0.002
	L4	8.33 \pm 2.16a	3.92 \pm 1.96a	0.91	1	0.39
<i>Cotesia sesamiae</i> (Coast)						
Larval mortality	L2	29.05 \pm 3.81a	16.66 \pm 4.40a	4.52	1	0.1
	L3	16.98 \pm 1.65a	15.00 \pm 2.88a	0.35	1	0.58
	L4	8.33 \pm 2.65a	13.33 \pm 3.66a	0.34	1	0.58
Pupal mortality	L2	14.05 \pm 0.84b	6.03 \pm 0.32a	78.94	1	<0.0001
	L3	20.55 \pm 2.42b	5.89 \pm 0.20a	3.86	1	0.04
	L4	8.33 \pm 4.16a	3.92 \pm 1.96a	0.05	1	0.81

Non-significant different means (\pm SE) are shown by identical letters within rows (i.e. comparisons between mortalities induced after stinging vs natural mortalities only), determined using analyse of variance (ANOVA) or Kruskal-Wallis nonparametric procedure depending on whether the data are normally distributed and homogeneous or not after Shapiro–Wilk and Bartlett tests, respectively at $P < 0.05$.

6.3.3 Parasitoid normal eggs and eggs encapsulated observations

No normal or encapsulated eggs were found in any of the FWA larval stages stung by the parasitoid species (Table 6.5). By comparison, the positive control hosts yielded both normal and eggs encapsulated (Table 6.5).

Table 6. 5: Mean number of eggs (\pm SE, n=5) laid by a given female parasitoid per sting and the number of eggs (\pm SE, n=5) encapsulated in *B. fusca* larvae

Parameters	Larval stages	Number of eggs/larva			Number of eggs encapsulated/larva	
		<i>Chilo partellus</i>	<i>Sesamia calamistis</i>	<i>Spodoptera frugiperda</i>	<i>Busseola fusca</i>	<i>Spodoptera frugiperda</i>
<i>Cotesia flavipes</i>	L2	16.34 \pm 1.73a		– ^d	9.16 \pm 1.82a	– ^e
	L3	25.48 \pm 3.34a		–	8.21 \pm 1.51a	–
	L4	37.61 \pm 3.94b		–	11.92 \pm 3.65a	–
<i>df</i>		2			2	
<i>F</i>		11.48			0.58	
<i>P</i>		0.001			0.57	
<i>Cotesia sesamiae</i> (Inland)	L2		17.14 \pm 2.20a	–		
	L3		19.10 \pm 2.08a	–		
	L4		27.81 \pm 2.07b	–		
<i>df</i>			2			
<i>F</i>			7.16			
<i>P</i>			0.008			
<i>Cotesia sesamiae</i> (Coast)	L2		16.34 \pm 2.38a	–	7.21 \pm 1.16a	–
	L3		27.25 \pm 2.22b	–	9.23 \pm 1.19a	–
	L4		31.94 \pm 2.73b	–	7.90 \pm 1.08a	–
<i>df</i>			2		2	
<i>F</i>			10.59		0.79	
<i>P</i>			0.002		0.47	

Neither normal eggs (–^d) nor eggs encapsulated (–^e) have been observed. Non-significant different means (\pm SE) are shown by identical letters within columns (i.e. larval stages comparisons), determined using Student Newman and Keuls (SNK), following ANOVA at $P < 0.5$.

6.3.4 Response of *Cotesia flavipes*, *C. sesamiae* inland choice between fall armyworm and maize stemborer species in Petri dish arena and in Y-tube olfactometer

Under two-choice conditions in the experimental petri dish arena, *C. flavipes* and *Cs*-Inland equally accepted FAW and *C. partellus* (L2: $\chi^2 = 3.37$, $df = 1$, $P = 0.06$; L3: $\chi^2 = 0.28$, $df = 1$, $P = 0.59$), and FAW and *S. calamistis* larvae (L2: $\chi^2 = 0.92$, $df = 1$, $P = 0.33$; L3: $\chi^2 = 0.01$, $df = 1$, $P = 0.89$), respectively (Fig. 1) (Figure 6.1).

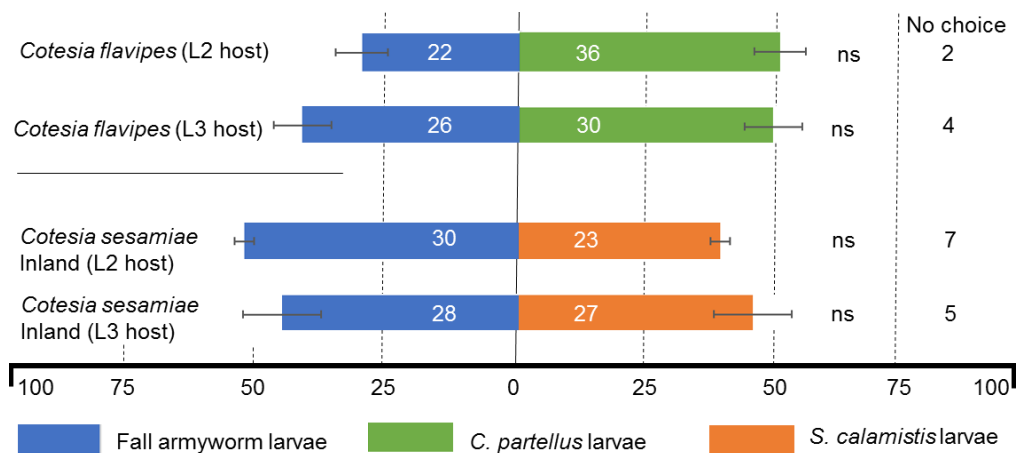


Figure 6. 1: Dual choice of *Cotesia* spp. between FAW larvae and maize stemborer species in Petri dish arena. On each bar, the number of parasitoids choosing a given host was mentioned and analysed using the two-tailed Fisher's exact probability 2 x 2 test. The number of parasitoids making no choice was also given.

In the Y-tube olfactometer, 83 – 93% of both *C. flavipes* and *Cs*- Inland females oriented significantly towards volatiles emitted by uninfested maize plants as compared to blanks (Figure 6.2, $\chi^2 = 10.8$, $df = 1$, $P = 0.001$ for *C. flavipes* and $\chi^2 = 8.53$, $df = 1$, $P = 0.003$ for *Cs*-Inland). The parasitoids showed a significant preference for plants infested by either FAW or stemborer larvae over uninfested plants (for *C. flavipes*: comparison uninfested vs *C. partellus* infested plants: $\chi^2 = 4.8$, $df = 1$, $P = 0.02$; comparison uninfested vs FAW infested plants: $\chi^2 = 8.53$, $df = 1$, $P = 0.003$) (for *Cs*-Inland: comparison uninfested vs *S. calamistis* infested plants: $\chi^2 = 10.8$, $df = 1$, $P = 0.001$; comparison uninfested vs FAW

infested plants: $\chi^2 = 4.8$, $df = 1$, $P = 0.02$). However, *C. flavipes* did not discriminate between the odours from plants infested by *C. partellus* and plants infested by FAW ($\chi^2 = 0.53$, $df = 1$, $P = 0.46$), while *Cs*-Inland showed marked preference to the odours of plants infested by FAW over that of plants infested by *S. calamistis* ($\chi^2 = 8.53$, $df = 1$, $P = 0.003$) (Figure 6.2).

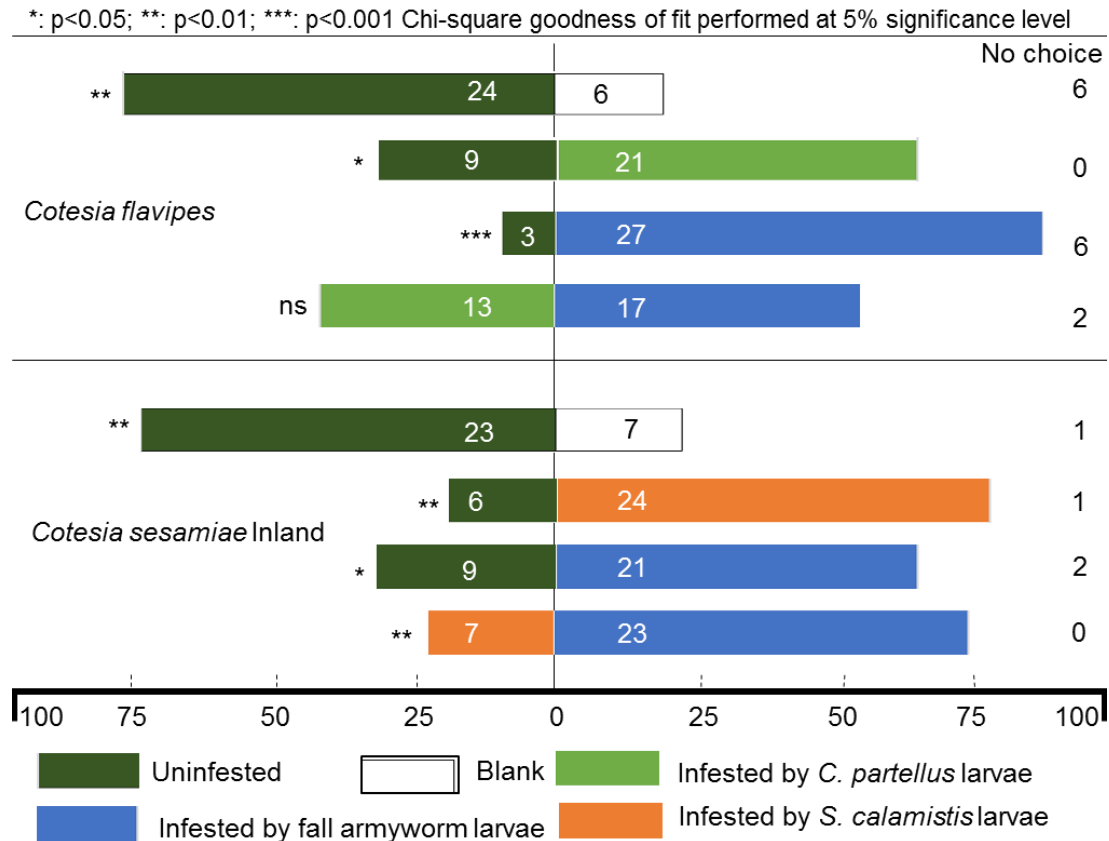


Figure 6. 2: Dual choice response of *Cotesia flavipes*, *C. sesamiae* inland to odour source from uninfested and different host infested maize plants in a Y-tube olfactometer. On each bar, the number of parasitoids choosing the odours was given as well as the number of parasitoids making no choice (n ranging from 30 to 36). The number of parasitoids making a choice was set to 100% to calculate the percentage of responding parasitoids. * = P<0.05; ** = P<0.01; *** = P<0.0001 according to Chi-square goodness of fit performed at 5% significance level.

6.4 Discussion

In the present study, parasitoids, *C. flavipes* and *C. sesamiae*, *Cs*-Inland and *Cs*-Coast populations of maize stemborers are found to exhibit searching behaviour on FAW larvae and then able to attack the larvae. For most of them, attack on non-host or new host larvae has been already reported (Obonyo *et al.*, 2008). However, in our study, escape and defense behaviours between the parasitoid and FAW larvae occurred mostly at the oldest FAW stage resulting in death of some female parasitoids. Parasitoid death following the aggressive reaction of the host in the response to the ovipositor insertion process has been already observed in *Cotesia* spp. by Takasu and Overholt (1997) and other parasitoids (e.g. Gordh, 1976). The time taken by the parasitoids for searching the FAW larvae increased with FAW larval stage. This could be due to the decreasing suitability and increased aggressiveness of the FAW with age of the larva. Chau and Mackauer (2000) working with the aphid parasitoid *Munroctonus paulensis* (Hymenoptera: Braconidae) showed that the total time needed by a female to capture, position, and parasitize an aphid varied with host instars, with fourth instar required nearly two fold of the time as that for the more suitable stages, first, second and third instar. The same authors, reported that increased in ovipositor insertion time increased the likelihood of parasitoids' death.

Cotesia flavipes and *Cs*-Inland attacked all larval instars of FAW tested while *Cs*-Coast mostly preferred the 2nd instar. On the other hand, *C. typhae* did not attack any instar of the host. This might be due to the higher host specificity of this parasitoid which has been previously documented (Kaiser *et al.*, 2017; Bichang'a *et al.*, 2018). Host habitat specificity could also played a key role in the behaviour of the four parasitoids in term of their attraction to and attack of FAW. *Cotesia flavipes* and *C. sesamiae* (both populations) share

the same maize ecosystem while *C. typhae* is predominately found on wild *Typha*- and *Cyperus*-habitats where its host *S. nonagrioides* is found in Kenya (Kaiser *et al.*, 2017).

Surprisingly, there was no significant difference in larval choice for ovipositor insertions under dual-choice conditions between FAW and either *C. partellus* larvae for *C. flavipes* or *S. calamistis* larvae for *Cs*-inland. Since older FAW larvae are frequently found in the bored holes and tunnels left by stemborers and even on corn with stemborer larvae in maize fields (Sokame B.M., Personal Observations), these two *Cotesia* species may evolve by time to successfully develop in FAW larvae. Other African native *Cotesia* species such as *C. icipe* Fernandez-Triana & Fiaboe (Hymenoptera: Braconidae) have been reported to be efficient on FAW larvae (Sisay *et al.*, 2018).

The Y-tube experiments confirmed preference of *Cs*- Inland for FAW to *S. calamistis*, while no preference was observed for *C. flavipes* between FAW and *C. partellus*. The results for *Cs*-Inland could be due to the fact that the odours emitted by plants infested by FAW were more intense than those of plants infested by *S. calamistis* since defoliation and plant damage by FAW larvae is higher than that caused by *S. calamistis* (Sokame *et al.*, 2020b). This is aligned with the finding by Gouinguéné *et al.* (2003) who reported a positive correlation between the intensity of the HIPVs emissions and the amount of damage inflicted by *Spodoptera littoralis* (Boisduval, 1833) (Lepidoptera, Noctuidae).

No successful parasitism of FAW larvae has been recorded by any of parasitoids tested, indicating that FAW is not suitable to all parasitoid species tested here. However, both larval and pupal nonreproductive mortality caused by the parasitoid species that attacked FAW larvae were significantly higher than natural mortality in the control treatment. The nonreproductive mortalities might be due to either mutilation or pseudoparasitism since

neither normal eggs nor eggs encapsulated were observed in FAW larva after being attacked by parasitoids (Abram *et al.*, 2019).

Those nonreproductive mortalities induced by native parasitoids on FAW may significantly regulate FAW population in the field mostly in the regions where stemborer species cohabitate, which serves as suitable host for the parasitoids and ensure its continuous propagation. For example, a model developed by Kaser *et al.* (2018) on biological control effects of nonreproductive host mortality caused by insect parasitoids predicts that native parasitoids may have large biological control impact on the population dynamics of the invasive agricultural pest, *Halyomorpha halys* Stål (Hemiptera: Pentatomidae) in areas where suitable native hosts coexist. Nevertheless, the fact that plants infested by FAW are attractive to *C. flavipes* and *Cs*-Inland and that these parasitoids equally stung FAW as compared to their respective natural and native hosts in dual choice, we can expect FAW to have also a negative impact on stemborer biological control already established before its invasion. It has been reported that exotic insect herbivore unsuitable to native parasitoids have the potential to disrupt existing native parasitoid-host interactions *via* two mechanisms: interference with the foraging behaviour of the parasitoids and costs associated with unsuccessful attacks of the exotic non-host (Chabaane *et al.*, 2015; Martorana *et al.*, 2017). When the parasitoids could not complete the development in the new host while they still respond to cues from plant-herbivore complex and ovipositing the unsuitable host, they might invest time and energy in unproductive foraging behaviour. This may directly affect the reproductive output of the parasitoids under field conditions (Hoogendoorn & Heimpel, 2002) leading to reduction of parasitism rates on its native hosts. In this respect, FAW may represent a sink or an evolutionary trap for stemborer

associated parasitoids that undergo a reduction of their populations. Indirectly, this might be an advantage for stemborer species which might exhibit significant outbreaks due to a lower demographic pressure by natural enemies (Abram *et al.*, 2014).

In conclusion, the present study showed that fall armyworm larvae are not acceptable to and not suitable for *Cotesia* spp. associated with lepidopteran stemborers in Kenya. However, *C. flavipes*, *Cs*- Inland and *Cs*-Costal are able to induce nonreproductive mortality of the host through possibly mutilation and pseudoparasitism. In the areas where stemborers co-exist with FAW, *Cotesia* spp. associated with stemborers could also contribute to regulate the FAW populations through host nonreproductive mortality. However, on the other hand, FAW can have a negative impact on stemborer biological control already established before its invasion. Hence, more studies are needed to confirm under field conditions the host nonreproductive mortality contribution against FAW larvae and its impact on stemborer biological control.

CHAPTER SEVEN

LARVAL DISPERSAL OF THE INVASIVE FALL ARMYWORM, *SPODOPTERA FRUGIPERDA* (LEPIDOPTERA: NOCTUIDAE) AND THE AFRICAN LEPIDOPTERAN CEREAL STEMBORERS

Abstract

In sub-Saharan Africa, the noctuid stemborers, *Busseola fusca* (Fuller) and *Sesamia calamistis* (Hampson), and crambid *Chilo partellus* (Swinhoe) are economically the most important lepidopteran cereal stemborers pest species that in addition to the recent invasion of fall armyworm, *Spodoptera frugiperda* (J.E. Smith), severely limit maize production. Recent surveys indicated that fall armyworm (FAW) larvae strongly interacts with maize stemborer species in maize fields. This might be due to differences in larval dispersal capacities of between species either through ballooning or crawling which results in the redistribution of insect population and infestations within or between plants in the fields. In this study, larval dispersal abilities between FAW and individual stemborer species was compared in order to elucidate future interactions between the existing stemborer communities and the newly invaded fall armyworm in the fields. Twenty potted maize plants with one eggs batch laid by of a given stemborer species (either *Busseola fusca*, *Sesamia calamistis* or *Chilo partellus*) and FAW were closely monitored in a greenhouse for ballooning activities. Following emergence, both the ballooning and the non-ballooning larvae were identified according to species and counted. FAW neonate larvae had significantly ($P < 0.05$) greater potential of ballooning off than those of stemborers

irrespective of species. For each species, females significantly ($P < 0.05$) dispersed than males and their survival rate was significantly higher than that of the non-ballooning larvae. In addition, plant-to-plant larval movements were studied in a 6.25 m² plots of caged maize plants in a completely randomized design with five replicates. FAW larvae showed a significant larger dispersal rate and plant damage potential than any of the stemborer species used. Therefore, FAW has a greater dispersal capacity ($P < 0.05$) than maize stemborer species within or between plants. These findings are critical in developing sampling protocols for monitoring pest densities in the field, choice and timing of appropriate pest management practices.

Keywords: *Busseola fusca*, *Sesamia calamistis*, ballooning activity, crawling activity, Lepidoptera, Noctuidae, Crambidae

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7.1 Introduction

Insect dispersal is an important adaptation that ensures survival in habitats with variable resources (Pannuti *et al.*, 2016). Most studies have mainly focused on insect flight as a mean of dispersal (Wyckhuys & Neil, 2006) but have shown little attention to other insect dispersal means such as crawling and ballooning off by the larvae (Rojas *et al.*, 2018; Pannuti *et al.*, 2016; 2015; Zalucki *et al.*, 2002). Moreover, little information is available on the significance of these larval dispersal mechanisms in insect dispersion.

Larval dispersal from the initial oviposition sites is common among species which lay eggs in batches such as the lepidopteran stemborers and the fall armyworm (Erasmus *et al.*, 2016; Zalucki *et al.*, 2002). Adaptive dispersal mechanisms enables larvae to switch feeding sites and move from impoverished food resources to the more fresh and richer sources. Adaptive dispersion also allows the larvae to escape disturbance such predation and change in location within the plant (Pannuti *et al.*, 2016). The larvae migrate from the oviposition plant to adjacent plants either through ballooning neonate larvae or as crawling older larvae (Schoonhoven *et al.*, 2005; Zalucki *et al.*, 2002). The ballooning larvae can migrate to other plants with the silk strand still attached to the original oviposition leaf. Sometimes several larvae spin-off in a row hanging on the same thread. They may also spin-off, and either continue again or climb back to the original plant (Schoonhoven *et al.*, 2005; Zalucki *et al.*, 2002) .

In sub-Saharan Africa, the noctuid stemborers, *Busseola fusca* (Fuller) and *Sesamia calamistis* (Hampson), and crambid *Chilo partellus* (Swinhoe) are the economically most important lepidopteran pest species that severely limit production of maize as a result of a continuous infestation of the crop throughout its growth stages (Oben *et al.*, 2015; Kfir *et al.*, 2002). In crop fields, most lepidopteran pest species occur as single species or a community of mixed species (Krüger *et al.*, 2008; Ong'amo *et al.*, 2006a; 2006b; Van den Berg *et al.*, 1991). Recently, in the existing lepidopteran infestation matrix, has been reported the invasion the fall armyworm, *Spodoptera frugiperda* (J.E. Smith) in sub-Saharan Africa, where it is reported to seriously limits maize yields (Fotso Kuate *et al.*, 2019; Cock *et al.*, 2017; Goergen *et al.*, 2016). Recent observations indicated that FAW strongly interacts with maize stemborer communities (Sokame B., unpublished results).

However, unlike the existing Lepidoptera stemborer communities, these interactions might be first conditioned by the dispersal capacity of the larval species. Among these species, the females of *B. fusca* and *S. calamistis* oviposit their eggs between the leaf sheath and the stem of plant, whereas, *C. partellus* and *S. frugiperda* deposit eggs directly on the leaf surfaces (Polaszek, 1998).

In this study, larval dispersal abilities (by both ballooning and crawling) of FAW and stemborers species were compared. In addition, the consequences of ballooning off on fitness parameters such as larval and pupal survival as well as development time and sex ratio were also determined. Stemborers and FAW display an aggregative behaviour in maize field spreading only short distances from the original oviposition site (Erasmus *et al.*, 2016; Ndjomatchoua *et al.*, 2016; Pannuti *et al.*, 2016; Calatayud *et al.*, 2014a; Van den Berg *et al.*, 1991; Ross & Ostlie, 1990). Therefore, the movements of larvae between adjacent plants and plant damage inflicted by the larvae were carried out in small, semi-controlled experimental plots.

7.2 Materials and methods

7.2.1 Plants and Insects

All experiments were carried out at the Duduville campus of the International Centre of Insect Physiology and Ecology, (ICIPE) Nairobi, Kenya. Maize plants of hybrid H513 (Simlaw, Kenya Seed Company, Nairobi, Kenya) were used. For the ballooning off experiments, the maize seeds grown in plastic pots (12 cm in height x 13 cm in diameter) in a greenhouse while for the plant-to-plant movement experiment, plots of 2.5 × 2.5 m covered with netted cage were used. Mean temperatures were approximately 31/17°C

(day/night) with a L12:D12 photoperiod. Plants were used for the experiments at the age of 4-6 weeks old and almost between 60 - 75 cm tall.

The pupae of *B. fusca*, *C. partellus*, *S. calamistis* and *S. frugiperda* were obtained from colonies reared at the Animal Rearing and Containment Unit (ARCU) at *icipe*, Nairobi, Kenya. Colonies were rejuvenated twice a year with field-collected larvae. Pupae were sexed, and males and females were kept separately in plastic containers (21 x 15 x 8 cm) until adult emergence. A cotton pad moistened with water was placed inside the container to maintain relative humidity at >80%. The insects were kept in a rearing room at a temperature of $25 \pm 0.05^\circ\text{C}$, RH of $58.5 \pm 0.4\%$, and a photoperiod of L12:D12.

For each species, emerged adult males and females were released in a mating cage ($40 \times 40 \times 63$ cm), at the onset of the scotophase. The mating status was checked at hourly intervals until the end of the scotophase. Pairs of moths that were mating were collected in plastic jars (8 cm high \times 5 cm in diameter). From these pairs, the gravid females were individually released in the aforementioned mating cages containing each one potted maize plant the following night. Egg deposition was checked the following day. For each species, each potted plant with one batch of eggs harboring a similar number of eggs per batch (see results) was used in the two following experiments.

7.2.2 Experiments to determine the larval movement on the plants (i.e. ballooning activities)

For each insect species, twenty potted maize plants with one egg-batch were placed in the greenhouse at $26 \pm 2.5^\circ\text{C}$ of temperature and 65 – 70% of relative humidity (Plate 7.1). The potted plants were positioned at distance of one-meter away from the other to avoid leaf

overlap of different plants. Five (*B. fusca* and *S. calamistis*) or three days (*C. partellus* and *S. frugiperda*) after oviposition, the plants were checked daily early morning for eggs hatching. At emergence, the newly hatched larvae were observed for ballooning activity every 5-10 min during the first hour, and every 20 min during the second and third hour following emergence. All larvae that left the maize plants by hanging on silk threads were collected and individually put on artificial diet in glass vial (7.5 x 2.5 cm).



Plate 7. 1: Potted maize plants with one egg batch each positioned one meter apart for ballooning activity of the newly hatched larvae

The experiments were stopped after three hours, when most of the larvae had entered the plant via the whorl or leaf sheath or had left the plant on silken threads. The plants were dissected, and the non-ballooning larvae were recorded from each plant. The egg batch was removed from the dissected plant, and the total number of eggs that hatched or

unhatched on each plant were recorded in order to estimate the egg hatching rate. The larvae found on the floor around each potted plant were removed and were considered as those which had left or dropped off the plant without spinning-off. Those larvae in addition to dead larvae which were not collected were also estimated after counting the total number of eggs hatched per batch.

For each egg batch and species, ballooning and non-ballooning larvae were kept separately on artificial diet in the laboratory rearing room (temperature = $25 \pm 2^\circ\text{C}$; RH = $60 \pm 10\%$; photoperiod of 12:12 [L:D] h) until pupation to estimate larval developmental time (Plate 7.2). Pupae were sexed according to the method described by Underwood (1994). These were kept in separate plastic containers (16 cm \times 10 cm) closed with perforated plastic lids until adult emergence to estimate pupal development time. Larval or pupal survival for each species was calculated by dividing the number of larvae that pupated or pupae that emerged by respectively, the number of the total larvae or pupae recorded for a given species.



Plate 7. 2: Rearing of Ballooning and non-ballooning larvae separately collected and inoculated on artificial diet in the laboratory

7.2.3 Field experiments to determine larval movement between adjacent plants (i.e. ballooning + crawling activities)

The study was conducted in a completely randomized design. For each species, there were five replicates each consisting of a caged 6.25m² plot. Mean temperatures were approximately 31/17°C (day/night) with a L12:D12 photoperiod. Each cage was 2.5m wide, 2.5m long and 2m high, supported with PVC pipes (3cm diameter) and covered with white insect net (mesh 16 or holes of 1.19mm). Maize was planted in two concentric circles as shown in Plate 6.3. Two seeds were planted per hole but reduced to one plant one week after germination. Following the results from dispersal of stemborer and FAW larvae by Erasmus *et al.* (2016), Pannuti *et al.* (2016), Ndjomatchoua *et al.*(2016), Van den Berg *et*

al. (1991), Ross & Ostlie (1990), two concentric circles of 40cm and 80cm radius were chosen (Plate 6.3).

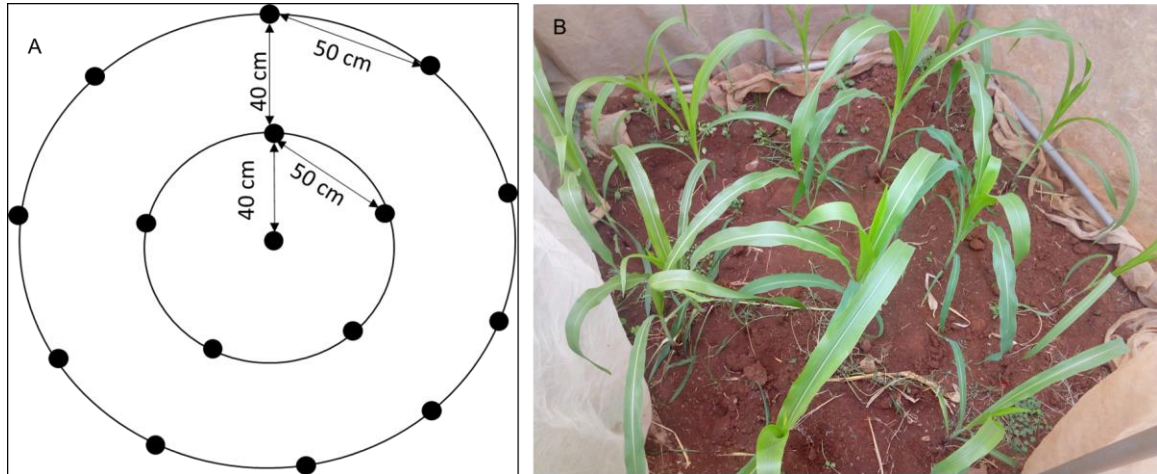


Plate 7. 3: Maize crop geometric (A) and plot of maize plants in the netting cage (B). The central point represents the initial point of infestation in the plot. Five maize plants were planted at 40cm and ten maize plants at 80 cm from the initial point of infestation

The plots were irrigated as required with a lateral overhead irrigation system. When the plants were four weeks old, one potted maize plant with one egg-batch obtained as described above was placed in the centre of the circles. The plant stage was chosen to be the most inflicted an optimum of leaf damage. After one week, the egg batch was removed from the plant, and the total number of eggs (hatched and not hatched) as well as the hatching rate were counted under a stereo microscope. Three weeks later at the plant's whorl stage, larval dispersal and leaf damage were assessed on each plant. The leaf damage was evaluated using the 1-9 damage scale described by Prasad *et al.* (2011) (1= No visible leaf injury or a small number of pin/ shot hole type of injury on a few leaves, 2= Small amount of shot hole type lesions on a few leaves, 3 = Shot hole injury common on several leaves, 4 = Several leaves with shot hole and elongated lesions, 5 = Several leaves with

elongated lesions, [2.5 cm], 6 = Several leaves with elongated lesions [>2.5 cm], 7 = Long lesions common on about one-half of the leaves, 8 = Long lesions common on about two-thirds of the leaves, and 9 = Most leaves with long lesions based on the type and amount of feeding). Infestation incidence was expressed as the percentage of plants damaged in each plot. Finally, the plants were uprooted and dissected to determine larval density per plant.

7.2.4 Data analysis

The proportion of ballooning larvae, sex ratio, survival rate, hatching rate, and infestation incidence were analyzed using generalized linear model (GLM) with the binomial error distribution due to the binary nature of these parameters (ballooning *vs* non-ballooning, female *vs* male, survival *vs* mortality, hatched *vs* non-hatched and infested *vs* uninfested) (Warton & Hui, 2011). Larval and pupal development time and number of eggs per batch were analyzed using GLM with the Poisson error distribution while the number of larvae per plant and leaf damage scoring were analyzed using GLM with a negative binomial error distribution because of the nature of the count data and the goodness of fit model. The means (\pm SE) were separated with Tukey's multiple comparisons tests, performed using the R package 'lsmeans' (Lenth, 2016). FAW and the stemborers species were taken as explicative variables in addition to the distance from the point of infestation in the between plants dispersal experiment. All the analysis were carried out in R version 3.5.1 (R Core Team 2018) and statistical results were considered significant when $P < 0.05$.

7.3 Results

7.3.1 Number of eggs per batch and eggs hatching rate of the fall armyworm and maize stemborer species

The average number of eggs per batch (mean \pm SE, n=25) obtained in this study was 64.6 \pm 4.1 for *B. fusca*, 60.1 \pm 4.6 for *S. calamistis*, 59.4 \pm 4.4 for *C. partellus* and 63.1 \pm 4.0 for FAW with hatching rates (mean \pm SE, n=25) of 91.0 \pm 1.6%, 94.8 \pm 0.83%, 95.1 \pm 0.64% and 91.9 \pm 0.89% respectively. Between species, the number of eggs per batch and the number of eggs hatched per batch were not significantly different (GLM: LR = 7.42, P = 0.06; LR = 2.72, P = 0.43 respectively). The proportion of the larvae found on the floor around the potted plants and those which died and were not collected represented (mean \pm SE %, n=20) 3.83 \pm 0.71 for *B. fusca*, 4.50 \pm 0.87 for *S. calamistis*, 3.37 \pm 0.58 for *C. partellus* and 9.27 \pm 1.01 for fall armyworm of the total eggs hatched.

7.3.2 Ballooning activity of the fall armyworm and maize stemborer species

The maize stemborers, *Busseola fusca*, *Sesamia calamistis* and *Chilo partellus* exhibited similar ballooning rates but they were significantly lower than those of FAW (GLM: LR = 99.69, P < 0.0001) (Figure 7.1). About 50% of the FAW larvae ballooned off the plant compared to only 30% of the stemborers.

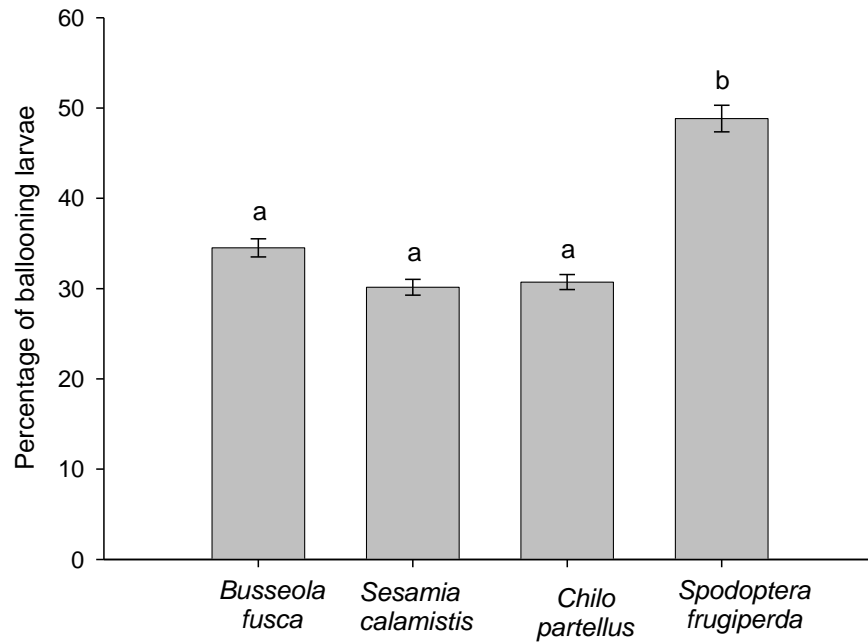


Figure 7. 1: Proportion of larvae (mean \pm SE) of each stemborer species and FAW larvae that left the maize plant by ballooning mechanisms. Non-significant differences are shown by identical letters determined using pairwise of lsmeans function with adjust = Tukey, following generalized linear model (GLM) with the simple binomial procedure.

For the ballooning or non-ballooning larvae, there was no significant difference of larva and pupa survival between species (Table 7.1).

Table 7. 1: Larval and pupal survivals, and developmental periods of the ballooning and non-ballooning larvae

Species	Survival rate (%)							
	Larvae				Pupae			
	Ballooning	Non-ballooning	LR	P	Ballooning	Non-ballooning	LR	P
<i>Busseola fusca</i>	89.68 ± 1.96 ^{aB}	61.55 ± 2.14 ^{aA}	56.95	<0.0001	91.47 ± 1.06 ^{aB}	69.03 ± 2.54 ^{aA}	56.95	0.004
<i>Sesamia calamistis</i>	91.08 ± 1.53 ^{aB}	58.98 ± 2.56 ^{aA}	157.92	<0.0001	89.14 ± 1.29 ^{aB}	65.98 ± 2.30 ^{aA}	54.17	<0.0001
<i>Chilo partellus</i>	90.50 ± 1.22 ^{aB}	57.84 ± 2.70 ^{aA}	114.62	<0.0001	90.16 ± 1.26 ^{aB}	63.88 ± 2.68 ^{aA}	61.73	<0.0001
<i>Spodoptera frugiperda</i>	85.81 ± 1.56 ^{aB}	63.04 ± 1.93 ^{aA}	82.06	<0.0001	89.19 ± 2.18 ^{aB}	62.09 ± 2.44 ^{aA}	89.15	<0.0001
LR	6.23	8.13			0.88	7.09		
P	0.10	0.07			0.82	0.06		
Development time (days)								
<i>Busseola fusca</i>	34.95 ± 0.18 ^{cA}	35.65 ± 0.15 ^{bA}	0.13	0.70	9.80 ± 0.18 ^{aA}	10.50 ± 0.26 ^{aA}	1.48	0.47
<i>Sesamia calamistis</i>	32.10 ± 0.51 ^{bcA}	34.40 ± 0.41 ^{bA}	1.59	0.20	10.10 ± 0.19 ^{aA}	11.15 ± 0.20 ^{aA}	1.03	0.30
<i>Chilo partellus</i>	29.85 ± 1.34 ^{bA}	31.80 ± 0.63 ^{bA}	1.23	0.26	10.35 ± 0.48 ^{aA}	12.15 ± 0.39 ^{aA}	2.88	0.08
<i>Spodoptera frugiperda</i>	20.65 ± 1.24 ^{aA}	22.65 ± 1.14 ^{aA}	1.84	0.17	10.40 ± 0.35 ^{aA}	11.55 ± 0.38 ^{aA}	1.20	0.27
LR	82.88	70.54			0.44	2.54		
P	<0.0001	0.003			0.93	0.46		

LR = Likelihood Ratio; Non-significant different means (\pm SE) are shown by identical letters within columns in lower case and between rows in upper case, determined using pairwise of lsmeans function with adjust = Tukey, following generalized linear model (GLM) with simple binomial procedure for survival rate and with Poisson for development time

However, for all species, ballooning larvae and pupae exhibited higher survival rates compared to those of non-ballooning larvae. Regardless of the pest species, there was no significant difference in the development time of both larvae and pupae between ballooning and non-ballooning larvae. However, the development time was for both ballooning and non-ballooning larvae was shorter for the FAW as compared to that of the stemborers (Table 7.1).

Moreover, regardless of the species the ballooning larvae that pupated produced a higher percentage of females than those of non-ballooning larvae (GLM: LR = 124.6, $P < 0.0001$ for *B. fusca*; LR = 17.2, $P < 0.0001$ for *S. calamistis*; LR = 16.1, $P < 0.0001$ for *C. partellus* and LR = 25.4, $P < 0.0001$ for FAW) (Figure 7.2).

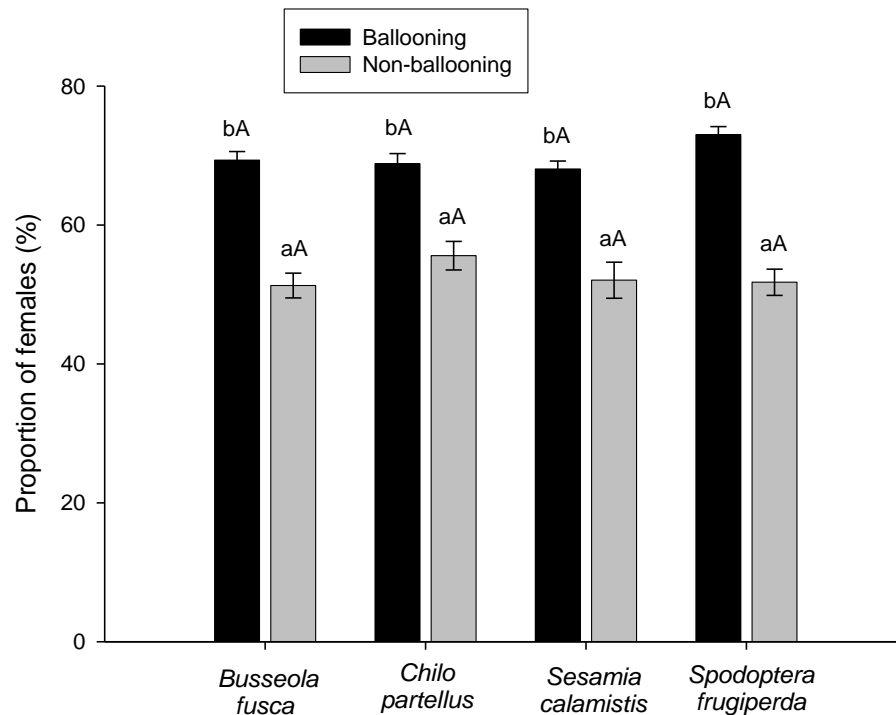


Figure 7. 2: Percentage of female (mean \pm SE) from the ballooning and the non-ballooning larvae activities of each stemborer species and FAW. Non-significant differences between ballooning vs non-ballooning are shown by identical letters in lower case and between

species in upper case, determined using pairwise of lsmeans function with adjust = Tukey, following generalized linear model (GLM) with simple binomial procedure.

However, there were no significant differences in percentage of females between insect species among ballooning (GLM: LR = 0.43, $P = 0.93$) and non-ballooning (GLM: LR = 0.10, $P = 0.99$) larvae (Figure 7.2).

7.3.3 Fall armyworm and maize stemborer species larval dispersal between maize plants

FAW had a significantly higher infestation incidence and number of larvae per plant than the stemborers (Table 7.2). In contrast to FAW, infestation incidence and number of larvae of the three stemborers was significantly higher at 40 cm than 80 cm long (Table 7.2).

Table 7. 2: Percentage of infested maize plants (mean \pm SE) and number of larvae per plant (mean \pm SE) according to the species and distance from the initial point of infestation.

Percentages of infested plants				
Species	40cm	80cm	LR	P
<i>B. fusca</i>	52 \pm 4.89 ^{aA}	30 \pm 3.83 ^{aB}	7.78	0.03
<i>S. calamistis</i>	52 \pm 4.89 ^{aA}	28 \pm 2.00 ^{aB}	4.1	0.04
<i>C. partellus</i>	58 \pm 2.00 ^{aA}	38 \pm 1.74 ^{aB}	3.26	0.04
<i>S. frugiperda</i>	100 \pm 0.00 ^{bA}	96 \pm 2.44 ^{aA}	0.24	0.62
LR	42.89	130.57		
P	< 0.0001	< 0.0001		
Number of larvae per plant				
Species	40cm	80cm	LR	P
<i>B. fusca</i>	1.00 \pm 0.22 ^{aA}	0.38 \pm 0.09 ^{aB}	8.02	0.004
<i>S. calamistis</i>	0.92 \pm 0.20 ^{aA}	0.38 \pm 0.09 ^{aB}	6.48	0.01
<i>C. partellus</i>	0.96 \pm 0.16 ^{aA}	0.50 \pm 0.10 ^{aB}	5.09	0.02
<i>S. frugiperda</i>	2.92 \pm 0.26 ^{bA}	2.36 \pm 0.14 ^{bA}	2.00	0.15
LR	18.20	78.98		
P	0.0003	< 0.0001		

LR = Likelihood Ratio; Non-significant differences are shown by identical letters within columns in lower case and between rows in upper case, determined using pairwise of lsmeans function with adjust = Tukey, following generalized linear model (GLM) with Poisson for percentage of infested plants and negative binomial procedure for number of larvae per plant.

The rating scale as indicators of the damages inflicted to plants by larvae, significantly decreased with the distance from the initial point of infestation for the stemborers but not for the FAW (GLM: LR = 4.75, $P = 0.02$ for *B. fusca*; LR = 4.16, $P = 0.03$ for *S. calamistis*; LR = 6.32, $P = 0.01$ for *C. partellus* and LR = 1.87, $P = 0.17$ for FAW). Damage inflicted was significantly lower for the stemborers compared to those of FAW (GLM: LR = 11.78, $P < 0.0001$ at 40 cm; LR = 258.48, $P < 0.0001$ at 80 cm) (Figure 7.3).

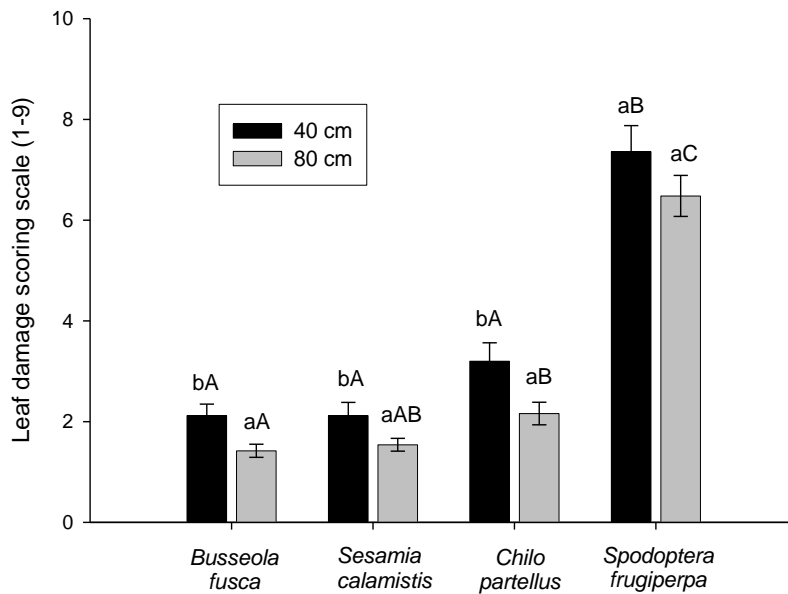


Figure 7. 3: Maize plant's damage scoring scale of each stemborer species and FAW. Non-significant differences are shown by identical letters between distance of 40 cm and 80 cm in lower case and between species in upper case, determined using pairwise of lsmeans function with adjust = Tukey, following generalized linear model (GLM) with the negative binomial procedure.

7.4 Discussion

The results obtained in this study showed that neonate larvae for each lepidopteran pest species studied ballooned away from the host on which they initially hatched. However, this

is the first time it has been systematically assessed for *S. calamistis* and FAW and its impacts on the biology of the insect documented. Ballooning activity has been recorded for several lepidopteran families including Cossidae, Geometridae, Lymantriidae, Noctuidae, Psychidae, and Pyralidae (Zalucki *et al.*, 2002).

Insect dispersal commonly leads to the selection of newly expanding leaves by neonates for their first meal (e.g., Joseph & Kelsey, 1994). However, the capacity of ballooning among neonate larvae mainly depends on the insect species (Zalucki *et al.*, 2002). It has been demonstrated that the percentage of first instar population that disperses by ballooning off the initial oviposition sites is usually variable ranging from 1% in *Ostrinia nubilalis* (Razze & Mason, 2012), 15–26% in *Lymantria dispar* (Diss *et al.*, 1996) to 93% in the common armyworm, *Mythimna convecta* (McDonald, 1991).

Ballooning activities of 4% and 55% have already been reported for *B. fusca* and *C. partellus*, respectively (Berger, 1989); in the present study it was 30% for both species, not so different from the mean values found in the previous studies. In the present study, FAW neonate larvae exhibited greater ballooning activity than the stemborer species. Several studies have suggested that in the cases where larval dispersal is limited, female generally selects for high-quality hosts (Bellota *et al.*, 2017; Gripenberg *et al.*, 2010). Thus, the higher dispersal abilities of the FAW larvae indicates that the pest is less involved in high-quality hosts assessment for oviposition and are also more polyphagous than any of the stemborer species explaining the extensive host range of the species that exceeds 350 plant species (Montezano *et al.*, 2018; Casmuz *et al.*, 2010). With such wide host range, dispersal of numerous larvae away from oviposition hosts is likely most adaptive for fall armyworm and it would further reduce intra-specific competition and mostly cannibalism (Andow *et al.*,

2015; Chapman *et al.*, 1999a). Neonate lepidopteran larvae disperse away from their natal plants for diverse reasons such as to avoid predation, competition and to find suitable hosts (Zalucki *et al.*, 2002). In gregarious species the risk of reduced larval fitness as a result of a rapid decline in host quality due to larval overcrowding. Thus, population density and host quality may mediate larval dispersal from natal hosts (Bernal *et al.*, 2015; Zalucki *et al.*, 2002; Berger, 1992).

For all species studied, the proportion of females that reached the pupal stage from the ballooning larvae was higher than that of non-ballooning larvae. Rhainds *et al.* (2002) reported that the larvae of *Metisa plana* Walker (Lepidoptera: Psychidae), a pest of the oil palm *Elaeis guineensis* Jacquin (Palmae), the sex ratio of ballooned larvae that attained the pupal stage was consistently female biased and exceeded 80.8%. It is possible that female-biased sex ratio might be important to ensure the perennity of the species (Rhainds *et al.*, 1998; Lagoy & Barrows, 1989). Sex-specific dispersal behaviour by male and female larvae have been reported in other lepidopteran species such as Lymantriidae (Harrison, 1994) and Psychidae (Rhainds *et al.*, 1998).

Moreover, for all species under study, ballooning larvae had a higher survival than the non-ballooning larvae in contrast to the lower survival rate of ballooning larvae that has already been reported for other species (Razze & Mason, 2012; Zalucki *et al.*, 2002). However, in the field, larval survival can be affected by abiotic factors such as ambient temperature, dislodgment by rainfall (Zalucki *et al.*, 2002), drowning and biotic factors such as unsuitability of the host plant (Razze & Mason, 2012; Zalucki *et al.*, 2002). Pannuti *et al.* (2015) reported similar higher survival rates of ballooning larvae than non-ballooning

larvae; while Reavey & Lawton (1991) have emphasized the contribution of larval behaviour to its fitness.

In addition to ballooning, neonates larvae may move between plants by lowering themselves on silk threads (Torres-vila *et al.*, 1997), whereas older larvae may migrate by crawling from one plant to another. This could have led to a decline in stemborers and fall armyworm densities on the oviposition plant and an increase in density on adjacent plants. This pattern is similar to that reported for the European corn-borer, *Ostrinia nubilalis* (Ross & Ostlie, 1990) and has already been reported for African stemborers (Ndjomatchoua *et al.*, 2016; Calatayud *et al.*, 2014a; Van den Berg *et al.*, 1991). In this study, both incidence and density of stemborer infestation decreased significantly with distance from the point of infestation for all stemborers, while those of FAW were not affected by the distance. In addition, as a pure leaf feeder, FAW larvae produced a higher leaf damage and dispersal potential than stemborers. The devastating character of FAW in maize fields has been reported in several studies (e.g. Fotso Kuate *et al.*, 2019; Sisay *et al.*, 2019; Goergen *et al.*, 2016).

In conclusion, FAW neonate larvae had greater potential of ballooning off from the initial oviposition sites than the stemborer larvae irrespective of species involved. A greater number of female larvae dispersed as compared to the males while their survival rate was higher than that of non-ballooning larvae. In addition, FAW larvae had higher potential to spread between plants than the maize African stem borers. These findings might help in the development of sampling plans for pests since larval dispersal directly influence the accuracy of scouting methods (Ross & Ostlie, 1990), larval survival information increase precision of economic thresholds (Ross & Ostlie, 1990) and information on larval dispersal is also critical to design effective pest management strategies (Malaquias *et al.*, 2017).

CHAPTER EIGHT

DENSITY-DEPENDENT AND DURATION OF RESOURCE UTILIZATION IN INTERACTION BETWEEN FALL ARMYWORM, *SPODOPTERA FRUGIPERDA* (LEPIDOPTERA: NOCTUIDAE) AND LEPIDOPTERAN MAIZE STEMBORERS

Abstract

In insect communities, the outcome of intra- and inter-specific competitions for food utilisation depends primarily on density and duration. Experimental manipulations of larval densities and durations of common diet feeding of the fall armyworm (FAW), *Spodoptera frugiperda*, and the lepidopteran African stemborers, *Busseola fusca*, *Sesamia calamistis* and *Chilo partellus*, were conducted to determine how the density and the duration of resource utilization affected larval survival and relative growth rates (RGR) in intra- and inter-specific interactions. Larvae at low (4 larvae), medium (8 larvae) and high (12 larvae) densities were fed on surrogate stems filled with artificial diet placed under laboratory conditions at constant temperature of 25°C for 5, 10, 15 and 20 days. Survival and relative growth rates of each species were assessed. Both intra- and interspecific competitions were observed among all the four species whereby interspecific competition was significantly stronger within the stemborers than between the FAW and the stemborers. For each species, survival and RGR were negatively related with either larval density or duration of resource utilization. The findings suggest that the spatio-temporal distribution of FAW will be driven by the distribution of the stemborer species across the agro-climatic zones.

Keywords: Fall armyworm, *Busseola fusca*, *Sesamia calamistis*, *Chilo partellus*,
Competition

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8.1 Introduction

Competitive intra- and interspecific interactions are pervasive in nature and represent a major structuring force in ecological communities (Cameron *et al.*, 2007; Gurevitch *et al.*, 1992). Resource availability varies spatially and temporally either within or across generations and leads to variation in the amounts of energy available to individuals (King & Roff, 2010). Ecological theory and empirical work indicate that competition should result in competitive exclusion when resources are limited. However, competition may be avoided via a number of mechanisms, including differential resource use, colonization-competition tradeoff across a patchy environment (Calcagno *et al.*, 2006), phenotypic plasticity (Fordyce, 2006), temporally varying condition-specific competition (Chesson, 2000), and population density (Ntiri *et al.*, 2017). Since density-dependent and temporal pattern of competitive interactions effects operate on insect community life traits, quantifying how variations in density and with time influence growth and survival rates could be used to predict changes in population growth rates that are spatially and temporally dependent.

The noctuid stemborers, *Busseola fusca* (Fuller) and *Sesamia calamistis* Hampson, and the crambid *Chilo partellus* (Swinhoe) are key pests of maize and sorghum in East and Southern Africa (Kfir *et al.*, 2002). Depending on altitude they occur as single species or mixed multi-species communities (van den Berg *et al.* 1991; Krüger *et al.*, 2008; Ong'amo *et al.* 2006).

It has been reported that *C. partellus* competitively displaced *B. fusca* in the maize fields in South Africa (Kfir, 1997; Rebe *et al.* 2004). Under laboratory conditions, Ntiri *et al.* (2016) found that *C. partellus* dominated *B. fusca* and *S. calamistis* in interspecific interactions while the interaction effect between the two noctuids was weak. Furthermore, larval density and temporal plasticity acting on larval survival and relative growth rates showed that survival and relative growth of the three species were significantly higher at low-density and the competition increased as the time of resource utilisation increased under either intraspecific or interspecific interactions (Ntiri *et al.*, 2017). In addition, larvae of the invasive fall armyworm *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera, Noctuidae) (FAW) showed frequent interactions with the stemborer community in the use of maize resources (Goergen *et al.*, 2016), which might lead to novel competitions between this new pest and stemborers.

Ntiri *et al.* (2017) elucidated the effects of larval density and duration on the intra- and interspecific resource utilization involving *B. fusca*, *S. calamistis* and *C. partellus*. In the present study FAW was included in order (i) to determine larval density effects on the intraspecific interaction within FAW and its interspecific interactions with *B. fusca*, *S. calamistis* and *C. partellus* when they utilize the same resource in a restricted space and (ii) to assess whether these interactions varied with time.

8.2 Materials and methods

8.2.1 Insects

Only second instar larvae (L2) were used in this study since younger FAW larval stages could not be reared on artificial diet (Da Silva *et al.*, 2013; Sokame *et al.*, 2020a). The stemborer and FAW larvae were provided by the Animal Rearing and Containment Unit

(ARCU) at *icipi*, Nairobi, Kenya. Larvae were reared on the artificial diet of Onyango and Ochieng'-Odero (1994) in plastic jars (16.5 × 9 cm) with about 200 ml per jar. After inoculation, the plastic jars were tightly sealed using perforated lids with galvanized mesh. They were covered with tissue paper and kept in the rearing room at 26 ± 1 °C, 60 ± 5% RH., and L12 and D12 photoperiod. Each colony was rejuvenated two times a year with larvae collected from the field. For the stemborers the diet consisted of a vitamins mix, maize leaf powder, brewer's yeast, bean powder, sucrose, ascorbic acid, sorbic acid, and methyl-paraben formaldehyde (Onyango and Ochieng'-Odero, 1994). For rearing of FAW larvae, the diet was complemented with wheat germ, milk and suprapen powder, whereas sucrose was removed. Agar was added to each type of diet to solidify the medium and hold the moisture.

8.2.2 Surrogate stems

Previous rearing studies showed that the surrogate stems yielded higher survival rates (Ntiri et al., 2016, Sokame et al., 2020a) because the maize plants deteriorated after only 5 to 7 days (Ntiri *et al.*, 2016). Therefore, surrogate stems were used. They consisted of a piece of polymerizing vinyl chloride (PVC) pipe with internal diameter 5cm and 30cm in length. Each pipe was divided into two lengthwise halves and then tight together using masking tape. One end was sealed using parafilm and then tight with masking tape. The entire surrogate stem was wrapped with aluminum foil except for the open end through which the pipe was filled with hot liquid diet. The pipe was filled up to half level, leaving about 300m³ free space. The following day when the diet had solidified, three quarters of the aluminum foil and masking tape were removed from the open to close end direction of the pipe. The

remaining was kept to later avoid the contamination of the diet from the bottom of the surrogates in contact of the holding containers.

8.2.3 Influence of the larval density of the interactions

The effect of density of larvae of *B. fusca* (Bf), *S. calamistis* (Sc), *C. partellus* (Cp) and FAW on survival and growth rates in single- or multiple-species infestations was evaluated. Thereby the surrogate stems were each infested with four (4), eight (8) or twelve (12) larvae involving either single-species or multi-species combinations (Table 9.1) giving a total of 33 treatments, which were replicated 10 times.

Table 8. 1: Single, pairings, three and four-species combinations treatments for each of the three larval infestation densities

Densities	Single-species				Multi-species							
	Bf	Sc	Cp	FAW	FAW +Bf	FAW +Sc	FAW +Cp	FAW+ Bf+Sc	FAW+ Sc+Cp	FAW+ Bf+Cp	FAW+Bf +Sc+Cp	
4 larvae	4	4	4	4	2+2	2+2	2+2	2+1+1	2+1+1	2+1+1	1+1+1+1	
8 larvae	8	8	8	8	4+4	4+4	4+4	2+3+3	2+3+3	2+3+3	2+2+2+2	
12 larvae	12	12	12	12	6+6	6+6	6+6	4+4+4	4+4+4	4+4+4	3+3+3+3	

Because of the low weight of L2, the wet mass of the group of larvae used was assessed in group before infestation (Nitri *et al.*, 2017).

After infestation, cotton wood was used to plug the open end of each surrogate stem fastened with a rubber band. The surrogate stems were placed upright in an incubator of mark of “Sanyo MIR 554, Tokyo-Japan” at 25°C, 70 ± 10% RH, and L12:D12 photoperiod. This temperature was shown to be the optimum for development of the stemborers (Khadioli *et al.*, 2014a,b). After 15 days, the experiment was terminated, and the stems were opened for assessment of the number and wet mass of live larvae of each species.

8.2.4 Influence of the duration of the interactions

This experiment was conducted to evaluate whether the duration of the interactions influences survival and growth rates of stemborer and FAW larvae in either single- or multiple-species infestations. Following the study of Sokame et al. (2020a), the single-species treatments consisted of 8 L2 was used. The multi-species treatments consisted of a) 4 L2 of each species in the following pairings: FAW+Bf, FAW+Sc, FAW+Cp; b) 3 L2 of each stemborer species and 2 L2 of FAW in the following combinations: FAW+Sc+Cp, FAW+Bf+Sc and FAW+Bf+Cp; c) 2 L2 of each species in following combinations: FAW+Sc+Bf+Cp. Each treatment was replicated ten times. The experiment was conducted in an incubator under the same conditions as for the larval density experiments. The number of larvae alive and their wet mass per surrogate stem were determined 5, 10, 15 or 20 days after the infestation.

8.2.5 Data analysis

The response variables calculated as interaction outcomes were survival and relative growth rate (RGR). The survival rate was evaluated as the number of larvae alive at the end of the experiment and expressed as percent of initial number of larvae inoculated. Survival data were analysed using a generalised linear model (GLM) with a binomial error distribution. Odds ratios (OR) with a 95% confidence interval (95% CI) were calculated for each treatment from the GLM results obtained. The RGR for each species was calculated using the following equation (Ojeda-Avila *et al.*, 2003):

$$\text{RGR} = \frac{\text{Mass per surviving larva} - \text{Initial mass per larva}}{\text{Number of days after infestation}}$$

RGR for a group of species was calculated as the sum of RGRs of each species in that community. RGRs of treatments were analyzed via analysis of variance (ANOVA). The

ANOVA was performed by constructing a general linear model with the *lm* function at 5% of level of significance. Significant differences were also separated using Tukey's multiple comparisons tests performed using the R package "lsmeans" (Lenth, 2016) with *p*-value adjustment method = false discovery rate (FDR) as addressed by Verhoeven *et al.* (2005). The RGR data were first tested for normality of their distribution using Shapiro–Wilk test and for homogeneity of variance using Bartlett test. All analyses were performed with R software version 3.5.1 (R Core Team, 2018).

8.3 Results

8.3.1 Intraspecific interaction of fall armyworm and stemborer species, *B. fusca*, *S. calamistis*, *C. partellus* and at different densities

Survival of all the four species was significantly ($P < 0.001$) higher at low density and decreased with density increasing from 4 to 12 larvae (Figure 9.1A). Between species, FAW had lower survival rate ($P = 0.001$) as compared to stemborer species, except at 4 larvae density (Figure 8.1A). Likewise, the RGR of each species was higher at low density and decreased from 4 larvae to 12 larvae densities ($P < 0.001$) and FAW exhibited a significant ($P = 0.001$) lower RGR than those of stemborer species (Figure 8.1 B).

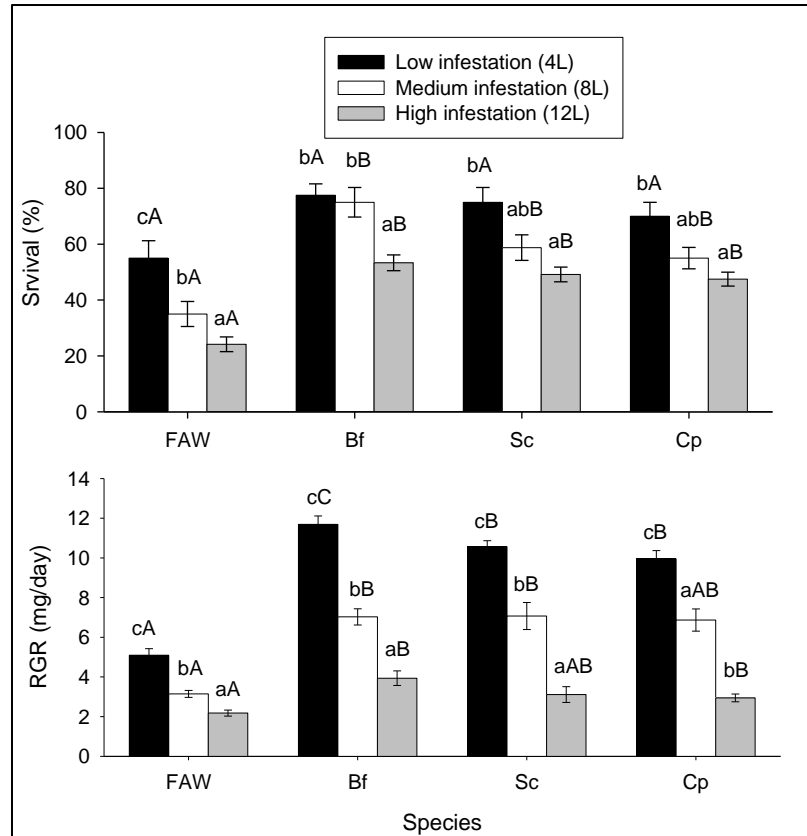


Figure 8. 1: Mean (\pm SE) survival (%) (A) and relative growth rate (RGR, mg/day) (B) of single infestations by fall armyworm (FAW), *Busseola fusca* (Bf), *Sesamia calamistis* (Sc) and *Chilo partellus* (Cp) at three different larval densities in artificial stem and growth media. Means with different letters are significantly different, determined using Tukey's multiple comparisons tests performed with lsmeans R package, following generalized linear model (GLM) with binomial error distribution for survival or the Student-Newman-Keuls following analysis of variance (ANOVA) for relative growth rate. Small letters were used to compare means between densities for each species and capital letters to compare means between species for each density.

8.3.2 Density dependent interspecific competitive interaction between fall armyworm and lepidopteran stemborers larvae

In two species combinations, FAW had higher ($P=0.001$) survival than that of *B. fusca* at all densities (Figure 8.2A) and dominated *S. calamistis* only at 8 larvae density (Figure 8.2B)

while the survival of *C. partellus* was higher ($P<0.001$) than that of FAW at all densities (Figure 9.2C). In three species combinations, *C. partellus* had higher ($P=0.001$) survival at all densities while FAW survival was only higher than that of either *B. fusca* or *S. calamistis* at low densities (Figure 8.2). However, in FAW+Sc+Bf combination, no significant ($P>0.05$) difference was revealed between survival rates of each species (Figure 8.2F). In four species combination, *C. partellus* had higher ($P=0.03$) survival than those of other species at all densities (Figure 8.2G).

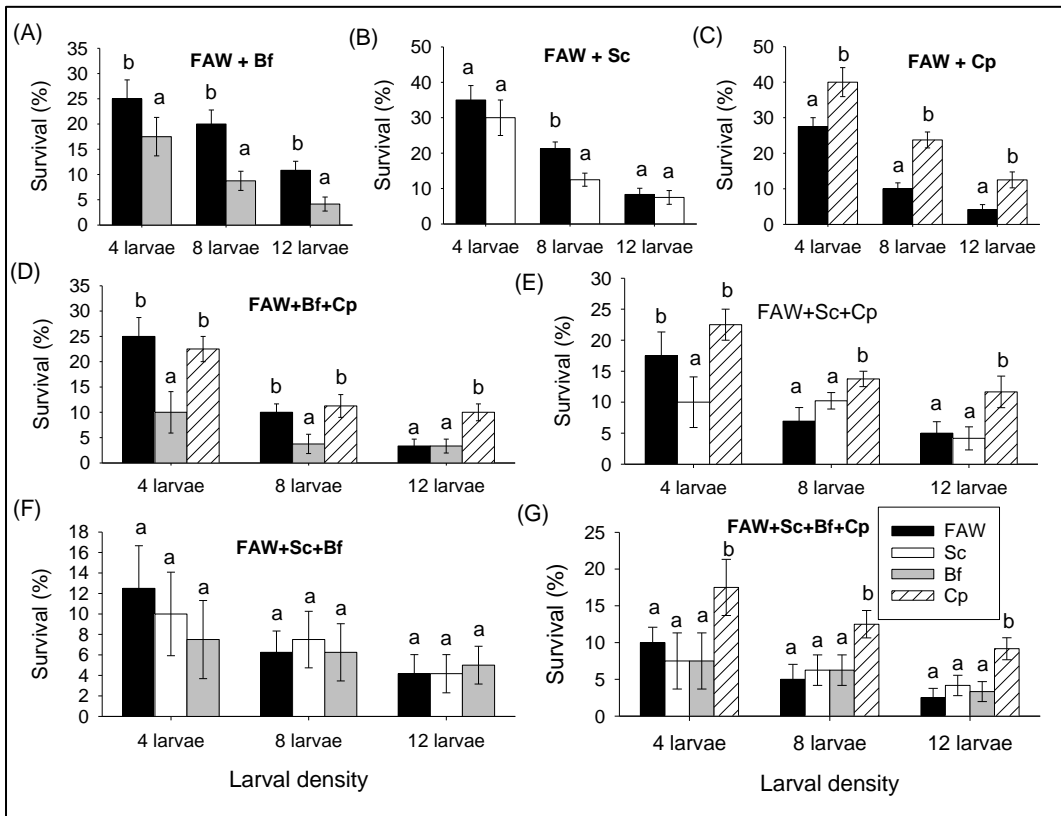


Figure 8. 2: Mean (\pm SE) of survival (%) in (A-C) two-, (D-F) three- and (G) four-species combinations of single infestations of fall armyworm (FAW), *Busseola fusca* (Bf), *Sesamia calamistis* (Sc) and *Chilo partellus* (Cp) at three different larval densities in artificial stem and growth media. Means with different letters are significantly different, determined using Tukey's multiple comparisons tests performed with lsmeans R package, following generalized linear model (GLM) with binomial error distribution.

The RGR of FAW was higher ($P < 0.001$) than that of *B. fusca* at 4 and 8 larvae densities (Figure 8.3A) and then that of *S. calamistis* at 4 larvae density (Figure 8.3B) while *C. partellus* had higher RGR ($P < 0.001$) than that of FAW at 12 larvae density (Figure 8.3C) in two species combinations. In three species combinations, the RGR of *C. partellus* was significantly higher ($P = 0.002$) at 12 larvae density in FAW+Bf+Cp (Figure 8.3D) and at all densities in FAW+Sc+Cp (Figure 8.3E) while no significant ($P > 0.05$) difference was revealed in FAW+Sc+Bf (Figure 8.3F). In four species combination, the RGR of *C. partellus* was significantly ($P < 0.001$) lower than those of other species at 4 larvae density while it was reversed at 8 and 12 larvae densities (Figure 8.3G).

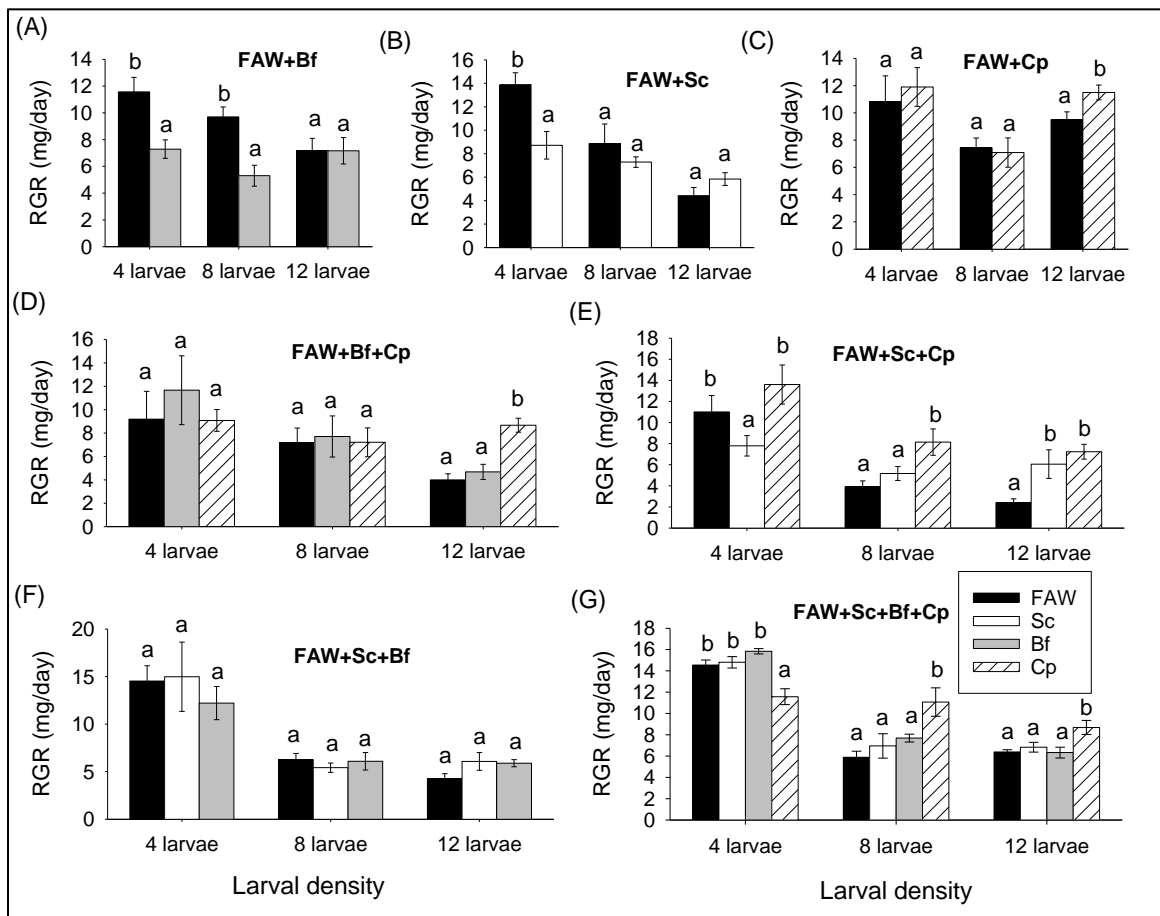


Figure 9. 3: Mean (\pm SE) of survival (%) in (A-C) two-, (D-F) three- and (G) four-species combinations of single infestations of fall armyworm (FAW), *Busseola fusca* (Bf), *Sesamia*

calamistis (Sc) and *Chilo partellus* (Cp) at three different larval densities in artificial stem and growth media. Means with different letters are significantly different, determined using the Student-Newman-Keuls following analysis of variance (ANOVA).

8.3.3 Single- and multi- species communities' interactions of fall armyworm and stemborer species, *B. fusca*, *S. calamistis*, *C. partellus* at different densities

Except FAW+Sc+Bf multi-species which had lower survival, there was no significant difference between the survival rates of FAW single species and multi-species combinations in which it was involved at all densities (Figure 8.4A). In contrast, except the comparison of Sc vs FAW+Sc and Cp vs FAW+Sc+Cp, the single species survival rates of stemborer were significantly ($P=0.03$) higher than those of their multi-species combinations with FAW (Figure 8.4A).

The RGR of FAW single species was significantly ($P<0.001$) lower than those of multi-species combinations in which it was involved at all densities, except FAW+Bf+Sc+Cp combination at 4 larvae density (Figure 8.4B). For stemborer species, at 4 larvae density, no significant ($P>0.05$) difference was revealed between the RGRs of single-species as compared to two species combinations with FAW while they were significantly ($P=0.004$) higher as compared to those of three and four multi-species combinations except Cp vs FAW+Sc+Cp (Figure 8.4B). However, at 8 larvae density, there was no difference ($P>0.05$) between RGR of stemborer single-species and those of their multi-species combinations with FAW while the 12 larvae density, the single species RGR of stemborer were significantly ($P=0.03$) higher than those of their multi-species combinations with FAW (Figure 9.4B).

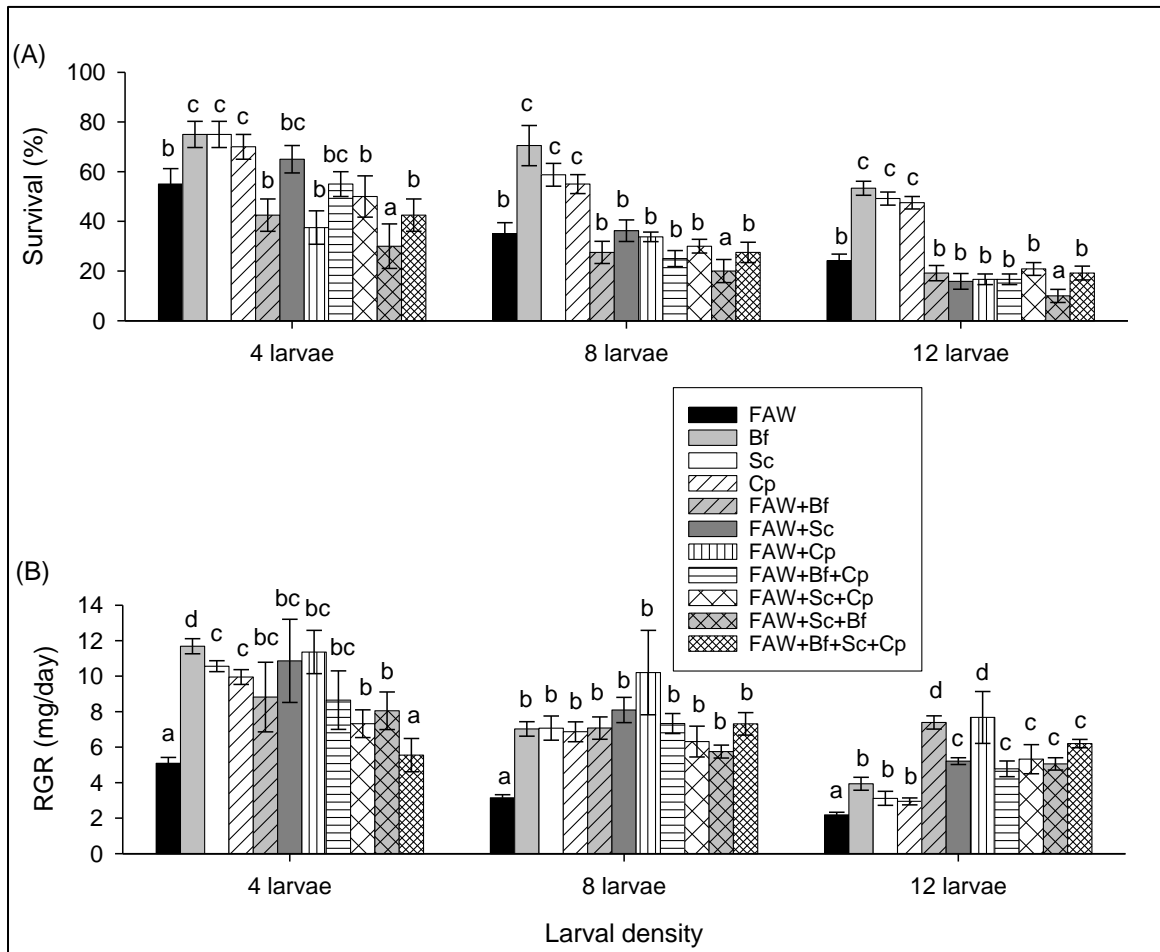


Figure 8. 4: Mean (\pm SE) survival (%) (A) and relative growth rate (RGR, mg/day) (B) of single-species infestations and combinations of fall armyworm (FAW), *Busseola fusca* (Bf), *Sesamia calamistis* (Sc) and *Chilo partellus* (Cp) at three different larval densities in artificial stem and growth media. Means with different letters are significantly different, determined using Tukey's multiple comparisons tests performed with lsmeans R package, following generalized linear model (GLM) with binomial error distribution for survival or the Student-Newman-Keuls following analysis of variance (ANOVA) for relative growth rates. Small letters were used to compare means between densities for each species and capital letters to compare means between species for each density.

8.3.4 Temporal plasticity of intraspecific interaction of fall armyworm and stemborer species, *B. fusca*, *S. calamistis*, *C. partellus* at different densities

For all the four species, survival of all the four species decreased with duration of infestation. However, this was significant ($P=0.01$) for FAW from 10 to 20 days, at 15 and 20 days for *S. calamistis* and at 15 days and 20 days for *C. partellus* and *B. fusca* respectively (Figure 8.5A). RGRs of FAW, *B. fusca* and *C. partellus* was higher significantly ($P<0.001$) at 10 and 15 days of infestation duration while it was only significant at 15 days for *S. calamistis* (Figure 8.5B).

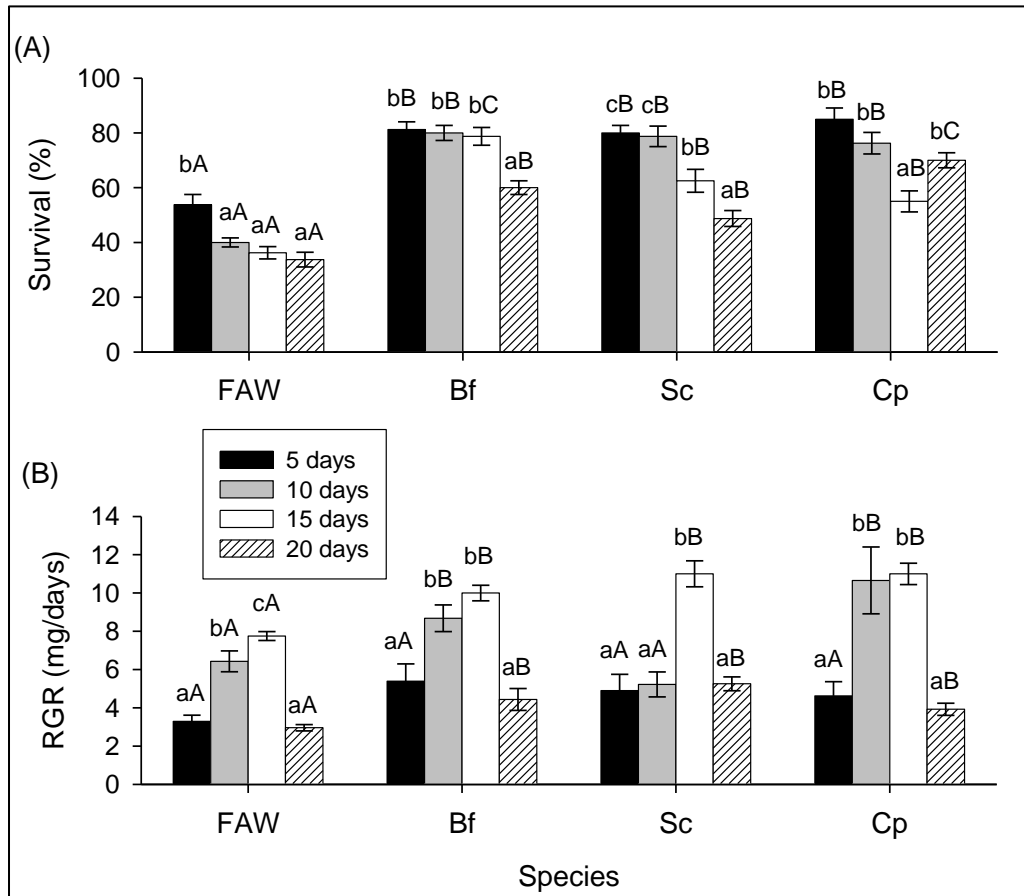


Figure 8. 5: Mean (\pm SE) survival (%) (A) and relative growth rate (RGR, mg/day) (B) of single infestations of fall armyworm (FAW), *Busseola fusca* (Bf), *Sesamia calamistis* (Sc)

and *Chilo partellus* (Cp) at various periods (days) after infestations in artificial stem and growth media. Means with different letters are significantly different, determined using Tukey's multiple comparisons tests performed with lsmeans R package, following generalized linear model (GLM) with binomial error distribution for survival or the Student-Newman-Keuls following analysis of variance (ANOVA) for relative growth rates. Small letters were used to compare means between days after infestation for each species and capital letters to compare means between species for each day after infestation.

8.3.5 Temporal plasticity of interspecific interaction between fall armyworm and stemborer species, *B. fusca*, *S. calamistis*, *C. partellus*

In pairing species combinations, FAW survived better ($P < 0.001$) than either *B. fusca* or *S. calamistis* at 15 and 20 days after infestation (Fig. 8.6A&B) while *C. partellus* had higher survival ($P < 0.001$) than that of FAW at all days after infestation (Figure 8.6C). In three species combinations, survival of *C. partellus* was significantly higher ($P < 0.001$) at all durations of infestation in multi-species that it was involved (Figure 8.6D&E) while there was no significant difference ($P > 0.05$) in FAW+Sc+Bf combination (Figure 8.6F). In four species combination, only *C. partellus* had higher ($P = 0.01$) survival at 10, 15 and 20 days after infestation (Figure 8.6G).

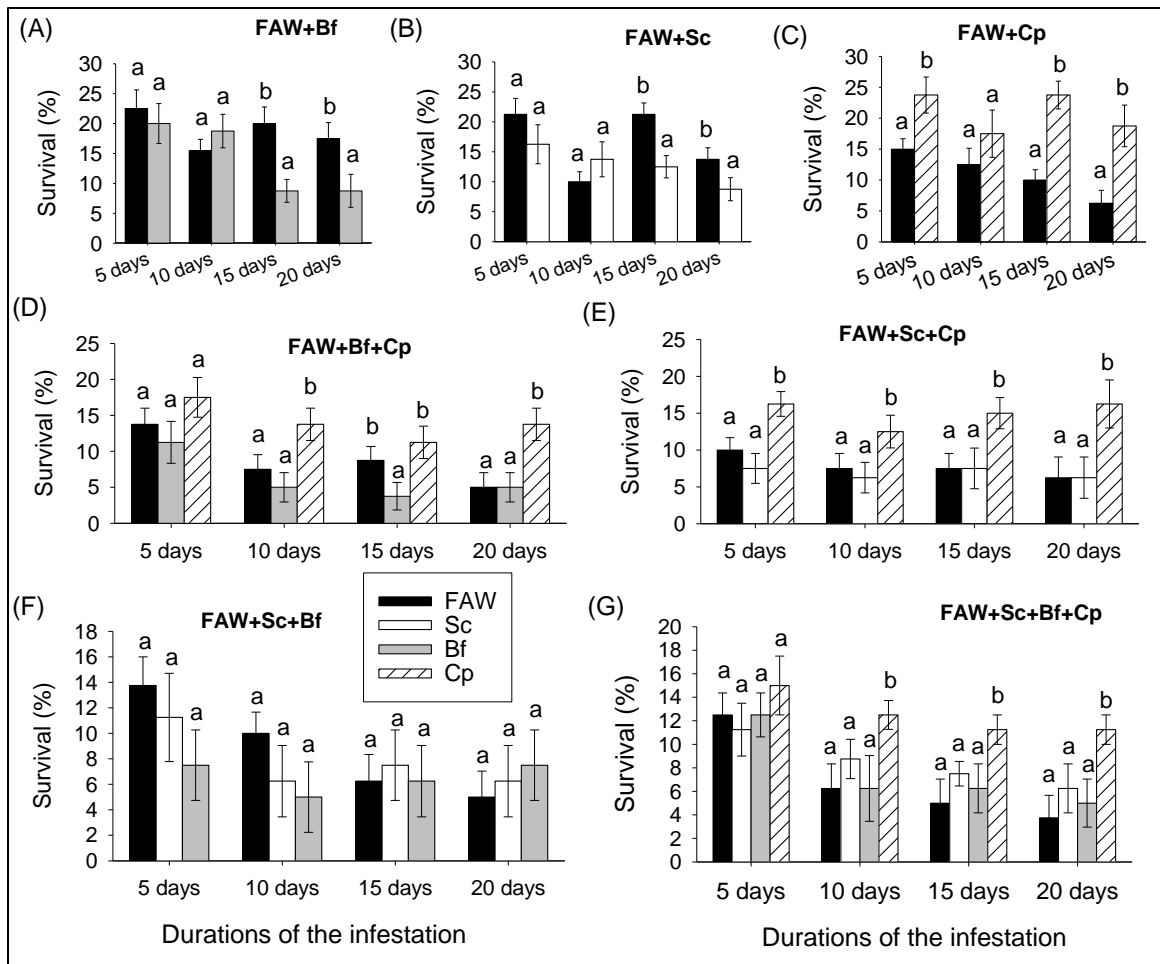


Figure 8. 6: Mean (\pm SE) of survival (%) in (A-C) two-, (D-F) three- and (G) four-species combinations of single infestations of fall armyworm (FAW), *Busseola fusca* (Bf), *Sesamia calamistis* (Sc) and *Chilo partellus* (Cp) at various periods (days) after infestations in artificial stem and growth media. Means with different letters are significantly different, determined using Tukey's multiple comparisons tests performed with lsmeans R package, following generalized linear model (GLM) with binomial error distribution

RGR of FAW was higher ($P < 0.001$) than that of *B. fusca*, at 5 days and 15 days after infestation (Figure 8.7A), and no significant difference ($P > 0.05$) was revealed in FAW+Sc combination (Figure 8.7B) while in FAW+Cp combination, RGR of FAW was higher ($P = 0.02$) at 5 days after infestation and it was reversed from 10 to 20 days after infestation (Figure 8.7C). In three species combinations, FAW and *C. partellus* at 10 days and *C.*

partellus at 20 days after infestation exhibited higher ($P<0.001$) RGRs (Figure 8.7D). Furthermore, RGRs of *S. calamistis* and *C. partellus* were significantly ($P<0.001$) higher than that of FAW at 15 and 20 days after infestation (Figure 8.7E) and it was also significant ($P<0.001$) for FAW and *B. fusca* at 10 days after infestation (Figure 8.7F). In four species combination, *C. partellus* had higher RGR ($P<0.001$) than those of other species at 15 days after infestation while it was significant ($P=0.02$) for all stemborer species at 20 days after infestation (Figure 8.7G).

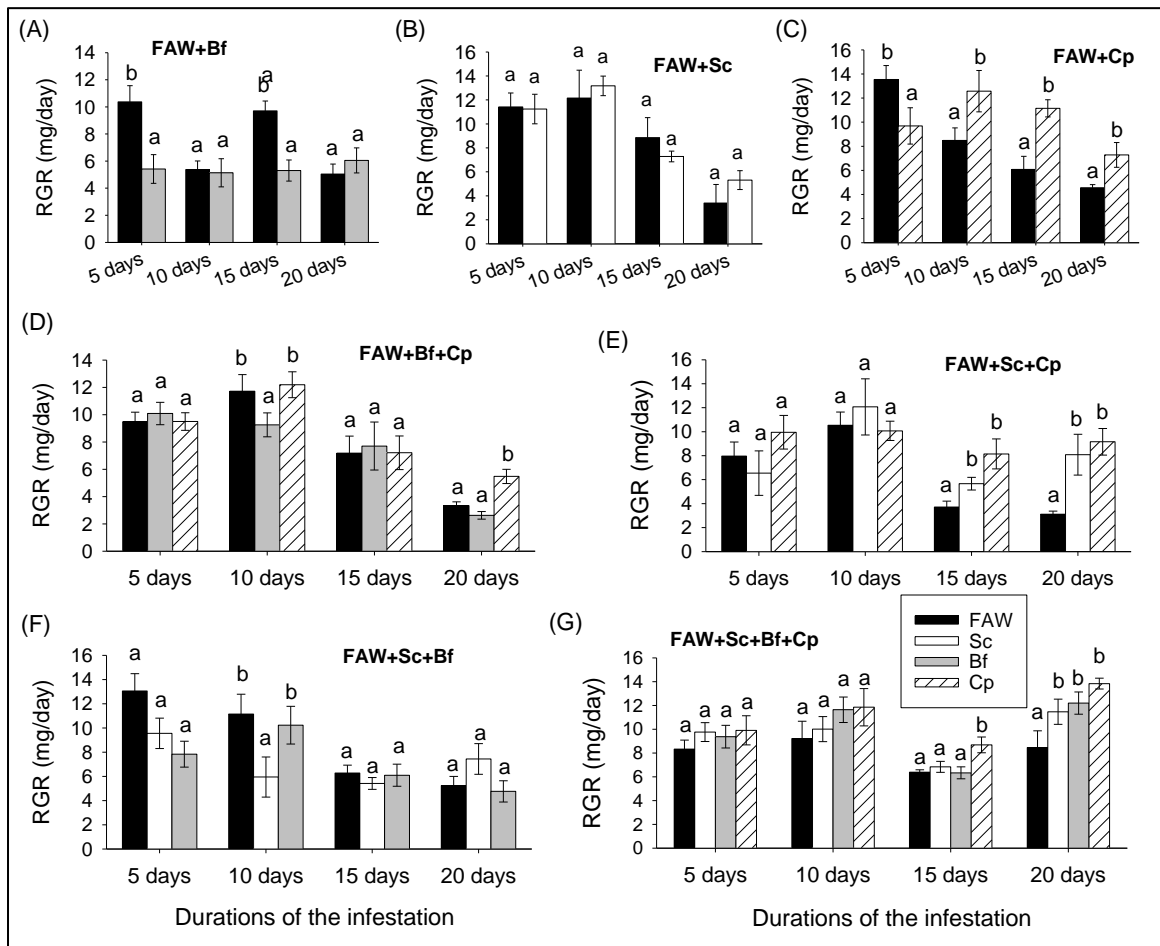


Figure 8. 7: Mean (\pm SE) of survival (%) in (A-C) two-, (D-F) three- and (G) four-species combinations of single infestations of fall armyworm (FAW), *Busseola fusca* (Bf), *Sesamia calamistis* (Sc) and *Chilo partellus* (Cp) at various periods (days) after infestations in

artificial stem and growth media. Means with different letters are significantly different, determined using the Student-Newman-Keuls following analysis of variance (ANOVA).

8.3.6 Single- and multi- species communities' interactions of fall armyworm and stemborer species, *B. fusca*, *S. calamistis*, *C. partellus* at different durations of interaction

Except FAW vs FAW+Sc+Bf comparison at all days after infestation and FAW vs FAW+Sc+Bf at 5 days after infestation and FAW vs FAW+Sc at 10 days after infestation where multi-species combinations exhibited lower survival, there was no significant ($P>0.05$) difference between FAW single species survival and those of multi-species combinations that it was involved (Figure 8.8A). In contrast, all stemborer single-species exhibited higher ($P=0.01$) survival than those of their multi-species combinations with FAW (Figure 8.8A).

Furthermore, except the comparisons of FAW vs FAW+Sc at 10 days and FAW vs FAW+Bf+Cp at 20 days after infestation, RGR of FAW single-species was significantly ($P<0.001$) lower than those of multi-species combinations in which it was implicated (Figure 8.8B). For stemborer species, when significant, RGRs of single-species communities were lower than the total RGRs of its given multispecies communities at 5 days after infestation and laterward reversed with the time to become higher ($P=0.01$) than those of their multi-species combinations with FAW from 15 days to 20 days after infestation (Figure 8.8B).

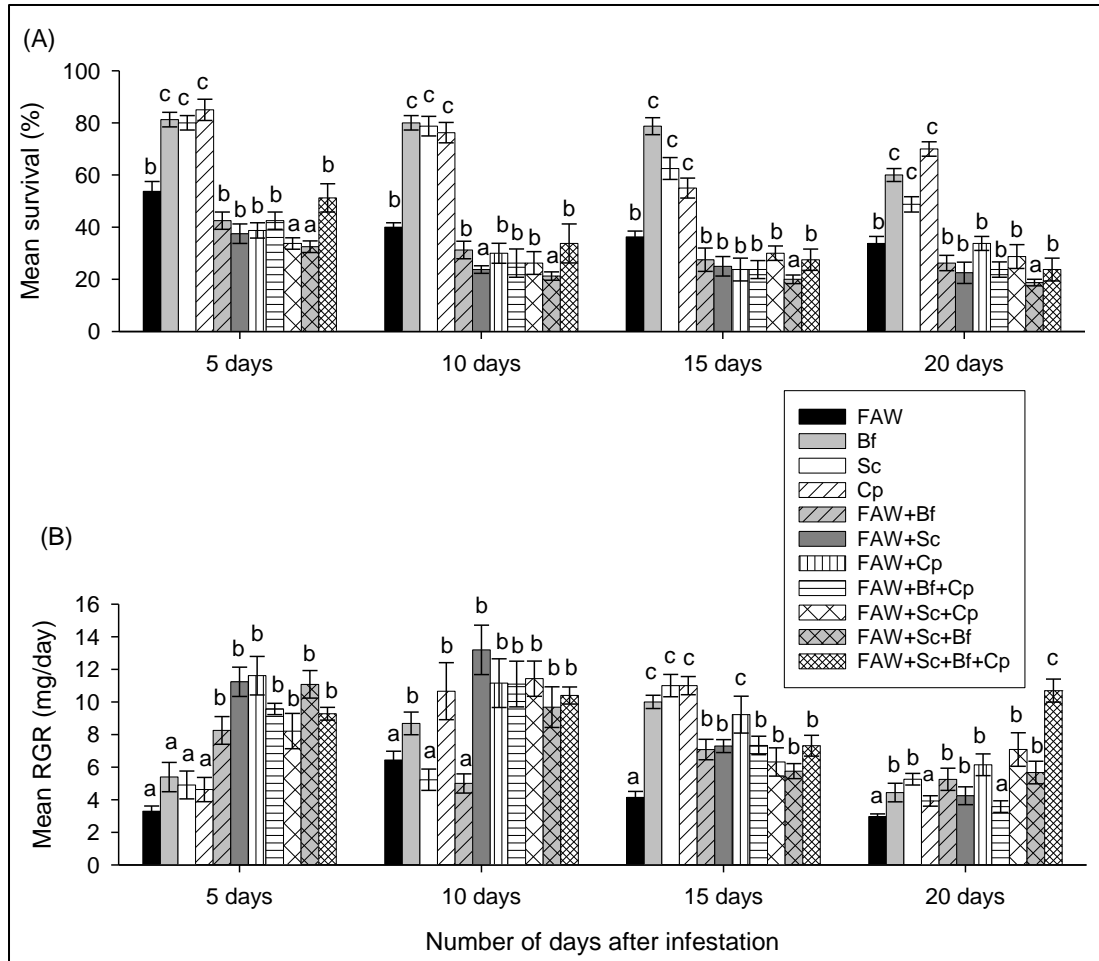


Figure 8. 8: Mean (\pm SE) survival (%) (A) and relative growth rate (RGR, mg/day) (B) of single-species infestations and combinations of fall armyworm (FAW), *Busseola fusca* (Bf), *Sesamia calamistis* (Sc) and *Chilo partellus* (Cp) at various periods (days) after infestations in artificial stem and growth media. Means with different letters are significantly different, determined using Tukey's multiple comparisons tests performed with lsmeans R package, following generalized linear model (GLM) with binomial error distribution for survival or the Student-Newman-Keuls following analysis of variance (ANOVA) for relative growth rates. Small letters were used to compare means between days after infestation for each species and capital letters to compare means between species for each day after infestation.

8.4 Discussion

The results of this study indicated that direct competition in the form of interference competition characterized also the interactions between FAW and maize stemborers community using the same resource plant though not the same niche on the plant as reported within maize stemborer communities (Ntiri *et al.*, 2017). While FAW larvae are pure foliar feeder stemborer larvae penetrate into the stem after feeding on the whorl leaves (*C. partellus*, *B. fusca*) or leaf sheath (*S. calamistis*), where after the direct competition between FAW and stemborer larvae ceases.

Under intraspecific interactions survival and RGR of both FAW and stemborers decreased with increasing larval density. Negative density-dependent intraspecific competitions is well reported for lepidopteran (Ntiri *et al.*, 2017; Gibbs *et al.*, 2004). Delong *et al.* (2014) reported an inverse relationship between population density and metabolic rate of species. The lower survival at higher larval density for FAW might be also due to the cannibalistic behaviour of this species as reported from field observations (Sarmiento *et al.*, 2002; Farias *et al.* 2001; Chapman *et al.* 2000) as well as under laboratory conditions (Bentivenha *et al.*, 2017 ; Da Silva & Parra , 2013; De Polanía *et al.*, 2009; Chapman *et al.*; 1999a; 1999b).

Under interspecific interactions, *C. partellus* yielded mostly higher survival and RGRs at all densities than the other species, while no clear trend was observed for FAW when paired with the noctuids. These results confirm the asymmetry of interspecific competition outcomes in phytophagous insects (Denno *et al.*, 1995; Kaplan & Denno, 2007). Asymmetrical competition between the noctuids and *C. partellus* has already been reported by Ntiri *et al.* (2016; 2017) and between FAW and stemborers by Sokame *et al.* (2020a).

The dominance of *C. partellus* over the noctuids at all densities indicates that it is a superior competitor in the use of resource. The reason might be that *C. partellus* has higher development rates than the noctuids (Khadioli et al., 2014 a and b). In addition, interspecific competition between species increased with increasing density indicating a density-dependent population regulation (Flockhart *et al.*, 2012; Underwood, 2010). Therefore, multiple infestations of cereal plants with low larval densities of each species at optimum conditions will very likely prolong their coexistence. Density-dependent organisms life traits are well investigated and are important factors regulating the growth of populations by affecting the phenotypic and genotypic expression of life-history traits (Agrew et al., 2002). Density affects the organisms survival, development time, their metabolic rates and the adult size (DeLong *et al.*, 2014; Muriu *et al.*, 2013;). The environment responses of species and their physiological functions have been also reported to be affected by density dependence (Tardy *et al.*, 2014).

Concerning duration of competitions, the intensity of intra- and inter-specific competition between FAW and the three stemborer species appeared to be plastic over a given time period. Although survival rates decreased with time, RGRs increased with time up till 15 days after infestation, after which it declined in either intraspecific or interspecific combinations. This temporal plasticity of competition between FAW and stemborers could be as the result of the fluctuations in food resources quality and availability as utilisation increased with time (Ayabe *et al.*, 2015;). Therefore, there was an increased need for food for development with time, as larvae moulted into next instars and developed to pupae. This has been reported within a community of lepidopteran stemborer species (Ntiri *et al.*, 2017) and in other species community (Fordyce, 2006). At early development stages, the size of

larvae enabled high survival and probably resulted in less contact between individuals. However, as body size increased with time, the increasing requirement and consumption of food increased the intensity of competition, and RGR started decreasing (Ntiri *et al.*, 2017). This could be one underlying influence of duration of interactions on competition outcomes. The trade-off between high larval RGR and low survival, that was observed as competition progressed, is a phenomenon already reported in other species (Nylin & Gotthard, 1998), and may be a response to specific nutrient needs in an increasing stressful competitive environment (Boggs, 2009). The time partitioning of the resource use appears as a key factor of the coexistence of these competing insect species.

In conclusion, this study showed that larval density and duration of interactions between larvae of FAW and stemborers, that utilise the same resource have a significant influence on the intra-and interspecific interactions outcomes within and between the species. These interaction findings are critical to predict the spatio-temporal distribution of the new invasive species depending on the distribution status of maize stemborer species across the agro-climatic zones.

CHAPTER NINE

INFLUENCE OF TEMPERATURE ON THE INTERACTION FOR RESOURCE UTILIZATION BETWEEN FALL ARMYWORM, *SPODOPTERA FRUGIPERDA* (LEPIDOPTERA: NOCTUIDAE), AND A COMMUNITY OF LEPIDOPTERAN MAIZE STEMBORERS LARVAE

Abstract

Intra- and interspecific interactions within communities of species that utilize the same resources are characterized by competition or facilitation. The noctuid stemborers, *Busseola fusca* and *Sesamia calamistis*, and the crambid stemborer, *Chilo partellus* were the most important pests of maize in sub-Saharan Africa before the recent “invasion” of fall armyworm (FAW), *Spodoptera frugiperda*, which currently seriously limits maize yields in Africa. This new pest is interacting with the stemborer community at the larval stage in the use of maize resources. From previous works on the influence of temperature on the larval intra- and interspecific resources utilization within the community of Lepidoptera stemborers involving *B. fusca*, *S. calamistis*, and *C. partellus*, there is a need to update these studies by adding the new pest, *S. frugiperda*, in order to understand the effect of temperature on the larval interactions of all these four species under the context of climate change. The influence of temperature on intra- and interspecific larval interactions was studied using artificial stems kept at different constant temperatures (15 °C, 20 °C, 25 °C, and 30 °C) in an incubator and assessing survival and relative growth rates of each species

in single and multi-species experiments. After the inclusion of FAW into the experiments, regarding relative growth rates, both intra- and interspecific competition was observed among all four species. With regard to survival rates, cannibalism can also explain the intra- and interspecific interactions observed among all four species. Interspecific competition was stronger ($P < 0.05$) between the stemborers than between the FAW and the stemborers. Similar to lepidopteran stemborers, temperature significantly ($P < 0.05$) affected both survival and relative growth rates of the FAW as well. Regardless of the temperature, *C. partellus* was superior ($P < 0.05$) in interspecific interactions shown by higher relative growth and survival rates. The results suggest that the FAW will co-exist with stemborer species along entire temperature gradient, though competition and/or cannibalism with them is weak. In addition, temperature increases caused by climate change is likely to confer an advantage to *C. partellus* over the fall armyworm and the other noctuids.

Keywords: Maize stemborers; fall armyworm; intra- and interspecific; interactions; temperature.

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9.1 Introduction

The noctuids *Busseola fusca* (Fuller) and *Sesamia calamistis* Hampson and the crambid *Chilo partellus* (Swinhoe) were considered as the economically most important pests of maize and sorghum in sub-Saharan Africa (Kipkoech *et al.*, 2006; Le Ru *et al.*, 2006a,b; De Groot, 2002; Kfir *et al.*, 2002; Overholt *et al.*, 2001; Polaszek, 1998) before the recent “invasion” of the fall armyworm (FAW), *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera, Noctuidae) in Africa, which currently limits seriously maize yields (Cock *et al.*, 2017;

Goergen *et al.*, 2016). In contrast to stemborers, the crop seems to be impacted by FAW at all growth stages from seedling to maturity (Cock *et al.*, 2017; Goergen *et al.*, 2016). FAW was first reported from the western region of Kenya in 2017 but by early 2018, it has been confirmed in more than 42 counties throughout the country (Sisay *et al.*, 2018). This new pest is showing at larval stages frequent interactions with the stemborer community in the use of maize resources (Sokame *et al.*, In press). In maize fields, second instar FAW larvae are commonly found in communities of mixed species with stemborer species in the whorl of the plants and, later on, it is frequent to find older FAW larvae in the bored holes and tunnels left by stemborers and to find a FAW larva feeding on corn with stemborer larvae (Sokame *et al.*, In press), which might lead them to compete with stemborer species, even with *S. calamistis* larvae which is known to feed in a short time on the leaf sheaths and to immediately bore into the stem (Holloway, 1998; Polaszek, 1998).

In 2016, both intra- and interspecific competition was studied at different larval stages and demonstrated for the *B. fusca*-*S. calamistis*-*C. partellus* communities with stronger interspecific competition recorded between the noctuids and the crambid than between the two noctuids (Ntiri *et al.*, 2016). It was also reported in America, interspecific competition between FAW with other species (Bentivenha *et al.*, 2017). As already stated by Ntiri *et al.* (2016), temperature is a crucial parameter among the abiotic factors driving directly the rate of growth and development, fecundity and mortality, resource utilization, and thus the interspecific interactions. Although unpredictability in rainfall is also a relevant effect of climate change which could be serious driver of caterpillar fitness, Ntiri *et al.* (2016) showed that temperature was the most significant abiotic factor influencing the composition of stemborer communities in Kenya.

From the work done by Ntiri *et al.* (2016) on the influence of temperature on the intra- and interspecific resource utilization within the community of Lepidoptera stemborers involving *B. fusca*, *S. calamistis* and *C. partellus*, there is a need to update this study by adding a new pest, *S. frugiperda*, in order to understand the effect of temperature on the interactions of all these four species under a context of climate change. In this study, competition within individuals of either FAW or of stemborers (*B. fusca*, *S. calamistis* and *C. partellus*) was considered as intraspecific while competition between FAW and a given stemborer larvae was considered as interspecific. The objectives of this study were to investigate the kind of intraspecific and interspecific interactions within and between FAW and stemborers and to evaluate the effect of temperature on these interactions under laboratory conditions.

9.2 Materials and Methods

9.2.1 Plants and Insects

Maize plants of hybrid H513 (Simlaw, Kenya Seed Company, Nairobi, Kenya) were grown in plastic pots of 12 cm in height and 13 cm in diameter in a greenhouse at the Duduville campus of International Centre of Insect Physiology and Ecology (*icipe*), Nairobi, Kenya. Mean temperatures were 29/17 °C (day/night) with an L12:D12 photoperiod. Plants used in experiments were between 4 to 5 weeks old (about 60– 75 cm of size), the earliest stage found to be infested in the field.

Larvae of stemborers (*B. fusca*: Bf, *C. partellus*: Cp, *S. calamistis*: Sc) and FAW were supplied by the Animal Rearing and Containment Unit (ARCU) at *icipe*. These colonies were rejuvenated twice a year with field-collected larvae. Larvae were reared in plastic jars of 16.5 cm length and 9 cm in diameter, filled with 200 mL of the artificial diet described by Onyango and Ochieng'-Odero (1994). For FAW larvae, the diet was modified by adding

wheat germ, milk powder, and Suprapen powder and removal of sucrose (Prasanna *et al.*, 2018). To prevent escape of larvae, the jars were tightly closed with tissue paper and perforated lids with a galvanized mesh. The jars were kept in a holding room at 26 ± 1 °C and RH of $62 \pm 5\%$.

9.2.2 Surrogate stems

Since larvae-infested maize plants kept in the incubator deteriorate after 5–7 days, the method developed by Ntiri *et al.* (2016) using surrogate stems filled with artificial diet was used in this experiment. They consisted of a piece of polymerizing vinyl chloride (PVC) pipe of 30 cm in length and 5 cm in diameter. This was cut into equal halves to allow opening of the stem for recovery of the larvae. The two halves were held together with masking tape and one end was covered with parafilm tightened with masking tape. The pipes were then half filled with artificial diet, leaving about 300 cm³ free space. Once the diet became solid in the pipe after 24 h, the masking tape and the aluminum foil covering the pipe were removed from the top to bottom up to three quarters length of the pipe.

9.2.3 Preliminary experiments: Effects of diets, instars, and rearing substrates on survival and relative growth rates of FAW larvae

The purpose was first to test if the stemborer diet of Onyango & Ochieng'-Odero (1994) was also suitable for FAW larvae. Since FAW larvae were shown to be reared on artificial diet from second instar (Da Silva & Parra, 2013), the first instar was reared on maize leaves whereas second and third instar larvae were reared on the stemborer diet (see results). Similar to the protocol of Ntiri *et al.* (2016), ten pipes were prepared with either stemborer or FAW rearing diets and each pipe was infested with either 8 s or third instar FAW larvae

using a small camel hair brush and kept in the holding room at 26 ± 1 °C and RH of $62 \pm 5\%$. After 15 days, the survival and relative growth rates of all larvae were evaluated. Since the fall armyworm is a leaf feeder and not a stemborer, the use of surrogate stems of Ntiri *et al.* (2016) might not be suitable. Thus, three different substrates consisting of entire maize plants, surrogate stems of Ntiri *et al.* (2016) (Plate 9.1A) and glass Petri dishes filled with artificial diet (Plate 9.1B) were tested under greenhouse conditions.





Plate 9. 1: Three different substrates consisting of entire maize plants, surrogate stems as described by Ntiri *et al.* (2016) (A) and glass Petri dishes (B) tested for the survival / growth performance suitability of the fall armyworm larvae under greenhouse conditions.

The surrogate stems and Petri dishes partially filled with the artificial diet of Onyango and Ochieng'-Odero (1994) (a diet found to be suitable also to fall armyworm larvae [see results]) as well as entire maize plants were each infested with 8 s instar FAW larvae. The plants were individually enclosed in a net (90 cm in height \times 33 cm in diameter) equipped with one-way drawstrings mesh cloth bag to restrict the larvae to the plant. The free end of the surrogate stem was plugged with cotton wool after infestation. The stems were placed upright in jar (8 cm in height \times 5 cm in diameter) per replicate. Each treatment was replicated ten times (ten maize plants, ten Petri dishes and ten surrogates). Temperature was recorded with a HOBO Temp/RH data logger (Onset, MA, USA). After 15 days, maize plants were dissected, surrogate stems and glass Petri dishes opened to record the number and the fresh mass of surviving larvae.

9.2.4 Influence of different constant temperatures on intra- and interspecific interactions within the FAW and a community of maize stemborer species

This experiment involved single species infestation of either fall armyworm or stemborers larvae and multi-species infestation of FAW and a community of stemborer larvae. They were conducted with surrogate stems of Ntiri *et al.* (2016), which were found to be suitable also to FAW larvae (see results). For all species, since second instar FAW larvae are commonly found in communities of mixed species with stemborer species in the whorl of the plants in the fields (Sokame *et al.*, In press) and that neonates of FAW are not able to feed on artificial diet (Da Silva & Parra, 2013), only second instar larvae were used for all the following infestations on artificial diet (Ntiri *et al.*, 2016). Following the protocol of Ntiri *et al.* (2016), the single-species infestation treatment consisted of 8 larvae. The multi-species infestation involved in four larvae per stem of each species for the FAW+Bf, FAW+Sc, FAW+Cp pairings, three larvae of each stemborers species and two larvae of FAW for the FAW+Sc+Cp, FAW+Bf+Sc, and FAW+Bf+Cp, and two larvae of each species for the FAW+Sc+Bf+Cp.

The surrogate stems, after infestation were plugged with cotton wool and placed in the jars in an upright position. The experiment was carried out in incubators (Sanyo MIR 554, Tokyo, Japan) (Plate 9.2) at four constant temperatures of 15, 20, 25, and 30 °C, with relative humidity $70 \pm 10\%$ and a photoperiod of L12:D12. Each treatment was replicated ten times. After 15 days, surrogate stems were opened to record the number and the mass of surviving larvae of each species.



Plate 9. 2: Position of surrogate stems in the jars kept in an incubator (Sanyo MIR 554, Japan)

9.2.5 Data Analysis

The outcomes of competition were evaluated through survival rates (proportion of the number of larvae alive after 15 days) and relative growth rates (RGR) as the response variables. The relative growth rate for each species was calculated using the equation of Ojeda-Avila *et al.* (2003):

$$\text{RGR} = \frac{\text{Mass per surviving larva} - \text{Initial mass per larva}}{\text{Number of days}}$$

For the species communities, RGR was performed as the mean of the RGR of all species in that community. For each treatment, survival rates were analyzed using the generalized linear models (GLM) with binomial error distribution. Significant differences were separated using Tukey's multiple comparisons tests performed using the R package

“lsmeans” (Lenth, 2016). For the comparison performed between treatments from the GLM results, Odd Ratio with a 95% confidence level interval (O.R. [95%CI]) was calculated. From each treatment, the differences between species RGR were analyzed via analysis of variance (ANOVA). The ANOVA was performed by constructing a general linear model with the *lm* function at 5% of level of significance. Significant differences were also separated using Tukey’s multiple comparisons tests performed using the R package “lsmeans” (Lenth, 2016) with *p*-value adjustment method = False Discovery Rate (FDR) as addressed by Verhoeven *et al.*, (2005). The RGR data were first tested for normality of their distribution using Shapiro–Wilk test and for homogeneity of variance using Bartlett test. All analyses were performed with R software version 3.5.1 (R Core Team, 2018).

9.3 Results

9.3.1 Effects of diets larval instars, and rearing substrates on survival and RGR of FAW larvae

The types of diet tested had no significant effect on survival rates (O.R. = 1.05 (0.85-2.02), $P = 0.86$) and RGRs ($F = 0.68$, $P = 0.41$) (Figure 9.1 A1 & B1), and similarly no differences in survival were found between the second and the third instars larvae (Survival : O.R. = 1.03 (0.95-1.92), $P = 0.87$; RGRs $F = 0.62$, $P = 0.43$) (Figure 9.1 A2 & B2).

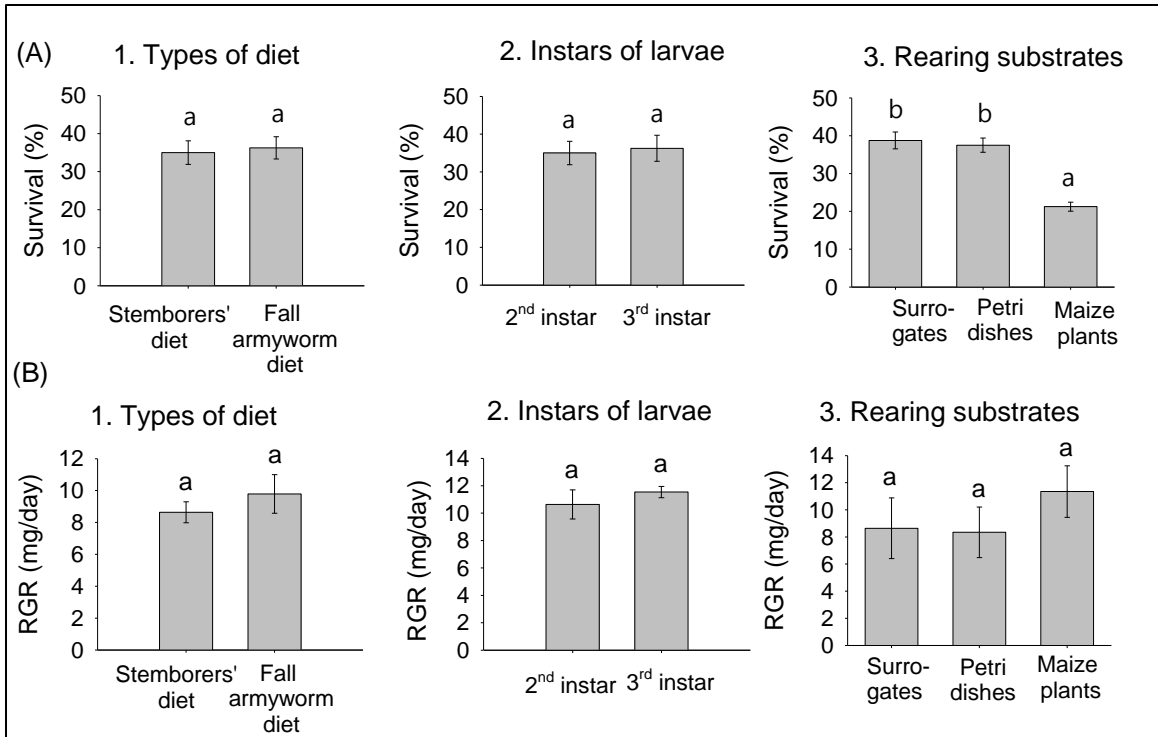


Figure 9. 1: Effects of diet type, larval stage and rearing substrates on the survival (A) and relative growth rate (B) of fall armyworm larvae. Means (\pm SE) with different letters are significantly different, determined using linear model (GLM) with binomial error distribution. Significant differences were separated using Tukey's multiple comparisons tests performed with lsmeans R package, following generalized linear model (GLM) with binomial errors distribution

While there were no statistical differences between rearing substrates for RGRs ($F = 2.56$, $P = 0.12$), higher larval survival rates were obtained for surrogate stems and Petri dishes than on maize plants (O.R. = 0.66 (0.47-0.93), $P = 0.01$) (Figure 9.1 A3 & B3). Therefore, stemborers' diet and surrogate stems were used for the subsequent experiments that determined the intra- and interspecific interactions using second instar FAW larvae.

9.3.2 The effect of temperature on survival and relative growth rates (RGR) of FAW and stemborer larvae

There was no statistical difference in survival rates of FAW larvae ($P = 0.29$) between temperatures but there was higher survival of *B. fusca* and *S. calamistis* at both 20 and 25°C compared to 15 and 30°C, while *C. partellus* had higher survival rate at both 15 and 20°C than 25 and 30°C (Figure 9.2A, ($P < 0.05$)). Between species at each temperature, the survival rate of FAW larvae was significantly ($P < 0.05$) lower than those of each stemborer species larvae except for *C. partellus* at 25°C (Figure 9.2A).

FAW had similar RGR at 15 and 20°C but later increased with increasing temperature (Figure 9.2B, $P < 0.001$). For each stemborer species, RGRs increased significantly ($P < 0.001$) with increasing temperature but levelled at 25 and 30°C (Figure 9.2B). There were no significant differences in RGRs between species at 15°C. When significant at other temperatures ($P < 0.001$), RGRs were lower for FAW than those of stemborers (Figure 9.2B).

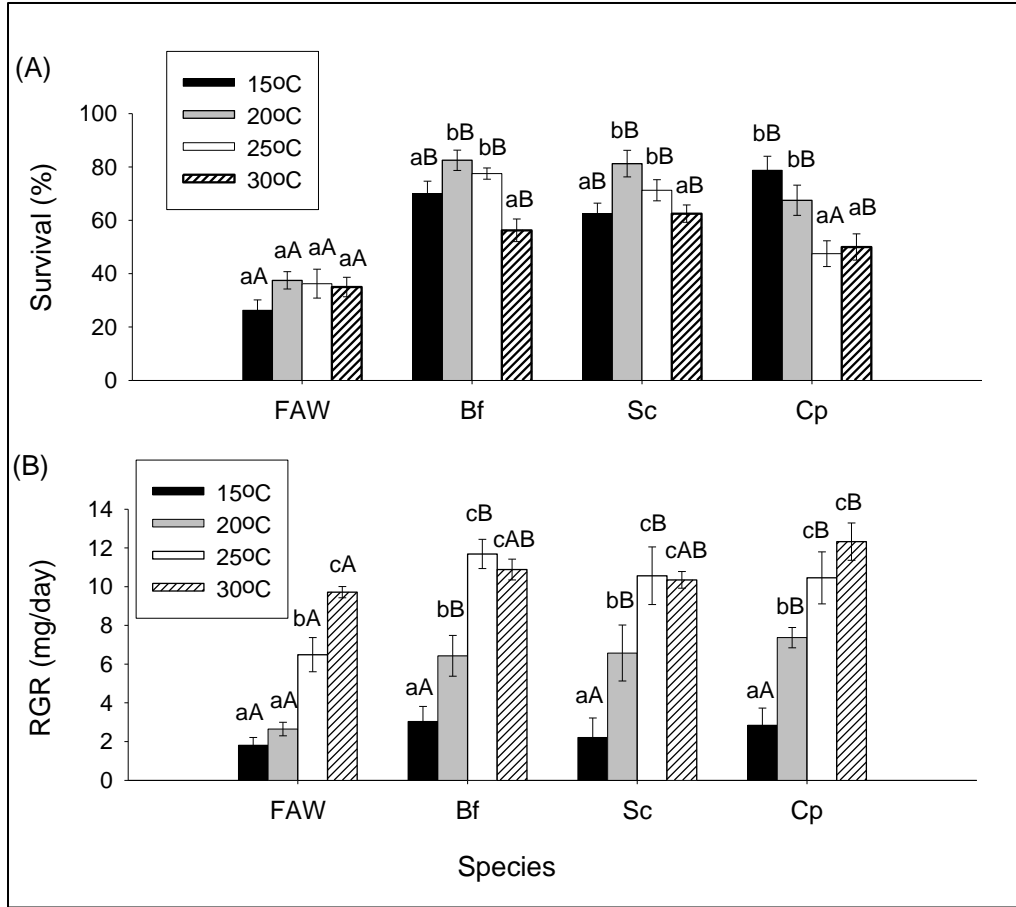


Figure 9. 2: Comparison of survival (A) and relative growth rate (B) of fall armyworm (FAW), *B. fusca* (Bf), *S. calamistis* (Sc) and *C. partellus* (Cp) in single-species combinations at various constant temperatures. Means (\pm SE) with different letters are significantly different, determined using Tukey’s multiple comparisons tests performed with lsmeans R package, following generalized linear model (GLM) with binomial error distribution for survival or the Student-Newman-Keuls following analysis of variance (ANOVA) for relative growth rates. Small letters were used to compare means between temperatures for each species and capital letters to compare means between species for each temperature.

9.3.3 Comparison of survival and RGR of FAW and lepidopteran stemborers in multi-species combinations under different constant temperatures

In pairwise combinations, larval survival of FAW was significantly ($P < 0.001$) higher than those of *B. fusca* at all temperatures (Figure 9.3A), and significantly ($P < 0.001$) higher than those of *S. calamistis* at 15°C, but decreased at 25°C (Figure 9.3B).

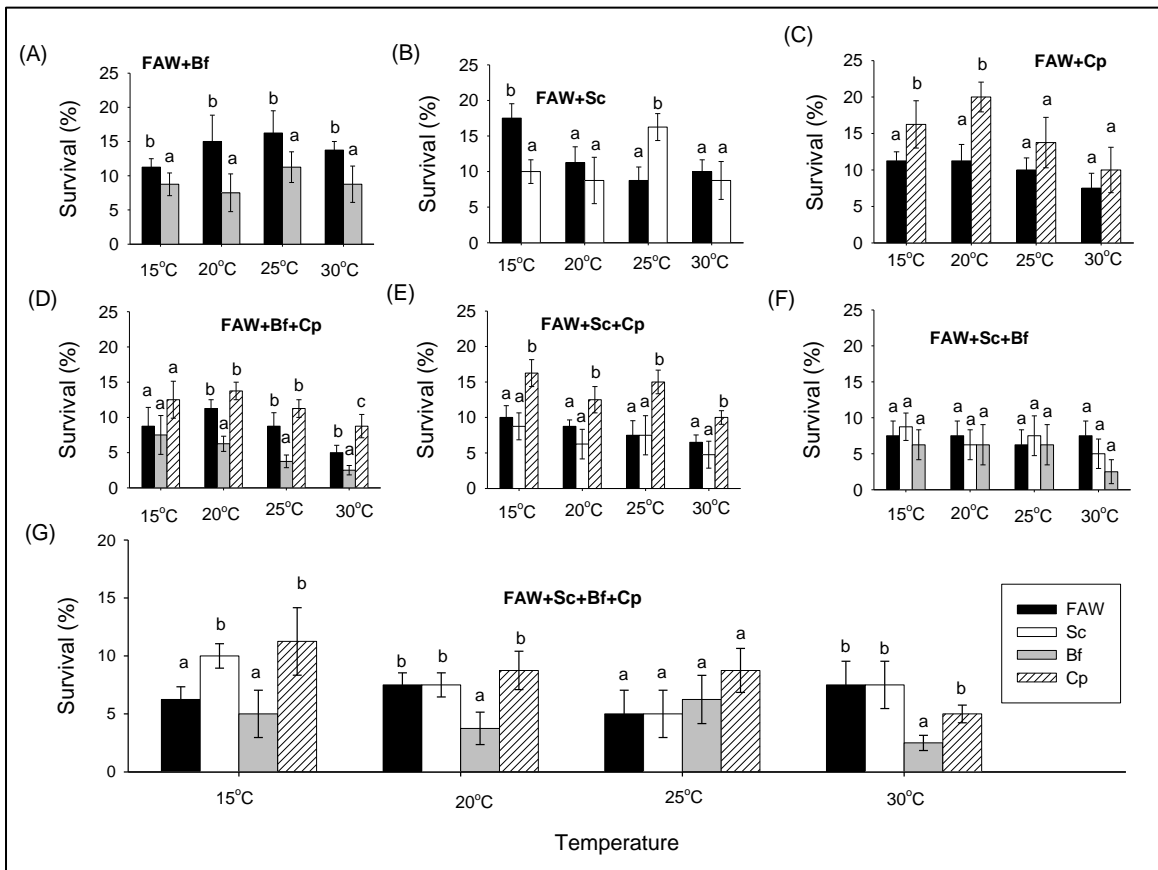


Figure 9. 3: Comparison of survival of fall armyworm (FAW), *B. fusca* (Bf), *S. calamistis* (Sc) and *C. partellus* (Cp) in multi-species combinations at different constant temperatures. Means (\pm SE) with different letters are significantly different, determined using Tukey's multiple comparisons tests performed with lsmeans R package, following generalized linear model (GLM) with simple binomial procedure.

In contrast, the survival of *C. partellus* dominated significantly ($P < 0.001$) those of FAW at 15 and 20°C (Figure 9.3C). In the three species combinations, *B. fusca* exhibited a significant ($P < 0.001$) lower survival rates in the FAW, *B. fusca* and *C. partellus* combination at 20, 25 and 30°C (Figure 9.3D) whereas *C. partellus* had the highest survival rate ($P < 0.001$) in the FAW, *S. calamistis* and *C. partellus* combination at all temperatures (Figure 9.3E). However, there was no significant difference ($P > 0.05$) between species when FAW was in combination with only noctuids (i.e. FAW + Sc + Bf) (Figure 9.3F) at all temperatures. In four species combination, *S. calamistis* and *C. partellus* had a significant higher survival rates ($P < 0.001$) than FAW and *B. fusca* at 15°C while *B. fusca* had the lowest survival rate at 20°C and 30°C (Figure 9.3G).

Except at 25°C, where *B. fusca* had the highest RGR, no significant difference ($P > 0.05$) was demonstrated in the FAW and *B. fusca* pairwise combination at all temperatures (Figure 9.4A). Likewise, no significant difference ($P > 0.05$) in RGR was found between species in the FAW and *S. calamistis* combination at all temperatures (Figure 9.4B), while in the FAW and *C. partellus* combination, FAW exhibited the highest ($P < 0.001$) RGR at 15 and 25°C (Figure 9.4C). In the FAW, *B. fusca* and *C. partellus* combination, FAW had the lowest RGR ($P = 0.001$) at 15°C but increased at 20°C together with *B. fusca* but it was similar at all other temperatures (Figure 9.4D). For the other three species combinations, there were no significant differences ($P > 0.05$) in RGRs between species at all temperatures (Figure 9.4E&F). In the combination of the four species, except at 20°C, *C. partellus* always exhibited the highest ($P = 0.001$) RGR (Figure 9.4G).

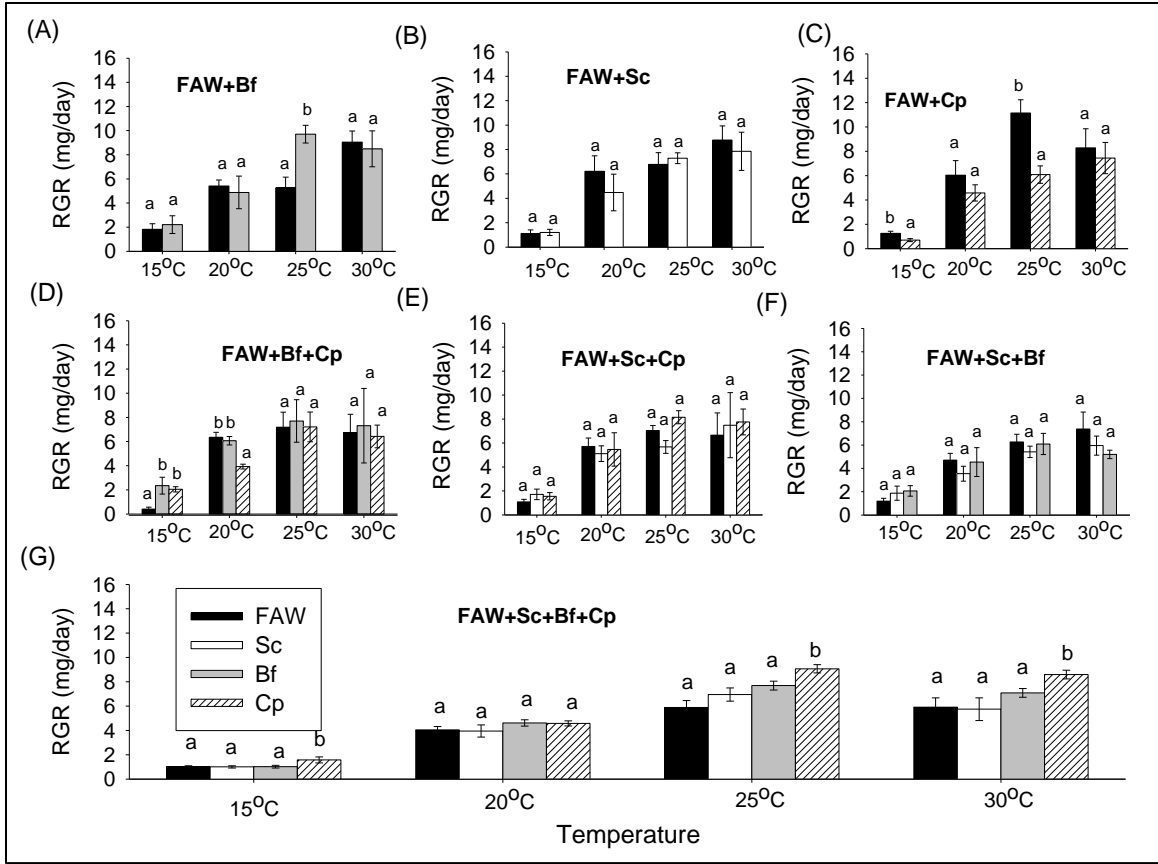


Figure 9. 4: Comparison of relative growth rates of fall armyworm (FAW), *B. fusca* (Bf), *S. calamistis* (Sc) and *C. partellus* (Cp) in multi-species combinations at various constant temperatures. Means (\pm SE) with different letters are significantly different, determined using the Student-Newman-Keuls following analysis of variance (ANOVA).

9.3.4 Comparison of survival and RGR between single and multi-species combinations of FAW and lepidopteran stemborers at different constant temperatures

When significant, survival and RGR of a single stemborer species were higher than the total survival and RGR of the corresponding multi-species communities and higher than that of FAW singly. Survival of *B. fusca*, *S. calamistis* and *C. partellus* singly tended to be higher than that of the total survival of the corresponding multi-species communities. For FAW, it

was higher ($P < 0.05$) than that of multi-species communities only at temperatures higher than 15°C (Figure 9.5A). Likewise, RGRs of single-species communities of *B. fusca*, *S. calamistis* and *C. partellus* tended to be higher than the total RGRs of the corresponding multispecies communities for all temperatures (Figure 9.5B). For FAW, it tended to be higher ($P < 0.05$) than that of multi-species communities only at 30°C, whereas it was lower at 20°C (Figure 9.5B).

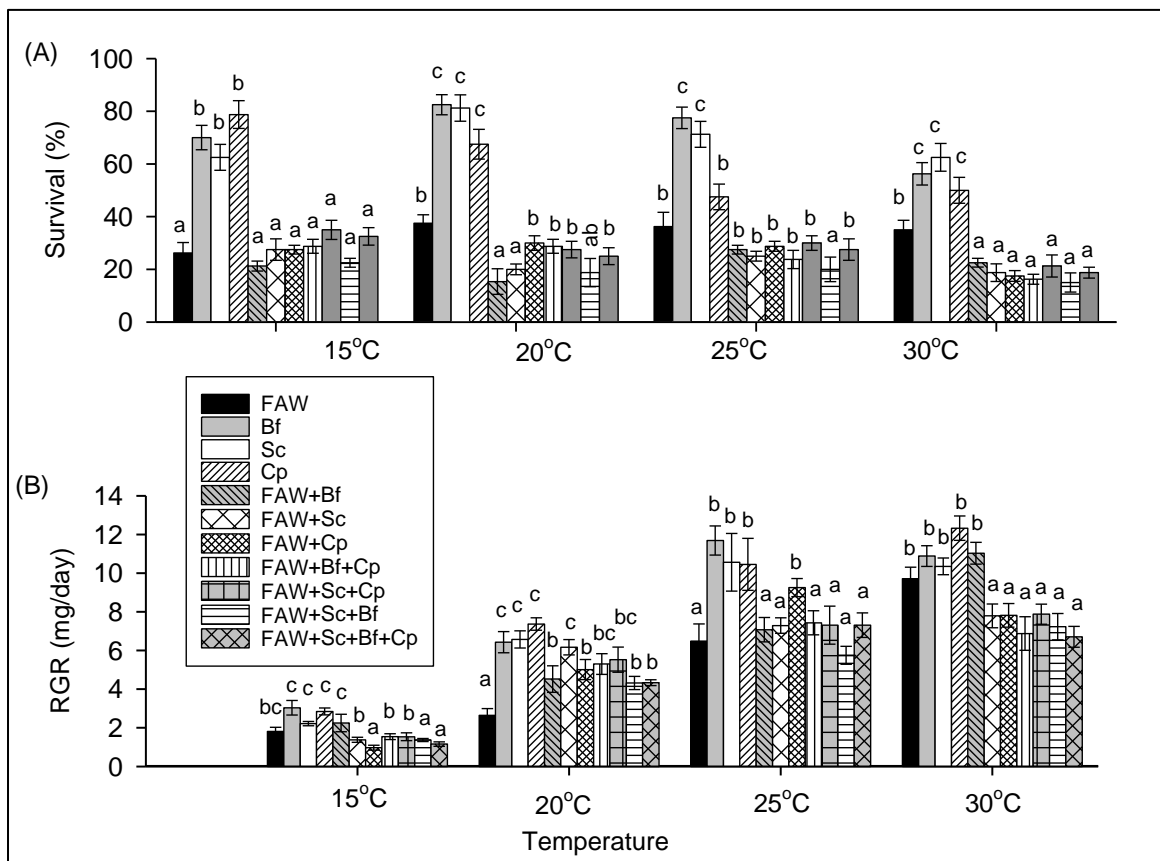


Figure 9. 5: Comparison of survival (A) and relative growth rate (B) between single-species and multi-species combinations of fall armyworm (FAW), *B. fusca* (Bf), *S. calamistis* (Sc) and *C. partellus* (Cp) under different constant temperatures. Statistical comparisons were made for single- and corresponding multi-species pairings (see Appendix 15 and 16). Means (\pm SE) were compared using Tukey’s multiple comparisons tests performed using with lsmeans R package, following generalized linear model (GLM) with simple binomial

procedure for survival or the Student-Newman-Keuls following analysis of variance (ANOVA) for relative growth rates.

9.4 Discussion

In intraspecific interactions, survival rates tended to decrease with increasing temperatures whereas the RGRs increased. This decrease of survival rates can be due to competition or rather cannibalism. In fact, FAW exhibited the lowest survival rates regardless of temperature. This might be due to the cannibalistic behavior of FAW well reported by several studies under field conditions such as, Sarmiento *et al.* (2002), Farias *et al.* (2001) and Chapman *et al.* (2000) and under laboratory conditions by De Polanía *et al.* (2009), Da Silva and Parra (2013), Chapman *et al.* (1999a,b), and Bentivenha *et al.* (2017). Cannibalism has been also reported in true stemborers such as the Southwestern corn borer *Diatraea grandiosella* (Dyer) and the European corn borer *Ostrinia nubilalis* (Hubner) (Lepidoptera: Crambidae) (Breden & Chippenales, 1989). Although it has been less frequently reported in African cereal stemborers, it occurs, for example, among *C. partellus* larvae of the same size at high larval densities (Bonhot & Overholt, 2001). Therefore, the decrease of survival rates in stemborers in our study can be also explained by cannibalism.

Larval survival and RGR of each stemborer tended to decrease under interspecific interactions regardless of the temperature. With regard to RGR, this indicates competitive resource utilization as already reported by Ntiri *et al.* (2016) between stemborer species. With regard to survival, as mentioned before, this could be due to competition or rather cannibalism. By contrast, compared to stemborers, RGRs of FAW across temperatures were less affected by interspecific than intraspecific interactions. The competition-relatedness hypothesis, which states that closely related species are more competitive (Denno *et al.*, 1995) might explain the higher competition among stemborers than between fall armyworm

and stemborers as FAW larvae have a different mode of feeding compared to the true stemborers. The lower interspecific competition between FAW and stemborers than within stemborers could be explained by the fact that in contrast to stemborers, which only feed on leaves up to the third larval instar stage (even earlier stage for *S. calamistis*), FAW is a pure foliar feeder; thus, as on live plants, FAW larvae remained on the surface whereas borer larvae penetrated into the diet, where after the direct competition ended between FAW and stemborer larvae. Similarly, Shi *et al.* (2008) reported no evidence of interspecific competition between the rice water weevil, a leaf feeder and rice stemborers at the tillering stage in contrast to the booting or earlier developmental stages of rice.

Regardless of the temperature, the pairwise interactions with FAW reduced survival of *B. fusca* but not of *S. calamistis* and *C. partellus*. This dominance in competitive systems can be the result of competitive inequalities between species (Pedruski *et al.*, 2015), but also the result of cannibalism inequalities between species. In fact, when FAW was reared together with *B. fusca* and *C. partellus*, it dominated over *C. partellus* in terms of survival but not when it was reared together with *S. calamistis* and *C. partellus*.

In the combinations involving all noctuids (fall armyworm, *B. fusca* and *S. calamistis*) and the crambid (*C. partellus*), the outcomes of interspecific competition were stronger in term of RGRs and skewed asymmetrically towards the crambid, suggesting a higher fitness of the crambid compared to the three noctuids. These results confirm the asymmetry of interspecific competition outcomes in phytophagous insects (Denno *et al.*, 1995; Kaplan & Denno, 2007) and the asymmetrical competition already showed by Ntiri *et al.* (2016) between noctuids, *B. fusca* and *S. calamistis* and the crambid, *C. partellus*. The superiority of *C. partellus* over other stemborer species has been well reported and discussed by Ntiri

et al. (2016). In addition, the competitive abilities of each species involved in a competition depend on its temperature tolerance limits for survival and development and thus the outcomes of interspecific competition are greatly affected by temperature (Ntiri *et al.*, 2016; Compos *et al.*, 2013). This was also the case for, the interactions between FAW and the stemborers. Except at 20 °C, *C. partellus* RGR always outcompeted FAW and other stemborers species when reared together; and this dominance over especially FAW was enhanced with increasing temperature. The effect of temperature on competitive abilities of interacting species has been reported between the three stemborer species used in this study (Ntiri *et al.*, 2016) who found that the competitive abilities of one of the competing species were enhanced by either low or high temperatures. The coexistence trend in FAW and *S. calamistis* combinations across temperatures might be due to their wider thermal tolerance. In the field, while *C. partellus* and *B. fusca* dominate within a limited thermal tolerance at the high and low temperature extremes, respectively, *S. calamistis* has a wider thermal tolerance by co-occurring with the two species along most of these temperature gradients (Guofa *et al.*, 2001; On'gamo *et al.*, 2006a,b). In addition to unpredictability in rainfall, the temperature increase caused by future climate change is likely to confer an advantage on *C. partellus* over FAW and the noctuids in the utilization of maize resources.

The overall weak competition from second instar between fall armyworm and stemborers indicates that FAW will be able to co-exist with stemborer species along the entire temperature gradient and add to the production constraints of cereal crops. However, with the expanding FAW invasion across agroecologies, studies need to be conducted in the fields along altitudinal gradient to validate the results of the laboratory studies and to predict the trends of population evolution of these species in different agro-climatic zones and how

it is likely to evolve with climate change for development of possible management strategies. Additional experiments are needed to understand such interactions at first instar larvae.

CHAPTER TEN

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

10.1 General discussion

Maize Lepidoptera stemborers, especially *Busseola fusca*, *Sesamia calamistis* and *Chilo partellus* are three main stemborer species which co-infest maize fields in Kenya (Ong'amo *et al.*, 2006a; Guofa *et al.*, 2001). These stemborers severely limit potentially attainable yields of maize by infesting the crop throughout its growth stages (Oben *et al.*, 2015; Van den Berg, 2010). Field infestation of stemborers ranges from 30 - 100 % and the resulting yield loss may reach up to 88 % (Kfir *et al.*, 2002). In Kenya, the estimated yield loss due to these stemborers is equivalent to Kshs 7.2 billion (US\$ 90 million) annually (Midingoyi *et al.*, 2016). Various control methods such as biological control, host plant resistance, manipulation of sowing dates and densities, residues management, cultural control, utilization of synthetic sex pheromones are used for their control (Kfir *et al.*, 2002) but the yield is still un-increasing. Moreover, several studies have been conducted to evaluate how host plant preferences are achieved in these stemborer species (Calatayud *et al.*, 2008a; Krüger *et al.*, 2008). However, the information on the drivers of these intraspecific and interspecific interactions (mainly oviposition selection) and their main carry-over source during non-cropping season remain understudied. Furthermore, it was recently reported in Africa the new species invasion from America, the Fall ArmyWorm, *Spodoptera frugiperda*, that may interfere with the existing maize stemborers larvae in maize fields

(Goergen *et al.*, 2016). This study sought to determine the driver mechanisms of off-season refugia, and female oviposition preference of maize stemborers within their community, as well as their larval co-habitation with the Fall ArmyWorm in order to improve an ongoing IPM-based maize pests control strategies.

The occurrence of maize stemborers and associated parasitoids study conducted in maize stem residues and wild grasses as a potential carry-over population to subsequent early-season maize plants during the non-cropping seasons showed that wild habitat had higher species diversity than maize residues habitat. Similarly, maize residues had a significant higher abundance of maize stemborer species such as *Busseola fusca*, *Sesamia calamistis*, and *Chilo partellus* and the associated parasitoid species (i.e., *Cotesia flavipes* and *Cotesia sesamiae*) than wild plants. The higher species diversity in wild habitat has already been largely reported in several studies in the literature (Ong'amo *et al.*, 2018; 2013; 2006a, 2006b; Mailafiya *et al.*, 2011; 2009; Otieno *et al.*, 2008; 2006; Matama-Kauma *et al.*, 2008; Ndemah *et al.*, 2007; Le Ru *et al.*, 2006a; 2006b). However, the results from this study showed that the stemborers and associated parasitoids species recorded in maize residues habitat being the most abundant species reported in maize fields in subsequent early-season with strong positive correlation. These results confirm the fact that maize residues constitute the main source of both maize stemborers and the associated parasitoids populations carried over to the subsequent early-season maize plant mostly in the areas with reduced wild habitats.

Busseola fusca, one of the main pests recovered in maize fields has already been reported to survive long dry season as a diapausing larvae in maize residues left in the field after harvest (Kfir, 1991; Kfir, *et al.*, 1989; Gebre-Amlak, 1988). The similarity between

stemborers and associated parasitoids species with regard to habit preference supposes that the parasitoids follow their lepidopteran stemborer hosts during the non-cropping seasons in diapausing larvae in maize residues left in the fields following harvest as previously reported (Polaszek, & Khan, 1998; Kfir, 1991; Kfir *et al.*, 1989;).

Chemical basis for the female moth oviposition site preference studied in a community of the noctuids, *Busseola fusca* (Fuller) and *Sesamia calamistis* Hampson and crambid *Chilo partellus* (Swinhoe) in laboratory and field conditions showed that all the gravid female moths significantly preferred the volatile organic compounds (VOCs) emitted by plants infested by con- or hetero-specific larvae over uninfested plants, and female moths did not systematically prefer VOCs emitted by plant infested by conspecifics. These results corroborated those reported earlier for several other lepidopteran phytophagous insects (Facknath, 2012; Viswanathan *et al.*, 2005), although they contrasted the fundamental concept of competition (Jaenike, 1978).

Chemical analysis with coupled gas chromatography/mass spectrometry showed that VOCs emitted by larvae-infested plants were compositionally richer than those emitted by uninfested plants regardless of the stemborer species. These results confirm those reported by Dicke & Van Loon (2000) and Ngi-Song *et al.* (2000). Some of these caterpillar-induced volatiles, have also been reported in other studies (e.g. Pinto-Zevallos *et al.*, 2016; De Moraes *et al.*, 2001). In addition, the intensity of VOCs emission varied with larvae species involved in maize infestation. *Sesamia calamistis* and *C. partellus* larvae induced a compositionally less richer VOCs profile than *B. fusca* larvae. The type of elicitors specific to the different stemborer larval species should thus be responsible for these variations in HIPVs by the different stemborer species. Several studies have shown that volicitin and β -

glucosidase are larval oral excretion elicitors that induce specific volatiles from the insect attacked plants (Gouinguéné *et al.*, 2001; Dicke & Van Loon, 2000).

Following the invasion of fall armyworm in stemborer communities, this study showed that the stemborer communities constituting of *Busseola fusca*, *Sesamia calamistis* and *Chilo partellus* species in addition to the fall armyworm (FAW) when utilising the same resources, are characterized by competitive interactions as previously reported in other insect communities (Bruno *et al.*, 2003; Denno *et al.*, 1995). The results showed that single-species infestation decreased while multi-species increased mainly at field level with the introduction of FAW as the combined effect caused by multi-species on crops is higher than that caused by individual species (Dangles *et al.*, 2009; Van den Berg *et al.*, 1991). The implications of that interactions is the increase of yield loss in maize fields. The maize pests temporal population dynamic in maize plants infestation showed that the incidence of infestations and larval densities increased varied between seasons depending on the species composition of the community and maize plant stage phenology. Competitive interaction was strong from maize reproductive to senescent stages when FAW main site, the whorl of the plant disappeared after tasseling and after which was distributed throughout the plant (CABI, 2017). This should increase the likelihood of interaction with the stemborers species, hence the decrease in the density of the stemborer larvae after tasseling stage in the fields. For the associated parasitoids, *Cotesia flavipes*, *C. sesamiae*, *Pediobius furrus* were the major species that were collected with overall, low stemborer parasitism of whereas no parasitoid was collected on fall armyworm larvae in maize fields. Performance studies carried out on *Cotesia flavipes*, *C. sesamiae* Inland, *C. sesamiae* Coastal and *C. typhiae* (Hymenoptera: Braconidae) in laboratory conditions with fall armyworm as a host showed

that except for *C. typhiae*, all the parasitoids accepted to sting the FAW larvae by insert their ovipositor to assess the suitability of the host but without laying the eggs. This has been reported in the parasitoid complex of the moth *Epinotia tedella* (Clerck) (Lepidoptera: Tortricidae) which rejects about 75% of the larvae probed (Munster-Swendsen, 2002). However, these parasitoids induced significant nonreproductive mortality of the host through mutilation and/or pseudoparasitism. Vinson & Iwantsch (1980) reported that during the suitability assessment process, the parasitoid might inject the viruses, venom, teratocytes, or other chemical/biological substances or mechanically damage the host leading to the nonreproductive mortality.

In a redistribution of insect population and infestations within or between plants, larval dispersal either through ballooning or crawling showed that FAW neonate larvae had greater potential of ballooning off the initial oviposition sites than those of stemborers irrespective of species studied. Furthermore, FAW appeared to have larger dispersal and plant damage potential than any of the stemborer species used in the study. This species-dependent ballooning capacity among neonate larvae has been reported in several studies (Zalucki *et al.*, 2002; Diss *et al.*, 1996). Several studies have suggested that in the cases where larval dispersal is limited, female selects for high-quality hosts (Bellota *et al.*, 2017; Gripenberg *et al.*, 2010;). Thus, the higher dispersal ability of FAW indicates that it is less involved for high-quality hosts assessment and are also more polyphagous than any of the stemborer species used. This clearly explains the expansive host range exceeding 350 plant species that has been associated with the species. (Montezano *et al.*, 2018; Casmuz *et al.*, 2010). With such wide host range, dispersal of numerous larvae away from oviposition hosts is most likely adaptive for the fall armyworm and it would further reduce the larval intra-

specific competition and cannibalism (Andow *et al.*, 2015; Chapman *et al.*, 1999a). For each species, more female larvae dispersed than males and their survival rate was higher than that of non-ballooning larvae. This female-biased sex ratio might be involved to ensure the perennity of the species (Rhainds *et al.*, 1998; Lagoy & Barrows, 1989). Pannuti *et al.* (2015) reported similar higher survival rates of ballooning larvae than non-ballooning larvae; while Reavey & Lawton (1991) emphasized the contribution of this larval behaviour to its fitness.

In restricted stems, the present study showed that temperature was an important factor that influence both the intra- and interspecific interactions between larvae of the three stemborer species and fall armyworm utilising the same resources. Temperature influenced both survival and relative growth rates of all species as has been previously reported by Ntiri *et al.* (2016) within maize stemborers species. However, in interspecific interactions the competitions were less between the fall armyworm and the stemborers than among stemborers across the temperatures used suggesting that FAW should co-exist with stemborer species along all the temperature gradients and add to the cereal crops production constraints in the sub-Saharan African region. This study also showed that negative density-dependence survival and RGR are functions of increased larval density and were the prevalent effects of all outcomes. Similarly, time partitioning of the resource use appears to be very important factor in the coexistence of these competing insect species. Agrew *et al.* (2002) showed that larval density-dependent competition is an important factor regulating the growth of population and furthermore, increased larval density leads to increased larval mortality. The temporal plasticity of competition of FAW and stemborers could result from

fluctuations in the quality and availability of food resources as utilisation its increased with time (Ayabe *et al.*, 2015).

The findings of this study have strong implications in the maize pest management strategies. The VOCs associated with larvae-infested plants identified open new avenues for developing specific attractants to trap female stemborers in the field for their control. On the other hand, the identification of sources of stemborer infestations and associated parasitoids provided in this study is essential for habitat pest management method in the agroecosystem. Similarly, the potential occurrence of both intraspecific and interspecific interactions within the stemborers community during prior and after introduction of FAW reported in maize fields as well as the importance of associated parasitoids are critical considerations in the development of effective management strategies of these insect pests. Larval dispersal is a major contributory factor to the spread of stemborer and mostly fall armyworm infestations in the fields and hence should be carefully considered when assessing pest management strategies. For instance, in order to predict the potential of the use of eggs parasitoids in lepidopteran biological control, Chapman *et al.* (1983) demonstrated that the potential for *Trichogramma* sp. to reduce the number of surviving larvae and infested plants is greater depending on how well migrating larvae establish on adjacent plants in maize fields. These results may also help the other IPM components, such as scouting and economic threshold. Larval dispersal can directly influence the accuracy of scouting methods (Ross & Ostlie, 1990) and larval survival information should increase precision of economic thresholds (Ross & Ostlie, 1990). The competition findings in this study are critical to predict the spatio-temporal distribution of the new invasive species (fall armyworm) depending on the distribution status of maize stemborer species across the agro-

climatic zones. Besides, temperature-dependent study has the advantage to predict the trends of population evolution of the studied species in different agro-climatic zones and how it is likely to evolve with climate change for development of possible and efficient pest management strategies.

10.2 Conclusion

This study highlighted that maize residues constitute a better refugia reservoir of maize stemborers and associated parasitoids during the non-cropping season as compared to wild habitat and thus might constitute the main source of both maize stemborers and associated parasitoids populations carry-over to subsequent early-season maize plant particularly when wild plants surrounding maize fields are scarce. The females of stemborer choose for oviposition preferentially VOCs emitted by plants infested by con- and hetero-specific larvae compared to VOCs of uninfested plants. Under interspecific choice, females did not systematically prefer VOCs emitted by plants infested by conspecific larvae. Larval feeding activity modified the whole volatile profiles of maize plants varying their emission intensity and composition depending on the stemborer species involved in the infestation. (*E*)- β -Farnesene, linalool, myrcene and β -bisabolene were the characteristics VOCs emitted by plants infested by *B. fusca* while α -zingiberene, sesquisabinene, indole and α -cis-bergamotene were the characteristics VOCs emitted by plants infested by *C. partellus* or *S. calamistis* and all were considered to have potential to attract conspecific and heterospecific females.

In maize fields, stemborers single-species infestation decreased while multi-species increased mostly at field level with the introduction of FAW and the overall incidence of

infestations and larval densities also increased. Competitive interaction between stemborers and FAW in the maize fields was strong from plant reproductive to senescent maize plant stages when FAW main site, the whorl of the plant disappeared after tasseling and got redistributed throughout the whole plant. Fall armyworm larvae were not suitable to improve population dynamics of maize stemborer associated *Cotesia* species parasitoids. In contrast, *Cotesia flavipes*, *C. sesamiae* Inland, *C. sesamiae* Costal could contribute to reduce fall armyworm population by inducing host nonreproductive mortality which is useful in inundative and conservative biological control. Fall armyworm had greater potential of larval dispersal either through ballooning or crawling than of the stemborers irrespective of the species in a redistribution of insect population and infestations within or between maize plant. In interspecific interactions competitions were lower between fall armyworm and stemborers than among stemborers suggesting that fall armyworm should co-exist with stemborer species and add to the production constraints of cereal crops. Temperature, larval density and duration of competitive interactions are the key factors that influence the magnitude of competitions between maize stemborer species and the fall armyworm larvae.

10.3 Recommendations

It is recommended that:

1. Systematic destruction of maize residues must be avoided, mostly in the areas with reduced wild habitat in order to preserve natural enemies for biological control;
2. Classical biological control of FAW to be designed independently of maize stemborer associated parasitoid species.
3. Augmentative biological control needs to be done for stemborer species since FAW became a trap for their associated parasitoids

4. Further study be conducted to develop female biased kairomonal lures from specific volatile signatures identified from both conspecific and heterospecific larval infested maize plants to trap female stemborers in the field;
5. Since the impact of FAW on maize stemborers communities in maize fields was conducted at the start of FAW introduction, further studies need to be conducted to determine the evolution of those interactions;
6. Larval dispersal findings should be carefully considered in the developing of sampling protocols for monitoring pest densities in the field and choice and timing of appropriate management practices of either stemborers or fall armyworm;
7. With the expanding FAW invasion across agroecologies, further studies need to be conducted in the fields along altitudinal gradient to conform laboratory study data and to predict the trends of population evolution of these species in different agro-climatic zones and how it is likely to evolve with climate change for development of possible management strategies.

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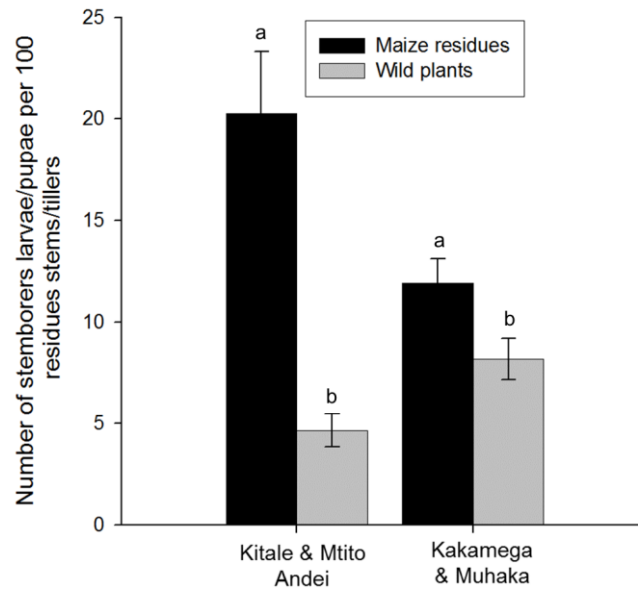
Appendices

Appendix 1: Stemborer species composition and total abundance in maize residues and wild plants during non-cropping seasons in different agro-ecological zones in Kenya (from Mailafiya, 2009).

Habitats	Total Number	Stemborer Species Composition					
		Bf	Sc	Cp	Co	Bse	Os
Kitale & Mito Andei							
Maize residues	1090	641	180	197	49	17	6 Es
Wild plants	146	6	4	2	7	86	41
<i>Sorghum arundinaceum</i> *	18	4	3	2	-	9	-
<i>Panicum maximum</i> *	29	2	-	-	-	26	1 Mn
<i>Panicum</i> sp. *	9	-	-	-	-	2	5 Ppi, 2 Mn
<i>Eleusine jaegen</i> *	34	-	-	-	2	30	2 Ppi
<i>Cynodon dactylon</i> *	17	-	1	-	4	-	7 Ssp, 5 Csp
<i>Cyperus</i> sp. †	2	-	-	-	-	-	2 Sv
<i>Echinochloa haploclada</i> *	20	-	-	-	1	7	12 Mni
<i>Rottboellia cochinchinensis</i> *	17	-	-	-	-	12	5 Esp
Kakamega & Muhaka							
Maize residues	453	126	141	138	27	15	6 Sp
Wild grasses	338	5	13	11	81	125	103
<i>Panicum maximum</i> *	107	-	6	2	76	6	17 Sp
<i>Panicum</i> sp. *	80	-	3	3	-	55	14 Csp, 5 Mn
<i>Cyperus</i> sp. †	33	-	-	-	-	9	24 Sno
<i>Sorghum arundinaceum</i> *	12	-	3	6	1	-	1 Ppi, 1 Es
<i>Pennisetum trachyphyllum</i> *	6	2	-	-	-	-	4 Sn
<i>Pennisetum purpureum</i> *	26	3	-	-	-	18	5 Ppi
<i>Cyperus dereilema</i> †	4	-	-	-	-	-	4 Ppi
<i>Cynodon</i> sp. *	3	-	-	-	-	-	2 Ppi, 1 Scs
<i>Scleria racemosa</i> †	39	-	-	-	-	35	1 Sv, 3 Mn
<i>Chloris gayana</i> *	5	-	-	-	-	-	5 Mn
<i>Setaria megaphylla</i> *	4	-	-	-	-	2	2 Bs
<i>Cynodon dactylon</i> *	19	-	1	-	4	-	8 Ssp, 6 Csp

Os = Other species. Noctuidae (Sn = *Sciomesa nyei*; Ppi = *Pirateolea piscator*; Sv = *Sciomesa venata*; Ssp = *Sciomesa* sp nov; Mn = *Manga nubifera*; Sp = *Sesamia poephaga*; Sno = *Sesamia nonagriodes*; Sc = *Sesamia calamistis*; Bse = *Busseola segeta*; Bs = *Busseola sensu*; Bf = *Busseola fusca*) / Crambidae (Co = *Chilo orichalcociliellus*; Cp = *Chilo partellus*; Csp = *Chilo* sp) / Pyralidae (Esp = *Ematheudes* sp.; Es = *Eldana saccharina*; Mni = *Mussidia nigrivenella*; Scs = *Schoenobius* sp). Wild grasses: * Poaceae, † Cyperaceae

Appendix 2: Number of *Busseola fusca*, *Sesamia calamistis* and *Chilo partellus* stemborer found per 100 maize residues or wild plants sampled in two agro-ecological zones (Kitale & Mtito Andei and Kakamega & Muhaka) during non-cropping seasons in 2006 and 2007.



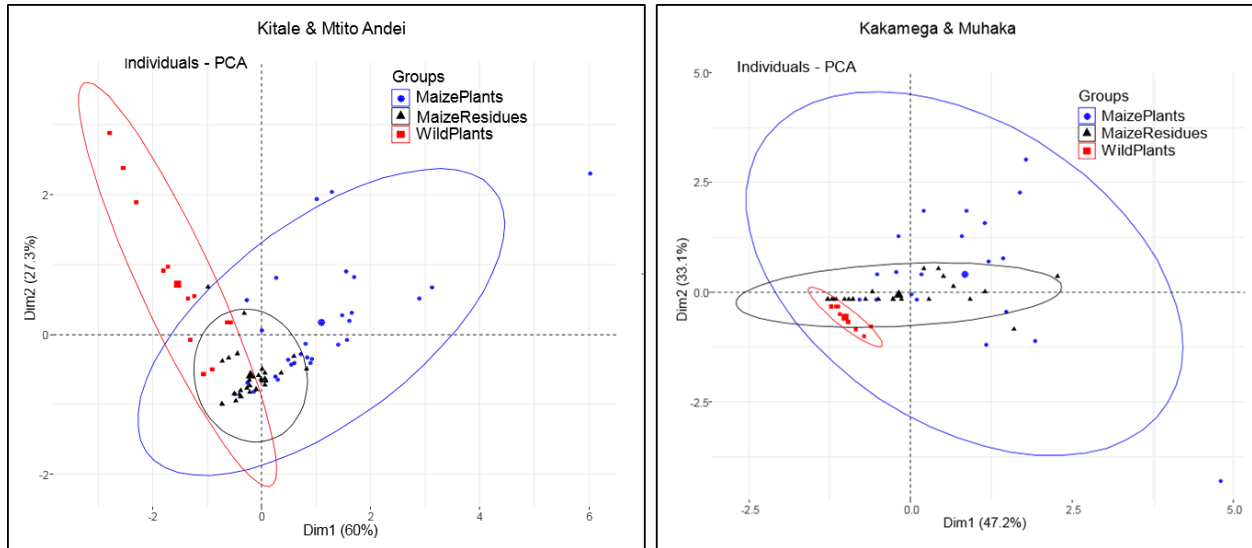
Significant differences at 5% level between maize residues vs. wild plants are shown by different letters determined using Tukey’s multiple comparisons tests with the R package “lsmeans”, following generalized linear model (GLM) with negative binomial error distribution.

Appendix 3: Parasitoids composition and abundance from stemborers species in maize residues and wild plants during the non-cropping seasons in different agro-ecological zones in Kenya (from Mailafiya, 2009).

Parasitoid Species	Borer Species	Borer Stages	Wild Plants	Total Number	Kitale & Mtito Andei		Kakamega & Muhaka	
					Maize Residues	Wild Plants	Maize Residues	Wild Plants
Hymenoptera: Braconidae								
<i>Cotesia flavipes</i>	Cp, Sc	larva	Sa	22	-	-	17	5
<i>Cotesia sesamiae</i>	Bf, Sc	larva	Sa	13	9	4	-	-
<i>Dolichogenidea polaskezi</i>	Bf	larva	-	1	1	-	-	-
<i>Macrocentrus sp.</i>	Tr	larva	Cr	1	-	-	-	1
Hymenoptera: Ichneumonidae								
<i>Syzectus sp.</i>	Co, Cp,	pupa	Sa, Pm	5	-	2	-	3
<i>Amouramorpha sp.</i>	Tr	larva	Cr	1	-	1	-	-
Hymenoptera: Eulophidae								
<i>Pediobius furvus</i>	Cp, Sc	pupa	Sa	3	-	-	3	-
Diptera: Muscidae								
<i>Atherigona sp</i>	Cp	larva	Pm	2	-	-	-	2
Diptera: Chloropidae								
<i>Chloropid sp</i>	Bf	larva	Sm	2	-	2	-	-
Diptera: Tachinidae								
<i>Sturmiopsis parasitica</i>	Bf	larva	Sa	2	1	1	-	-
<i>Siphona (Meigen) sp.</i>	Bf, Mn	larva	Pm	2	2	-	-	-

Borer species: Bf = *Busseola fusca*, Sc = *Sesamia calamistis*, Cp = *Chilo partellus*, Co = *Chilo orichalcocillielus*, Tr = Tortricidae, Mn = *Manga nubifera*; Plant species: Sa = *Sorghum arundinaceum*, Pm = *Panicum maximum*, Cr = *Cyperus rotundus*, Sm = *Setaria megaphylla*. No. = Total number of parasitized larvae recorded for a given parasitoid species, - absent.

Appendix 4: Principal component biplot showing the relationship between abundance of stemborer species in maize plants of cultivated fields during cropping season and those in either maize residues or wild plant during the non-cropping season in two agro-ecological zones of Kenya (Kitale & Mtito Andei and Kakamega & Muhaka).



Appendix 5: The Morista–Horn similarity index between maize plants versus maize residues and between maize plants versus wild plants in the carry-over of lepidopteran maize stemborers and associated larval/pupal parasitoid species

Morista-Horn Index (CmH)				
Agro-Ecological Zones	Lepidopteran Maize Stemborer Species		Associated Larval/Pupal Parasitoid Species	
	Maize Fields vs. Maize Residues	Maize Fields vs. Wild Plants	Maize Fields vs. Maize Residues	Maize Fields vs. Wild Plants
Kitale & Mtito Andei	0.95	0.04	0.98	0.68
Kakamega & Muhaka	0.99	0.14	0.97	0.63

The value close to 1 indicates a greater similarity between the two habitats and vis versa.

Appendix 6: Mean relative ratios (\pm SE) of VOCS detected in both uninfested and maize plants infested by *Busseola fusca*, *Sesamia calamistis* or *Chilo partellus* larvae by dynamic head space VOCs collection system

RT (min)	Compound name	Class of compound	Uninfested	Infested by <i>B. fusca</i>	Infested by <i>S. calamistis</i>	Infested by <i>C. partellus</i>	Kruskal-Wallis	
							χ^2	<i>P</i>
7.91	(<i>E</i>)-2-Hexenal [†]	Green leaf volatile	0.00 \pm 0.00 b	1.26 \pm 0.77b	3.041 \pm 1.37ab	5.76 \pm 1.52 a	8.82	0.03*
8.08	(<i>Z</i>)-3-Hexen-1-ol [†]	Green leaf volatile	0.00 \pm 0.00 b	0.00 \pm 0.00 b	1.62 \pm 0.67 a	0.00 \pm 0.00 b	9.98	0.02*
9.26	Anisole [†]	Cyclic hydrocarbon	0.00 \pm 0.00 a	0.01 \pm 0.01 a	0.00 \pm 0.00 a	0.00 \pm 0.00 a	3.00	0.39
9.82	α -Pinene [†]	Monoterpenoid	25.66 \pm 1.93 a	11.41 \pm 4.69 b	11.17 \pm 4.70 b	6.08 \pm 3.75 b	11.55	0.01*
10.44	Cumene	Cyclic hydrocarbon	0.00 \pm 0.00 a	0.38 \pm 0.38 a	0.71 \pm 0.71 a	0.00 \pm 0.00 a	2.11	0.55
10.64	Sabinene [†]	Monoterpenoid	0.00 \pm 0.00 a	2.10 \pm 1.74 a	0.00 \pm 0.00 a	0.00 \pm 0.00 a	6.31	0.10
10.60	Mesitylene	Cyclic hydrocarbon	2.27 \pm 1.39 a	1.59 \pm 0.41 a	2.16 \pm 0.63 a	0.73 \pm 0.45 a	3.01	0.39
11.03	Myrcene [†]	Monoterpenoid	0.00 \pm 0.00 b	7.62 \pm 2.62 a	3.86 \pm 0.10 b	5.83 \pm 0.75 a	8.82	0.03*
11.45	(<i>Z</i>)-3-Hexenyl acetate [†]	Green leaf volatile	0.23 \pm 0.16 a	0.02 \pm 0.02 a	0.07 \pm 0.05 a	0.00 \pm 0.00 a	3.05	0.38
11.50	(<i>E</i>)-3-Hexenyl acetate [†]	Green leaf volatile	0.00 \pm 0.00 a	1.37 \pm 1.37 a	0.00 \pm 0.00 a	2.00 \pm 1.25 a	3.80	0.28
11.72	Limonene [†]	Monoterpenoid	7.50 \pm 3.13 a	1.23 \pm 0.50 a	1.49 \pm 0.66 a	0.72 \pm 0.44 a	2.69	0.45
12.25	γ -Terpinene [†]	Monoterpenoid	8.35 \pm 4.21 a	0.65 \pm 0.65 a	0.00 \pm 0.00 b	0.00 \pm 0.00 b	7.77	0.05*
12.98	Linalool [†]	Monoterpenoid	0.00 \pm 0.00 a	13.63 \pm 3.44 b	0.00 \pm 0.00 a	12.66 \pm 6.04 a	10.20	0.02*
13.16	n-Nonanal [†]	Green leaf volatile	11.79 \pm 2.08 a	0.00 \pm 0.00 c	5.35 \pm 1.41b	5.65 \pm 0.78 b	15.50	<0.001*
16.17	Indole [†]	Alkaloid	0.00 \pm 0.00 b	2.54 \pm 0.64 a	0.00 \pm 0.00 b	0.00 \pm 0.00 b	14.03	<0.001*
17.10	α -Ylangene	Sesquiterpene	1.25 \pm 1.24 a	1.84 \pm 0.76 a	3.54 \pm 1.00 a	0.00 \pm 0.00 a	6.89	0.08
17.08	α -Amorphene	Sesquiterpene	3.95 \pm 3.95 a	0.00 \pm 0.00 a	0.57 \pm 0.57 a	0.63 \pm 0.63 a	1.14	0.77
17.30	α -Muurolene	Sesquiterpene	0.00 \pm 0.00 a	0.00 \pm 0.00 a	0.00 \pm 0.00 a	2.75 \pm 1.69 a	6.31	0.10
17.46	7-epi-Sesquithujene	Sesquiterpene	3.52 \pm 0.88 a	0.35 \pm 0.21 b	0.19 \pm 0.19 b	0.09 \pm 0.01 b	14.37	<0.001*
17.48	Sesquithujene	Sesquiterpene	0.00 \pm 0.00 a	0.50 \pm 0.50 a	0.73 \pm 0.73 a	1.28 \pm 1.28 a	1.14	0.77
17.75	γ -Muurolene	Sesquiterpene	0.00 \pm 0.00 a	0.54 \pm 0.53 a	1.61 \pm 1.04 a	0.62 \pm 0.62 a	2.61	0.46
17.76	α -Cedrene	Sesquiterpene	14.14 \pm 3.42 a	2.53 \pm 0.65 b	8.12 \pm 3.36 a	10.76 \pm 3.07 a	8.92	0.03*
17.78	(<i>E</i>)-Caryophyllene [†]	Sesquiterpene	0.00 \pm 0.00 b	1.58 \pm 0.65 a	0.00 \pm 0.00 b	3.28 \pm 0.58 a	13.57	<0.001*
17.81	α -cis-Bergamotene	Sesquiterpene	4.37 \pm 0.40 a	3.32 \pm 0.12 a	7.57 \pm 0.63 b	6.29 \pm 1.22 b	9.93	0.02*
17.93	α -trans-Bergamotene	Sesquiterpene	0.00 \pm 0.00 a	1.25 \pm 1.25 a	0.00 \pm 0.00 a	0.00 \pm 0.00 a	3.00	0.39
18.18	Sesquisabinene	Sesquiterpene	3.81 \pm 2.26 b	3.37 \pm 1.62 b	13.33 \pm 3.67 a	11.19 \pm 6.40 a	7.83	0.04*
18.15	(<i>E</i>)- β -Farnesene [†]	Sesquiterpene	0.04 \pm 0.04 c	24.65 \pm 4.59 a	12.17 \pm 7.60 b	12.39 \pm 5.49 b	8.23	0.04*
18.30	(<i>Z</i>)- β -Farnesene	Sesquiterpene	0.00 \pm 0.00 a	0.31 \pm 0.12 a	1.21 \pm 0.56 a	0.37 \pm 0.37 a	6.46	0.09
18.38	α -Zingiberene	Sesquiterpene	0.00 \pm 0.00 c	3.23 \pm 0.20 b	9.43 \pm 4.63 a	8.97 \pm 3.12 a	7.96	0.05*
18.88	δ -Amorphene	Sesquiterpene	9.85 \pm 4.91 a	0.56 \pm 0.56 a	1.85 \pm 1.14 a	0.64 \pm 0.64 a	4.28	0.23
18.89	β -Bisabolene [†]	Sesquiterpene	0.00 \pm 0.00 c	10.04 \pm 4.14 a	5.08 \pm 2.48 b	0.00 \pm 0.00 c	8.16	0.04*
19.03	β -Sesquiphellandrene	Sesquiterpene	0.00 \pm 0.00 b	3.24 \pm 0.11 a	2.45 \pm 1.05 a	0.53 \pm 0.52 b	11.21	0.01*
19.14	(<i>E</i>)- γ -Bisabolene	Sesquiterpene	0.00 \pm 0.00 a	2.11 \pm 1.29 a	0.00 \pm 0.00 a	0.00 \pm 0.00 a	6.31	0.10
19.53	(<i>E,Z</i>)-Geranyl linalool	Sesquiterpene	0.00 \pm 0.00 a	0.41 \pm 0.41 a	1.46 \pm 0.90 a	0.76 \pm 0.76 a	2.61	0.46

VOC identified using three databases of the ChemStation software; National Institute of Standards and Technology Mass Spectra database of 2008 (NIST), Adams2 (1995) and Chemoecol. The identifications were tentative and only based on spectra and retention times matches. Compounds with [†] were further identified by matching the retention time and mass spectra of the VOC with authentic standards injected under the same conditions as the samples. Mean relative amount of each compound is expressed as a percentage of the total emission, i.e. the sum of all 14 VOCs for the uninfested maize plants, 29 for the plants infested by *B. fusca*, 24 for the plants infested by *S. calamistis* and 22 for the plants infested by *C. partellus*; SE = standard error. Five replicates were carried out in each case. Only the compounds showing significant differences for $P < 0.05$ between maize plants status (Kruskal-Wallis test) are indicated by * on their P-value. Means followed by different letters in the same row were significantly different by dunn.test, $p < 0.05$.

Appendix 7: Mean relative ratios (\pm SE) of VOCs detected in uninfested maize plants and maize plants infested by *Busseola fusca*, *Sesamia calamistis* or *Chilo partellus* larvae by SPME collection system

RT (min)	Compound name	Class of compound	Uninfested	Infested by <i>B. fusca</i>	Infested by <i>S. calamistis</i>	Infested by <i>C. partellus</i>	Kruskal-Wallis χ^2	<i>P</i>
9.28	Anisole [†]	Cyclic hydrocarbon	0.00 \pm 0.00 a	2.02 \pm 1.47 a	0.65 \pm 0.41 a	1.86 \pm 1.86 a	2.57	0.46
11.05	Myrcene [†]	Monoterpenoid	0.00 \pm 0.00 a	4.87 \pm 0.38 b	6.34 \pm 0.73 b	6.12 \pm 1.18 b	9.57	0.02*
11.25	4,8-Dimethyl-1,3-(<i>Z</i>),7-nonatriene	Monoterpenoid	0.00 \pm 0.00 a	5.49 \pm 2.12 b	6.86 \pm 1.21 b	6.89 \pm 1.84 b	7.65	0.04*
11.44	(<i>Z</i>)-3-Hexenyl acetate [†]	Green leaf volatile	0.00 \pm 0.00 a	0.00 \pm 0.00 a	0.00 \pm 0.00 a	1.04 \pm 1.04 a	3.00	0.39
11.75	Limonene [†]	Monoterpenoid	2.79 \pm 1.34 a	0.00 \pm 0.00 b	1.04 \pm 0.39 a	0.00 \pm 0.00 b	10.25	0.03*
11.83	Benzyl alcohol	Green leaf volatile	7.33 \pm 2.22 a	2.59 \pm 0.76 a	1.05 \pm 0.48 a	3.08 \pm 1.51 a	7.34	0.06
12.98	Linalool [†]	Monoterpenoid	0.00 \pm 0.00 a	10.29 \pm 1.23 b	8.30 \pm 0.98 b	5.54 \pm 1.51 b	10.47	0.01*
13.15	n-Nonanal [†]	Green leaf volatile	13.29 \pm 1.08 a	6.82 \pm 0.85 b	4.78 \pm 0.79 b	4.67 \pm 0.89 b	10.12	0.02*
14.56	Methyl salicylate	Green leaf volatile	3.77 \pm 1.43 a	0.58 \pm 0.58 b	0.00 \pm 0.00 c	0.00 \pm 0.00 c	8.30	0.04*
16.05	Thymol [†]	Monoterpenoid	3.09 \pm 1.17 a	1.86 \pm 0.25 a	1.42 \pm 0.32 a	1.68 \pm 0.44 a	2.80	0.42
16.16	Indole [†]	Alkaloid	0.00 \pm 0.00 c	5.44 \pm 0.77 b	8.79 \pm 0.58 a	12.35 \pm 0.96 a	14.01	0.002*
17.09	Butyl butanoate	Green leaf volatile	40.25 \pm 2.00 a	19.30 \pm 2.40 b	16.42 \pm 2.96 b	15.28 \pm 3.92 b	8.93	0.03*
17.56	7-epi-Sesquithujene	Sesquiterpene	4.01 \pm 1.44 a	0.96 \pm 0.96 a	0.77 \pm 0.25 a	1.26 \pm 0.79 a	3.31	0.35
17.72	α -Cedrene	Sesquiterpene	7.06 \pm 0.97 a	4.35 \pm 1.19 a	2.67 \pm 0.58 a	2.98 \pm 1.02 a	7.54	0.06
17.73	β -Cedrene	Sesquiterpene	3.49 \pm 0.22 a	1.04 \pm 0.60 b	0.00 \pm 0.00 b	0.12 \pm 0.12 b	11.43	0.01*
17.79	(<i>E</i>)-Caryophyllene [†]	Sesquiterpene	0.00 \pm 0.00 a	4.35 \pm 1.89 b	2.23 \pm 0.49 b	6.65 \pm 2.05 b	10.91	0.01*
17.83	α -cis-Bergamotene	Sesquiterpene	2.22 \pm 1.29 c	7.29 \pm 0.21 b	7.44 \pm 1.00 b	12.04 \pm 1.11 a	12.35	0.01*
18.08	Geranyl acetone	Monoterpenoid	3.17 \pm 1.16 a	1.35 \pm 1.35 a	0.00 \pm 0.00 a	0.89 \pm 0.89 a	4.53	0.21
18.13	(<i>E</i>)- β -Farnesene [†]	Sesquiterpene	1.54 \pm 0.88 c	11.80 \pm 4.33 a	6.90 \pm 0.76 b	6.99 \pm 0.65 b	11.73	0.01*
18.35	α -Zingiberene	Sesquiterpene	0.00 \pm 0.00 a	8.93 \pm 5.56 b	10.82 \pm 3.84 b	5.66 \pm 2.80 b	8.09	0.04*
18.83	β -Bisabolene [†]	Sesquiterpene	2.64 \pm 0.45 a	9.46 \pm 0.82 b	5.68 \pm 0.61 b	1.86 \pm 0.19 a	12.66	0.01*
19.00	β -sesquiphellandrene	Sesquiterpene	0.00 \pm 0.00 a	1.29 \pm 0.76 a	1.08 \pm 0.38 a	1.35 \pm 0.50 a	4.36	0.23
19.55	(<i>E,Z</i>)-Geranyl Linalool	Sesquiterpene	0.00 \pm 0.00 a	0.83 \pm 0.83 a	0.48 \pm 0.29 a	0.97 \pm 0.61 a	2.57	0.46
20.10	epi-Cedrol	Sesquiterpene	3.04 \pm 1.21 a	1.94 \pm 0.24 a	1.27 \pm 0.18 a	1.56 \pm 0.59 a	2.80	0.42

VOC identified using three databases of the ChemStation software; National Institute of Standards and Technology Mass Spectra database of 2008 (NIST), Adams2 (1995) and Chemoecol. The identifications were tentative and only based on spectra and retention times matches. Compounds with [†] were further identified by matching the retention time and mass spectra of the VOC with authentic standards injected under the same conditions as the samples. Mean relative amount of each compound is expressed as a percentage of the total emission, i.e. the sum of all 14 VOCs for the uninfested maize plants, 29 for the plants infested by *B. fusca*, 24 for the plants infested by *S. calamistis* and 22 for the plants infested by *C. partellus*; SE = standard error. Five replicates were carried out in each case. Only the compounds showing significant differences for $P < 0.05$ between maize plants status (Kruskal-Wallis test) are indicated by * on their P-value. Means followed by different letters in the same row were significantly different by dunn.test, $p < 0.05$

Appendix 8: Mean relative ratios (\pm SE) of VOCs detected in maize plants infested with 5 or 12 larvae of *Busseola fusca*, *Sesamia calamistis* or *Chilo partellus* with the densities of 5 and 12 larvae by dynamic headspace VOCs collection system

RT (min)	Compound name	Infested by <i>B. fusca</i>		Infested by <i>S. calamistis</i>		Infested by <i>C. partellus</i>	
		5 Larvae	12 Larvae	5 Larvae	12 Larvae	5 Larvae	12 Larvae
7.91	(E)-2-Hexenal [†]	0.00 \pm 0.00 a	1.26 \pm 0.77a	10.45 \pm 5.02 a	3.04 \pm 1.37 a	2.76 \pm 0.31 a	5.76 \pm 1.52 a
8.08	(Z)-3-Hexen-1-ol [†]	0.00 \pm 0.00 a	0.00 \pm 0.00 a	1.53 \pm 0.94 a	1.62 \pm 0.67 a	0.00 \pm 0.00 a	0.00 \pm 0.00 a
9.26	Anisole [†]	0.00 \pm 0.00 a	0.01 \pm 0.01 a	0.00 \pm 0.00 a	0.00 \pm 0.00 a	0.00 \pm 0.00 a	0.00 \pm 0.00 a
9.82	α -Pinene ^{†*}	9.40 \pm 5.75 a	11.41 \pm 4.69 a	20.68 \pm 1.53 a	11.17 \pm 4.70 b	13.80 \pm 8.46 a	6.08 \pm 3.75 b
10.44	Cumene	0.00 \pm 0.00 a	0.38 \pm 0.38 a	1.18 \pm 0.73 a	0.71 \pm 0.71 a	0.00 \pm 0.00 a	0.00 \pm 0.00 a
10.64	Sabinene [†]	2.36 \pm 1.06 a	2.10 \pm 1.74 a	0.00 \pm 0.00 a	0.00 \pm 0.00 a	0.00 \pm 0.00 a	0.00 \pm 0.00 a
10.60	Mesitylene	0.78 \pm 0.78 a	1.59 \pm 0.41 a	0.00 \pm 0.00 a	2.16 \pm 0.63 a	0.64 \pm 0.64 a	0.73 \pm 0.45 a
11.03	Myrcene [†]	6.86 \pm 3.25 a	7.62 \pm 2.62 a	3.58 \pm 1.05 a	3.86 \pm 0.10 a	2.28 \pm 0.31 a	5.83 \pm 0.75 a
11.45	(Z)-3-Hexenyl acetate [†]	0.00 \pm 0.00 a	0.02 \pm 0.02 a	1.52 \pm 0.65 a	0.07 \pm 0.05 a	0.00 \pm 0.00 a	0.00 \pm 0.00 a
11.50	(E)-3-Hexenyl acetate [†]	0.00 \pm 0.00 a	1.37 \pm 1.37 a	0.00 \pm 0.00 a	0.00 \pm 0.00 a	2.00 \pm 0.31 a	2.00 \pm 1.25 a
11.72	Limonene [†]	1.37 \pm 1.37 a	1.23 \pm 0.50 a	0.41 \pm 0.41 a	1.49 \pm 0.66 a	0.00 \pm 0.00 a	0.72 \pm 0.44 a
12.25	γ -Terpinene [†]	0.10 \pm 0.03 a	0.65 \pm 0.65 a	0.00 \pm 0.00 a	0.00 \pm 0.00 a	0.00 \pm 0.00 a	0.00 \pm 0.00 a
12.98	Linalool ^{†*}	9.63 \pm 1.33 a	13.63 \pm 3.44 b	2.60 \pm 1.61 a	0.00 \pm 0.00 a	10.20 \pm 10.20 a	12.66 \pm 6.04 a
13.16	n-Nonanal ^{†*}	5.00 \pm 0.04 a	0.00 \pm 0.00 b	20.56 \pm 4.03 a	5.35 \pm 1.41 b	8.00 \pm 2.2.55 a	5.65 \pm 0.78 a
16.17	Indole ^{†*}	0.09 \pm 0.04 a	2.54 \pm 0.64 b	0.00 \pm 0.00 a	0.00 \pm 0.00 a	0.00 \pm 0.00 a	0.00 \pm 0.00 a
17.10	α -Ylangene	3.09 \pm 1.31 a	1.84 \pm 0.76 a	3.24 \pm 1.98 a	3.54 \pm 1.00 a	0.00 \pm 0.00 a	0.00 \pm 0.00 a
17.08	α -Amorphene	1.45 \pm 1.45 a	0.00 \pm 0.00 a	0.00 \pm 0.00 a	0.57 \pm 0.57 a	0.00 \pm 0.00 a	0.63 \pm 0.63 a
17.30	α -Muuroolene	1.78 \pm 1.78 a	0.00 \pm 0.00 a	0.00 \pm 0.00 a	0.00 \pm 0.00 a	0.00 \pm 0.00 a	2.75 \pm 1.69 a
17.46	7-epi-Sesquithujene [*]	1.06 \pm 0.93 a	0.35 \pm 0.21 a	10.84 \pm 0.84 a	0.19 \pm 0.19 b	11.76 \pm 1.80 a	0.09 \pm 0.01 b
17.48	Sesquithujene	0.00 \pm 0.00 a	0.50 \pm 0.50 a	0.20 \pm 0.07 a	0.73 \pm 0.73 a	0.00 \pm 0.00 a	1.28 \pm 1.28 a
17.75	γ -Muuroolene	0.80 \pm 0.80 a	0.54 \pm 0.53 a	0.00 \pm 0.00 a	1.61 \pm 1.04 a	0.00 \pm 0.00 a	0.62 \pm 0.62 a
17.76	α -Cedrene [*]	1.50 \pm 0.21 a	2.53 \pm 0.65 a	0.00 \pm 0.00 a	8.12 \pm 3.36 b	0.00 \pm 0.00 a	10.76 \pm 3.07 a
17.78	(E)-Caryophyllene [†]	0.00 \pm 0.00 a	1.58 \pm 0.65 a	0.00 \pm 0.00 a	0.00 \pm 0.00 a	2.24 \pm 1.37 a	3.28 \pm 0.58 a
17.81	α -cis-Bergamotene	4.85 \pm 2.44 a	3.32 \pm 0.12 a	4.97 \pm 0.62 a	7.57 \pm 0.63 a	5.35 \pm 0.29 a	6.29 \pm 1.22 a
17.93	α -trans-Bergamotene	1.64 \pm 1.64 a	1.25 \pm 1.25 a	0.00 \pm 0.00 a	0.00 \pm 0.00 a	0.00 \pm 0.00 a	0.00 \pm 0.00 a
18.18	Sesquisabinene	4.45 \pm 1.85 a	3.37 \pm 1.62 a	2.82 \pm 1.31 a	13.33 \pm 3.67 b	3.35 \pm 0.84 a	11.19 \pm 6.40 b
18.15	(E)- β -Farnesene ^{†*}	0.16 \pm 0.12 a	24.65 \pm 4.59 b	8.08 \pm 0.03 a	12.17 \pm 7.60 a	10.10 \pm 0.06 a	12.39 \pm 5.49 a
18.30	(Z)- β -Farnesene	0.00 \pm 0.00 a	0.31 \pm 0.12 a	0.00 \pm 0.00 a	1.21 \pm 0.56 a	0.10 \pm 0.06 a	0.37 \pm 0.37 a
18.38	α -Zingiberene [*]	4.87 \pm 1.14 a	3.23 \pm 0.20 a	2.82 \pm 0.88 a	9.43 \pm 4.63 b	4.93 \pm 0.66 a	8.97 \pm 3.12 b
18.88	δ -Amorphene	2.93 \pm 1.24 a	0.56 \pm 0.56 a	0.00 \pm 0.00 a	1.85 \pm 1.14 a	2.25 \pm 2.07 a	0.64 \pm 0.64 a
18.89	β -Bisabolene [†]	8.55 \pm 1.14 a	10.04 \pm 4.14 a	2.44 \pm 1.00 a	5.08 \pm 2.48 a	0.68 \pm 0.48 a	0.00 \pm 0.00 a
19.03	β -Sesquiphellandrene	1.18 \pm 1.84 a	3.24 \pm 0.11 a	1.25 \pm 1.25 a	2.45 \pm 1.05 a	0.00 \pm 0.00 a	0.53 \pm 0.52 a
19.14	(E)- γ -Bisabolene	2.17 \pm 1.36 a	2.11 \pm 1.29 a	0.00 \pm 0.00 a	0.00 \pm 0.00 a	0.00 \pm 0.00 a	0.00 \pm 0.00 a
19.53	(E,Z)-Geranyl linalool	2.13 \pm 1.89 a	0.41 \pm 0.41 a	1.01 \pm 1.01 a	1.46 \pm 0.90 a	0.00 \pm 0.00 a	0.76 \pm 0.76 a

VOC identified using three databases of the ChemStation software; National Institute of Standards and Technology Mass Spectra database of 2008 (NIST), Adams2 (1995) and Chemoecol. The identifications were tentative and only based on spectra and retention times matches. Compounds with [†] were further identified by matching the retention time and mass spectra of the VOC with authentic standards injected under the same conditions as the samples. Mean relative amount of each compound is expressed as a percentage of the total emission, Regardless the species, all the volatile compounds recorded from the plants infested with 12 larvae were also revealed in the plants infested with 5 larvae. SE = standard error. Five replicates were carried out in each case. Only the compounds showing significant differences for $P < 0.05$ between 5 and 12 densities larvae feeding upon maize plants (Mann–Whitney U Test) are indicated by*.

Appendix 9: Results of GLM with binomial error distribution analysis comparing proportion of infested maize plants prior and after fall armyworm “invasion” in relation to rain seasons

Localities	Seasons	LR	Z-value	P-value
Makutano	Long rain season	3.97	1.987	0.040
	Short rain season	7.26	2.680	0.007
Murang’a	Long rain season	11.64	3.393	0.0006
	Short rain season	8.73	2.938	0.003

Appendix 10: Results of Generalized Linear Model (GLM) with binomial error distribution analysis comparing proportion of infested maize plants prior and after fall armyworm “invasion” relative to different developmental stages of maize plants.

Localities	Maize plant developmental stage	Likelihood Ratio (LR)	Z-value	P-value
Makutano	Pre-tasseling	18.116	4.207	<0.0001
	Reproduction	0.20095	0.448	0.654
	Senscence	0.0576	0.240	0.810
Murang’a	Pre-tasseling	16.886	4.081	0.0006
	Reproduction	0.80924	0.899	0.369
	Senscence	1.9855	1.405	0.160

Appendix 11: Publications and presentations from this thesis

Publications

Sokame B.M., Rebaudo F., Malusi P., Subramanian S., Kilalo D.C., Juma G., Calatayud P-A. (2020) Influence of temperature on the interaction for resource utilization between Fall Armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae), and a community of lepidopteran maize stemborers larvae. *Insects*, 11, 73. <https://doi.org/10.3390/insects11020073>

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