BIO-ECOLOGICAL STUDIES OF AMARANTHS LEPIDOPTERAN DEFOLIATORS AND DEVELOPMENT OF IPM TECHNOLOGIES FOR THEIR MANAGEMENT

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Bio-ecological studies of amaranth's lepidopteran defoliators and development of IPM technologies for their management

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DECLARATION

This Thesis is my original work and has not been submitted for a degree in any other University.

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To my late daughter Gloria Afi Agbodzavu, sorry for not being there for you

May your soul rest in eternal peace

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF APPENDICES

LIST OF ABREVIATIONS AND ACRONYMS

a.s.l	Above sea level
AIVs	African Indigenous Vegetables
ANOVA	Analyse of Variance
COI	Cytochrome c oxidase I
COII	Cytochrome c oxidase II
cyt b	Mitochondrial cytochrome b
DD	Degree days
DNA	Deoxyribonucleic acid
GPS	Global Position System
HORTINLEA	Horticultural Innovation and Learning for Improved Nutrition and Livelihood in East Africa
icipe	International Centre of Insect Physiology and Ecology
IPM	Integrated Pest Management
ITRA	Togolese Institute of Agronomic Research (*Institut Togolais de Recherche Agronomique*)
ITS	Internal transcribed spacer
JKUAT	Jomo Kenyatta University of Agriculture and Technology
KARLO	Kenya Agricultural, Livestock and Research Organization
L: D	Light-Darkness photoperiod proportion

LSD	Least Significant Difference
mt DNA	Mitochondrial DNA
NaClO	Sodium hypochlorite
NCBI	National Center of Biotechnology Information
PAA	Phenylacetaldehyde
PCoA	Principal Coordinates Analysis
PCR	Polymerase chain reaction
ppm	Particles per million
rDNA	Ribosomal DNA
RH	Relative humidity
RNA	Ribonucleic acid
rRNA	Ribosomal RNA
SE	Standard error
SNK	Student-Newman and Keuls
UK	United Kingdom
U.S.A	United States of America

ABSTRACT

One of the most important and largely consumed African Indigenous Vegetables (AIV) is amaranth. It is known to have a high nutritional value, agronomical assets and economic attributes. Amaranth production is however constrained by numerous biotic factors such as insect pests, occurring as a complex of species among which lepidopteran defoliators are found to be the most destructive. Investigations on their bio-ecological and control options were conducted. Field experiments were conducted from September 2015 to June 2017 in two agro-ecology zones (mid and high altitudes) in Kenya using two species of amaranth (*Amaranthus dubius* L. *and Amaranthus cruentus* L.). Results indicated that three lepidopteran species namely *Spoladea recurvalis* Fabricius (Lepidoptera: Crambidae), *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) and *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) were the most important. In mid-altitude, *S. recurvalis* was the most significant defoliator followed by the two other *Spodoptera* species while in high altitude *S. recurvalis* was of minor importance. Population dynamics studies revealed that *S. recurvalis* was more abundant from December to February where the mean infestation rate reached 24 larvae per plant and the incidence of 100 % during the outbreak. *Spodoptera exigua* and *S. littoralis* were present throughout the year with variable population levels. Leaf damages ranged from 0.85 to 62.45 % according to amaranth species, the site and the season. Tested attractants were neither able to significantly reduce the damage level nor induce significant catch in traps. A total of four indigenous parasitoids were found associated to *S. recurvalis* including *Apanteles hemara* (Hymenoptera: Braconidae), *Atropha tricolor* (Hymenoptera: Ichneumonidae), *Phanerotoma* sp. (Hymenoptera: Braconidae), and *Schoenlandella testacea* (Kriechbaumer) (Hymenoptera: Braconidae) while four others that were found associated with the *Spodoptera* species were *Chelonus curvimaculatus* Cameron (Hymenoptera: Braconidae), *Coccygidium luteum* Brulle (Hymenoptera: Braconidae), *Charops ater* Szepligeti (Hymenoptera: Ichneumonidae) and *Cotesia icipe* Fernandez-Triana & Fiaboe (Hymenoptera: Braconidae) which was a newly discovered species during the present work, with variable level of field parasitism rates. The results of laboratory experiments on the performance of the newly discovered parasitoid *C. icipe* showed a high potential for the use of this species. On *S. littoralis* a single female of *C. icipe* parasitized 42.99 ± 2.66 % of the 50 exposed larvae in 24 hours whereas a cohort of five females parasitized 85.59 ± 1.46 % of same host density. On *S. exigua* parasitism rates were 9.72 ± 0.76 % and 59.53 ± 3.1 % for a single and cohort of 5 females released respectively in the same conditions. Life history studies of *A. hemara* on *S. recurvalis* revealed that the parasitoid was a good candidate in the management of *S. recurvalis*. The temperatures range for its development was from 15 and 30°C. However, the total developmental time was affected by temperature. Female *A. hemara* developmental times were 50.5 ± 0.29, 21.78 ± 0.17, 12.88 ± 0.14 and 9.13 ± 0.06 days at 15, 20, 25 and 30 °C respectively. No pre-oviposition period was observed. Aiming at a successful biological control program for *S. recurvalis* in the sub-region, the genetic taxonomy studies were conducted using the cytochrome c oxidase subunit gene (COI). Results showed that populations of *S. recurvalis* from

Kenya and Tanzania as well as data available in the Genbank from Pakistan, Florida, India, Japan, Greece, Canada, China and Costa Rica, belong to the same species. This represents an important result that may have implications for a future biological program in the way that unlike the current finding, cryptic species usually lead to the failure of the management options.

Keywords: *Apanteles hemara*, biological control, *Cotesia icipe*, fecundity, genetic taxonomy, *Spoladea recurvalis*, *Spodoptera* spp..

CHAPTER ONE

GENERAL INTRODUCTION

1.1 Background of the study

African Indigenous Vegetables (AIVs) are composed of many vegetable species (Abukutsa-Onyango *et al.*, 2007). In East Africa particularly in Kenya and Tanzania, more than two hundred species are known to communities (Maundu, 1996; Lotter *et al.*, 2014). AIVs are among the most important vegetables produced and traded in rural, peri-urban and urban markets for underprivileged groups. One of the important and largely consumed AIVs is amaranth, because of the high nutritional value of some species that are important sources of food, as either vegetable or grain (Srivastava, 2011). The plant is also used for medicinal purposes (Kumar *et al.*, 2014).

Despite nutritional and medicinal values of this crop and the increasing importance of demand, crop yield and quality, especially those produced by smallholder farmers remain far below their potential due to numerous biotic constraints, especially insect pests (Banjo, 2007). Lepidopteran defoliators mainly leafworms (*Spodoptera littoralis* Boisduval (Noctuidae), *Spodoptera exigua* Hübner (Noctuidae)) and leaf webbers (*Spoladea recurvalis* Fabricius (Crambidae), *Udea ferrugalis* Hübner (Crambidae)) can lead to total crop losses.

The management of these lepidopteran defoliators is based mainly on the use of synthetic chemical insecticides with frequencies of up to twice a week which are generally not only inefficient but also detrimental to environmental, animal and human health (Clarke-Harris & Fleischer, 2003). The use of botanical extracts (Baskar *et al.*, 2011; Ladhari *et al.*, 2013), entomopathogen fungi (Anand & Tiwary, 2009; Asi *et al.*, 2013) and different parasitoids (Swezey, 1925; 1929; Narayanan *et al.*, 1957; Bhattacherjee & Ramdas Menon, 1964; Hafez *et al.*, 1980; Ruberson & Whitfield, 1996; Sertkaya *et al.*, 2004; Kedar & Kumaranag, 2013) have also been reported.

Direct use of these biocontrol agents has not apparently passed into practice, and no information is available on the parasitoids complex associated with the pest in East Africa as well as their potential role in regulating the population of lepidopteran defoliators. The objective of this study is, therefore, to assess the bio-ecology of the lepidopteran defoliators and develop an IPM package suitable for small scale amaranth farmers in East Africa.

1.2 Problem statement

Amaranths are reported to be the second most economically important AIV in East Africa after leafy cowpea. However, this crop is facing numerous biotic constraints affecting the crop yield and quality especially insect pests. A recent countrywide survey carried out on amaranths in Kenya and Tanzania during the two rainy seasons of the year 2014 in the context of the HORTINLEA (Horticultural Innovation and Learning for Improved Nutrition and Livelihood in East Africa) project, has revealed the presence of different species of lepidopteran defoliators such as *S. littoralis*, *S. exigua* (leafworms), *S. recurvalis* and *U. ferrugalis* (leaf webbers) (Mureithi *et al.*, 2015). Different levels of yield losses due to this complex of lepidopteran species have been reported. Kahuthia-Gathu (2011) reported up to 100% of yield loss due to *S. recurvalis* alone while Othim *et al.* (2018a) reported 54.81 % of yield loss when screening different lines of amaranth. Management of these pests has been through the use of chemical pesticides which are inefficient. Moreover, their inefficiency, their negative impact on the environment and their accessibility by small-scale farmers is also a concern.

Currently, scanty information only exists regarding the seasonal dynamics of these lepidopteran defoliators and the performance of their associated natural enemies in East Africa. Similarly, no information on the effect of agro-ecological conditions and farming practices on these pests exist. Also, no data regarding the effect of temperatures on the development of *Apanteles hemara* Nixon (Hymenoptera: Braconidae), a parasitoid of *S. recurvalis* as well as the genetic diversity of *S. recurvalis* from the region is available. Furthermore, no integrated management approach has been developed against these lepidopteran defoliators. There is,

therefore, a need to document the agro-ecology of these pests and develop an adequate IPM strategy to tackle these lepidopteran defoliators, since the demand of AIVs has constantly been rising and its production is providing food and nutritional security as well as a sustainable income generation while closing the gender gap.

1.3 Justification

Different studies have shown that leafworms and leaf webbers can be controlled in various parts of the world by various natural enemies such as the larval parasitoids *M. rufiventris*, *H. didymator, A. delhiensis*, *A. ruficrus* and the egg-larval parasitoid *C. inanitus* (Hafez *et al.*, 1980; Ruberson & Whitfield, 1996; Sertkaya *et al.*, 2004). However, no information exists on the parasitoid complex associated with these lepidopterans in East Africa. In addition, different attractants mainly Phenylacetaladehyde (Landolt *et al.*, 2011; Landolt *et al.*, 2013) and sex pheromones (Deng *et al.*, 2004) have been reported to attract various lepidopteran defoliators in other parts of the world but nothing is known about their effect on the lepidopteran defoliators in East Africa and their potential use in attract and kill technologies against the pests. In order to set up eco-friendly management approaches, basic information on the biology and ecology of the pests and their associated natural enemies and genetic data of the pests are needed to lay a solid foundation for developing efficient integrated pest management (IPM) strategies against the amaranth pests, with particular focus on lepidopteran defoliators.

1.4 Research questions

The main research questions of this study are:

1. What is the effect of season, agro-ecology and attractants on the population dynamics of amaranth lepidopteran pests and their damages, and their associated natural enemies?
2. What is the host range of *Cotesia icipe* Fernández-Triana & Fiaboe (Hymenoptera: Braconidae) within the complex of amaranth lepidopterans and its performances on *S. littoralis*?

3. What is the performance of *C. icipe* on *S. exigua* in terms of acceptability and suitability?
4. What is the effect of temperatures on the development, the survival and the reproduction of *A. hemara* on *S. recurvalis*?
5. What is the genetic difference between Kenyan and Tanzanian populations of *S. recurvalis* in one hand and with populations from other countries on another hand?

1.5 Research hypotheses

1. Lepidopteran defoliators, their damages and their associated parasitoids performance in the field are not affected by agro-ecology, seasonality and attractants.
2. There is no host for *C. icipe* within the commonly found amaranth lepidopterans in Kenya, and it is not effective in controlling *S. littoralis*.
3. *Cotesia icipe* is not efficient for biological control of *S. exigua*.
4. Temperature has no effect on the development, survival and reproduction of *Apanteles hemara* on *S. recurvalis*.
5. There is no genetic difference between Kenyan and Tanzanian populations of *S. recurvalis* in one hand and with populations from other countries on another hand.

1.6 Research objectives

1.6.1 General objective

The goal of the present study is to elucidate the bio-ecology of amaranth Lepidopteran defoliators and develop an IPM package suitable for small-scale amaranth farmers in East Africa.

1.6.2 Specific objectives

1. To determine the influence of climate and attractants on amaranth lepidopteran pests, their host and natural enemies.
2. To assess the host range of *C. icipe* on commonly found amaranth lepidopteran defoliators in Kenya, *S. littoralis*, *S. exigua*, *H. bipunctalis*, *S. recurvalis* and *U. ferrugalis*, as well as its effectiveness as a biological control agent of *S. littoralis*.
3. To assess the performance of *C. icipe* on *S. exigua* in terms of acceptability, aggressiveness and suitability.
4. To study the effect of different temperatures on the development, the survival and the reproduction of *Apanteles hemara* on *Spoladea recurvalis*.
5. To study the genetic taxonomy of *Spoladea recurvalis* from Kenya and Tanzania.

CHAPTER TWO

LITERATURE REVIEW

2.1 Origin, taxonomy and distribution of Amaranth species

Most of amaranth species originated in Western, Central and South America (Tubene & Myers, 2008). It is one of the oldest food crops in the world with evidence of cultivation dating back to over 6000 years in Puebla, Mexico (Iturbide & Gispert, 1994). The genus *Amaranthus* comprises about 60 - 70 species among which, 40 are native to America. Among the 70 species, 17 are vegetable amaranths with edible leaves, and three are grain amaranths with edible seeds (Xu & Sun, 2001; Grubben & Denton, 2004; Ebert *et al.*, 2011) while the remaining species are ornamental plants and others are weeds (Anon, 1984).

Grain amaranth is not commonly cultivated in Africa (Grubben & Denton, 2004). More recently, however, a few farmers have taken the growing of grain amaranth more seriously and are supplying millers and supermarkets in Zimbabwe, Kenya, Uganda and Ethiopia (Achigan-Dako *et al.*, 2014). Leafy vegetable amaranths are plants of African, Southeast Asian, and Central American origin, which include species such as *Amaranthus tricolor*, *A. lividus*, *A. dubius*, *A. gangeticus*, *A. blitum*, and *A. hybridus* (Tubene & Myers, 2008) probably because leafy vegetables are cheap and readily affordable to many low-income communities in rural, peri-urban and urban areas.

Amaranthus species are widely distributed throughout the tropical and sub-tropical region of the world (Anon, 1984). In Sub-Saharan Africa, both introduced (10 species), and native species (8 species) (Brenan, 1981; Glen, 2002) exist. Introduced species come through germplasm collection from Indian, China, Central and South American. Cultivated form of various species are grown in India, Malaysia, Indonesia, Burnea, Philippines, Taiwan, China, South Pacific, Tropical and southern Africa, the Carribean, Central and southern America and in the tropics generally, Greece, Italia and Russia (Venter *et al.*, 2007). Table 2.1 resumes uses and areas of origin of *Amaranthus* species.

Table 2.1: Uses and areas of origin of *Amaranthus* species

Species	Use	Area of origin
Amaranthus blitum (*A. lividus*, *A. oleraceus*)	Vegetable, ornamental	Asia
Amaranthus caudatus (*A. edulis*, *A. mategazzianus*)	Grain, vegetable, ornamental	South America (Andes)
Amaranthus cruentus (*A. paniculatus*)	Grain, vegetable	Central America (Guatemala)
Amaranthus dubius	Vegetable	South America
Amaranthus hybridus	Vegetable	South America
Amaranthus hypochondriacus (*A. leucocarpus*, *A. leucosparma*, *A. flavus*)	Grain, vegetable	Central America (Mexico)
Amaranthus retroflexus	Vegetable	North America
Amaranthus spinosus	Vegetable	Asia
Amaranthus tricolor (*A. gangeticus*, *A. mangostanus*)	Vegetable, ornamental	Asia
A. viridis (*A. ascendens*, *A. gracilis*)	Vegetable	Africa

Source: Grosz-Heilman *et al.* (1990)

According to Das (2014) amaranth species can be classified into three categories, which represent more or less use-groups: (i) vegetable *Amaranthus* that includes for instance *Amaranthus tricolor* var. tricolor, *Amaranthus tricolor* var. *tristis*; (ii) grain *Amaranthus* which *includes Amaranthus hypochondriacus*, *A. caudatus*, *A. cruentus*; and (iii) weed Amaranthus with members such as *Amaranthus spinosus*, *A. viridis*, *A. retroflexus*, *A. graecizans*, *A. dubius*, and *A. hybridus*.

2.2 Nutritional, medicinal and economic importance of amaranth vegetable

Most of the amaranth species are harvested in the wild as a food resource. Only some species are grown for their leaves and their seeds commonly found in tropical markets of Africa (Wu *et al.*, 2000). The seed of grain amaranth is important for its

relatively high nutritive value especially protein content compared to major food grains like maize, wheat, oat, barley and rye (Luis, 1992). Depending on cultivation conditions and properties of the species, this crop is used as food, fodder, medicinal, and ornamental plant in many countries (Yudina *et al.*, 2005). Amaranth foliage is an excellent source of bio-available iron, up to 57 ppm and vitamin A, averaging 250 ppm (Rangarajan & Kelly, 1994), calcium, magnesium and zinc (Kamga *et al.*, 2013). It is also a high source of protein (Segura-Nieto *et al.*, 1994).

Amaranth plant is used as febrifuge, antipyretic, laxative and diuretic. Besides its culinary value, it is used for treating nausea, appetiser, biliousness, galactagogue, haematinic, stomachic, flatulence, anorexia, blood diseases, burning sensation, leucorrhoea, leprosy and piles (Kumar *et al.*, 2014). Phytochemical investigations prove that it is a rich source of alkaloids, flavonoids, glycosides, phenolic acids, steroids, amino acids, terpenoids, lipids, saponin, betalain, b-sitosterol, stigmasterol, linoleic acid, rutin, catechuic tannins and carotenoids (Akubugwo *et al.*, 2007; Mensah *et al.*, 2008). In Malaysia, *A. spinosus* is used as an expectorant and to relieve breathing in acute bronchitis. Some tribes in India apply *A. spinosus* to induce abortion (Grubben & Denton, 2004).

In Kenya and Uganda where there is an expanding middle class and a growth in urban market demand, the commercial production of indigenous vegetables such as *Solanum nigrum*, *Cleome gynandra* and *Amaranthus* spp. is also increasing through the efforts of commercial producers and through direct links to supermarkets who are beginning to offer contracts to supply indigenous vegetables to them (Abukutsa-Onyango *et al.*, 2007). In Kenya, Abukutsa-Onyago (2003) showed that AIVs offer a significant opportunity for the poor people in western Kenya to earn a living because indigenous leaf vegetable production can be done with little capital investment.

2.3 Insect pests of amaranth vegetable

Many insects' species belonging to different orders are found on amaranth. They are mainly lepidopterans, hemipterans, coleopterans and orthopterans (Kagali *et al.*, 2013). However, lepidopterans are the most devastating of leaves. Based on this reason, the study will be focused on them.

2.3.1 Bio-ecology of amaranth lepidopteran defoliators

Bio-ecology is the study of the relationships between organisms and their environment; it is also defined as the science that deals with the interrelations of communities of animals and plants with their environment. Bio-ecology is interested in studying the diversity, distribution, a number of organisms, as well as competition between them within and among ecosystems.

2.3.1.1 Spodoptera littoralis

Spodoptera littoralis larvae damage many agricultural plants. The species is very polyphagous. It attacks plants belonging to 44 different families including grasses, legumes, crucifers and deciduous fruit trees. Adults feed on nectar, and females oviposit on the leaves of plants. Depending on the climate of the region, *S. littoralis* can have from two to seven generations per year and does not undergo diapause (Salem & Salama, 1985). Adult moths are capable of long-distance dispersal flights. Between two and five days after emergence, *S. littoralis* females lay 1000-2000 eggs in egg masses of 100-300 on the lower leaf surface of the plant. The eggs hatch in about four days in warm conditions. The larvae pass through six instars in 15-23 days at 25-26°C. The pupal period is about 11-13 days at 25°C. The longevity of adults is about 4-10 days (Brown & Dewhurst, 1975).

Damage of *S. littoralis* consists of feeding scars and skeletonizing caused by feeding on the undersides of the leaves (Plate 2.1). On newly infested hosts, young larvae feed at numerous small feeding points that eventually spread over the entire leaf. Older instars chew large holes or wholly consume leaves (Sullivan, 2014).

Plate 2.1: Damages of *Spodoptera littoralis* on *Amaranthus dubius* under laboratory conditions

Photo: M. K. Agbodzavu

2.3.1.2 *Spodoptera exigua*

As *S. littoralis*, *S. exigua* is polyphagous. It feeds on wide host range including soybean, sugar beet, cabbage, cauliflower, brussel sprouts, tomato, maize, cotton, lettuce, peanut, alfalfa, shallot, pastures crops, and various wild hosts (Abdullah *et al.*, 2000). Seasonal activity varies considerably according to climate. In warm

locations, all stages can be found throughout the year, although the development rate and overall abundance are reduced during the winter months (Tingle & Mitchell, 1977). Eggs of *S. exigua* are laid in clusters of 50 to 150 eggs per mass. Eggs hatch in two to three days during warm weather. There are usually five instars (Capinera, 1999) lasting for 9.7 days at a constant 30°C (Fye & McAda, 1972). Pupation occurs in the soil. Duration of the pupal stage is six to seven days during warm weather. Mating occurs soon after the emergence of the moths, and oviposition begins within two to three days (Capinera, 1999). Larvae feed on both foliage and fruit. Young larvae feed gregariously and skeletonize foliage. As they mature, larvae become solitary and eat large irregular holes in foliage (Plate 2.2) (East *et al.*, 1989).

Plate 2.2: Damages of *Spodoptera exigua* on *Amaranthus dubius* in field

Photo: M. K. Agbodzavu

2.3.1.3 *Spoladea (Hymenia) recurvalis*

The Beet webworm (*S. recurvalis*) is a cosmopolitan pest of tropical and subtropical regions, but in more recent times has been reported from more temperate countries including Belgium and Denmark (Bailey, 2007). Pulse crops attacked include adzuki beans, mung beans, navy beans and soy beans. Other crops attacked include silverbeet and beetroot (*Beta vulgaris*), *Trianthema postulacastrum*, cockscomb (*Celosia* spp.), goosefoot (*Chenopodium* spp.), *Portulaca* spp., and *Amaranthus* spp. (Capinera, 2001). In Hawaii, *S. recurvalis* is active throughout the year, and about ten generations occur annually (Capinera, 2001). It cannot survive winter because they have no hibernation stage and are less tolerant of cold temperatures (Miyahara, 1991). Its oviposition commences three days after mating. Each female has oviposition period of 3 days and a fecundity rate of 224.90 eggs. Eclosion occurs at 4.2 days after oviposition and the mean duration of larval instars is 22.1 days; pre-pupa (2 days) and pupa (9.7days). The total developmental period averaged 32.7 days (Seham *et al.*, 2006).

The caterpillar rolls the leaf into distinctive leaf shelter and voraciously feed on the green matter. Severe attack results in complete skeletonisation and drying up of the leaves within a short time (Plate 2.3).

Plate 2.3: Damages of *Spoladea recurvalis* on *Amaranthus dubius* under field conditions; Photo: M. K. Agbodzavu

Other synonyms of *Spoladea recurvalis*

- *Phalaena recurvalis* Fabricius, *1794*
- *Hydrocampa albifacialis* Boisduval, *1833*
- *Hymenia diffascialis* Hübner, 1825
- *Hymenia exodias* Meyrick, 1904
- *Nacoleia ancylosema* Dognin, 1909
- *Odezia Hecate var. formosana* Shiraki, 1910
- *Phalaena angustalis* Fabricius, 1787
- *Pyralis fascialis* Stoll, 1782
- *Phycis recurvella* Zincken, 1818
- *Spoladea animalis* Guenée, 1854
- *Zinckenia fascialis* (Cramer)
- *Zinckenia recurvalis* Fabricius

2.3.2 Management of Lepidopteran defoliators

2.3.2.1 Chemical control

Studies have shown that *S. littoralis* and *S. exigua* have developed resistance to organophosphorus, synthetic pyrethroid and other insecticides with the appearance of cross-resistance in many cases (Cobb & Bass, 1975; Abo-El-Ghar *et al.*, 1986; Zhou *et al.*, 2011). Indiscriminate use of insecticides is disruptive to naturally occurring biological control agents, leading to outbreaks and also detrimental to environmental, animal and human health. This awareness has therefore triggered a strong interest in looking for an alternative strategy of control which is eco-friendly, sustainable and cost-effective.

2.3.2.2 Cultural and interference methods

Alternative host plant management is very important in insect pest management because these plants play an important role in population increases and outbreaks of cosmopolitan polyphagous insect pest. Despite the existence of various reports on host plants of these lepidopteran defoliators in various countries over the World (Saeed *et al.*, 2010; Mehrkhou *et al.*, 2015), there is a conspicuous lack of sound data on this ecological dimension of these pests in Kenya where nothing was so far done. Also, pheromone and phenylacetaldehyde-based attractants have been developed and used in monitoring, mass trapping and sexual communication disruption of lepidopteran defoliators (Wakamura *et al.*, 1989; Landolt *et al.*, 2011). However, there is no information related to the efficacy of such a design on the East African population of leafworms and leaf webbers and on how this efficacy would vary according to different agro-ecology and season. This study, therefore, comes to fill this gap of knowledge which is crucial for the development of a sustainable management strategy.

2.3.2.3 Biological control

Different parasitoids have been reported on *S. littoralis* and *S. exigua*. *Microplitis rufiventris* Kokujev (Hymenoptera: Braconidae) can attack and develop on earlier instars of *S. littoralis* larvae (late first to third) (Hegazi *et al.*, 1991). The egg-larval parasitoid *Chelonus inanitus* L. (Hymenoptera: Braconidae) induces in *S littoralis* two major developmental effects, namely a precocious onset of metamorphosis followed by a developmental arrest in the prepupal stage (Kaeslin *et al.*, 2005) and reduction in food consumption of the parasitized larvae (Morales *et al.*, 2007). Another parasitoid of *S. littoralis* is *Hyposoter didymator* (Thunberg) (Hymenoptera: Ichneumonidae), a solitary endoparasitoid (Ingram, 1981; Morales *et al.*, 2007). On *S. exigua*, the braconid larval parasitoids *M. rufiventris* and *Meteorus ictericus* Nees; the egg-larval parasitoid *Chelonus obscuratus* Herrich Schäffer (Hymenoptera: Braconidae); the gregarious braconid larval parasitoid *Apanteles ruficrus* Haliday were reported (Sertkaya *et al.*, 2004).

The larva of *S. recurvalis* has also been reported to be parasitized under field condition by *Apanteles delhiensis* and *Apanteles hemara* (Nayar *et al.*, 1976; Peter & Balasubramanian, 1984).

It is important to note that most of these studies on parasitoids have been carried on "major crops" such as cotton, tobacco, soybeans, tea, maize etc. Little has been done regarding parasitoids associated with these Lepidopteran defoliators on indigenous vegetables especially on amaranth in East Africa except the work reported by Kagali *et al.* (2013) and Kahuthia-Gathu (2011) which were basic on the matter. It is therefore important to document and promote their use.

In term of entomopathogens utilization, treatment with *Lecanicillium lecanii, Isaria fumosorosea* (formerly *Paecilomyces fumosoroseus*), *Nomuraea rileyi, Bacillus thuringiensis, Trichoderma harzianum* and *Metarhizium anisopliae* has been used on *S. littoralis* and *S. exigua* (El-Hawary & Ame, 2009; Meshrif *et al.*, 2011; Han *et al.*, 2014). On *S. littoralis*, it was shown that more than 80% of larval or pupal mortality could be obtained depending on the pathogen species and the conidial concentration. On *S. exigua*, both *M. anisopliae* FT83 and *P. fumosoroseus* FG340 effectively controlled the moth at 20-30°C. In work carried by Jung & Kim (2006), they have shown that *Xenorhabdus* sp. or *Photorhabdus temperate* can be applied to kill *S. exigua* by oral treatment in a mixture with *B. thuringiensis*. On *S. recurvalis*, two entomopathogenic fungi *Isaria farinosa* (formerly *Paecilomyces farinosus*) and *Fusarium monilliformae* var. *subglutinans*, were assayed against larvae in the laboratory and the results indicated potential for *Paecilomyces* as a biological control agent (Kuruvilla & Jacob, 1980). Also, *B. thuringiensis* subsp. kurstaki has been tested and showed high mortality against third-instar larvae (Gangwar et al., 1980). However, direct use of these biocontrol agents to control *S. littoralis* and other lepidopteran defoliators has not apparently passed into practice extensively, except for *B. thuringiensis* for which commercial formulation exist (Kamel *et al.*, 2010); also, many studies regarding entomopathogen fungi use have been done separately and not in an integrated approach with other control practices. This study therefore, not only will document the potential use of parasitoids in amaranth lepidopteran defoliators' management, but also evaluate their use in an integrated approach;

integrated pest management being defined as selecting, integrating, and implementing complimentary pest management tactics to maintain pests at economically acceptable levels while minimizing negative ecological and social impacts of pest management activities (Gent *et al.*, 2009).

2.4 Contribution of molecular tools to integrated pest management

2.4.1 Accurate species, subspecies and populations identification

Precise identification of insect pests and their associated natural enemies has long been recognized as an essential first step in developing successful biological control programs; and lack of adequate identification approaches can lead to failure of biological control programs (Rosen, 1986; Gariepy *et al.*, 2007). Nowadays, many cryptic insect species cannot be identified with the conventional morphological features, and there is a need to support pest and natural enemies' identification with integrated molecular approaches. For example, some cryptic insect species such as the encyrtid endoparasitoid *Ageniaspis citricola* Logvinovskaya (obtained from Australia and Taiwan) (Hoy *et al.*, 2000; Alvarez & Hoy, 2002), the western flower thrips *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) populations across California (Rugman-Jones *et al.*, 2010) and *Rhynchophorus ferrugineus* (Olivier) (Coleoptera: Curculionidae) were previously considered like single species but which in fact are not (Rugman-Jones *et al.*, 2013). That fact could only be revealed through the use of molecular tools. Also, some cases of biological control programs failure due to the use of the wrong strain of natural enemies are reported in Stiling (1993): *Apanteles glomeratus* (Hymenoptera: Braconidae) failed to control *Pieris rapae* (Lepidoptera: Pieridae) in New Zeeland, *Lixophaga diatraeae* (Diptrea: Tachnidae) failed to manage *Chilo sacchariphagus* (Lepidoptera: Crambidae) in Madagascar and *Bessa harveyi* (Diptrea: Tachnidae) couldn't control *Pristiphora erichsonii* (Hymenoptera: Tenthredinidae) in Western Canada. Relevant information on pest identity, therefore, a prerequisite for pest management.

2.4.2 Molecular marker used for genetic taxonomy studies in insect species

Molecular markers are useful for identifying or distinguishing taxa that are not well-studied, very small, members of cryptic species complexes (Tixier *et al.*, 2006; Smith *et al.*, 2008), and which are different geographical populations of the same species (Wei *et al.*, 2013; Chen *et al.*, 2014). However, part of the challenge of finding suitable genetic markers for ecological research involves identifying which regions of the genome have levels of variability that are appropriate to the questions being addressed (Freeland, 2005). Different genes have been used to evaluate the genetic divergence of closely related insect species. The taxonomic level at which specific genes or nucleotide regions are useful varies across taxa (Alvarez & Hoy, 2002). The two regions most often targeted for sequencing in insect systematic are mitochondrial DNA (mtDNA) and nuclear ribosomal DNA (rDNA) (Caterino *et al.*, 2000). For mtDNA, the most commonly used genes include cytochrome oxidase I and II (COI, COII), the 16S and 12S subunits of rDNA. Besides, when sequencing nuclear rDNA, the 18S and 28S subunits of rRNA and the first and second internal transcribed spacer regions (ITS 1 and ITS 2) are commonly used (Gariepy *et al.*, 2007). In the current study, the mitochondrial DNA gene (COI) was used to confirm the specific identity of *S. recurvalis* populations from Kenya and Tanzania.

CHAPTER THREE

INFLUENCE OF CLIMATE AND ATTRACTANTS ON AMARANTH LEPIDOPTERAN PESTS, THEIR HOST AND NATURAL ENEMIES

Abstract

Field experiments were conducted in two agro-ecology zones (mid and high altitudes) in Kenya using two species of amaranth (*Amaranthus dubius and A. cruentus*) for five consecutive seasons. The objective was to study the diversity, seasonality and damage due to lepidopteran defoliators on amaranth, their associated natural enemies as well as the effect of farming practices on damage reduction. Three lepidopteran species were found to be most important which included, *Spoladea recurvalis*, *Spodoptera exigua* and *Spodoptera littoralis*. In mid-altitude, *Spoladea recurvalis* was the most significant defoliator followed by the two other *Spodoptera* species while in high altitude, *S. recurvalis* was of minor importance. Population dynamics studies revealed that *S. recurvalis* was more abundant from December to February where the mean infestation rate reached 24 larvae per plant during the outbreak. *Spodoptera exigua* was present throughout the year, unlike *S. littoralis*. Leaf damages varied according to the amaranth species, the season and the site with the highest damages (62.45 %) recorded on *A. dubius* during the first season in Thika. Tested attractants (Phenylacetaldehyde, *S. exigua* and *S. recurvalis* pheromones) were neither able to reduce the damage level significantly nor induce significant catch in traps. A total of four indigenous parasitoids were found associated to *S. recurvalis* namely *Apanteles hemara*, *Atropha tricolor*, *Phanerotoma* sp., and *Schoenlandella testacea* while four others were found associated with the *Spodoptera* species: *Chelonus curvimaculatus*, *Coccygidium luteum*, *Charops ater* Szepligeti and *Cotesia icipe* which was a newly discovered species during the present work, with variable level of field parasitism rates. This work set the baseline for developing an integrated management approach against the major amaranth lepidopteran defoliators.

Keywords: Attractants, *Cotesia icipe*, damages, leaf webbers, parasitism, population dynamics.

3.1 Introduction

Amaranth is one of the most commercialized indigenous vegetables in Africa. It is exploited economically as a vegetable, grain and ornamental. It is a multiple purposes crop which is used as food for human consumption and feed for animal consumption (Mlakar *et al.*, 2009), and has medicinal values (Akubugwo *et al.*, 2007; Mensah *et al.*, 2008; Kumar *et al.*, 2014). Furthermore, amaranth species are important sources of nutrients in the African diet, because they are excellent sources of vitamins A, B complex, C, E, iron and calcium (Abukutsa-Onyango, 2014). Agronomic advantages reported on amaranth species include short growth period, the ability to produce seed under tropical conditions, respond well to organic fertilizers and can tolerate both biotic and abiotic stress (Maundu, 1997; Abukutsa-Onyango, 2002).

However, despite nutritional and medicinal values of these crops and the increasing importance of demand, crop yield and quality, especially those produced by smallholder farmers remain far below their potential due to numerous biotic constraints (Gokowski & Ndumbe, 1997; Schippers, 2000; Ebert *et al.*, 2011). One of the greatest limiting factors in increasing the productivity of amaranths is insect pests (Gokowski & Ndumbe, 1997; Schippers, 2000; Banjo, 2007) especially lepidopteran defoliators (Kahuthia-Gathu, 2011).

Many species of lepidopteran defoliators have been reported on Amaranth in Kenya: *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae)., *S. exigua* (Boisduval) (Lepidoptera: Noctuidae)., *S recurvalis* Fabricius (Crambidae), *Udea ferrugalis* Hübner (Crambidae) (Kahuthia-Gathu, 2011; Mureithi *et al.*, 2015) and *Herpetogramma bipunctalis* (Lepidoptera: Crambidae) (Kagali *et al.*, 2013). *Spoladea recurvalis* larvae feed on the epidermis and the palisade tissues of the leaves when they are young but later on roll up a leaf or fasten two or more leaves together with the help of silken threads they produce and feed from the margin, remaining hidden underneath (Bhattacherjee & Ramdas Menon, 1964; Pande, 1972). Leaf damage caused by *S. recurvalis* larvae decreases the quality of vegetable crops, making them less marketable and leading to economic loss. Complete leaf

skeletonization occurs in severe infestations (Chang & Ramasamy, 2016). The same behaviour was observed for *U. ferrugalis* and *H. bipunctalis* which are also leaf webbers. On newly infested hosts, *S. littoralis* young larvae feed at numerous small feeding points that eventually spread over the entire leaf. Older instars chew large holes or wholly consume leaves (Sullivan, 2014). Young larvae of *S. exigua* feed gregariously and skeletonize foliage. As they mature, larvae become solitary and consume large irregular holes in foliage (East *et al.*, 1989). Currently, major knowledge gaps exist on the diversity and seasonal dynamics of these lepidopteran defoliators and their associated natural enemies in East Africa, the effect of agro-ecological conditions and farming practices on these pests. Information about the relative importance of key pest species in a given area is the prerequisite for priority setting in the development of integrated pest management strategies (Coppel & Mertins, 1977; Ulrichs & Mewis, 2003). There is, therefore, a strong need for documenting the agro-ecology of these pests. The objective of this paper was to determine the influence of climate and attractants on amaranth pests, their host and natural enemies.

3.2 Materials and methods

3.2.1 Survey for amaranth Lepidopteran defoliators and their associated parasitoids in farmers' fields

Surveys were carreied out in farmers' fields in two agro-ecological zones of Kenya based on altitude: mid-altitude (1,000 - 1,800 m a.s.l) and high altitude (>1,800 m a.s.l) (Hassan, 1998; Makokha *et al.*, 2001) to get data on amaranth lepidopteran defoliators and their associated parasitoids richness. The surveys were carried out in March and April 2015 representing the long rainy season; in July and August 2015 representing the dry cool season, in February 2016 representing the dry hot season and in October and November 2016 for the short rainy season. Areas covered were Kitengela (mid-altitude 1540-1545 m a.s.l), Mwea (mid-altitude 1172 -1210 m a.s.l), Yatta (mid-altitude 1173-1184 m a.s.l), Limuru (high altitude 1881-1912 m a.s.l) and Kimende (high altitude 2243 m a.s.l). The latitude, longitude, and elevation of each study site were determined with a GARMIN eTrex 12 portable Global Positioning

System (GPS) gadget (Garmin International, Inc. Kansas, U.S.A). During each farm visit, amaranth leaves showing lepidopteran damages were picked randomly and placed in plastic boxes (15 cm × 7 cm × 5 cm) lined with a paper towel to absorb excessive humidity. An opening was made on the top of the plastic boxes and closed with a fine mesh to allow ventilation. Plastic boxes were kept in a cooler box for transportation into the laboratory at *icipe* where they were kept at 25 ± 2°C, 75 ± 5% R.H and a photoperiod of 12L: 12D hours. Collected larvae were fed with amaranth leaves daily. All emerged lepidopteran species, and parasitoids were recorded. Lepidopteran species identification was done using voucher specimens previously identified at the National Museum of Kenyan. Parasitoids were identified at the Natural History Museum, UK and Canadian National Collection of Insects, Canada.

3.2.2 Field experiments

3.2.2.1 Study sites

Experimental plots were set-up in two agro-ecological zones of Kenya as indicated in section 4.2.1. At mid-altitude, experiments were conducted at KARLO Kandara in Muranga county (1508 m; 01° 00.1617'S; 037° 04.715'E) and at high altitude at KARLO Tigoni (2114 m; 01° 08.9587'S; 036° 41.0171'E) in Kiambu County. The two agro-ecological zones are characterised by a bimodal distribution of rains which are highly variable in timing, duration and intensity. In Thika, the weather is generally warm. Precipitation averages 840 mm and the driest month is usually July. The highest amount of precipitation occurs in April. The average annual temperature is 19.8 °C. March is the warmest month with an average of 21.3 °C. The lowest average temperatures in the year occur in July when it is around 17.8 °C (http://en.climate-data.org/location/5812/ of 20 May 2016). In Tigoni, the average annual rainfall is 1096 mm. Long rains season occurs between March and May while short rains season is between October and December (Jaetzold *et al.*, 2006). The mean annual air temperature is 18°C and ranges between 12 and 24°C (Muthoni & Kabira, 2011). The soils at Thika are rhodic nitisols (Adamtey *et al.*, 2016). They have a clay texture and show gradual to diffuse soil horizon boundaries. The colour is often dark red, dusky red or dark reddish brown. The soils are friable or very

friable and are porous throughout. They have marked structure stability. The chemical properties of these soils vary widely. The organic matter content, cation exchange capacity (CEC) and percentage base saturation range from low to high. These soils are known to have a high degree of phosphorus sorption (NAAIAP & KARI, 2014). The soil type at KARLO Tigoni is nitisol which is inherently acidic (Jaetzold *et al.*, 2006; Muthoni, 2016). Soils are well drained, shallow, dark reddish brown, and moderately fertile (Makokha *et al.*, 2001).

3.2.2.2 Nursery raising, transplanting and plots management

Seedlings were established on-site using the two amaranth species. At 4-6 weeks old, the seedlings were transplanted in the field at the rate of one plant per hole in rows spaced at 50 cm and 40 cm within rows. Manure was applied on the seedlings' beds at the rate of 1 kg per square meter and 200-250g per planting hole. Plots were under irrigation and were watered twice per week at Tigoni and thrice at Thika. Gapping was done one week after transplanting; the first weeding was done two weeks after and repeated one month later. No pesticide was applied.

The first season ran from September 2015 to March 2016, the second season from March 2016 to July 2016, the third season from July 2016 to December 2016, the fourth season from December 2016 to March 2017 and the fifth season from April 2017 to June 2017. During the first season, data were collected up to December, and *A. dubius* were pruned to rejuvenate it because of the sudden outbreak of *S. recurvalis* when the crops were already old. Data collection was continued up to March 2016. For the following seasons, the half of each subplot were uprooted (5 m x 10 m) and transplanted with new seedlings while still collecting data on the previous season crops for one more month after which the data collection was shifted onto the new crop.

3.2.2.3 Experimental layout and treatments

Two generally most cultivated leafy amaranth species in Kenya (broad and narrow leaves) were used in this experiment. The broad-leaved (*Amaranthus dubius* Mart. ex Thell., 1912) commercially termed "Dubious" was purchased from Simlaw Seeds

Company Ltd, Kenya and the narrow-leaved *Amaranthus cruentus* (Nees, 1834), "Terere" was purchased from East African Seed Co. Ltd, Kenya. At each site, three plots (referred to as main plots) were selected distant of 50 m from each other. The dimensions of each main plot were 40 m x 25 m. Each main plot was split into six subplots (experimental units), and each amaranth species was randomly allocated to three subplots within a main plot; a subplot corresponded therefore to a replicate. Dimensions of each subplot were 10 m x 10 m and separated by 5 m within a main plot. The experiment was designed as a split plot with attractants and amaranth species as first and second factors respectively (Figure 3.1). The factor attractants had three levels: Phenylacetaldehyde (PAA), pheromones and control (without attractant) set in traps and was assigned to the main plots; the factor amaranth species had two levels as stated above and was assigned to the subplots. Completely randomized designed (CRD) was then used at the main plots level and randomized complete block design was used at the subplots level.

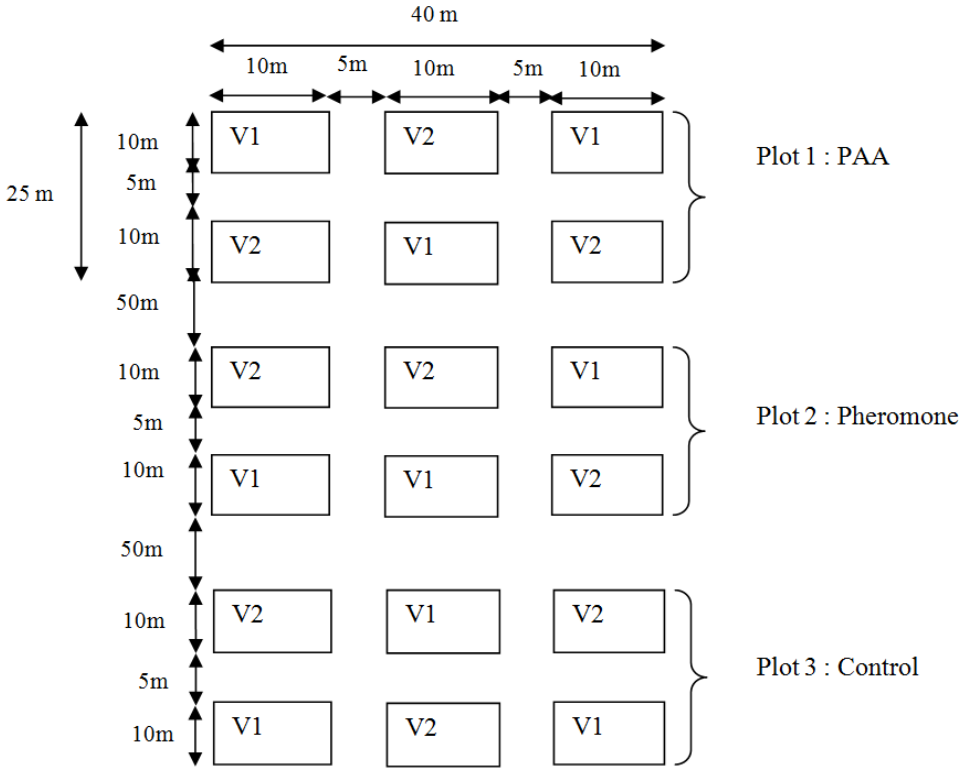

Plate 3.1: Schematic representation of the experimental design

V1: *Amaranthus dubius;* V2*: Amaranthus cruentus*, PAA: Phenylacetaldehyde

Standard plastic universal moth traps, Unitraps (International Pheromone System Ltd, United Kingdom) comprising with green lids, yellow cones, white bucket (Plate 3.1) and red Delta trap (Russell IPM Ltd, United Kingdom) (Plate 3.2) were used. Unitrap and Delta traps were used because they were reported to be effective in monitoring and capturing moths (LóPez, 1998; Chen *et al.*, 2015). About four millilitres of PAA (Sigma-Aldrich Chemie GmbH, Germany) were loaded onto a cotton ball and placed in the Unitrap's trap cage. This amount of PAA was loaded onto the cotton ball because it was used in previous experiments carried by Landolt *et al.* (2013) and it successfully trapped different moth species. *Spoladea recurvalis* pheromone (Pest control PVT. Ltd, biocontrol research laboratories, Bangalore, India) was designed as an impregnated septa rubber and was directly loaded in the Unitrap's cage. *Spodoptera exigua* pheromone designed as a hollow wire was cut at one end and placed on the sticky card in the Delta trap (Plates 3.3 and 3.4). Each trap was set in the middle of a sub-plot. Insecticide strips (10% of 2, 2-dichlorovinyl dimethyl Phosphate; International Pheromone System Ltd, United Kingdom) was introduced in Unitraps to kill moths that were caught (Meagher, 2002). The lures were replaced fortnightly, and the insecticide strips on a monthly basis.

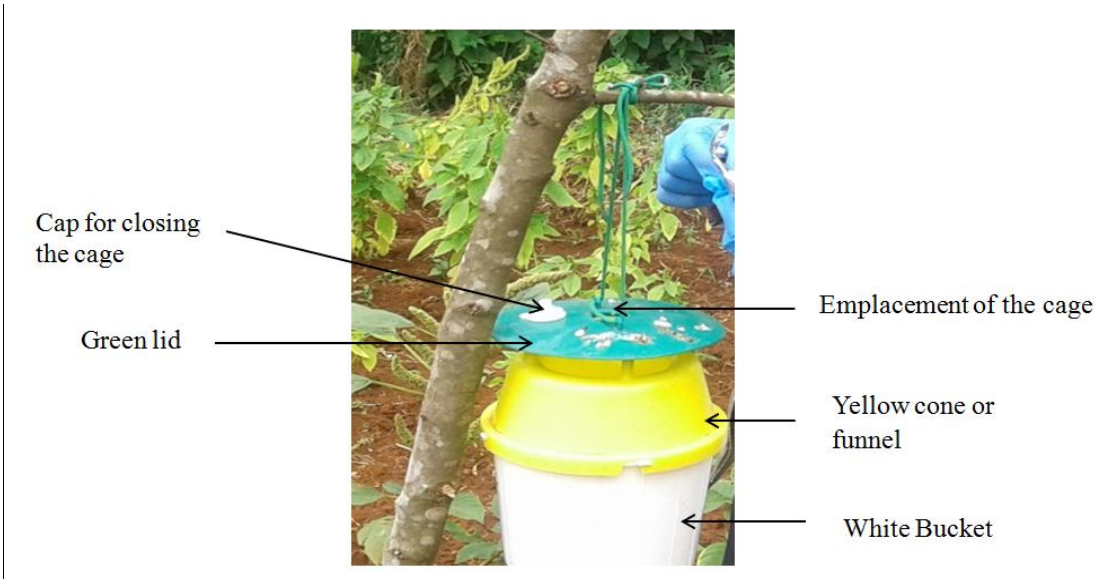

Plate 3.2: Unitrap

Photo: M. K. Agbodzavu

Plate 3.3: Delta trap with its different components

Photo: M. K. Agbodzavu

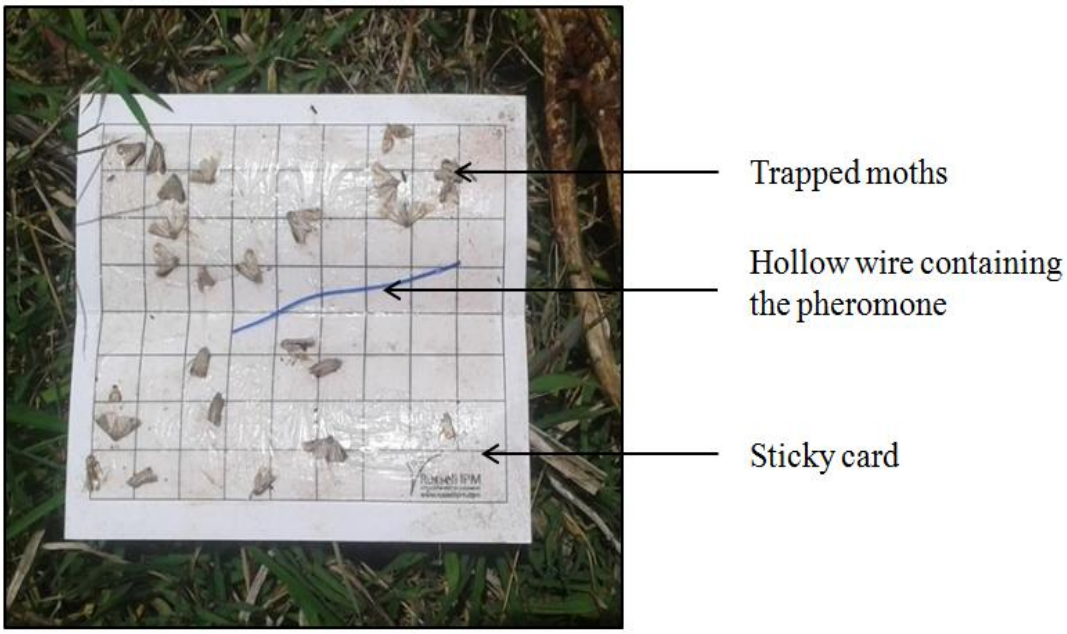

Plate 3.4: Sticky card showing trapped moths (*Spodoptera exigua*) and the pheromone wire

Photo: M. K. Agbodzavu

3.2.2.4 Sampling and data collection

Biweekly field samplings were carried out. Each sub-plot was divided into four quadrants from which ten plants were randomly selected and examined. All encountered larvae were collected, put in plastic jars (4.5 m x 2.5 cm), provided with fresh amaranth leaves picked from the plot they were collected from, labelled and taken to the laboratory for rearing until either adult moths or parasitoids emerge. At the same time, any parasitoid cocoons encountered were collected. For the cryptic insects hiding inside the plants' canopy, the beating method was used whereby a 32 m x 23.5 cm tray sprayed with a layer of 70% ethyl alcohol was placed below the plant canopy. The plant was then tapped ten times to dislodge insects that were hiding on the leaves and flowers of amaranth plants. A fine camel brush (No. 1) was used to pick the insects that fell on the tray and transferred them into insect collection vial containing 70% ethyl alcohol, preserved for identification.

The leaf damage was determined every sampling date by visually approximating the area damaged versus the undamaged area (Saini, 2011). This was done on five leaves randomly selected from the ten sampled plants as described above. Border's rows were avoided during the sampling process (buffer zone).

Once the samples were brought to the laboratory, cocoons/pupae, and larvae were isolated individually in a plastic vial (20 ml) containing amaranth leaves, plugged with cotton wool, and rear till adulthood. Emerged adult moths and parasitoids were collected and identified. Specimens caught in the traps were removed from the Unitraps and transferred into a plastic jars while the ones from the Delta traps were carried with the sticky card to the laboratory, every fortnight (Meagher & Nagoshi, 2004) for counting.

For alternative host plants, scouting on surrounding weeds and crops was done every month starting from the vicinity of the plot till 10 m on the diagonal. Plants were visually examined for the presence of either *S. littoralis*, *S. exigua*, *U. ferrugalis* and *S. recurvalis* larvae.

3.3 Data analyses

Lepidopteran species diversity on amaranth crop across different seasons and agro-ecology zones were analyzed with experimental fields' data using the Shannon Diversity index. *BioFTF* package (Di Battista *et al.*, 2017) was used to calculate the diversity indices and plot the diversity profile graphics. This tool is a scalar measure that reflects the information provided by the biodiversity profile and allows for ordering communities with different richness and evenness. *BioFTF* calculates a parameter called surface area under the diversity profile which is used for ordering communities (Di Battista *et al.*, 2017). Abundance was calculated as the number of individuals of given species divided by the total number of collected insects multiplied by 100. Incidence was computed as the number of plant hosting a given species divided by the total number of sampled plants and multiplied by 100. Infestation rate was calculated as a mean number of insects of a given species per plant. Parasitism rate was quantified as a proportion of parasitized larvae for a given species per season. Larvae were not separated into instars when recording parasitism and only parasitoids arrived at maturity were considered in the calculation. Since the leaves/plants were sampled over time, to avoid pseudo-replication, damages and incidences were averaged, and the averages were used as the data for analysis (Hurlbert, 1984). Data were analyzed separately for each site before being compared for both sites. Damage data were arcsine transformed, tested for normality and homogeneity of variance and then subjected to two-way ANOVA to assess the effect of attractant and amaranth species. The *sp.splot* function in agricolae package for the variance analysis of a split-plot design was used. Means were separated using the LSD test. The effect of the season on damage levels was also studied separately in one-way ANOVA. Wilcoxon test was used to compare the mean number of catches with *S. exigua* pheromone and the damages between sites because the data were not normally distributed in the compared groups. Results were considered significant at $p < 0.05$. All the analyses were performed in R software version 3.5.1 (R Core Team, 2018).

3.4 Results

3.4.1 Richness of Lepidopteran and associated parasitoids in farmers' fields

A total of five lepidopteran leaf defoliators and eight associated parasitoid species were recorded during farmers' field surveys (Table 3.1). Species composition in mid-altitude sites represented by Yatta, Mwea and Kitengela was higher than in high altitude represented by Limuru and Kimende. In mid-altitude, *S. exigua*, *S. littoralis*, *S. recurvalis* and *H. bipunctalis* were the lepidopteran species encountered whereas parasitoids were the recently described *Cotesia icipe* (Hymenoptera: Braconidae) (Fiaboe *et al.*, 2017), *Coccigydium luteum* (Hymenoptera: Braconidae), *Charops ater* (Hymenoptera: Ichneumonidae), *Chelonous curvimaculatus* (Hymenoptera: Braconidae), *Apanteles hemara* (Hymenoptera: Braconidae), *Atropha tricolor* (Hymenoptera: Ichneumonidae), *Phanerotoma* sp. (Hymenoptera: Braconidae) and *Schoenlandella testacea* (Hymenoptera: Braconidae). Yatta was the richest site within the mid-altitude zone in terms of lepidopteran leaf defoliators and parasitoids (Table 3.1). In high altitude, particularly in Limuru, *U. ferrugalis*, *S. recurvalis* and *S. exigua* were recorded, and only *C. ater* were recorded as a parasitoid (Table 3.1). In Kimende where amaranth production is scarce, no lepidopteran leaf defoliator was registered.

Cotesia icipe, *C. luteum*, *C. ater*, and *C. curvimaculatus* were associated with the leafworms *S. littoralis* and *S. exigua* while *A. hemara*, *A. tricolor*, *Phanerotoma* sp. and *S. testacea* parasitized *S. recurvalis. Udea ferrugalis* was attacked only by *A. hemara*. *Phanerotoma* sp. and *C. curvimaculatus* are egg-larval endoparasitoids while *Cotesia icipe*, *C. luteum*, *C. ater*, *A. hemara*, *A. tricolor* and *S. testacea* are larval endoparasitoids.

Table 3.1: Different species of lepidopteran defoliators and parasitoids recorded in two agro-ecological zones in Kenya

Altitude / agro-ecology	Sites	Season	Lepidopteran species	Parasitoids
Mid altitude	Kitengela	Long rainy season	*Spodoptera exigua, S. littoralis, Spoladea recurvalis, Herpetogramma bipunctalis*	*Cotesia icipe, Coccygidium leteum, Charops ater, Atropha tricolor*
		Dry cool season		
		Dry hot season	*S. recurvalis, S. exigua*	*C. icipe, C. ater, C. leteum*
		Short rainy season	-	
	Mwea	Long rainy season	*S. recurvalis, S. exigua, S. littoralis, H. bipunctalis*	*A. tricolor, C. icipe, C. ater*
		Dry cool season		
		Dry hot season	*S. recurvalis, S. exigua, S. littoralis*	*C. icipe, C. ater, Chelonus curvimaculatus*
		Short rainy season	*S. recurvalis, S. exigua, S. littoralis*	*Apanteles hemara, Phanerotoma* sp., *A. tricolor, C. leteum*
	Yatta	Long rainy season	*S. exigua, S. littoralis, S. recurvalis, H. bipunctalis*	*C. icipe, C. ater, A. tricolor, C. leteum, A. hemara, C. curvimaculatus, Schoenlandella testacea*
		Dry cool season	*S. recurvalis*	*A. hemara*
		Dry hot	*S. exigua, S. littoralis, S. recurvalis, H. bipunctalis*	*A. hemara, C. icipe, A. tricolor, C. luteum, C. ater, C. curvimaculatus*
		Short rainy season	*S. littoralis, S. exigua; S. recurvalis*	*C. icipe, C. ater, C. leteum, Phanerotoma* sp.
High altitude	Limuru	Long rainy season	*Udea ferrugalis, S. recurvalis*	*C. ater*
		Dry cool season	*U. ferrugalis, S. recurvalis*	
		Dry hot season	*0*	
		Short rainy season	*Udea ferrugalis, S. exigua*	
	Kimende	Long rainy season	-	
		Dry cool season	-	
		Dry hot season	0	
		Short rainy season	0	

3.4.2 Diversity of Amaranth lepidopteran in Thika and Tigoni and across seasons

In Kandara, Thika season 5 and 3 had higher species richness, followed by season 2, 1 and 4 in Thika. Considering the Shannon Diversity index, season 5 had the highest amaranth lepidopteran species diversity. It was followed by seasons 2, 3, 4 and 1 (Table 3.2). In Tigoni, Season 1 had the highest species richness followed by season 2, 4, 3 and 5. Shannon indice ranked season 2 as the most diverse followed by season 4, 1, 5 and 3 (Table 3.2).

When the agro-ecology zones were considered, the high-altitude zone had the highest pest species richness and more diverse in terms of amaranth Lepidopteran (Table 3.3).

Table 3.2: Biodiversity ranking of different seasons on experimental sites in Thika and Tigoni

Experimental site	Season	Richness	Shannon index
Thika	1	5	0.129082
	2	5	1.231748
	3	6	1.197664
	4	4	0.909989
	5	6	1.327926
Tigoni	1	7	0.812066
	2	6	1.010847
	3	5	0.484477
	4	5	0.980934
	5	4	0.792697

Table 3.3: Biodiversity ranking of different agro-ecology zones

Agro-ecology zone	Richness	Shannon
High altitude (Tigoni)	8	0.993112
Mid-altitude (Thika)	7	0.430774

3.4.3 Relative abundance of collected insects from experimental fields

From all treatments, 116,645 individual insects belonging to eight orders were identified. A total number of 76,460 insects (47,033 on *A. dubius* and 29,427 on *A. cruentus*) were collected in Thika while 40,185 (23,392 on *A. dubius* and 16,793 on *A. cruentus*) were sampled from Tigoni.

The relative abundance of these different orders recorded on both sites is presented on Figure 3.1 and 3.2.

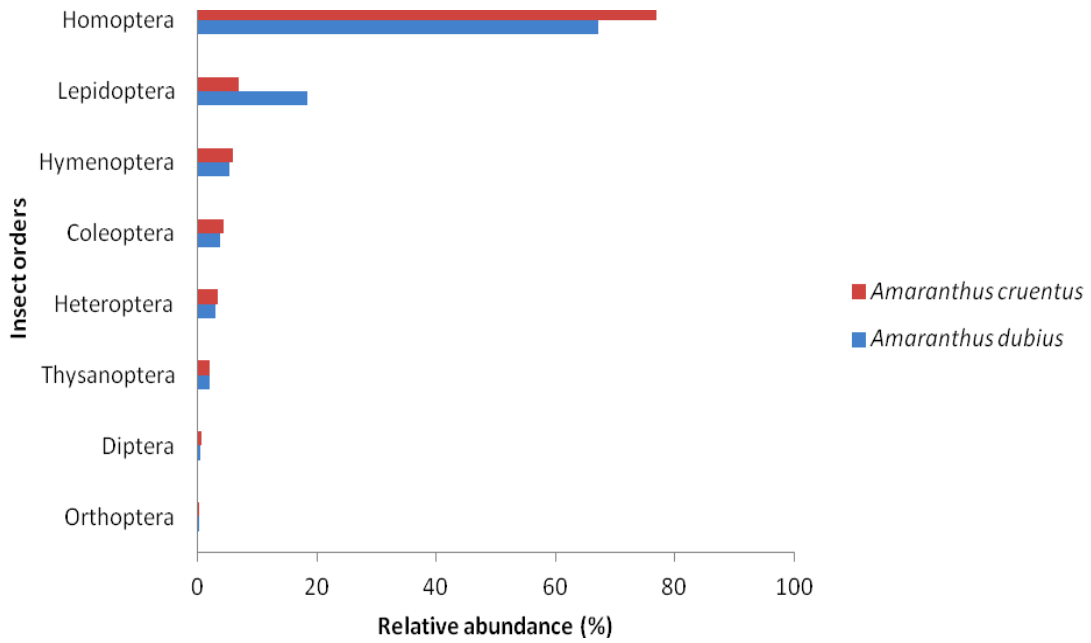

Figure 3.1: Abundance of different insect orders on *Amaranthus dubius* and *Amaranthus cruentus* collected from Thika

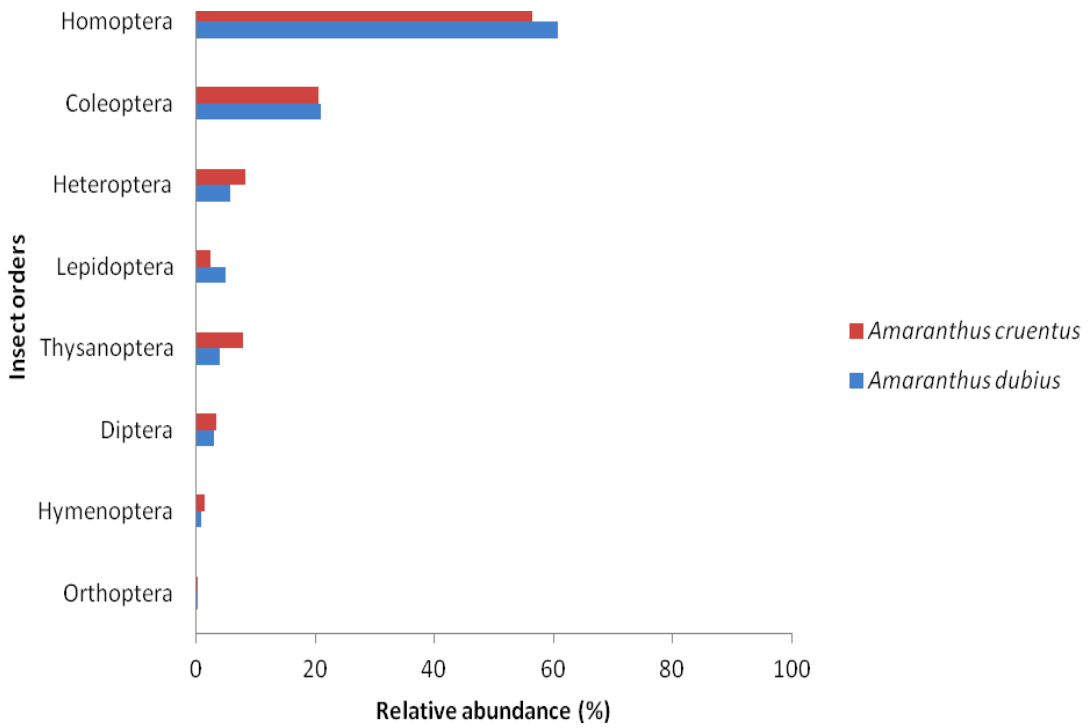

Figure 3.2: Abundance of different insect orders on *Amaranthus dubius* and *Amaranthus cruentus* collected from Tigoni

The most abundant order was Homoptera regardless of the amaranth species at Thika and Tigoni. The order was represented by aphids' species, *Aphis gossypii*, *Myzus persicae* (Aphididae), leafhoppers (Cicadellidae) and mealybugs (Pseudococcidae). This was secondly followed by Lepidoptera species in Thika and Coleopterans at Tigoni. Coleopterans were represented by flea beetles, ladybird beetles and stem weevils (*Hypolixus* sp.). The third position in terms of abundance was occupied by Hymenopterans (ants) in Thika while in Tigoni it was Heteroptera (*Cletus* sp., *Nezara viridula*). In Tigoni, Lepidoptera order occupied the fourth position in terms of abundance on *A. dubius*. Different Lepidoptera species were recorded on both sites. Their abundance is presented on Figure 3.3 and 3.4 and Table 4.4.

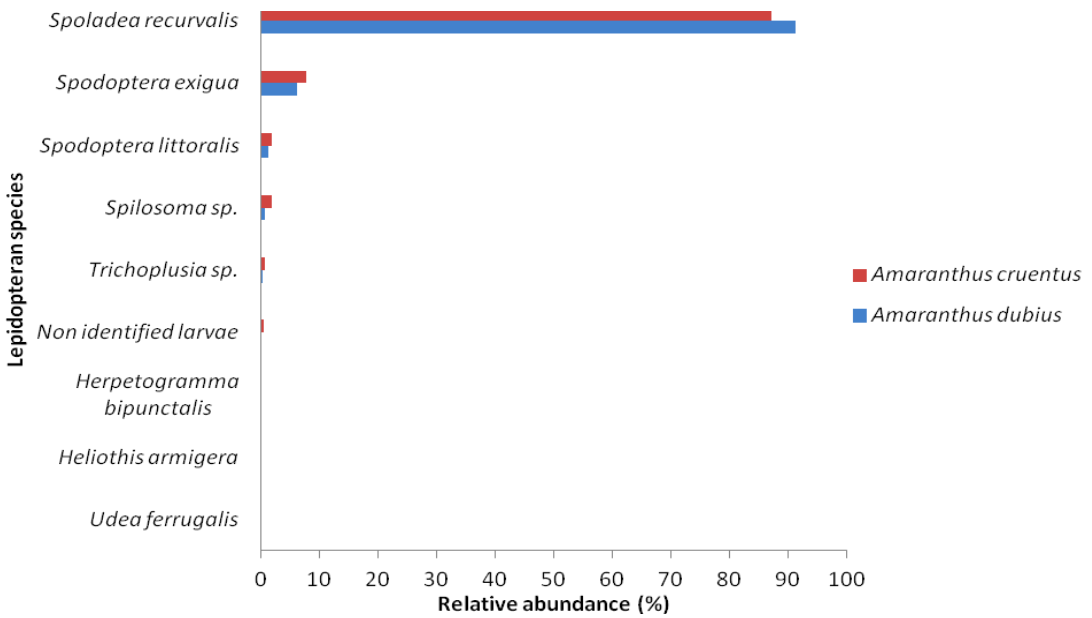

Figure 3.3: Abundance of different lepidopteran species on *Amaranthus dubius* and *Amaranthus cruentus* in Thika

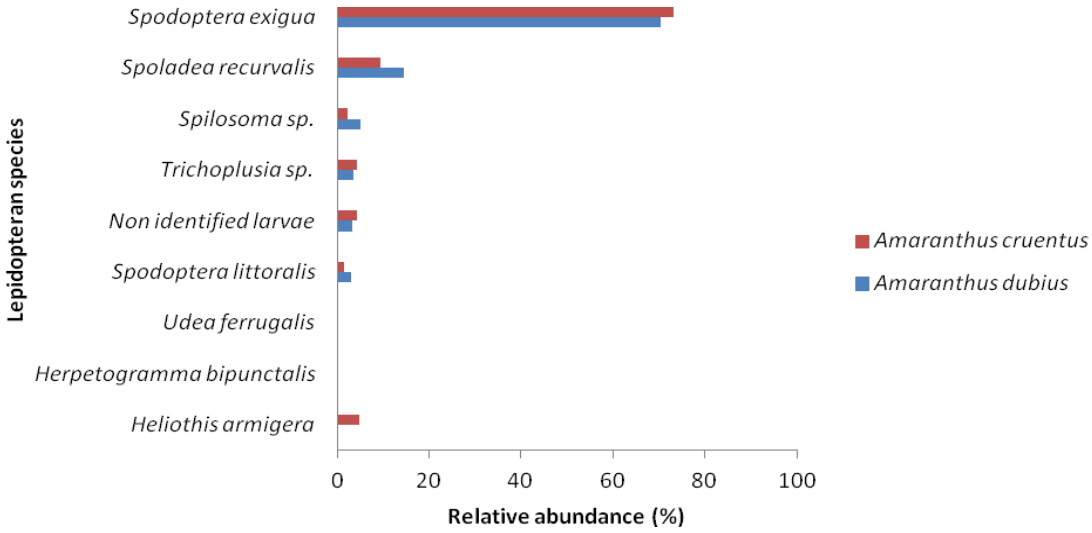

Figure 3.4: Relative abundance of different lepidopteran species of *Amaranthus dubius* and *Amaranthus cruentus* in Tigoni

Spoladea recurvalis represented the most abundant lepidopteran in Thika, followed by *S. exigua*, *S. littoralis*, *Trichoplusia* sp. and *Spilosoma* sp. The other two spotted species *Herpectogramma bipunctalis* and *Heliothis armigera* were minor (Figure 3.3). At Tigoni, *S. exigua* was the most abundant followed by *S. recurvalis*. Three other species, *Spilosoma* sp., *Trichoplusia* sp. and *S. littoralis* had an intermediate importance as well as *Heliothis armigera* on *A. cruentus*. *Herpetogramma bipunctalis* and *U. ferrugalis* were minor (Figure 3.4).

There was a significant difference in the relative abundance of *S. littoralis* (χ^2 = 16.68, *df* = 1, $p < 0.0001$), *S. exigua* (χ^2 = 3468.3, *df* = 1, $p < 0.0001$), and *S. recurvalis* (χ^2 = 4019, *df* = 1, $p < 0.0001$) in Thika as compared to Tigoni on *A. dubius*. However, the relative abundance on *A. cruentus* of *S. littoralis* recorded on both sites was not significantly different (χ^2= 0.12, *df* = 1, $p = 0.732$) (Table 3.4).

Table 3.4: Comparison between relative abundances of different lepidopteran species recorded in Thika and Tigoni on two species of amaranth

Lepidoptera species	*Amaranthus dubius*		χ^2	*df*	*p*	*Amaranthus cruentus*		χ^2	*df*	*p*
	Thika	Tigoni				Thika	Tigoni			
Spodoptera littoralis	1.31[b] (113)	2.93[a] (33)	16.68	1	< 0.0001	1.91[a] (39)	1.51[a] (6)	0.12	1	0.732
Spodoptera exigua	6.27[b] (541)	70.43[a] (793)	3468.3	1	< 0.0001	7.79[b] (159)	73.12[a] (291)	940.76	1	< 0.0001
Spoladea recurvalis	91.2[a] (7871)	14.56[b] (164)	4019	1	< 0.0001	87.07[a] (1778)	9.55[b] (38)	1047.60	1	< 0.0001
Udea ferrugalis	0[b] (0)	0.18[a] (2)	7.89	1	0.005	0[a] (0)	0.25[a] (1)	0.83	1	0.362
Herpetogramma bipunctalis	0.02[a] (2)	0.09[a] (1)	0.08	1	0.781	0 (0)	0 (0)	-	-	-
Spilosoma sp.	0.68[b] (59)	4.97[b] (56)	153.71	1	< 0.0001	1.91[a] (39)	2.26[a] (9)	0.07	1	0.791
Trichoplusia sp.	0.4[b] (35)	3.64[a] (41)	130.78	1	< 0.0001	0.68[b] (14)	4.27[a] (17)	31.34	1	< 0.0001
Heliothis armigera	0 (0)	0 (0)	-	-	-	0.05[b] (1)	4.77[a] (19)	85.75	1	< 0.0001
Non identified larvae	0.12[b] (10)	3.2[a] (36)	195.03	1	< 0.0001	0.59[b] (12)	4.27[a] (17)	35.41	1	< 0.0001

Means followed by the same lower case letters in the same row were not significantly different with proportion test at $p < 0.05$.

Note: Comparisons were done between sites for the same lepidopteran species and the same amaranth species. Relative abundances were tested.

Figures in brackets represent absolute abundance.

3.3.4 Seasonal dynamics of lepidopteran larval populations

The seasonal dynamics of major lepidopteran species recorded on both sites and on both species are presented on Figures 3.5 - 3.8. *Spoladea recurvalis* outbreaks occurred only during the first crop season in Thika, starting from December and ended January. The highest abundance occurred in December with the highest mean infestation rate reaching 7.93 ± 0.92 and 24 ± 2.50 (mean ± SE) larvae per plant on *A. cruentus* and *A. dubius* respectively (Figure 3.5 and 3.6). The same trend was observed at Tigoni though the infestation rate was 72 and 120 folds lower on *A. cruentus* and *A. dubius* respectively. The highest infestation rate recorded on that site was 0.11 ± 0.04 and 0.20 ±0.03 larva per plant on *A. dubius* and *A. cruentus* respectively (Figure 3.7 and 3.8). *Spoladea recurvalis* was absent in February-March and July- November in Thika (Figure 3.5 and 3.6). No consistent trend was observed in Tigoni (Figure 3.7 and 3.8). *Spodoptera exigua* was present throughout the year on both sites but its abundance and infestation rate differed according to the site and to the season. Heavy infestation occurred from February to August with a peak in May at Thika (Figure 3.5) and from September to November at Tigoni (Figure 3.7). *Spodoptera littoralis* was generally present from December to March and from May to August at Thika (Figure 3.5). At Tigoni, it was more present in September and October (Figure 3.7). *Spilosoma* sp. appeared consistently on both sites from April to July (Figure 3.5 and 3.7). *Trichoplusia* sp. appears in November to January and April-June on both sites (Figure 3.5 and 3.7).

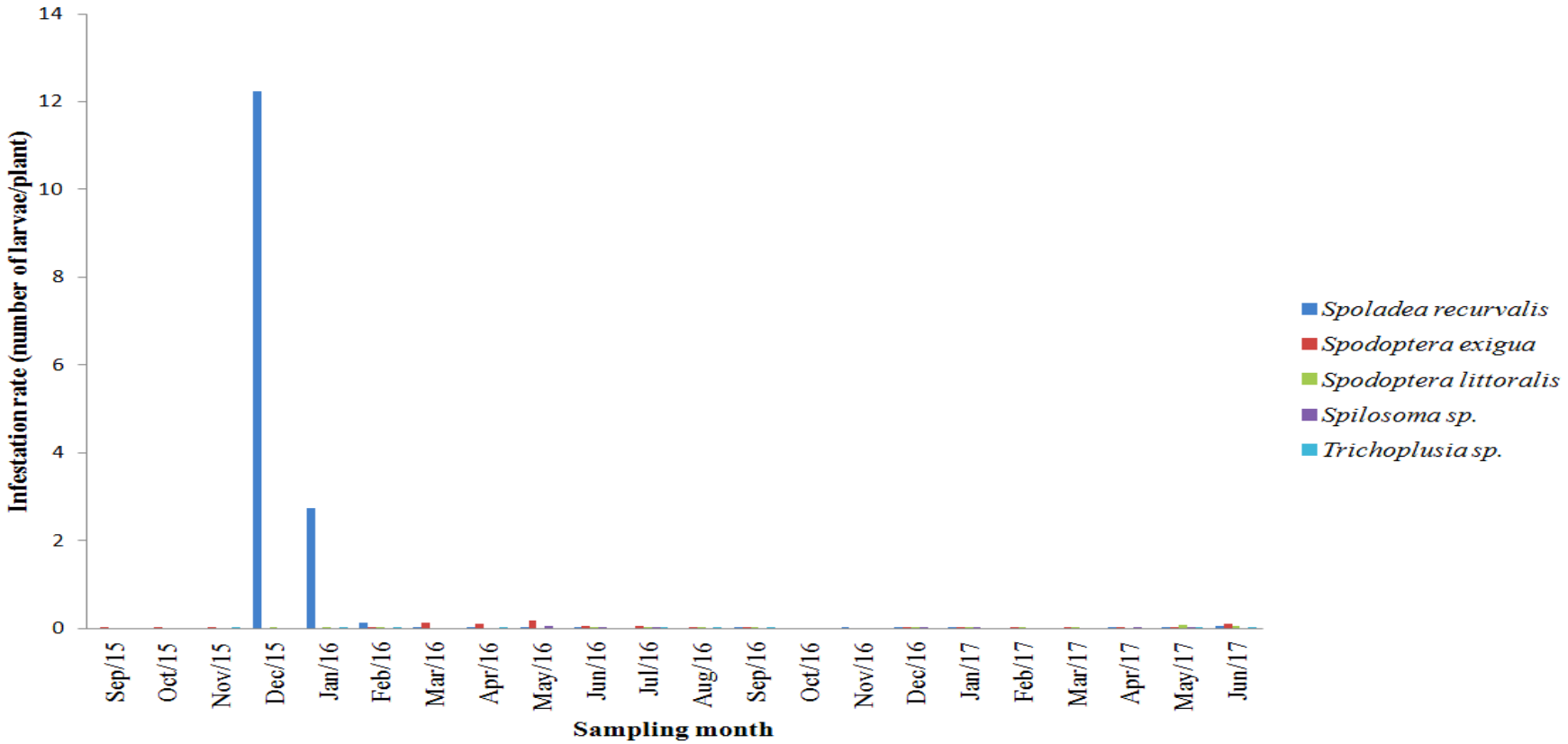

Figure 3.5: Seasonal dynamics of major lepidopteran species on *Amaranthus dubius* in Thika

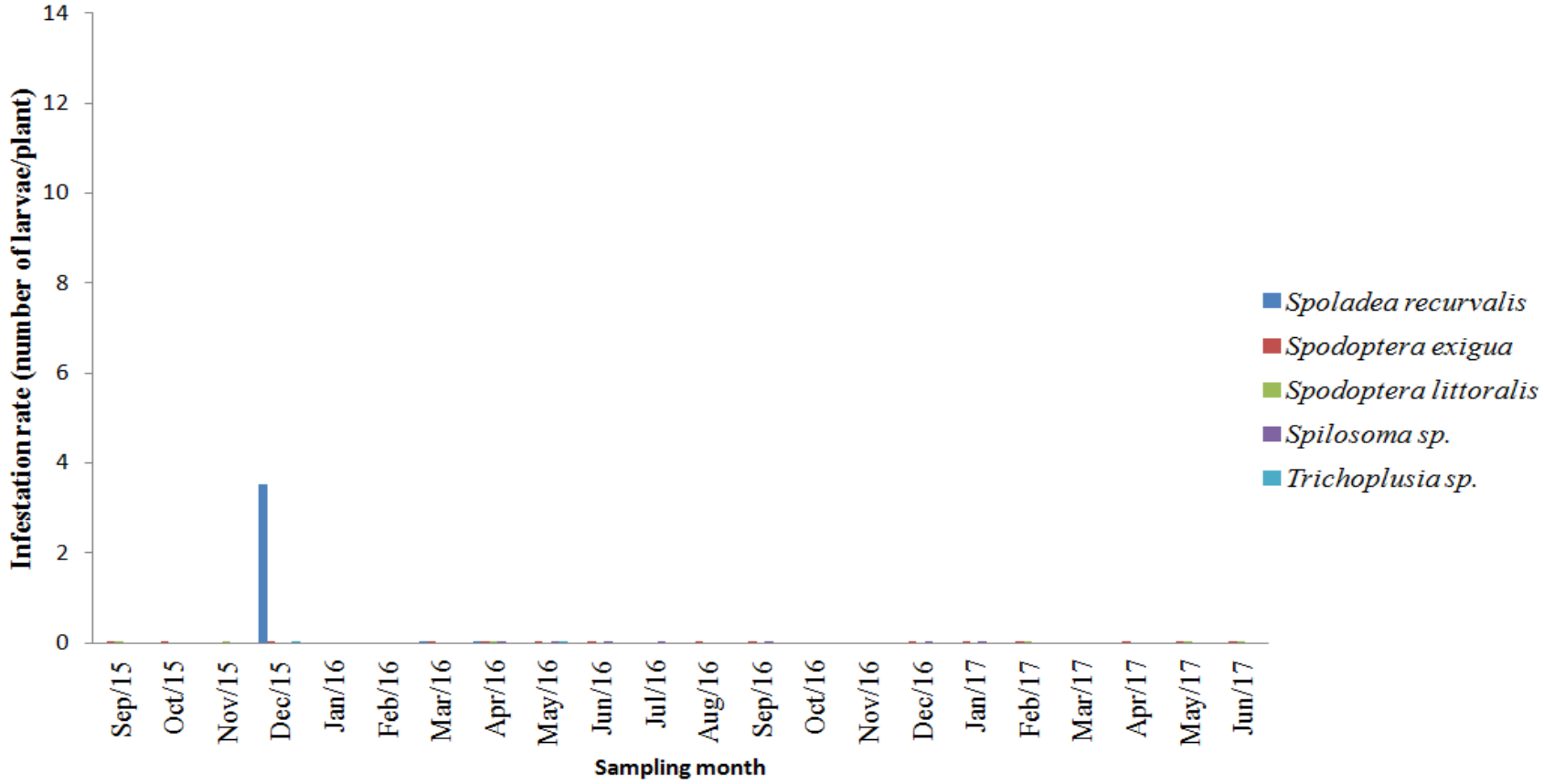

Figure 3.6: Seasonal dynamics of major lepidopteran species on *Amaranthus cruentus* in Thika

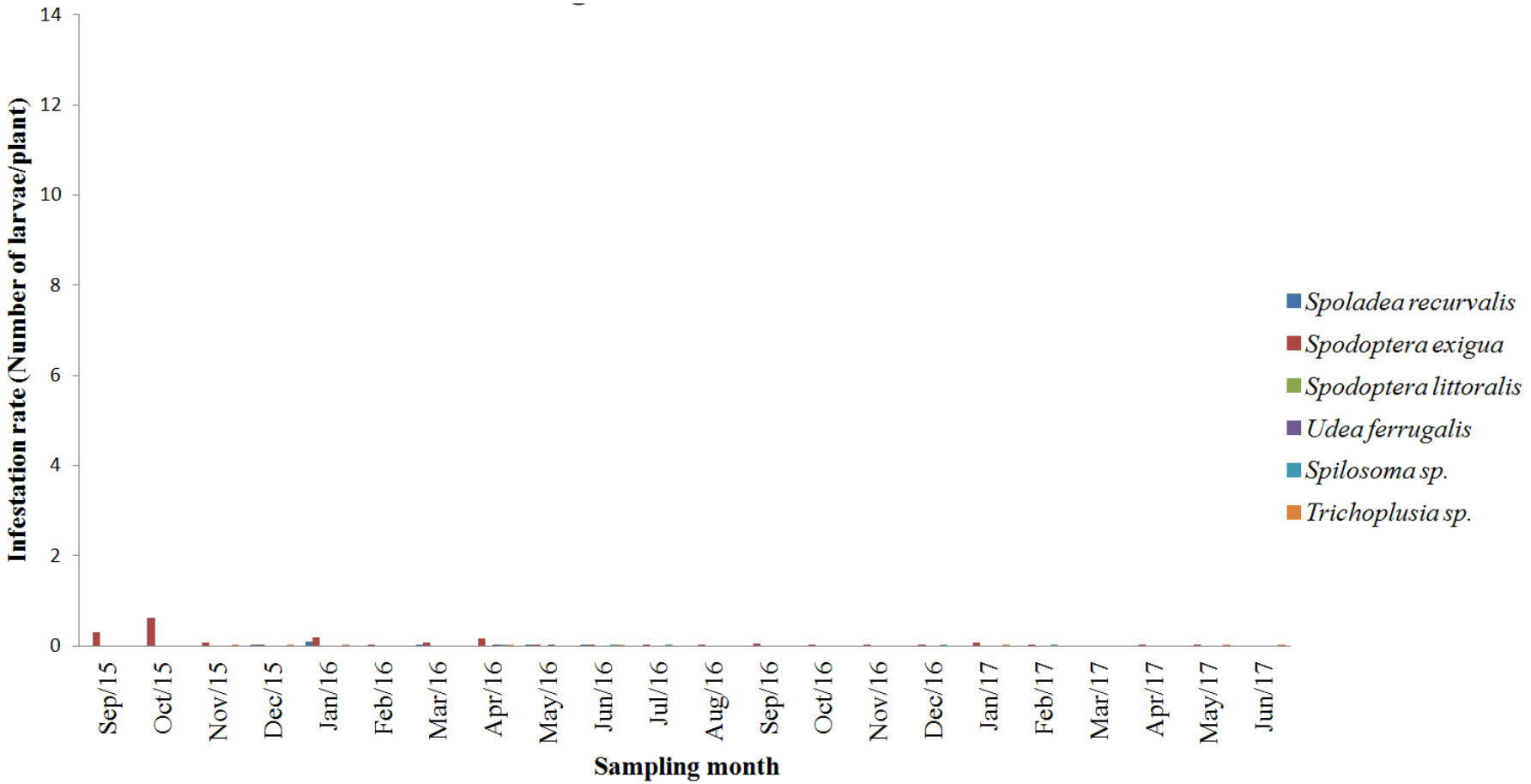

Figure 3.7: Seasonal dynamics of major lepidopteran species on *Amaranthus dubius* in Tigoni

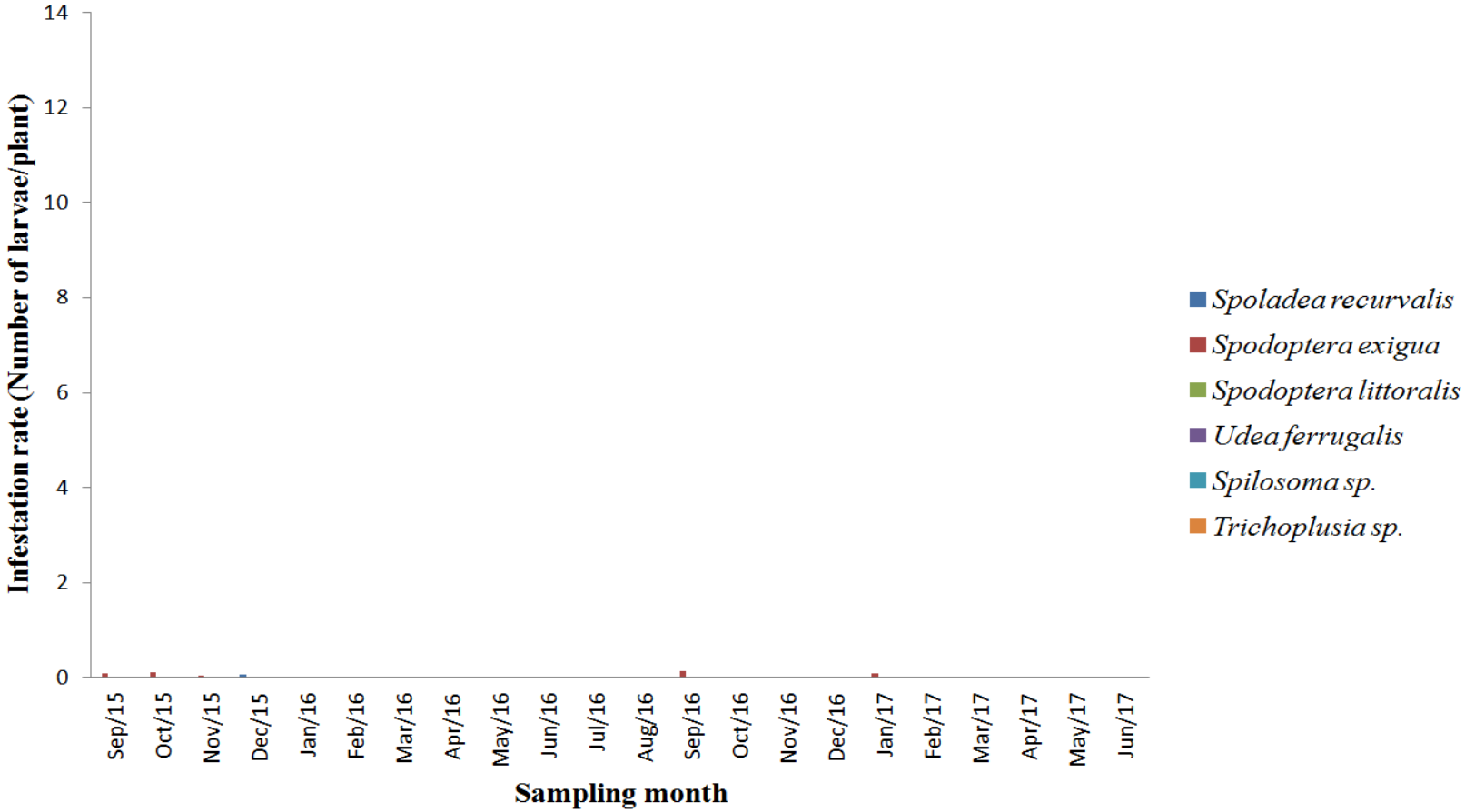

Figure 3.8: Seasonal dynamics of major lepidopteran species on *Amaranthus cruentus* in Tigoni

3.3.5 Seasonal incidence of different lepidopteran species

Incidences were highly variable according to the site, the amaranth species and across seasons, (Tables 3.5). The highest incidence was recorded with *S. recurvalis* in the first season on *A. dubius* at Thika. It reached 100 % during the outbreak though the seasonal mean was 19.49 %. It was followed by *S. exigua* which registered a seasonal mean of 9.17 % during the first season on *A. dubius* at Tigoni and by *S. littoralis* which registered a seasonal mean of 3.75 % on *A. dubius* during the fifth season at Thika. The other two species recorded a seasonal mean incidence less than 1% throughout the five crop seasons (Tables 3.5).

Table 3.5: Incidence (%) (mean ± SE) of collected lepidopteran species on *Amaranthus dubius* and *Amaranthus cruentus* on experimental plots in Thika and Tigoni

Season	Lepidopteran species	Thika		Tigoni	
		Amaranthus dubius	*Amaranthus cruentus*	*Amaranthus dubius*	*Amaranthus cruentus*
S1	*Spoladea recurvalis*	19.49± 5.23	5.06± 2.12	1.67± 0.77	0.70± 0.58
	Spodoptera exigua	2.18± 0.84	0.19± 0.116	9.17± 2.01	2.50± 0.94
	Spodoptera littoralis	0.51± 0.25	0.13± 0.09	0.51± 0.32	0±0.00
	Spilosoma sp.	0.0±0.00	0.0±0.00	0.0±0.00	0.06±0.064
	Trichoplusia sp.	0.32±0.16	0.06±0.06	0.51±0.25	0.0±0.00
S2	*Spoladea recurvalis*	1.05± 0.30	0.42± 0.19	0.42± 0.19	0.21± 0.14
	Spodoptera exigua	7.40± 1.26	2.92± 0.63	3.33± 0.89	0.94± 0.39
	Spodoptera littoralis	0.21± 0.14	0.21± 0.21	0.10± 0.10	0.10± 0.10
	Spilosoma sp.	1.67±0.41	0.73±0.28	0.31±0.23	0.0±0.00

	Trichoplusia sp.	0.21±0.14	0.29±0.14	0.21±0.14	0.10±0.10
S3	*Spoladea recurvalis*	0.34± 0.20	0.0±0.00	0.0±0.00	0.0±0.00
	Spodoptera exigua	1.17± 0.33	0.17± 0.12	1.83± 0.48	1.75± 1.57
	Spodoptera littoralis	0.42± 0.17	0.0±0.00	0.33± 0.26	0±0.00
	Spilosoma sp.	0.25±0.18	0.17±0.11	0.17±0.12	0.0±0.00
	Trichoplusia sp.	0.25±14	0.0±0.00	0.0±0.00	0.0±0.00
S4	*Spoladea recurvalis*	0.32± 0.23	0.0±0.00	0.0±0.00	0.0±0.00
	Spodoptera exigua	2.92± 0.85	0.52± 0.21	1.77± 0.97	1.77± 1.14
	Spodoptera littoralis	1.46± 0.47	0.10± 0.10	0.10± 0.10	0.10± 0.10
	Spilosoma sp.	0.10±0.10	0.42±0.24	0.10±0.10	0.0±0.00
	Trichoplusia sp.	0.0±0.00	0.0±0.00	0.10±0.10	0.0±0.00
S5	*Spoladea recurvalis*	2.92± 0.99	0.0±0.00	0.0±0.00	0.14± 0.14
	Spodoptera exigua	4.58± 1.43	2.22± 0.70	1.11± 0.41	0.83± 0.35
	Spodoptera littoralis	3.75± 2.26	1.39± 1.25	0.27± 0.19	0±0.00
	Spilosoma sp.	0.42±0.22	0.0±0.00	0.0±0.00	0.0±0.00
	Trichoplusia sp.	0.42±0.30	0.0±0.00	0.42±0.22	0.28±0.19

3.3.6 Fluctuations of moth catch using Phenylacetaldehyde, *Spodoptera exigua* pheromone and developed *Spoladea recurvalis* pheromone

Many species of moths were caught in traps containing Phenylacetaldehyde (PAA) but which we did not find feeding on amaranth such as (*Anyma octogueae* Guenèe (Lepidoptera: Noctuidae), *Chrysodexis acuta* (Walker) (Lepidoptera: Noctuidae), *Eulocastra aethiops* (Distant) (Lepidoptera: Noctuidae), *Traminda pallida* (Warren) (Lepidoptera: Geometridae), *Anomis sabulifera* Guenèe (Lepidoptera: Erebidae), etc. Some other species collected in PAA traps were of minor importance namely *Heliothis armigera* Hübner (Lepidoptera: Noctuidae) and *Plusia* sp. (Lepidoptera: Noctuidae). *Spoladea recurvalis* which was a major defoliator of amaranth was caught but represented 2.01 % and 0.31 % of the total catches of the moth at Thika and Tigoni respectively. Similarly, catches of *S. exigua* were very low and represented 2.68 and 0.78% for Thika and Tigoni respectively.

Spoladea recurvalis pheromone rarely and accidentally caught some moths with no importance to the targeted crop. However, *S. exigua* pheromone regularly caught adults of *S. exigua* and fluctuations of adults across the seasons are presented on Figures 3.9 and 3.10 for Thika and Tigoni respectively.

The fluctuation of *S. exigua* adults showed a seasonal variability at the two sites with high catches in 2017 (Figures 3.9 and 3.10). Both sites had a sharp increase of moths from April 2007 following the onset of the rainy season. The maximum catches were 23.33 ± 2.51 moths per trap at Thika and 41.66 ± 2.08 moths per trap at Tigoni (mean ± SE). There was a highly significant difference ($W = 272$, $df = 1$, $p = 0.003$) in the mean number of catches in Tigoni (12.09 ± 1.92) as compared to Thika (5.88 ± 1.11).

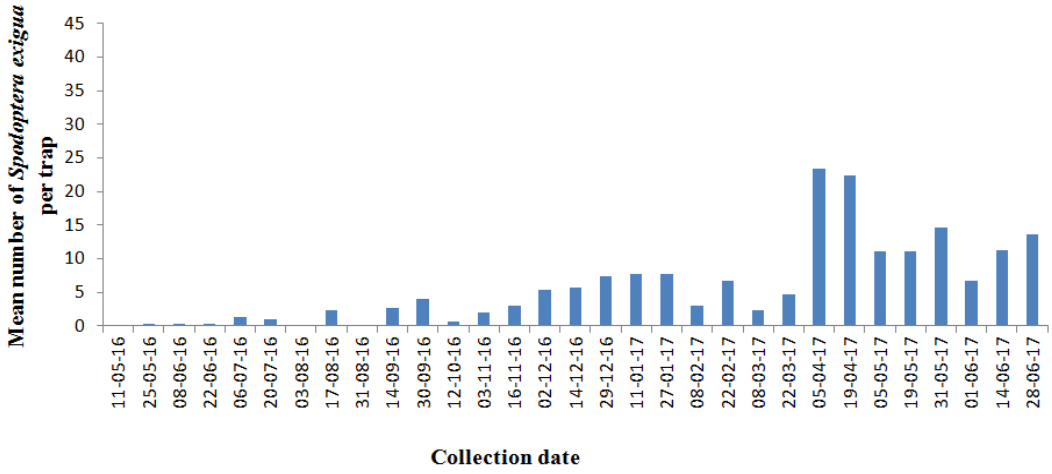

Figure 3.9: Seasonal *Spodoptera exigua* pheromone trap captures in Thika

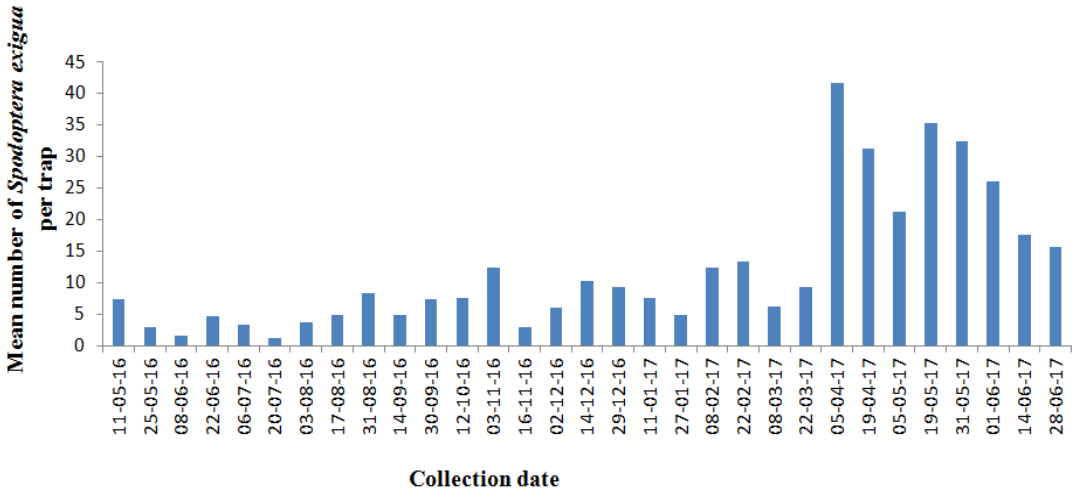

Figure 3.10: Seasonal *Spodoptera exigua* pheromone trap captures in Tigoni

3.3.7 Damage variations

Damage registered on the control plot (plots without any crop protection measure) varied from 0.85 to 62.45 % on *A. dubius* and from 0.63 to 28.77% on *A. cruentus* at Thika per sampling date. It varied from 1.35 to 39.52% on *A. dubius* and from 0.80 to 31.92 % on *A. cruentus* at Tigoni.

When considering sites separately, there was no significant difference ($F = 0.16$, $df = 2$, $p = 0.859$, in the mean damages recorded in the control plots as compared to the plots having Phenylacetaldehyde and plots which received the combination of *S. exigua* pheromone and the developed *S. recurvalis* pheromone at Thika (Figure 3.11). The same trend was obtained at Tigoni site ($F = 19.07$, $df = 2$, $p = 0.050$, Figure 3.11). However, there was a significant difference in the level of damage recorded on the two tested species of amaranth at Thika ($F = 6.77$, $df = 1$, $p = 0.040$, Figure 3.12). On the contrary, there was no significant difference in the damage caused on both amaranth species at Tigoni ($F = 0.95$, $df = 1$, $p = 0.367$, Figure 3.12). The interaction between the attractant and the species was not significant on both sites (Thika: $F = 0.079$, $df = 2$; $p = 0.925$; Tigoni: $F = 0.39$; $df = 2$, $p = 0.694$).

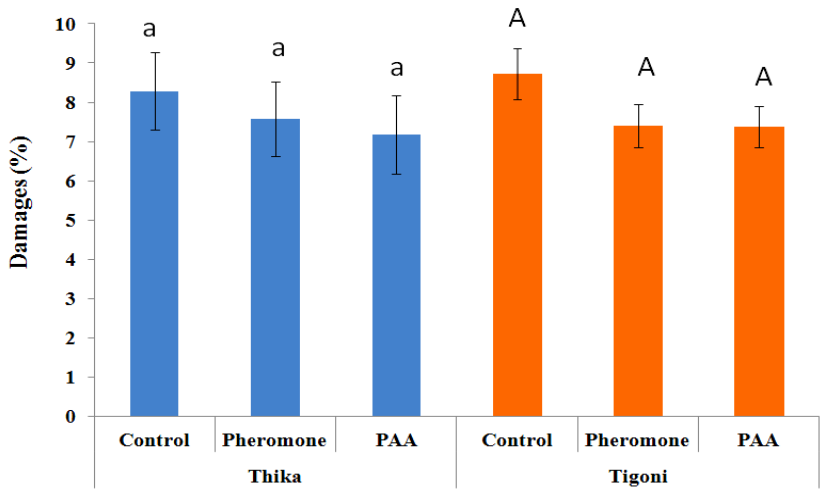

Figure 3.11: Mean damage of amaranth leaves at Thika and Tigoni per attractants

Different letters denote statistically significant difference according to LSD test at $p < 0.05$.

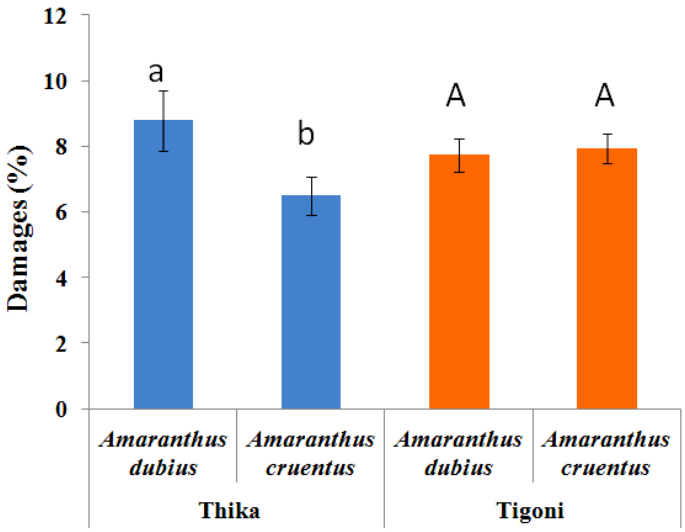

Figure 3.12: Mean damage per amaranth species at Thika and Tigoni in five seasons

Different letters denote statistically significant difference according to LSD test at $p < 0.05$.

There was a significant difference in the damage levels when considering the seasons on both sites (Thika: $F = 19.77$, $df = 4$, $p < 0.0001$; Tigoni: $F = 57.31$, $df = 4$, $p < 0.0001$). Seasons one and two were significantly different from seasons three, four and five (Table 3.6).

Table 3.6: Level of damage (mean ± SE) in different cropping seasons

Season	Damages (%)	
	Thika	Tigoni
I	13.7 ± 1.35a	10.84 ± 0.49a
II	10.96 ± 1.33a	11.47 ± 0.38a
III	4.85 ± 0.22b	5.37 ± 0.31b
IV	5.68 ± 0.77b	5.81 ± 0.41b
V	4.28 ± 0.51b	5.69 ± 0.41b

Means followed by different letters in the same column were significantly different, $p < 0.05$.

There was no significant difference among the sites regarding the damage ($W = 98$, $df = 1$, $p = 0.5668$). Thika recorded 7.96 ± 1.05 while Tigoni 7.84 ± 0.76 % of damage.

3.3.8 Parasitoids and parasitism rate

Three species of parasitoids were recorded in the experimental field trials on both sites: *A. hemara*, *C. icipe* and *C. ater*. The parasitism rates obtained were highly variable. Where high parasitism rates were obtained, the abundance of the pest was quite low (Table 3.7) which could be due either to the low prevalence of the pest or the high pressure of the parasitoid.

Table 3.7: Parasitism rate of recovered parasitoids according to the season, the sites and the amaranth species

Season	Parasitoids	Lepidopteran species	Thika		Tigoni	
			Amaranthus dubius	*Amaranthus cruentus*	*Amaranthus dubius*	*Amaranthus cruentus*
			Parasitism rate (%)			
S1	*Apanteles hemara*	*Spoladea recurvalis*	7.9	2.7	3.3	0.0
	Cotesia icipe	*Spodoptera exigua*	0.0	0.0	0.4	0.0
		Spodoptera littoralis	0.0	0.0	22.2	-
	Charops ater	*Spodoptera exigua*	0.0	0.0	15.7	4.2
		Spodoptera littoralis	11.1	50.0	0.0	-
S2	*Apanteles hemara*	*Spoladea recurvalis*	0.0	25.0	0.0	50.0
	Cotesia icipe	*Spodoptera exigua*	0.0	0.0	0.0	0.0
		Spodoptera littoralis	0.0	0.0	0.0	0.0
	Charops ater	*Spodoptera exigua*	0.0	0.0	0.0	0.0
		Spodoptera littoralis	0.0	100.0	0.0	0.0
S3	*Apanteles hemara*	*Spoladea recurvalis*	75.0	-	-	-
	Cotesia icipe	*Spodoptera exigua*	0.0	0.0	0.0	0.0
		Spodoptera littoralis	0.0	-	0.0	-

		Spodoptera exigua	0.0	0.0	0.0	0.0
	Charops ater	*Spodoptera littoralis*	16.7	-	0.0	-
S4	*Apanteles hemara*	*Spoladea recurvalis*	0.0	-	-	-
	Cotesia icipe	*Spodoptera exigua*	0.0	0.0	0.0	0.0
		Spodoptera littoralis	21.4	0.0	0.0	0.0
	Charops ater	*Spodoptera exigua*	0.0	0.0	0.0	0.0
		Spodoptera littoralis	28.6	0.0	0.0	0.0
S5	*Apanteles hemara*	*Spoladea recurvalis*	23.8	-	-	100.0
	Cotesia icipe	*Spodoptera exigua*	0.0	0.0	0.0	0.0
		Spodoptera littoralis	0.0	0.0	0.0	-
	Charops ater	*Spodoptera exigua*	0.0	0.0	0.0	0.0
		Spodoptera littoralis	0.0	0.0	0.0	-

S1: Season one; S2: Season 2; S3: Season three; S4: Season 4; S5: Season 5.

3.3.9 Alternative host plants of amaranth lepidopteran defoliators

During the entire data collection period, only one wild host plant was recorded in farmers' fields for *S. recurvalis*. It was *Achyranthes aspera* L. (Amaranthaceae) commonly known as devil's horsewhip (Plate 3.5 A) collected at Nyeri Kiawana area: S 00° 12.12', E 036° 49.964', 1960 m a.s.l. One cultivated alternative host plant was also found in Mwea (S 01° 14.245', E 037° 27.948', 1180 m a.s.l). It was spinach (*Spinacia oleracea* L.) (Amaranthaceae) (Plate 3.5 B). *Apanteles hemara* was found parasitizing *S. recurvalis* on the two alternative host plants. No alternative host was found for *S. littoralis* and *S. exigua* during this study.

(A)

(B)

Plate 3.5: *Achyranthes aspera* (A) and *Spinacia oleracea* (B) severely damaged by Spoladea recurvalis

3.4 Discussion

Knowledge about species diversity and abundance is essential for the development of integrated pest management strategies. In the present study, we established the baseline data on the diversity, distribution, seasonality and alternative hosts of amaranth lepidopteran defoliators and their associated natural enemies in two agro-

ecological zones of Kenya. Lepidopteran foliage feeders have been reported as one of the major insect group constraining the production of vegetable amaranths in several parts around the world (García *et al.*, 2011; Aderolu *et al.*, 2013; Othim *et al.*, 2018a). Many species of Lepidoptera were found feeding on vegetable amaranths during this study but the most important are *S. recurvalis*, *S. exigua* and *S. littoralis* based on their larval infestation and incidence rates and feeding activities. While in Thika representing the mid-altitude *S. recurvalis* is the main concern, in high altitude, it was not a major problem but rather *S. exigua*. Both species were nevertheless present on both sites along with *S. littoralis* causing losses to the crop. The higher infestation rate of *S. recurvalis* at Thika as compared to Tigoni could be explained by the difference in environmental conditions at the two sites. Thika is warmer than Tigoni and is, therefore, more conducive for the reproduction and the survival of *S. recurvalis*. According to studies conducted by Lee *et al.* (2013), high emergence rate of adult *S. recurvalis* was obtained at 25°C with a low death rate for different larval instars. However, as the temperature decreases, the emergence rate also decreases with an increase in the death rate. No adult emergence was observed at 17°C. In a study conducted by Yamada & Koshihara (1976) they reported that in Japan, *S. recurvalis* mainly occurs in the warm region and it occurs especially in the summer/autumn seasons. In Tigoni, the temperature can drop up to 12°C (Muthoni & Kabira, 2011) which cannot allow survival of *S. recurvalis*. Therefore, few adults and larvae encountered at high altitude might be migratory individuals from mid or low altitude since *S. recurvalis* is known to be a long-distance migrating insect (Yamada *et al.*, 1979; Miyahara, 1997). On the contrary, *S. exigua* is able to reproduce and survive within a range of 15 to 34°C, showing its higher plasticity as regards to environmental conditions. The presence of *S. littoralis* on both sites even though in lower numbers could also be explained by the same reason (Baker & Miller, 1974).

During the five seasons of data collection, *S recurvalis* outbreak occurred only once and during the first season, wiping completely away the foliage of the amaranth crops. It is likely that massive rainfall preceding that cropping season had driven this serious outbreak. However, to ascertain this fact, long term observations are

necessary to establish a positive correlation between the quantity of rainfall and the occurrence of *S. recurvalis* outbreak.

The fact that *S. recurvalis* larvae were found on *A. dubius* over most of the months in Thika and the fact that at least one wild alternate and cultivated host were recorded supports the hypothesis that this pest maintains permanent populations on other crops or wild alternative hosts, awaiting favourable conditions to multiply. Studies carried by Bhattacherjee and Ramdas Menon (1964) in India showed that adults *S. recurvalis* shift from amaranth to *Gomphrena* flowers and spinach from July to October. In January and February, they are known to become very scarce, but with the advent of summer, there is again an increase in their number on grasses and various summer vegetables. Subsequently, they shift to amaranth and multiply very rapidly. Similar behaviour was observed by Pande (1972). However, there are studies showing that *S. recurvalis* is long-distance migrating insect as mentioned earlier. In Japan, it is thought to be one of the lepidopterous insects that migrate overseas from subtropical and tropical regions, together with *Cnaphalocrocis medinalis* (Lepidoptera: Crambidae) during the rainy season (Yamada *et al.*, 1979; Miyahara, 1997). *Spoladea recurvalis* has also been known to invade, through a transoceanic migration, New Zealand from Australia, together with other Lepidoptera (Fox, 1978). Therefore, the initial infestation of cultivated crops can also be due to the invasion of migrating individuals looking for more conducive ecology or environments. Presence of *S. exigua* was noted year-round at both sites. The reason for the continuous presence of *S. exigua* on *Amaranthus* as compared to *S. littoralis* could be the host plant preference. The presence during the crop seasons of a most preferred host can trigger the displacement of *S. littoralis* from amaranth. Thöming *et al.* (2013) showed the existence of plant preference hierarchies among ovipositing females of *S. littoralis*.

Pheromone traps are a convenient means of obtaining a "snapshot" of the adult of insect population density at a given time and place (Pair *et al.*, 1986) but can also be used as one of pest management options (Wyatt, 1997; Malo *et al.*, 2001). In the current study, PAA was not able to provide this information about targeted pests. It

was a generalist moths' attractant which was capturing mostly moths of no importance to the crop under investigation. Contrary to Landolt *et al.* (2011) who reported a mean trap of up to 27 of *S. recurvalis* in Waimea, Hawaii, the number of *S. recurvalis* trapped in the present study never exceeded three moths per trap. It, therefore, couldn't be used either as a mean of monitoring or mass trapping of *S. exigua*, *S. littoralis* or *S. recurvalis* which are the major defoliators of amaranth. The same way, *S. recurvalis* pheromone was not efficient in trapping the population of *S. recurvalis* under investigation though it is reported to be efficient with India populations (Dr Bhanu personal communication, 2017). This inefficiency may be explained by the difference in environmental conditions since further studies showed the genetic homogeneity of Kenyan populations of *S. recurvalis* and the ones from India (Agbodzavu *et al*., in press). Only *S. exigua* pheromone was efficient enough to provide accurate information about the seasonality of *S. exigua*. It confirmed the presence of *S. exigua* year-round with a high abundance in April after the onset of the rainy season. Goergen *et al.* (2016) reported that severe outbreaks of armyworms usually coincide with the onset of the wet season, mainly when the new cropping season follows a long period of drought. Also, according to Sharma *et al.* (2002) experiments, rainfall, maximum and minimum relative humidity were positively associated with oriental armyworm moth catches.

Damages recorded were variable, with the season mean lower than 15%. However, during the first season where *S. recurvalis* outbreak happened, damage was high on *A. dubius* showing the higher destructive potential of this pest as compared to the *Spodoptera* species on amaranth. The impact on yield losses of *Spodoptera* species cannot be neglected. Although the quantitative losses due to *Spodoptera* species were not as high as for *S. recurvalis*, the quality of the harvestable vegetable is affected, making them unmarketable. Attractants used in this study were not able to reduce the damages due to lepidopteran defoliators feeding.

Different parasitoids were identified during this study. In the field experiments, only three parasitoids were identified whereas in the farmer fields, up to eight species were identified. These results showed that even within the same agro-ecological

zone, there is a difference in the distribution of the parasitoids as well as in the lepidopteran species. Presence of *Apanteles* sp. as one of the major parasitoid of *S. recurvalis* has been reported in different studies (Bhattacherjee & Ramdas Menon, 1964; Peter & Balasubramanian, 1984; Kedar & Kumaranag, 2013). However, its parasitisation by *Phanerotoma* species and *Schoenlandella testacea* has never been reported. The same way, data on the biology of *C. ater* on *S. littoralis* and *S. exigua* is missing crucially in the literature, therefore, opening an avenue for research works.

This study showed that the most important lepidopteran defoliators on leafy amaranths were *S. recurvalis*, *S. exigua* and *S. littoralis*. However, their relative importance depends on the agro-ecology zone, the season and the amaranth species. In high altitude, *S. exigua* was the predominant species while in the mid-altitude it was *S. recurvalis*. In the view of implementing an integrated management approach of these defoliators, the focus should not target one species but the species complex. Damages recorded were also variable, with the highest occurring with *S. recurvalis* outbreak. Tested *S. recurvalis* pheromone and Phenylacetaldehyde were not efficient in attracting the moths. However, *S. exigua* pheromone was efficient in catching adult' individuals though not able to reduce the leaf damage level. Adding *S. littoralis* pheromone in the field, having an efficient *S. recurvalis* pheromone and increasing the number of traps baited with *S. exigua* pheromone might induce a reduction in the damage. The discovering of a new species of *Cotesia* is a tremendous finding of this work. Its efficiency in managing other *Spodoptera* species will be needed as *Spodoptera* species constitute highly polyphagous pests species of worldwide economic importance (Rose *et al.*, 2000; Goergen *et al.*, 2016).

CHAPTER FOUR

PERFORMANCE OF THE NEWLY IDENTIFIED ENDOPARASITOID *Cotesia icipe* FERNANDEZ-TRIANA AND FIABOE ON *Spodoptera littoralis* (BOISDUVAL)

Abstract

Cotesia icipe is a solitary koinobiont larval endoparasitoid, recently discovered in Kenya and new to science, that parasitizes select lepidopteran herbivores of amaranth. Its host range was investigated on five commonly encountered amaranth lepidopteran defoliators. *Cotesia icipe* accepted, successfully and aggressively parasitized the amaranth noctuid defoliators *Spodoptera littoralis* and *S. exigua*, but failed to parasitize *Herpetogramma bipunctalis*, *Spoladea recurvalis* and *Udea ferrugalis* all in Crambidae family. On *S. littoralis*, *C. icipe* was highly efficient, with 95 % of females successfully ovipositing during 2 hrs of exposure. Parasitism rate and larval and pupal non-reproductive mortalities were significantly higher at higher parasitoid density. A single female of *C. icipe* parasitized 42.99 ± 2.66 % of the 50 exposed larvae for oviposition in 24 hrs; whereas a cohort of five females of *C. icipe* conferred 85.59 ± 1.46 % parasitism rate. The efficiency ratio per female was much higher in single releases than in cohort releases while a balanced sex ratio was obtained in F1 offspring regardless of the density of female released. The potential use of *C. icipe* for conservation and augmentative biological control of *S. littoralis* in amaranth as well as its possible use against other Noctuid moths is discussed.

Keywords: Amaranth, biological control, host range, Noctuidae, non-reproductive mortality, parasitism.

4.1 Introduction

Amaranth, *Amaranthus* spp. (Caryophyllales: Amaranthaceae) is generally grown for its leaves and grains. Amaranth leafy vegetables, despite their economic importance and their high nutrition potential coupled with their medicinal attributes, have

received little research attention until the last decade (Priya *et al.*, 2007; Venskutonis & Kraujalis, 2013).

Amaranth crop production is seriously hampered by a complex of insect pests of which lepidopteran defoliators are the most destructive. The most important species include *Herpetogramma bipunctalis* (F.) (Lepidoptera: Crambidae), *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae), *Pholisora catullus* (F.) (Lepidoptera: Hesperiidae), *Spoladea recurvalis* (F.) (Lepidoptera: Crambidae), *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), *S. eridania* (Stoll) (Lepidoptera: Noctuidae), *S. littoralis* (Boisduval) (Lepidoptera: Noctuidae) and *Udea ferrugalis* (Hübner) (Lepidoptera: Crambidae) (Ellis, 2004). *Herpetogramma bipunctalis* and *S. recurvalis* are leaf webbers while the other *Spodoptera* species are leafworms. Leaf-webbing larvae typically roll the leaf into distinctive leaf shelters and voraciously feed on the green matter. Severe attacks result in rapid and complete skeletonisation and desiccation of leaves (James *et al.*, 2010). Within leafworms, *S. littoralis* is one of the most damaging species, where the young larvae feed at numerous sites and eventually spread over the entire leaf. Older instars chew large holes or wholly consume leaves (Sullivan, 2014).

In Kenya, lepidopteran defoliators are an increasingly serious constraint for sustainable amaranth production (Kahuthia-Gathu, 2011). A recent countrywide survey carried out in Kenya during the long raining season of 2014 revealed that lepidopteran defoliators, including *S. littoralis*, are the most devastating pest of vegetable amaranth, causing foliage damage ranged between 20 and 40% (Othim *et al.*, 2017). This pest belongs to the genus *Spodoptera*, which includes several economically important species in Africa (Brown & Dewhurst, 1975) that attack various cash and traditional food crops (Capinera, 2008; Khedr *et al.*, 2015). *Spodoptera littoralis* management has solely been through the use of insecticides. However, due to increased reports of pesticide resistance (Mosallanejad & Smagghe, 2009) and with the recommended pre-harvesting interval (PHI) usually not followed by farmers, integrated pest management (IPM) options, including biological control,

that are safe, sustainable and environmentally friendly are crucial for sustainable amaranth productivity.

Different biological agents such as the entomopathogenic fungi (Ahmed & El-Katatny, 2007), entomopathogenic nematodes (Atwa, 2013; Shairra & Noah, 2014), viruses (Jones *et al.*, 1993) and parasitoids (Vojtech *et al.*, 2005; Depalo *et al.*, 2010; Hatem *et al.*, 2016) have proven effective against *S. littoralis*. During surveys of amaranth lepidopteran parasitoids in central Kenya, a new species of *Cotesia* named *C. icipe* Fernandez-Triana & Fiaboe was frequently found parasitizing *S. littoralis* larvae (Fiaboe *et al.*, 2017). The genus *Cotesia* is one of the most diverse genus of the subfamily Microgastrinae (Hymenoptera: Braconidae), with almost 300 species already described (Yu *et al.*, 2016). *Cotesia* is known to encompass key biological control agents of economically important pests in agricultural ecosystems. Some few examples are *C. flavipes* Cameron, *C. sesamiae* (Cameron) and *C. vestalis* (Haliday) which were used against various key pests across the continent (Omwega *et al.*, 2006; Gounou *et al.*, 2009; Kahuthia-Gathu *et al.*, 2017). *Cotesia icipe* may become a key component of an IPM approach for *S. littoralis* population suppression. However, there is currently no study on its performance on *S. littoralis* using amaranth as a host food plant.

The purpose of this study was to assess the host range of *C. icipe* on commonly found amaranth lepidopteran defoliators in Kenya, *S. littoralis*, *S. exigua*, *H. bipunctalis*, *S. recurvalis* and *U. ferrugalis*, as well as its effectiveness at two different densities as a biological control agent of *S. littoralis*.

4.2 Materials and Methods

4.2.1 Host plant

The study was conducted at the International Centre of Insect Physiology and Ecology (*icipe*), Nairobi, Kenya (1.22170°S; 36.89648°E). Host plant used throughout the laboratory experiments were 6 to 7 week-old *A. dubius* Mart. ex Thell

(variety Dubious) grown on a mixture of soil and compost (4:1) in a screen house environment free of any pest infestation (Plate 4.1).

Plate 4.1: *Amaranthus dubius* growing in a screen house at *icipe*

4.2.2 Lepidopteran pests colonies

Laboratory rearing was carried out at 25 ± 2 °C, 50-70 % RH and a photoperiod of 12:12 (L: D) h and the experiments were conducted at 25 ± 1 °C, 50 ± 6 % R.H and a photoperiod of 12:12 (L: D) h.

Larvae of *S. littoralis, S. exigua, S. recurvalis*, and *H. bipunctalis* were initially collected from Yatta, Machakos County (01.23044°S; 037.45789°E) and Mwea, Kirinyaga County (0.6309°S; 37.35117°E) while *U. ferrugalis* were collected from Limuru (01.20318°S; 036.71871°E) in Kiambu County and reared for at least five generations prior to experiments. Adult moths were placed in cages (40 x 40 x 45 cm) constructed of Perspex materials with netting materials fitted on the back side and a sleeve on the front side. They were fed with a 1:9 honey: water solution soaked in cotton and hung on the wall of the cage. Moths were provided with potted amaranth plants for oviposition. Plants were removed after 24 hrs exposure to the moths and transferred to wooden holding cages (50 x 50 x 60 cm), ventilated on the sides and at the top until the eggs hatched. First instar larvae, kept under the same conditions as adults, were allowed to feed on seedlings for five to seven days and

were then transferred with the leaves into ventilated plastic boxes (15 x 7 x 5 cm) lined with a paper towel to absorb excess moisture. Larvae were fed with fresh amaranth leaves until pupation. During the process, plastic boxes were monitored daily to remove wastes and to change the paper towel when it was wet. Pupae were incubated under similar rearing conditions until adult emergence. For *S. recurvalis*, *H. bipunctalis* and *U. ferrugalis*, folded leaves containing pupae were held in a cage until adults' emergence (Plate 4.2).

4.2.3 *Cotesia icipe* colony

The colony of *C. icipe* originated from naturally infested *S. littoralis* larvae collected from Yatta, Kenya (01.23044°S, 037.45789°E, 1173 masl). Preliminary observations in *icipe* laboratory showed that second-instar larvae were more readily accepted for oviposition by *C. icipe* and were, therefore, used for rearing and the experiments. Plants heavily infested with second-instar larvae of *S. littoralis* were introduced in ventilated perspex cages containing 2 to 3 days old mated *C. icipe* adults. *Cotesia icipe* adults were fed on honey spread on strips of paper. Parasitoids were, therefore, exposed to their host for 24 hrs to allow oviposition. After 24 hrs of exposure, amaranth plants with larvae were harvested and incubated in plastic boxes. Fresh amaranth leaves were regularly provided as needed until parasitoid cocoons were formed. Cocoons were collected, placed in opened Petri dishes (90 x 12 mm), and kept in a cage till emergence (Plate 4.2).

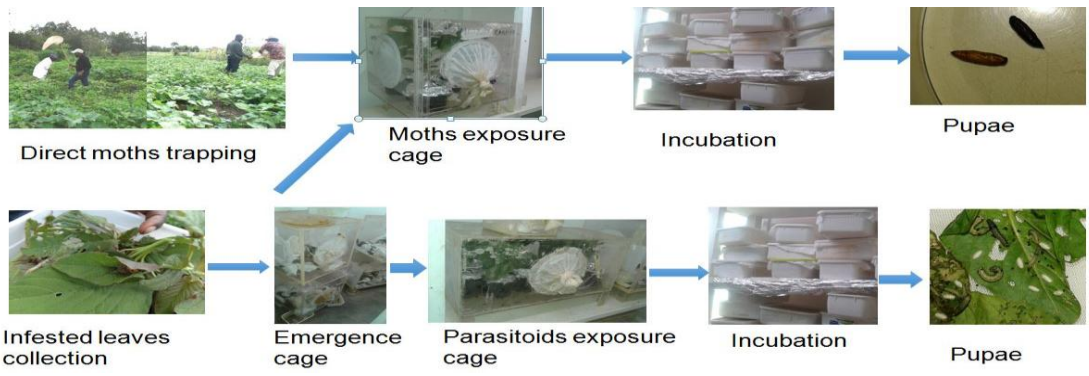

Plate 4.2: Schematic representation of lepidopteran and parasitoid species rearing process; Photo: AIV-IPM project

4.2.4 Hosts range and acceptability experiments

Potted plants were infested with host larvae 24 hrs prior to the release of the parasitoids to allow them to settle and establish a feeding site. Six to seven week-old potted plants were each infested with 30 second instar larvae of the five species (*S. littoralis*, *S. exigua*, *H. bipunctalis*, *S. recurvalis* and *U. ferrugalis*), and were placed in separate cages (Plate 4.3). Thereafter, host larvae were exposed to a single two to three day-old naive mated *C. icipe* female originating from the stock culture. This experiment was replicated 20 times for each host. Acceptance of the parasitoid was determined by the number of oviposition attempts by the female during a two hrs exposure regime. Each larval probe was assumed to be an oviposition attempt (Chabi-Olaye *et al.*, 2013). The time taken before first and second oviposition attempts was recorded. The experiment was discontinued when a female spent one hr without oviposition attempts and used to assess host range. Hosts on which the female *C. icipe* did not attempt to oviposit, in any of the replicates in the first hr of observation, were considered unacceptable hosts while those with effective oviposition attempts were considered as acceptable to the parasitoid. Parasitoids were removed from larval cages two hrs after exposure for accepted hosts; however, in the present study, acceptability levels were measured only for *S. littoralis*. Leaves with *S. littoralis*' larvae were harvested and incubated in plastic boxes and monitored for either parasitoid or *S. littoralis* emergence. Fresh amaranth leaves were supplied to the larvae until pupation or parasitoid cocoon formation. Successful oviposition was defined as the occurrence of at least one cocoon of the parasitoid during incubation. To determine whether the parasitoid was solitary or gregarious, 50 parasitized larvae were isolated, each in a separate vial plugged with cotton wool and held until parasitoid emergence or host pupation. The number of parasitoid cocoons per larva was recorded.

Plate 4.3: Typical set up for acceptability and suitability studies

Photo: M. K. Agbodzavu

4.2.5 Host suitability of *Spodoptera littoralis* for *Cotesia icipe*

One potted amaranth plant was caged and infested with 50 second instar *S. littoralis* larvae for 24 hrs to allow the larvae to settle and establish a feeding site. Two different parasitoid densities were tested. In the first set of experiments, a cohort of 2 males and 5 naive mated females of *C. icipe*, aged 2 to 3 days, were released in the cage and allowed to oviposit on the larvae for 24 hrs. In the second cohort of the experiment, a male and a naive mated female of *C. icipe*, aged 2 to 3 days were released in the cage and monitored. After 24 hrs, the larvae were harvested and handled as described in section 4.2.4. To observe the developmental stages of the parasitoid, each collected cocoon was placed in a gelatine capsule (2.20 cm height, 0.7 cm diameter and 0.8 cm^3 volume). The intervals from exposure until the cocoon formation (Plate 4.4) and from cocoon formation until adult emergence were recorded. The control consisted of plants infested with 50 larvae but not exposed to parasitoid, to assess natural mortality of the hosts under the same rearing conditions. This experiment was replicated 20 times.

Data collected included the number of parasitoid cocoons, the parasitoid's larval and pupal developmental time, the non-reproductive mortality, and the F1 sex ratio. In addition, the relative fitness of the emergent parasitoids as a function of body size was determined by measuring the length of the adult hind leg and forewing (Plate 4.5) from 30 randomly selected parasitoids of each sex (Othim *et al.* 2017).

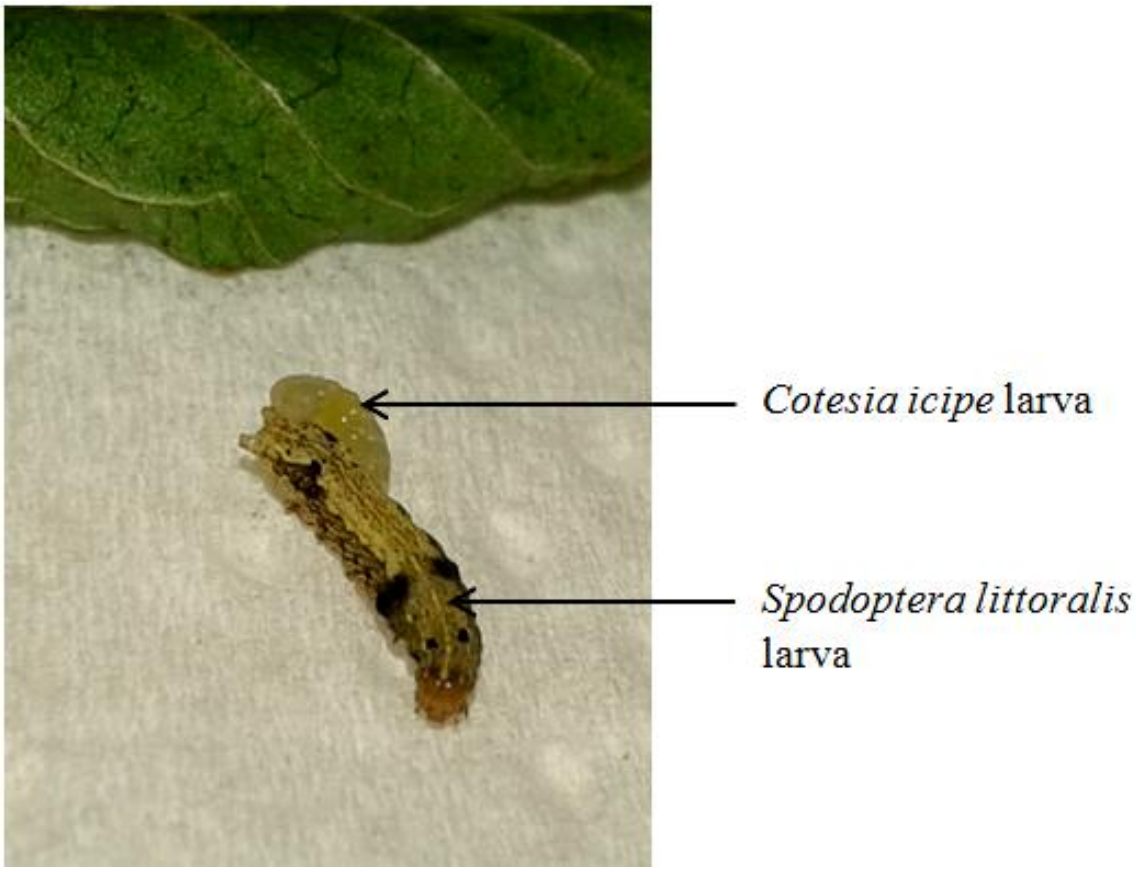

Plate 4.4: Parasitized larva of *Spodoptera littoralis* showing exiting *Cotesia icipe*

Photo: M. K. Agbodzavu

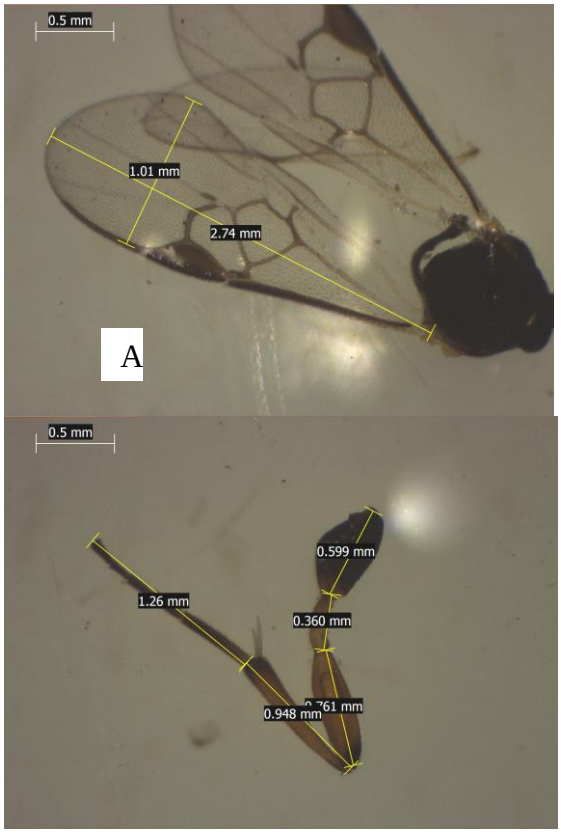

Plate 4.5: Measurements of *Cotesia icipe* forewing (A) and foreleg (B) reared on *Spodoptera littoralis*

Photo: M. K. Agbodzavu

4.2.6 Data analyses

All data were checked for normality and homogeneity of variance using Shapiro-Wilk and Bartlett tests respectively. The time before the first oviposition attempt, parasitoid egg-larval development time, forewing length, parasitism rate, the significance of non-reproductive mortality, as well as non-reproductive larval mortality were analysed using a Wilcoxon test (SAS Institute Inc., Cary, NC, USA). Cocoon developmental time and the hind leg tibia length of the F1 adults of both female and male *C. icipe* were analysed using an independent samples t-test while the total development time, non-reproductive pupal mortality were analysed using a Welch Two Samples *t*-test. Parasitism rate was calculated as the percentage of the

number of parasitoid cocoons divided by the sum of pupae of host and parasitoid cocoons. Host larval mortality in the presence and absence of the parasitoid were calculated as described by Othim *et al.* (2017).

Host larval mortality in the presence of the parasitoid (HLMp) was calculated as follow:

$$\text{HLMp} = \frac{\text{Ni} - (\text{Npc} + \text{Nhp})}{\text{Ni} - \text{Npc}} \text{ X } 100$$

Where: Ni: the number of larvae exposed at the beginning

Npc: the number of parasitoid cocoons

Nhp: the number of host pest pupae

The larval mortality in the control (LMc) was calculated as:

$$\text{LMc} = \frac{\text{Ni} - \text{Nhp}}{\text{Ni}} \text{ X } 100$$

While the pupal mortalities (PM) in the presence of the parasitoid and the control were calculated as:

$$\text{PM} = \frac{\text{Nhp} - \text{Ne}}{\text{Nhp}} \text{ X } 100$$

Where: Ne: the number of emerged host adults

The actual non-reproductive (induced) host mortality (NRM) was calculated using the Abbott's formula (Abbott, 1925) as follow:

$$\text{NRM} = \frac{(\text{P} - \text{Pc}) \text{ x } 100}{(100 - \text{Pc})}$$

Where P is the percentage of mortality in the presence of the parasitoid and Pc is the percentage of mortality in the controls.

The significance of non-reproductive mortality was assessed by comparing natural mortalities in the control with mortalities in presence of the parasitoid. Sex ratio was examined using a χ^2 test. For all data analyses, *p*-values of < 0.05 were considered significant. All analyses except Wilcoxon test were carried out in R version 3.4.1 (R Core Team, 2017).

4.3 Results

4.3.1 Host range and host acceptability of *Cotesia icipe*

Herpetogramma bipunctalis, *S. recurvalis* and *U. ferrugalis* were not acceptable hosts for oviposition by *C. icipe*. However, the noctuids *S. littoralis* and *S. exigua* were accepted for oviposition. After two hrs of exposure, 95 % of female *C. icipe* oviposited successfully on *S. littoralis* larvae. Upon release into the cage, an active search and escape behaviour was observed between the parasitoid and its host. The female parasitoid started moving actively in search of the larvae by probing host larvae frass left on the leaf surface with its abdomen, even in the absence of the larvae and drumming the leaves surface by its antennae. Once the parasitoid detected the presence of the silk thread, it intensified the searching by moving faster, increasing the vibration of its antennae and started the ‘‘sting’’ behaviour by bending its abdomen aside, exposing and introducing its ovipositor in the web in search of the host. When oviposition was attempted, the sting usually happened near the thoracic segments of the larvae. Upon inserting its ovipositor, a female quickly removed it just after and moved away from the larva. No marking behaviour was observed. After the ovipositor was removed, the stung larva lifted its head and stayed immobilized and seemingly ‘‘paralysed’’ for about 5 seconds before resuming movement.

The time taken for 1st host encounter was 11 ± 2.78 min and significantly (χ^2 = 10.35, *df* = 1, *p* = 0.001) higher than the 3.7 ± 1.04 min taken before the second oviposition attempt. The number of oviposition attempts during the period of observation was 28.6 ± 3.56 per female. When 3rd instar and later were offered, the larvae aggressively defended themselves and often escaped the oviposition attempt by

shaking vigorously their head and thorax. Although the females were sometimes observed attacking host larvae more than once, only one parasitoid cocoon was obtained per larva.

4.3.2 Host suitability of *Spodoptera littoralis* for *Cotesia icipe*

Total parasitoid developmental time did not differ significantly (t = 0.02, df = 26.88, p = 0.983) between the single and cohort female releases (Table 4.1). However single female parasitoid release resulted in significantly shorter larval developmental time (χ^2 = 4.00, df = 1, p = 0.045) and significantly longer pupal developmental time (t = 2.56, df = 38, p = 0.015) compared to cohort females release (Table 4.1). The larvae started spinning cocoons three to four hrs after emerging from the host larvae. Feeding activities of parasitized larvae declined within three days parasitization, and a conspicuous colour change occurred from the 5th to 8th day of incubation at which time the larval colour changed from green to yellow-green and was associated with reduced growth and movement.

Table 4.1: Developmental time of the endoparasitoid *Cotesia icipe* (mean ± SE) under single and cohort female releases

Parameters	Density of parasitoids release		χ^2	t	df	p
	Single female release	Cohort of 5 females release				
Larval development time (days)	8.7 ± 0.16[b]	9.0 ± 0.07[a]	4.00		1	0.045
Pupal development time (days)	4.6 ± 0.08[a]	4.3 ± 0.08[b]		2.56	38	0.015
Total development time (days)	13.3 ± 0.18[a]	13.3 ± 0.08[a]		0.02	26.88	0.983

Means followed by same lower case letters in the same row were not significantly different either by Wilcoxon rank sum test, Two Sample *t*-test or Welch Two Sample *t*-test at $p < 0.05$.

The parasitism rate was significantly higher ($\chi^2 = 29.29$, $df = 1$, $p < 0.001$) when five female parasitoids were used than when a single female was used. Similarly, the efficiency ratio per female was higher in the single release compared to the cohort release. Once offered 50 larvae of *S. littoralis*, a single female *C. icipe* parasitized 42.99 ± 2.66 % of the larvae, and a cohort of five females parasitized 85.59 ± 1.46 % of larvae (Table 4.2). Both the single and cohort female parasitoid releases resulted in significant larval and pupal non-reproductive mortalities (Single: Larval- χ^2 = 6.27, df =1, p = 0.012, Pupal- t = -2.681, df = 38, p = 0.011; Cohort: Larval - χ^2 =29.3288, df =1, p < 0.001, Pupal- t = -3.25, df = 22.07, p = 0.004). The non-reproductive larval and pupal mortalities in the cohort release were significantly higher than in a single female release (Table 4.2). A balanced sex ratio was obtained in the F1 offspring regardless of the parasitoid density (Table 4.3).

Table 4.2: Parasitism rate (mean ± SE) and non-reproductive mortality (mean ± SE) under single and cohort female releases of the endoparasitoid *Cotesia icipe* on 50 host larvae after 24 hours

Parameters	Single female release	Cohort of 5 females release	χ^2	*t*	*df*	*p*
Parasitism rate (%)	42.99 ± 2.66[a]	85.59 ± 1.46[b]	29.29	-	1	< 0.001
Larval non reproductive mortality (%)	17.92 ± 6.34[a]	68.94 ± 3.87[b]	22.93.0	-	1	< 0.001
Pupal non reproductive mortality (%)	7.39 ± 3.54[a]	26.92 ± 8.47[b]		2.30	27.59	0.029

Means followed by same lower-case letters in the same row were not significantly different by Wilcoxon rank sum test and two-sample *t*-test at $p < 0.05$.

Table 4.3: F1 offspring sex ratio under single and cohort female releases of the endoparasitoid *Cotesia icipe* on 50 host larvae after 24 hours

	Sex ratio (%)	
Sex	Single female release	Cohort of 5 females release
Male	52[a]	48[a]
Female	48[a]	52[a]
χ^2	0.26	0.56
df	1	1
p	0.611	0.454

Means followed by same lower-case letters in the same column were not significantly different by χ^2 test at $p \leq 0.05$

Both the parasitoid density and the sex had significant effects on the hind tibia length of *C. icipe* progenies. The hind tibia length of female progenies was significantly longer for the single female release compared to cohort release. However, the parasitoid density did not affect the size or fitness of the male progeny (Table 4.4). Female *C. icipe* obtained at both high and low densities had longer forewings and hind tibia than their male counterparts (Tables 4.4). The parasitoid density did not affect male wing length while female progenies had a significantly longer forewing in cohort versus single release (Table 4.4).

Table 4.4: Mean hind tibia length (mm) (mean ± SE) of both sexes at different parasitoid release densities of the endoparasitoid *Cotesia icipe* on 50 host larvae after 24 hours

Density of female parasitoid release	Female	Male	*t*	χ^2	*df*	*p*
	Hind tibia lengths (mm)					
Single female release	0.92 ± 0.01^{Bb}	0.87 ± 0.01^{Aa}	4.9 3		5 8	< 0.001
Cohort of 5 females release	0.89 ± 0.01^{Ba}	0.87 ± 0.01^{Aa}	2.0 9		5 8	 0.041
t	-3	-0.17				
df	58	58				
p	0.004	0.866				
	Forewing lengths (mm)					
Single female release	2.81±0.02^{Ba}	2.72±0.02^{A} ^{a}		5.60	1	0.018
Cohort of 5 females release	3.10 ± 0.04^{Bb}	2.71±0.02^{A} ^{a}		27.0 3	1	<0.001
χ^2	20.6181	0.111				
df	1	1				
p	<0.001	0.739				

Means followed by the same upper (lower) case letters in the same row (column) were not significantly different at $p \leq 0.05$.

4.4 Discussion

Numerous successes have been achieved through the release of *Cotesia* species either through a classical or augmentative biological program against Crambidae, Noctuidae and Pieridae. Some of the best-known examples are *C. flavipes*, *C. marginiventris* (Cresson), *C. rubecula* (Marshall), *C. kazak* (Telenga) and *C vestalis* (Overholt *et al.*, 1997; Gillespie *et al.*, 1999; Cameron *et al.*, 2006; Herlihy & Driesche, 2013). In all these cases, the *Cotesia* species were reported to cause parasitism ranging between 25- 90 %, and were key components in IPM programs.

This work reports on novel biological data on a new species of *Cotesia* recently named as *C. icipe*. The time spent by the female parasitoid to make its first oviposition attempt was significantly longer than the time it took before the second oviposition attempt, suggesting that *C. icipe* underwent a learning process allowing it to easily detect the volatiles directly associated with the host. This is in line with Othim *et al.* (2017) who reported that experience can increase the responsiveness of *Apanteles hemara* Nixon (Hymenoptrea: Braconidae) on *S. recurvalis* and *U. ferrugalis*.

Cotesia icipe frequently searched for a host in the frass and faeces' excreted on the plant by the host larvae, in the web threads of the host larvae or the larvae when antennated. The first hypothesis to explain these observations is that the frass and the faeces, as well as the silk threads and the larvae themselves, were emitting volatile organic compounds that were directing and attracting the parasitoid. It is well known that female parasitoids while searching for hosts, use a blend of airborne semiochemicals often referred to as herbivore-induced plant volatiles (HIPVs) (Turlings *et al.*, 1991; Agelopoulos *et al.*, 1995; Hare, 2011). Further studies are warranted to find out which HIPVs were used by *C. icipe* to locate the larvae on infested amaranth plants by *S. littoralis* and their use for early parasitoid recruitment in amaranth fields to enhance biological control. The second hypothesis is that in addition to olfactory stimuli, texture and shape of the host larvae and silk thread,

perceived through visual and tactile stimuli may help the female *Cotesia* in host location (Wackers and Lewis 1994).

Currently, the only known hosts of *C. icipe* are *S. littoralis* and *S. exigua*. It is, however, possible that the parasitoid has a wider host range among *Spodoptera* species or even other lepidopteran species. Although certain species of *Cotesia* have been found parasitizing more than one family of Lepidoptera, such as *C. flavipes* attacking species of Crambidae, Pyralidae and Noctuidae (Getu *et al.*, 2003; Jiang *et al.*, 2004; Rossi *et al.*, 2014; Kaiser *et al.*, 2017), other species such as *C. rubecula* are restricted to one family. *Cotesia icipe* did not show any attractiveness toward the three Crambidae species tested in the current study. This was further exemplified by the failure to rear *C. icipe* on the three Crambidae species under laboratory conditions while the parasitoid completed development on *S. littoralis*. However, it remains clear that the full host range potential of this parasitoid is still unknown and requires further studies to unravel the breadth.

Solitary (Gao *et al.*, 2016a) and gregarious species (Vos & Vet, 2004) are found in the genus *Cotesia*; *Cotesia icipe* has consistently proven to be a solitary koinobiont endoparasitoid. The results of this study showed that, although the parasitoid was observed to oviposit more than once in a host larva when parasitized larvae were singly isolated in vials, each yielded only one parasitoid cocoon, suggesting the solitary nature of *C. icipe*.

Cotesia icipe had a relatively shorter total developmental time (13.3 days) compared to other species of *Cotesia* such as *C. flavipes* on *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) (17.9 days) (Ngi-Song *et al.*, 1995) and *Cotesia sesamiae* (Cameron) on *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) (16.2 days) (Ndemah *et al.*, 2012). However, this developmental time is similar to the one of *C. marginiventris* (12-13 days) (Kunnalaca & Mueller, 1979), *C. plutellae* (12.6 days) (Shi *et al.*, 2002), *C. chilonis* (Munakata) (12.5 days) (Hailemichael *et al.*, 2009), *C. rubecula* (13.48 days) (Harvey *et al.*, 1999) and *C. ruficrus* (Haliday) (13.2 days) (McCutcheon *et al.*, 1983). Having a parasitoid with relatively short life cycle such

as *C. icipe*, is advantageous in mass rearing and for quick population build up in the field.

While using a single female, the parasitism rate obtained was 42.99 %. This finding is similar to parasitism rates of related *Cotesia* species (*C. kazak*), suggesting their high efficacy on suitable hosts. For instance, Jalali *et al.* (1988) showed that using one mated female of *C. kazak* for 24 hrs, it parasitized 50.5 % of *H. armigera* 4-6 days-old larvae at a density of 25 larvae/plant. The parasitism rate obtained with a single female on *S. littoralis* within 24 hrs exposure is relatively high, suggesting *C. icipe* is a good candidate for conservation or augmentative biological control. When five females cohort were released, the parasitism rate was even higher at 85.59 %. These results demonstrate the necessity of carrying out functional and numerical response studies for optimization of *C. icipe*'s densities in biological control programs. The results of the present study further showed significant non-reproductive larval and pupal mortalities both in single and cohort female releases, which further enhance the performance of this parasitoid in pest control.

In the present study, the sex ratio of the parasitoid was constantly balanced. This is likely the first study reporting a balanced sex ratio in *Cotesia* species. Most known studies have reported either female-biased sex ratio such as for *C. flavipes* (Veiga *et al.*, 2013; Trevisan *et al.*, 2016), *C. glomerata* in field clusters (Tagawa, 2000), or male-biased in *C. plutellae* (Kawaguchi & Tanaka, 1999; Heimpel & Lundgren, 2000), *C. melanoscela* (Ratzeburg) (Kolodny-Hirsch, 1988) and *C. vestalis*, both in inbred crosses and outcrosses (De Boer *et al.*, 2007).

This study is the first report on some aspects of the biology of the new species of *Cotesia*. While the crambid amaranth leaf webbers tested were not attacked by the parasitoid, *C. icipe* accepted and developed successfully on *S. littoralis* and *S. exigua*. The high parasitism coupled with the high non-reproductive mortality rate and the reduction of food consumption by the parasitized larvae suggest that *C. icipe* has potential as a biological control agent of *S. littoralis* in an IPM program for amaranth. Further studies are needed to determine optimal densities for use in biological control as well as to assess the performance of the parasitoid under field

conditions and on other host plants and other *Spodoptera* species such as *S. exempta* and *S. frugiperda*.

CHAPTER FIVE

ACCEPTABILITY AND SUITABILITY OF *Spodoptera exigua* (HÜBNER) FOR *Cotesia icipe* FERNANDEZ-TRIANA AND FIABOE ON AMARANTH

Abstract

The beet armyworm *Spodoptera exigua* is a polyphagous insect that is distributed worldwide and was recently reported as an important pest on African indigenous vegetables. *Cotesia icipe* is a recently described parasitoid, reported from various Afrotropical countries. This work investigated the performance of *C. icipe* on *S. exigua* infesting *Amaranthus dubius* under laboratory conditions. *Cotesia icipe* was aggressive on the host and successfully oviposited on *S. exigua* with 70 % of parasitoid females ovipositing after 2 h of exposure. Parasitoid densities significantly affected the parasitism rate and the non-reproductive larval mortality. Parasitism rate was 9.7 ± 0.8 % and 59.5 ± 3.1 % for a single and cohort of 5 females released respectively when offered 50 host larvae. The cohort female release resulted in significantly higher larval non-reproductive mortality than the single release. However, there was no significant difference between parasitoid release densities in regards to pupal non-reproductive mortality. The larval and pupal mortalities in the presence of *C. icipe* were significantly higher than the natural mortalities at both parasitoid release densities. The parasitoid sex ratio was female biased for the cohort females but balanced when a single female was released. The hind tibia and forewing lengths were not affected by the density of female parasitoids, but there were variations according to sex. The implication of these findings on the potential use of *C. icipe* for biological control of *S. exigua* in amaranth production systems is discussed.

Keywords*:* Biological control, non-reproductive mortality, parasitism; parasitoid density.

5.1 Introduction

Amaranth species are one of the most important African indigenous vegetables (Pasquini *et al.*, 2009), valued for their high content of protein, minerals, and vitamins and the central role they play in human nutrition. Their production is, however, constrained by many insect pests of which *Spodoptera* species are the most important (Ellis, 2004; Mureithi *et al.*, 2017). The genus *Spodoptera* (Lepidoptera: Noctuidae) contains highly polyphagous pests species of worldwide economic importance (Rose *et al.*, 2000; Goergen *et al.*, 2016). One representative of this group is the species *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae). This pest has a nearly cosmopolitan distribution (Amaldoss & Hsue, 1989; Ruberson & Whitfield, 1996) and has been reported in Africa, North America, Central America and Caribbean, Europe and Oceania (Amaldoss & Hsue, 1989; Ruberson, Herzog, Lambert, & Lewis, 1994, Baron, 2007). The beet armyworm, *S. exigua*, is a generalist herbivore with over 90 known host plant species, including a number of economically important crops, such as cotton, corn, soybeans, rice and amaranth among other crops (Kim *et al.*, 2009; Mehrkhou *et al.*, 2012; Mehrkhou *et al.*, 2015). Young larvae feed gregariously on terminal clusters, seedlings, and stems of host crops. Leaves may be skeletonized and almost completely consumed (Bohmfalk *et al.*, 1999; McDougall *et al.*, 2013).

Different management options to control this pestiferous insect exist such as use of pheromones (Deng *et al.*, 2004; Acín *et al.*, 2010; Mujiono *et al.*, 2015), host-plant resistance (Eigenbrode *et al.*, 1993; Eigenbrode & Trumble, 1994; Guo *et al.*, 2011), biological control by the use of nuclear polyhedrosis virus (Zamora-Avilés *et al.*, 2017), use of pesticides (Moulton *et al.*, 2000; Moulton *et al.*, 2002) and parasitoids releases (Efil & Kara, 2004; Liu & Li, 2006). Several parasitoid species have been reported to parasitize *S. exigua* in many parts of the world (Stapel *et al.*, 1997; Cai *et al.*, 2012; Ghazali *et al.*, 2014). However, pesticide applications are the most frequently used management option to control *S. exigua*, although not recommended due to its detrimental effect to the natural enemies, leading to more frequent pest outbreaks (Ruberson *et al.*, 1994). Overuse of pesticides can also result in the

development of insecticide resistance in the pest (Ahmad & Arif, 2010; Che *et al.*, 2013). Therefore, there is an urgent need to identify alternative control methods to the chemical application, which is ecologically acceptable and is compatible with other management options within an overall Integrated Pest Management strategy.

Cotesia icipe Fernandez-Triana & Fiaboe (Hymenoptera: Braconidae) is a recently described parasitoid species that has been found parasitizing *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) in amaranth fields in central Kenya. Its current known distribution is the Afrotropical zone: Madagascar, Saudi Arabia, South Africa and Yemen (Fiaboe *et al.*, 2017). However, little is currently known about its bio-ecology especially its performance on different hosts. The present work, therefore, reports on the performance of this parasitoid on *S. exigua* in terms of acceptability, aggressiveness and suitability on *Amaranthus dubius* Mart. ex Thell.

5.2 Materials and methods

5.2.1 Host plants

The study was conducted at the International Centre of Insect Physiology and Ecology (*icipe*), Nairobi, Kenya (1.22170°S, 36.89648°E, 1602 masl). *Amaranthus dubius* was used throughout as the host plant for laboratory experiments. The variety (Dubious) used was obtained from Simlaw Seeds Company, Nairobi, Kenya. Seedlings were raised in trays in the screenhouse maintained at ambient conditions. A mixture of soil and compost (4:1) was used to fill 10 cm diameter plastic pots (628 cm^3) into which the amaranth seedlings were transplanted individually two weeks after germination and watered daily. Six to seven weeks old plants were used for experiments.

5.2.2 *Spodoptera exigua* colonies

Larvae of *S. exigua* were collected in farmers' fields during a survey at Yatta, Machakos County (01.23044°S, 037.45789°E, 1173 masl) and Mwea, Kirinyaga County (0.6309°S, 37.35117°E, 1197 masl) in May and June 2014. Emergent adult

moths were placed in cages (40 × 40 × 45 cm) made up of Perspex material with netting material (150 x 150 μm mesh) fitted on the back side and a sleeve on the front side. They were fed with 10 % honey/water solution soaked in cotton and hung with masking tape on the wall of the cage. Adults of *S. exigua* were provided with potted amaranth plants for egg laying at 25 ± 2 °C, 50 – 70 % RH and a photoperiod of 12:12 hrs (L:D). Plants were removed after every 24 hrs and transferred to wooden holding cages (50 × 50 × 60 cm), ventilated on the sides and at the top until the eggs hatched. First instar larvae were allowed to feed on the seedlings for 5 - 7 days and then transferred with the leaves into ventilated, rectangular plastic boxes (15 × 7 × 5 cm) lined with a paper towel to absorb excess moisture. Larvae were fed with fresh amaranth leaves until pupation. During the experiment, those plastic boxes were monitored daily to remove the larval faecal matter and change the paper towel when it was wet. Pupae were incubated under similar rearing conditions until adult emergence. Colony of *S. littoralis* was established following the same procedure.

5.2.3 *Cotesia icipe* colony

The colony of *C. icipe* originated from *S. littoralis* collected from Yatta, reared and maintained on *S. littoralis* larvae. Plants infested with second instar larvae of *S. littoralis* were introduced in ventilated perspex cages as described above in section 6.2.2 containing 2 - 3 days old mated *C. icipe* adults. Preliminary observations showed that second-instar larvae were more readily accepted for oviposition by *C. icipe* and were therefore used (Agbodzavu *et al.*, 2018). The parasitoids were fed on honey and hosts were offered to them for oviposition for a period of 24 hrs. After 24 hrs of exposure, the amaranth plants with exposed larvae were harvested and incubated in plastic boxes lined with paper towel. Fresh amaranth leaves were provided to the *S. littoralis* larvae ad-libitum till the parasitoid cocoons were formed. Cocoons were collected, placed in open Petri dishes (90 x 12 mm), and held in a cage till emergence. The rearing was conducted under the same conditions as for the host.

5.2.4 Searching and oviposition behaviour and acceptability of *Spodoptera exigua* for *Cotesia icipe*

All the bioassays to assess the host acceptability of *C. icipe* were set up in the laboratory at 25 ± 1 °C, 60 ± 5 % R.H and a photoperiod of 12:12 (L:D) hrs. Potted amaranth plants, each infested with 30 second-instar larvae of *S. exigua*, were placed in separate cages 24 hrs before the onset of the experiment. Host larvae were exposed to a single 2 - 3 days old naive mated female of *C. icipe* originating from the stock culture, in a clear Perspex cage (20 × 20 × 30 cm) with a netting material (150 x 150 µm mesh) fixed at the top for ventilation. The searching and oviposition behaviour of the parasitoid was noted by visual observation. Acceptance of the parasitoid was determined by the oviposition attempts of the female *C. icipe*, which was observed and recorded during a 2 hrs exposure time. The searching time before the first oviposition and between the first and the second oviposition attempts were recorded. The experiment was terminated when a female spent 1 hr without any oviposition attempt. The parasitoids were removed from larvae cage 2 hrs after exposure. The exposed larvae were removed with the leaves and incubated in plastic boxes. Fresh amaranth leaves were supplied to the larvae ad-libitum until pupation or parasitoids cocoon formation. This experiment was replicated 20 times. Successful oviposition was determined as the occurrence of at least one cocoon during incubation (Othim *et al.*, 2017).

5.2.5 Host suitability of *Spodoptera exigua* for *Cotesia icipe*

A single potted amaranth plant was caged and artificially infested with 50 second-instar larvae of *S. exigua* for 24 hrs to allow for larvae to settle and establish a feeding site. The infested amaranth plant was then introduced into another cage as described in section 6.2.4. Two different parasitoid's densities were tested. In the first set of experiments, a cohort of 2 males and 5 naive mated females of *C. icipe*, aged 2 - 3 days were released in the cage and offered the larvae for 24 hrs for oviposition. In the second set of experiment, a single male and single naive mated female of the same age were released in the cage. After 24 hrs, the larvae were

removed and handled as described in section 6.2.4. To observe the developmental stages of the parasitoid, each cocoon collected was isolated in a gelatine capsule (2.20 cm height, 0.7 cm diameter) and kept in a Petri dish according to the date of collection and per replicate. The development was checked once a day. The time that elapsed from the exposure until the cocoon formation as well as from cocoon formation till adults' emergence of the parasitoid was recorded. The control comprised of plants artificially infested with 50 second-instar larvae but not exposed to the parasitoid, to assess natural mortality of the hosts under the same rearing conditions. This experiment was replicated 20 times.

Data collected included the number of parasitoid cocoons, the development time of the parasitoid, number of host pupae, number of emerged host adults and F1 sex-ratio of the parasitoids. In addition, the fitness of the parasitoids' progeny was determined by measuring the lengths of adult hind leg and forewing from 20 randomly selected parasitoids of each sex. The forewing and the hind leg of both female and male were cut off from the insect body and placed in distilled water in an open Petri dish. Digital photographs of the insect parts were taken using a calibrated *Leica* microscope EZ4D (Leica Microsystems Ltd, Switzerland) connected to a laptop computer (Hewlett-Packard Company, Cupertino California). Measurements were made using *Leica* Software version 3.3.0. According to Kermani *et al.* (2014), forewing and the hind leg tibia measurements give an indication of an individual's body size.

5.2.6 Data analyses

All data were checked for normality and homogeneity of variance using Shapiro-Wilk and Bartlett tests respectively. The time before the first and the second oviposition attempt were normally distributed but failed to meet the condition of homogeneity of variance; they were therefore compared using Welch Two Samples t-test. The parasitoid egg-larval development time, the cocoon developmental time and the total development time were analysed using the Wilcoxon test because they were not normally distributed. The pairwise comparison of the hind leg tibias and the

forewings lengths of the F1 adults of *C. icipe* when considering the sex in one hand and the density of female parasitoids, on the other hand, were done using *t*-test, Welch Two Samples *t*-test and Wilcoxon test. The efficiency ratio per female which is the ratio between the parasitism rate and the total number of released female parasitoids was analysed using independent samples t-test because it was normally distributed with homogeneity of the variance. Parasitism rate was calculated as the percentage of the number of parasitoid cocoons divided by the sum of pupae of the host and parasitoid cocoons. Host larval mortality in the presence of the parasitoid (HLMp) was calculated as follow (Othim *et al.* 2017):

$$\mathrm{HLMp} = \frac{\mathrm{Ni} - (\mathrm{Npc} + \mathrm{Nhp})}{\mathrm{Ni} - \mathrm{Npc}} \mathrm{X}\ 100$$

Where: Ni: the number of larvae exposed at the beginning

Npc: the number of parasitoid cocoons

Nhp: the number of host pest pupae

The larval mortality in the control (LMc) was calculated as:

$$\mathrm{LMc} = \frac{\mathrm{Ni} - \mathrm{Nhp}}{\mathrm{Ni}} \mathrm{X}\ 100$$

While the pupal mortalities (PM) in the presence of the parasitoid and in the control were calculated as:

$$\mathrm{PM} = \frac{\mathrm{Nhp} - \mathrm{Ne}}{\mathrm{Nhp}} \mathrm{X}\ 100$$

Where: Ne: the number of emerged host adults

The actual non-reproductive host mortality was calculated using the Abbott's formula (Abbott, 1925) as follow:

$$NRM = \frac{(P - Pc) \times 100}{(100 - Pc)}$$

Where P is the percentage of mortality in the presence of the parasitoid and Pc is the percentage of mortality in the controls.

Because the parasitism rates and non-reproductive larval mortalities were not normally distributed and homogeneous, the Wilcoxon test was used to compare single and cohort release in relation to the parasitism rates and non-reproductive mortalities except for pupal non-reproductive mortality where Welch Two Samples *t*-test was used because it was normally distributed without homogeneity of variance.

The significance of larval and pupal non-reproductive mortality was assessed by comparing larval and pupal natural mortality in the control with mortality in presence of the parasitoid using independent samples *t*-test except the case of cohort release where the larval mortality was compared with Wilcoxon test. Sex ratio significance was assessed using a Chi-square test. All statistical analyses were done in R statistical software version 3.4.1 (R Core Team, 2017).

5.3 Results

5.3.1 Searching and oviposition behaviour of *Cotesia icipe* on *Spodoptera exigua*

After initial resting following release into the experimental cage, *C. icipe* became frequently agitated, flying and landing on various plant parts sporadically, and drumming its antennae on the leaf surface. *Cotesia icipe* was also seen probing the frass resulting from *S. exigua* feeding, using its ovipositor, in search of host larvae. The vibration of the antennae increased when the parasitoid approached the host. Once the larva was located, the female *C. icipe* started the ‘‘sting’’ behaviour, raising its wings, bending its abdomen aside, exposing and thereby introducing its ovipositor into the larva. It made the sting by standing close to the larva but not climbing onto it. *Cotesia icipe* attacked the larva at the thoracic segments by inserting its ovipositor for ca. five seconds before flying away. The attack was followed by a resting time of the parasitoid. Temporary paralysis of the attacked host larva was observed

immediately after the parasitoid attack before the larva resumed normal movement. As a defence mechanism, whenever larvae sensed the presence of the parasitoid or when they were attacked, they shook their body vigorously and dropped off the leaves on a silk thread to the ground. *Cotesia icipe* was observed to follow the web thread until the ground, although often was unable to parasitize such escaping larvae on the ground. *Cotesia icipe* was often observed to revisit already parasitized larvae and attempt another oviposition.

5.3.2 Acceptability of *Spodoptera exigua* for *Cotesia icipe*

The time taken by the female parasitoid to make the first oviposition attempt was significantly higher (t = 5.49, df = 21.28, $p < 0.0001$) than the time taken before the occurrence of the second oviposition attempt. The parasitoid spent 23.75 ± 3 min from the time it was released to the occurrence of the first oviposition attempt. The second oviposition attempt took place 6.8 ± 0.73 min after the first one. The mean number of attempts during the 2 h of observation was 16.45 ± 1.74, with 70 % of released female parasitoids making a successful oviposition attempt.

5.3.3 Host suitability of *Spodoptera exigua* for *Cotesia icipe*

There was no significant difference between the single and cohort parasitoid releases with regard to larval, pupal and total developmental times (Table 5.1). During larval development of *C. icipe*, a change in *S. exigua* colouration was observed 3 - 4 days after parasitism. The larvae turned from pale green to yellowish with retarded growth and reduced movement compared to un-parasitized larvae. Moreover, a remarkable reduction in feeding activities was noticed from day three onwards. Once the parasitoid larvae egressed from the *S. exigua* larvae, they spun cocoons within 3 to 4 h near their hosts which died within one day. The egression from the host larvae occurred from the abdominal segments when the host larvae were at their 3rd to 4th instar.

Table 5.1: Developmental time (mean ± SE) of the endoparasitoid *Cotesia icipe* under single and cohort of 5 females releases

Parameters	Density of parasitoids release		*W*	*df*	*p*
	Single female release	Cohort of 5 females release			
Larval development time (days)	9.5 ± 0.15[a]	9.8 ± 0.07[a]	226.5	1	0.460
Pupal development time (days)	4.9 ± 0.14[a]	4.8 ± 0.07[a]	174.5	1	0.481
Total development time (days)	14.4 ± 0.24[a]	14.6 ±0.06[a]	217	1	0.653

Means followed by same lower-case letters in the same row, did not differ significantly at $p < 0.05$ (Wilcoxon rank sum test).

The parasitism rate and larval non-reproductive mortalities were significantly higher in the cohort female parasitoid release compared to the single release. However, parasitoid release density didn't significantly affect the induced pupal non-reproductive mortality (Table 5.2). The efficiency ratio per female was also significantly higher in the cohort of 5 females release (11.91%) compared to the single female release (9.72 %) (t = 2.23, df = 38, p = 0.032). The single female parasitoid release was, however, sufficient to achieve significant larval (t = -3.91, df = 38, $p < 0.001$) and pupal non-reproductive mortalities (t = -6.9, df = 38, $p < 0.001$) when compared to natural mortality (Table 5.3). Similarly, the cohort release resulted in significant larval (W = 0, df = 1, $p < 0.0001$) and pupal (t = -2.93, df = 25.18, p = 0.007) non-reproductive mortalities when compared to natural mortality (Table 5.3).

Table 5.2: Parasitism rate and the non-reproductive mortality (mean ± SE) under single and cohort female releases of the endoparasitoid *Cotesia icipe* on 50 host larvae after 24 h

Parameters	Single female release	Cohort of 5 females release	*W*	*t*	*df*	*p*
Parasitism rate (%)	9.72 ± 0.76[a]	59.53 ± 3.1[b]	400		1	< 0.0001
Larval non-reproductive (%)	10.22 ± 1.88[a]	66.14 ± 3.03[b]	400		1	< 0.0001
Pupal non-reproductive mortality (%)	11.31 ± 1.43[a]	11.25 ± 4.08[a]		-0.01	23.60	0.988

Means followed by same lower-case letters in the same row are not significantly different at $p < 0.05$ (Wilcoxon rank sum test and Welch Two Sample *t*-test)

Table 5.3: Comparison between mortality (mean ± SE) in presence and absence of the endoparasitoid *Cotesia icipe* at different release densities on 50 host larvae after 24 h at 25 ± 1°C, 60 ± 5% R.H and a photoperiod of 12:12 hrs (L: D)

Treatments	Larval mortality	Pupal mortality
Single female parasitoid release		
Mortality in presence of *Cotesia icipe*	39.9 ± 1.19 a	22.42 ± 1.22 a
Natural mortality in control	32.7 ± 1.39 b	12.44 ± 0.78 b
t	-3.91	-6.9
p	< 0.001	< 0.0001
df	38	38
Cohort of 5 female parasitoids release		
Mortality in presence of *Cotesia icipe*	77.61 ± 1.85 a	25.27 ± 3.16a
Natural mortality in control	32.9 ± 1.42b	15.27 ± 1.29b
Wilcoxon test/Welch t-test	$W = 0$	$t = -2.93$
p	< 0.0001	0.007
df	1	25.18

For each release density, means followed by same letters in the same column are not significantly different at $p < 0.05$ (Wilcoxon rank sum test, *t*-test and Welch Two Sample *t*-test)

The sex ratio of the F1 progeny of *C. icipe* when a single female was released was balanced ($\chi^2 = 0.13$, $df = 1$, $p = 0.722$), with 48% male and 52% female. However, when a cohort of 5 females were released, the sex ratio was significantly female biased ($\chi^2 = 3.90$, $df = 1$, $p = 0.048$), with 45 % male and 55% females.

The hind tibia length was significantly longer in the female progeny in the single ($W = 312$; $df = 1$; $p = 0.002$) and cohort female releases ($t = 3.03$; $df = 38$; $p = 0.004$) compared to their male counterpart. Similarly, the forewing length of the female progenies was significantly longer than for the males in the single ($t = 2.22$; $df = 29.98$; $p = 0.034$) female and cohort release ($t = 2.75$; $df = 38$; $p = 0.009$). There was

no significant difference between release densities in relation to female and male progeny sizes (Table 5.4).

Table 5.4: Mean hind tibia and forewing lengths (mm) (mean ± SE) of both sexes at different *parasitoid* release densities of the *endoparasitoid Cotesia icipe* on 50 host larvae after 24 h

Density of female parasitoid release	Sex		*W*	*t*	*df*	*p*
	Female	Male				
Hind tibia lengths (mm)						
Single female release	0.81 ± 0.01^{Aa}	0.76 ± 0.01^{Ba}	312		1	0.002
Cohort of 5 females release	0.80 ± 0.01^{Aa}	0.75 ± 0.01^{Ba}		3.03	38	0.004
W	160.5	137				
df	1	1				
p	0.291	0.090				
Forewing lengths (mm)						
Single female release	2.42 ± 0.01^{Aa}	2.38 ± 0.01^{Ba}		2.22	29.98	0.034
Cohort of 5 females release	2.45 ± 0.02^{Aa}	2.39 ± 0.02^{Ba}		2.75	38	0.009
W	252.5	202				
df	1	1				
p	0.159	0.968				

Means followed by same upper (lower) case letters in the same row (column) are not significantly different at $p < 0.05$ (Wilcoxon rank sum test, Two Sample *t*-test or Welch Two Sample *t*-test)

5.4 Discussion

Within the Braconidae family, the subfamily Microgastrinae is the most conspicuous single group of parasitoids of Lepidoptera in the world, both in species richness and in economic importance (Inanç & Cetin Erdogan, 2004). It encompasses species which have been used successfully in agricultural insect pests' management (Kipkoech *et al.*, 2009; Soul-kifouly *et al.*, 2016; Pratt *et al.*, 2017). *Cotesia icipe*, a newly described parasitoid from central Kenya, with a significant biological control potential of lepidopteran pests, adds to the species complex of this important parasitoids group (Fiaboe *et al.*, 2017). Although this parasitoid is important on *S. littoralis* and this host played an important role in the rearing of the species for the experiment, *C. icipe* was also able to successfully parasitize and complete its development on *S. exigua*, a related *Spodoptera* species.

In the acceptability study, *C. icipe* took a longer time to make the first oviposition attempt compared to the second oviposition attempt. This difference in timing is also reported for *Cotesia kariyai* (Watanabe) (Hymenoptera: Braconidae) and could be a result of the experience gained from being in contact with the host / damaged leaves (Potting *et al.*, 1997; Fukushima *et al.*, 2001). *Cotesia icipe* was observed to use its antennae and ovipositor when searching for its host. This behaviour is typical of many parasitoid species, as they are known to use their antennae first to encounter sensory stimuli; the antennae are endowed with many distant and contact chemoreceptors and mechanoreceptors. To a lesser extent, their ovipositors also contain contact (taste) receptors to assist with the identification of suitable oviposition sites (Gullan & Cranston, 2014). It was reported by Wang & Keller (2002) that *Cotesia plutellae* (Kurdjumov) (Hymenoptera: Braconidae) also uses antennae and ovipositor in locating its host as was observed in *C. icipe*. Introduction of the ovipositor in the frass in the absence of larvae suggests that this parasitoid uses chemical cues in locating the host. *Cotesia icipe* also used the web thread to locate the host larvae indicating that contact cues may be an additional strategy in host searching. The temporary paralysis observed soon after parasitism suggests that *C. icipe* injects toxins in the larvae during the attack. Previous studies have documented

the injection of a cocktail of proteinaceous and non-proteinaceous compounds by different parasitoids, whose role is to suppress the immune system of their host insect, retard its development; and also viruses or virus-like particles that contribute to host manipulation (Hayakawa, 1994; Moreau & Asgari, 2015).

Contrary to the venom of ectoparasitoids, endoparasitoids venom usually does not have a permanent paralytic effect on the host (Desneux *et al.*, 2009) but in some cases causes a rather transient paralysis. In those cases, the host normally recovers in a few minutes or within one hour after parasitism (Moreau *et al.*, 2002; Desneux *et al.*, 2009). This transient paralysis could increase oviposition success by interfering with the host's defence (Desneux *et al.*, 2009).

Dropping to the ground using the silk thread to avoid parasitoid attack is a typical defensive behavior in many lepidopteran larvae: *Udea ferrugalis* (Hübner) (Lepidoptera: Crambidae) and *Spoladea recurvalis* (F.) (Lepidoptera: Crambidae) exposed to *Apanteles hemara* Nixon (Hymenoptera: Braconidae) (Othim *et al.*, 2017) and *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) larvae exposed to *Diadegma semiclausum* Hellen (Hymenoptera: Ichneumonidae) and *C. plutellae* (Wang & Keller, 2002) display the same behaviour. The strategy used by *C. icipe* which involved pursuing the host pest has been reported in *C. plutellae* (Wang & Keller, 2002). The strategy of these *Cotesia* species seems to be less efficient compared to the strategy used by *D. semiclausum* which waits near the silk thread for a suspended host to climb back upwards to the leaf, then attacks it (Wang & Keller, 2002).

Reduction in the feeding of host larvae following parasitism by solitary parasitoids, as observed in the present study, has been documented for various lepidopteran species. (Morales *et al.*, 2007) reported that larvae of *S. littoralis* parasitized by *Chelonus inanitus* L. (Hymenoptera: Braconidae) a solitary egg-larval parasitoid or *Hyposoter didymator* (Thunberg) (Hymenoptera: Ichneumonidae) a solitary larval endoparasitoid consumed significantly less food than un-parasitized larvae from the 2^{nd} or 4^{th} day onwards, respectively. Rahman (1970) also reported that larvae parasitized by the solitary endoparasitoid *Apanteles rubecula* Marsh. (Hymenoptera:

Braconidae) generally consume less food than un-parasitized larvae during their development. Similar reduction in host feeding potential was also reported for *Spilosoma obliqua* (Walker) (Lepidoptera: Arctiidae) following parasitism by the gregarious parasitoid *Apanteles obliquae* Wilkinson (Hymenoptera: Braconidae) which significantly reduced food consumption by the larval host (Sultana, 2003). The reduction in the feeding could be explained by the profound changes that occur in the host, such as behaviour, metabolism, endocrine events, and immune defence induced by the parasitoid (Rossi *et al.*, 2014). However, in one reported instance, the solitary larval endoparasitoid *C. plutellae* increased larvae feeding behaviour in *P. xylostella* following the attack (Shi *et al.*, 2002).

The parasitism rate obtained for *C. icipe* on *S. exigua* when using a single female (9.72 %) was lower than that obtained for the same parasitoid on *S. littoralis* (42.99 %) (Agbodzavu *et al.*, 2018). It was also relatively lower compared to the parasitism rates reported for other parasitoids on *S. exigua*. Li *et al.* (2015) reported parasitism rate of 68.05 % from a single female of *Microplitis similis* Lyle (Hymenoptera: Braconidae), a solitary endoparasitoid when exposed to thirty newly-moulted 3rd instar of *S. exigua.* When a cohort of 20 *S. exigua* larvae of different instars was exposed to a single female of *Meteorus pulchricornis* (Wesmael) (Hymenoptera: Braconidae), a parasitism rate ranging from 20 to 70 % was reported according to the larval instar stage (Liu & Li, 2006). Similarly, when a single female of *Cotesia marginiventris* (Cresson) (Hymenoptera: Braconidae), was used on 60 second instar larvae of *S. exigua*, 67.8 % parasitism was obtained (Riddick, 2004). From the current study, when a cohort of 5 females of *C. icipe* were released, there was more than five-fold increase in parasitism compared to the single female use, suggesting that the optimal density of the parasitoid release need to be documented. Additional mortality due to the non-reproductive larval and pupal mortality should also be considered in the evaluation of the performance of *C. icipe* in an integrated management approach for *S. exigua.*

When using a single female, the sex ratio was balanced; however, with a cohort of 5 females the progeny were female-biased. In parasitoids, a balanced or female-biased

sex ratio infers stability and higher efficiency, as only females directly contribute to the mortality of the pest (Ode & Heinz, 2002; Chow & Heinz, 2005). Moreover, a female-biased sex ratio is conducive for mass rearing of the biological agent in the laboratory for augmentative field release. Generally, the female progeny obtained in this study also had higher fitness compared to their male counterparts. It has been reported that on average, female parasitoids tend to be larger than males primarily due to the female larvae feeding longer than males (MacKauer, 1996; Harvey & Strand, 2003). The fitness of a female parasitoid positively influences the success of host parasitism. Gao *et al.* (2016b) reported that when the parasitoid *Sclerodermus pupariae* Yang & Yao (Hymenoptera: Bethylidae) encountered bigger or more active hosts, large females had greater advantages to subdue host larvae than the smaller sized females. Moreover, the same authors found that generally, large female parasitoids have greater reproductive potential because of larger volume of the spermatheca than small females.

This study provides the first detailed report on the acceptability and the suitability of *S. exigua* for *C. icipe*. These findings demonstrate that at optimal population density, *C. icipe* has a great potential to significantly reduce beet armyworm damage in Amaranth farming systems. Inundative releases of parasitoid can be one of the components of an integrated pest management strategy for *S. exigua*. Further research is warranted to determine the effect of rearing host and learning on *C. icipe*'s performance on both *S. exigua* and *S. littoralis* in order to increase the parasitoid's efficacy through augmentative and conservation biological control programs. Studies to assess the effect of temperature on the performance of the parasitoid are also warranted. Functional and numerical response studies should also be carried out to establish the optimal densities of *C. icipe* required in efficient biological control programs. At a time where a closely related pest species, *Spodoptera frugiperda* (Smith) is causing havoc across the African continent, this study will contribute considerably in advancing IPM against *Spodoptera* species.

CHAPTER SIX

TEMPERATURE-DEPENDENT DEVELOPMENT, SURVIVAL AND REPRODUCTION OF *Apanteles hemara* NIXON (HYMENOPTERA: BRACONIDAE) ON *Spoladea recurvalis* F. (LEPIDOPTERA: CRAMBIDAE)

Abstract

The temperature-dependent development of *Apanteles hemara*, a larval endoparasitoid of *Spoladea recurvalis* was studied in the laboratory at six constant temperatures (10, 15, 20, 25, 30 and 35°C), a photoperiod of 12L:12D and relative humidity of 60-70%. Different models were used to estimate the development threshold (Tmin), the thermal constant (K), the lethal temperature (Tmax) and the optimum temperature. Developmental time decreased significantly with increasing temperature within the range of 15 - 30°C. Survival was hindered at 10 and 35°C. The immature stage mortality, parasitism, emergence rate, adult longevity, sex ratio and fecundity were affected by temperature. The development threshold (Tmin) and the thermal constant (K) calculated by linear model and the lethal temperature (Tmax) determined by Lactin-1 model gave estimated values of 10.30°C, 185.18DD and 35.00°C respectively for the total immature development. The estimated value of the optimum temperature using the Taylor model was 31.76°C. This study is the first to report on the effect of temperature on developmental parameters of *A. hemara* giving an insight to its biology. It forms the basis in the development of phenology modelling study and mass rearing for the use of this biological control agent in pest management.

Keywords: Amaranth, sex ratio, developmental thresholds, models, fecundity.

6.1 Introduction

Spoladea recurvalis F. is a lepidopteran species belonging to the family Crambidae feeding on different crops especially on amaranth species (Chang & Ramasamy, 2016; Othim *et al.*, 2017). The larvae wrap and roll amaranth leaves into shelters

from which they feed skeletonizing the foliage and leaving frass on leaves often leading to entire foliage loss (Bhattacherjee & Ramdas Menon, 1964; Pande, 1972; James *et al.*, 2010). Chemical control of *S. recurvalis* is inefficient (Clarke-Harris & Fleischer, 2003; Clarke-Harris *et al.*, 2004). Moreover, their inefficiency, the use of insecticides is considered as non-environment friendly strategy and costly for small-scale farmers. In a study carried by Macharia (2015) in Kenya, it was reported an increase of pesticide-related acute illness by over 70%. This situation is, therefore, creating negative consumer sentiment around the use of insecticides which puts pressure on growers to use alternative control measures, such as natural enemies (Wohlfarter & Addison, 2014).

Biological control, whether using the introduction, conservation or augmentation approaches, is facilitated when the climatic responses of biocontrol agents are known, especially temperature (Roy *et al.*, 2002). Poor ecological adaptability of a parasitoid to the environment in the field is reported to be one of the factors explaining the failure of biological programs. Stiling (1993) meta-analyses on natural enemy failures, estimated that approximately 35% of biocontrol introductions or programs might have been unsuccessful because of climate-related factors (Hoddle *et al.*, 2014). Failure of *Trichogramma chilonis* Ishii (Hymenoptera: Trichogrammatidae) in Uttar Pradesh was due to the high temperature and low humidity prevailing during April-July (Tiwari & Tanwar, 2001). In a study carried out by Llacer *et al.* (2006), it was reported that overwintering of *Quadrastichus citrella* Reina and La Salle (Hymenoptera: Eulophidae) in Spain might present a barrier, especially in areas like Valencia, where average winter temperatures are around 11ºC and could account for the low recovery rates observed on *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae). It is also demonstrated that *Asecodes hispinarum* Boucek (Hymenoptera: Eulophidae) was unable to control the coconut hispine beetle, *Brontispa longissima* Gestro (Coleoptera: Chrysomelidae) in Central Vietnam because the parasitoid failed to emerge at 30ºC whereas temperatures during the hot season in that area fluctuate from 25 to 35 ºC (Htwe *et al.*, 2013).

It is thus important to document how a parasitoid will respond to a range of temperatures to predict its survival and establishment in a given area and the best timing of the release. *Apanteles hemara* Nixon (Hymenoptera: Braconidae) is also called *Apanteles proalastor* Hedqvist 1965; *Apanteles bulgaricus* Balevski and Tobias 1980 and *Apanteles caboverdensis* Hedqvist 1965 (Yu, 2016). Detailed information on the biology of this parasitoid is scarce. The only available literature is just based on report of its presence in different regions such as in Cape Verde Islands where it was recovered from its host *S. recurvalis* on *Achyranthes aspera* L. (Amaranthaceae) (Papp, 1996), in India where 11% of field parasitism rate was noted on the same host (Peter & Balasubramanian, 1984) and from Qazvin province, Iran (Ghahari *et al.*, 2012). Work done by Othim *et al.* (2017) showed high parasitism rate of the parasitoid in laboratory conditions, making it a good potential candidate for an augmentative or classical biological control for other areas where *S. recurvalis* represents a serious threat to vegetable production. The objective of this research was to assess the effect of a range of temperatures on the biology of *A. hemara* on its preferred host *S. recurvalis*.

6.2 Materials and methods

6.2.1 Source and rearing of *Spoladea recurvalis* and *Apanteles hemara*

The insects used in this experiment were reared at the insectaries of *icipe* in Nairobi. *Spoladea recurvalis* were collected from a field survey on amaranth lepidopteran pests in Narok county, Transmara (0° 35′ 32.892″ N, 3° 0′ 49.14″ E), and Yatta, Machakos County (01° 08.295′ S, 037° 25.892′ E) in May and June 2014. *Apanteles hemara* was established from pupal samples collected during a survey conducted in Yatta, Machakos County (01° 07.878′ S, 037° 33.274′ E) in June 2014. Field insects' materials were later on collected from Yatta to infuse the laboratory colonies. Both *S. recurvalis* and *A. hemara* were reared according to the method described by Othim *et al.* (2017).

The moths were placed in transparent ventilated perspex rearing cages (40 × 40 × 45 cm) with a sleeve and a netting material at the back. They were fed on 10 % honey

solution soaked in cotton wool as a source of food and provided with potted amaranth plants for oviposition at 25 ± 2°C, 50–70% RH, and 12:12 L:D (photoperiod). The plants were removed after every 24 h and held in separate holding wooden cages (50 × 50 × 60 cm) with ventilation on the sides and at the top until the eggs hatched. Newly hatched larvae were left to feed on the live plants for 3 days and transferred into ventilated plastic boxes (15 × 7 × 5 cm) lined with a paper towel to absorb excess moisture. Fresh amaranth leaves were supplied to the larvae as a food source until pupation. The pupae were incubated under similar rearing conditions until adult emergence.

Apanteles hemara adults were provided with potted plants containing three-day-old larvae of *S. recurvalis* in a cage for oviposition. The exposed larvae were removed daily and placed in ventilated plastic boxes (15 × 7 × 5 cm) lined with a paper towel. Fresh amaranth leaves were added into the boxes as required until pupation. The parasitoid pupae were harvested and transferred in clean Petri dishes (9-cm diameter), into a perspex cage under similar conditions for adult emergence. Effect of different temperatures on the developmental time of *Apanteles hemara*

Newly emerged adults of *A. hemara* were isolated from the stock culture in separate cages (20 x 20 x 25 cm) and allowed to mate for two to three days. Cages were made up of Perspex materials with fine netting materials (150 x 150 μm mesh) fitted on the back side to allow efficient air circulation inside the cages. A sex ratio of 1 male to 2 females was used. They were fed with a drop of honey smeared on paper and hung with masking tape on one internal face of the cage and also with 10 % of honey solution soaked in cotton and kept in an opened Petri dish (90 x 12 mm).

Two to three-day-old mated naive female (with no oviposition experience) was introduced in a cage as indicated above. The cage contained 20 larvae (3-4 days old on their second instar) of *S. recurvalis* kept in an opened Petri dish for parasitization which was observed visually. One female parasitoid was used for ten host larvae. Once the female parasitoid introduced and removed its ovipositor from a host, this host larva was collected using a fine camel brush, isolated in a vial (20 ml) plugged

with cotton wool, and introduced in the incubator (Environmental chambers, SANYO MIR-553 and MIR-554, Sanyo Electrical Ltd., Tokyo, Japan) (Plate 6.1), at either 10, 15, 20, 25, 30 and 35°C under a relative humidity (RH) ranging from 60-70%. To maintain this range of RH, two plastic boxes (15 × 7 × 5 cm) containing water were placed on the lower shelf of the incubator and refilled as needed. Every two days, fresh amaranth leaves were provided to the larvae until parasitoid cocoon formation, host pupation and the subsequent emergence of parasitoid or host adults or death of larvae. A total of 200 exposed larvae for parasitization were used for each temperature. For those with effective parasitization, the developmental time of each stage of the parasitoid, the number of cocoons, the number of cocoons from which adult wasps emerged and the sex ratio of the emerged adults were determined.

Plate 6.1: Vials with parasitized larvae placed in an incubator

Photo: M. K. Agbodzavu

6.2.2 Longevity of *Apanteles hemara* adults

Before the emergence of adults' parasitoid, a drop of honey was put on the internal wall of the vial to allow them to start feeding once they emerged from the cocoons. Vials were kept in an incubator set at the same conditions as mentioned in section 7.2.2. They were followed daily at the same time. Mortality was recorded until all parasitoid adults died.

6.2.3 Fecundity of *Apanteles hemara*

Due to host larvae limitations (difficulties of having continuous and sustainable colonies in laboratory conditions), fecundity of *Apanteles hemara* was studied only at 20 and 25 °C. Newly emerged female was coupled with a male of the same age in individual containers (12 cm in diameter, 6.5 cm in height) which had their top covered in the middle with fine mesh for ventilation (Plate 6.2). A drop of honey was put on the wall of the container to allow the adults to feed. Each couple of wasp were provided with 20 three-to four-days-old larvae of *S. recurvalis* daily until the female parasitoid died. Fresh amaranth leaf was hung on the top of the container as a food source for the exposed larvae. New container was used for daily exposure of the larvae. A total of 10 and 15 pairs (replicates) of wasps were used for 20 and 25°C respectively. Daily exposed larvae were isolated individually in vials plugged with cotton wool and reared on amaranth leaves until the emergence of either a parasitoid or a moth as described in section 7.2.2. The parasitoid lifespan was measured and divided into pre-oviposition period, an oviposition period and a post-oviposition period. The post-oviposition period refers to the time when a parasitoid ceased to parasitize hosts until the death of the parasitoid. Two fecundity parameters were calculated, realized fecundity as the number of parasitized larvae that developed in a cocoon (whether it developed into an adult parasitoid or not) over the life-span of the parasitoid, and fertility as the number of adult parasitoids that emerged from cocoons (emergence rate) (Murillo *et al.*, 2012).

Plate 6.2: Containers placed on an incubator shelf to study *Apanteles hemara* fecundity

Photo: M. K. Agbodzavu

6.2.4 Data analyses

The developmental durations for each life stage and adult longevity were compared between temperatures with Dunnett test using *dunn.test* package and between sex with Wilcoxon test because data were not normally distributed. Where data were normally distributed between sex (adult longevity), an independent samples *t*-test was used. Larval and pupal mortality, as well as the parasitism rate and the emergence rate of the parasitoid, were compared between temperatures with proportion test. Sex ratio was examined at each temperature using a chi-square test.

Development rate was calculated as the inverse median development time (development rate = 1/median development time) (Régnière, 1984), for each immature stage (egg-larval and pupal) and plotted against temperature. The degree-day model states that the relationship between development rate r (T) (1/development time in days) vs. temperature can be described by a linear equation: $r(T) = a + bT$, where T is the rearing temperature, a is the intercept and b is the slope of the linear function. The lower threshold temperature Tmin (Tmin = $-a/b$) and the thermal constant K (i.e., the number of degree-days above the lower threshold required to complete development, DD) ($K = 1/b$) were calculated based on the linear equation (Mathieu *et al.*, 2014). The data points for extreme temperatures (nonlinear points) were excluded. This is because linear functions cannot correctly capture the

development rate at extreme temperatures. For that reason, three non-linear models, Taylor, Lactin-1 and Ratkowsky were used to describe the relationship between temperature and development rates. Different models were fitted to the data to select the one with the best fit. Taylor function is defined as follows *rT = Rm * exp(-1/2 * ((T - Tm)/To)^2),* rT is the development rate, T the temperature, Rm the maximum development rate, Tm the optimum temperature, and To the rate at which development rate falls away from Tm (Taylor, 1981). Lactin-1 model is defined as *rT = exp(aa * T) - exp(aa * Tmax - (Tmax - T)/deltaT)* where rT is the development rate, T the temperature, and aa, Tmax, and deltaT fitted parameters. The Ratkowsky model is formulated as rT = (cc * (T - T1) * (1 - exp(k * (T - T2))))^2 where rT is the development rate, T the temperature, T1 and T2 the minimum and maximum temperatures at which rate of growth is zero, cc the slope of the regression and k a constant (Ratkowsky_83: Ratkowsky Equation of Development Rate. Retrieved from https://rdrr.io/cran/devRate/man/ratkowsky_83.html). The *devRate* package for R (Rebaudo *et al.*, 2017) was used to quantify the relationship between development rate and temperature. All the analyses were performed in R software version 3.5.1 (R Core Team, 2018).

6.3 Results

6.3.1 Immature developmental time of *Apanteles hemara* at different temperatures

Apanteles hemara larvae reared at constant 10°C and 30°C failed to complete development and died before emergence from the host, thus these data were only analyzed from individuals that successfully emerged from *S. recurvalis*. At 15°C, the egg-larval developmental time of male parasitoids was not statistically different from females (W = 11.5, df = 1, p = 0.794). The same trend was observed at 20°C (W = 435.5, df = 1, p = 0.059) and 30°C (W = 744, df = 1, p = 0.786). It was however significantly different at 25°C (W = 619, df = 1, p = 0.024) in favour of females. Pupal developmental time between sex was not significant at 15 °C (t = 0.099, df = 7, p = 0.924) and 30°C (W = 827, df = 1, p = 0.385). It was rather significant at 20°C

(W = 952.5, df = 1, p < 0.0001) as well as at 25°C (W = 763.5, df = 1, p < 0.0001). The total immature developmental time was not significantly different between males and females at 15°C (t = 0.355, df = 4.35, p = 0.739) and at 30°C (W = 812, df = 1, p = 0.476) but was significantly different at 20°C (W = 1003.5, df = 1, p < 0.0001) and 25°C (W = 811.5, df = 1, p < 0.0001) (Table 6.1).

Immature developmental time varied greatly among tested temperatures. There was a highly significant difference between males (χ^2 = 115.39, df = 3, p < 0.0001) and females (χ^2 = 86.783, df = 3, p < 0.0001) egg-larval developmental time as function of temperatures. The same way, there was a highly significant difference in males pupal (χ^2 = 106.32, df = 3, p < 0.0001) and females (χ^2 = 82.509, df = 3, p < 0.0001) developmental time when temperatures were compared. There was a highly significant difference in males (χ^2 = 116.47, df = 3, p < 0.0001) and females (χ^2= 116.47, df= 3, p <0.0001) total developmental time across all the temperatures

Table 6.1: Developmental time (mean ± SE in days) of immature stages of *Apanteles hemara* reared on *Spoladea recurvalis*

Temperature	Sex		W	t	df	p
	Male	female				
	Egg-larval developmental time (days)					
15°C	28.20 ± 0.73^{aA} (5)	28.50 ± 0.65^{aA} (4)	11.5		1	0.794
20°C	12.81 ± 0.09^{aB} (42)	12.57 ± 0.08^{aB} (27)	435.5		1	0.059
25°C	7.31 ± 0.09^{bC} (29)	7.7 ± 0.13^{aC} (33)	619		1	0.024
30°C	5.25 ± 0.08^{aD} (51)	5.2 ± 0.07^{aD} (30)	744		1	0.786
χ^2	115.39	86.783				
df	3	3				
p	<0.0001	<0.0001				
	Pupal developmental time (days)					
15°C	21.80 ± 1.74^{aA} (5)	22.00 ± 0.41^{aA} (4)		0.099	7	0.924
20°C	7.88 ± 0.12^{bB} (42)	8.96 ± 0.14^{aB} (27)	952.5		1	< 0.0001
25°C	4.41 ± 0.09^{bC} (29)	5.18 ± 0.11^{aC} (33)	763.5		1	< 0.0001

		3.93 ± 0.08^{aD}				
30°C	3.84 ± 0.06^{aD} (51)	(30)	827		1	0.385
χ^2	106.32	82.509				
df	3	3				
p	< 0.0001	< 0.0001				
Total developmental time (days)						
		50.50 ± 0.29^{aA}			4.3	
15°C	50.00 ± 1.38^{aA} (5)	(4)		0.36	5	0.739
		21.78 ± 0.17^{aB}	1003.			
20°C	20.45 ± 0.1^{bB} (42)	(27)	5		1	0.0001
	11.72 ± 0.12^{bC}	12.88 ± 0.14^{aC}				
25°C	(29)	(33)	811.5		1	0.0001
30°C	9.10 ± 0.08^{aD} (51)	9.13 ± 0.06^{aD} (30)	812		1	0.476
χ^2	116.47	86.51				
df	3	3				
p	<0.0001	<0.0001				

Means followed by same lower (upper) case letters in the same row (column) are not significantly different at $p < 0.05$ (Dunnett test, Wilcoxon rank sum test, two-sample *t*-test or Welch two-sample *t*-test).

6.3.2 Temperature-dependent development models of *Apanteles hemara* and estimated values

Estimated parameters and graphs of different models (linear regression, Taylor, Lactin-1 and Ratkowsky) of the effect of temperature on the development rate of *A. hemara* are presented in Table 6.2 and in Appendix 2 respectively. The fit of all tested non-linear models for the dependence of development rates of *A. hemara* on temperature was significant for the egg-larval and total developmental time. For egg-larval development, $F = 25.33$ and $p < 0.0001$ for Taylor model, $F = 6.57$ and $p = 0.010$ for Ratkowsky and $F = 229.5$ and $p < 0.0001$ for Lactin-1. For total developmental time $F = 55.24$ and $p < 0.0001$ for Taylor model, $F = 22.62$ and $p < 0.0001$ for Ratkowsky and $F = 602.8$ and $p < 0.0001$ for Lactin-1. For pupal development time, only Lactin-1 fit was significant ($F = 118.1$, $p < 0.0001$) (Table 6.2). Base on R^2, Lactin-1 was retained as best fits the data for larval, pupal and total development rates. Lactin-1 model estimated the upper threshold temperature for development at 35.00°C for larval, pupal and total development stage.

Using the linear model, the lower (Tmin) and the sum of effective temperatures (K) for development were 10.06, 10.13 and 10.30°C in one hand and on another hand 106.38, 75.76 and 185.18 DD for the egg-larval stage, pupal stage and total developmental time respectively (Table 6.2).

Table 6.2: Model parameters of linear regressions, Taylor, Lactin-1 and Ratkowsky models for temperature effect on *Apanteles hemara* immature stages' development rate

Model	Formula	Parameters	Egg-larval stage	Pupal stage	Total development
Linear regression	r T = a + bT	a	-0.0966***	-0.1337***	-0.0556***
		b	0.0094***	0.0132***	0.0054***
		R^2	0.98	0.96	0.98
		K (DD)	106.38	75.76	185.18
		Tmin (°C)	10.06	10.13	10.30
Taylor_81	rT ~ Rm * exp(-1/2 * ((T - Tm)/To)^2)	Rm	0.20 ± 0.002***	0.26 ± 0.002***	0.1.10 ± 0.0007**
		Tm (°C)	31.76 ± 0.31***	29.58 ± 0.25***	30.78 ± 0.20***
		To (°C)	8.27 ± 0.20***	7.43 ± 0.21***	7.92 ± 0.14***
		R^2	0.057	0.005	0.116
		AIC	-2664.40	-2039.90	-3289.57
		BIC	-2648.20	-2023.70	-3273.37
Lactin1_95	rT ~ exp(aa * T) - exp(aa * Tmax - (Tmax - T)/deltaT)	aa	0.22 ± 0.001***	0.20 ± 0.002***	0.21 ± 0.001***
		Tmax (°C)	35.00 ± 0.007***	35.00±0.010 ***	35.00±0.007***
		deltaT (°C)	4.62 ± 0.03***	4.89 ± 0.047***	4.73 ±0.03***

Model	Formula	Parameters	Egg-larval stage	Pupal stage	Total development
		R^2	0.27	0.16	0.49
		AIC	-3868.77	-2959.88	-4585.75
		BIC	-3851.02	-2942.125	-4568.00
Ratkowsky_83	$rT \sim (cc * (T - T1) * (1 - \exp(k * (T - T2))))^2$	cc	0.02 ± 0.0003***	0.03 ± 0.0006***	0.02 ± 0.0001***
		T1	7.13 ± 0.21***	7.59 ± 0.32***	7.26 ± 0.16***
		T2	35.04 ± 0.07***	35.09 ± 0. ***	35.06 ±0.05***
		k	0.49 ± 0.02***	0.35 ± 0.01***	0.42 ± 0.009 ***
		R^2	0.01	0.0006	0.035
		AIC	-4293.92	-3289.17	-5256.06
		BIC	-4271.73	-3266.98	-5233.87

Significance code: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1.

rT is the development rate, a: y intercept, b: slope, K: thermal constant (DD), Tmin: lower development temperature threshold, T the temperature, Rm the maximum development rate, Tm the optimum temperature, To the rate at which development rate falls away from Tm, T the temperature, aa, Tmax, and deltaT are fitted parameters T1 and T2 the minimum and maximum temperatures at which rate of

growth is zero, cc the slope of the regression k a constant; R^2 coefficient of determination; AIC: Akaike information criterion; BIC: Bayesian information criterion

6.3.3 Immature stage mortality, parasitism and emergence rates

Immature mortality recorded at each temperature for egg-larval and pupal stages of *A. hemara* is presented in Table 6.3. Since *A. hemara* is an endoparasitoid, the host larval mortality was assimilated to the parasitoid egg-larval mortality. There was a high significant difference in the mortality recorded at different temperatures for the egg-larval (χ^2 = 45.49, *df* = 3, $p < 0.0001$) and pupal (χ^2 = 106.50, *df* = 3, $p < 0.0001$) stages. The highest egg-larval morality occurred at 15°C while the lowest was recorded at 30°C. Similarly, the highest pupal mortality was recorded at 15°C, but the lowest was recorded at 25°C.

Table 6.3: Effect of constant temperatures on *Apanteles hemara* larval and pupal morality when reared on *Spoladea recurvalis*

Temperature	Developmental stage	Mortality (%)
15°C	Larval stage	69.00[a]
20°C		54.00[b]
25°C		65.00[ab]
30°C		38.50[c]
χ^2		45.49
df		3
p		<0.0001
15°C	Pupal stage	84.48[a]
20°C		24.18[b]
25°C		1.59[c]
30°C		25.45[b]
χ^2		106.50
df		3
p		<0.0001

Means followed by different letters were significantly different by proportion test, $p < .05$.

Parasitism (χ^2 = 37.28, *df* = 3, $p < 0.0001$) and adult emergence (χ^2= 106.50, *df* = 3, $p < 0.0001$) rates were significantly affected by temperatures. The highest parasitism rate was obtained at 30°C and the lowest at 15°C. Highest adult emergence rate occurred at 25°C while lowest still at 15°C (Table 6.4).

Table 6.4: Effect of constant temperatures on *Apanteles hemara* parasitism and emergence rates when reared on *Spoladea recurvalis*

Temperature	Parasitism (%)	Emergence (%)
15°C	29.00 (n*=58)[a]	15.52 (n=9)[a]
20°C	45.50 (n=91)[b]	75.82 (n=69)[b]
25°C	31.50 (n=63)[a]	98.41 (n=62)[c]
30°C	55.00 (n=110)[b]	74.55 (n=81)[d]
χ^2	37.28	106.50
df	3	3
p	<0.0001	<0.0001

* Numbers n in brackets represent the number of collected cocoons and emerged adults

Means followed by different letters in the same column were significantly different by proportion test, $p < 0.05$.

6.3.4 Adult longevity and sex ratio of *Apanteles hemara* at different temperatures

Adult longevity of *A. hemara* was significantly influenced by temperature. Adult longevity decreased with increase in temperature from 15°C to 25°C. An increase was however noted at 30°C. There was no significant difference in the longevity of females' *A. hemara* as compared to their conspecific males at the same temperature (Table 6.5).

Table 6.5: Effect of constant temperatures on adult longevity (mean ± SE in days) of *Apanteles hemara* when reared on *Spoladea recurvalis*

Temperature	Sex		*W*	*t*	*df*	*p*
	Male	female				
	Adult longevity (days)					
15°C	24.6 ± 5.63^{aA}	22.5 ± 8.77^{aAB}		-0.21	7	0.840
20°C	15.57 ± 1.59^{aB}	14.81 ± 1.96^{aA}	525			0.609
25°C	9.41 ± 0.83^{aC}	9.88 ± 0.92^{aC}		0.37	60	0.711
30°C	18.96 ± 1.2^{aA}	20.97 ± 1.5^{aB}	878			0.270
χ^2	24.4996	22.5539				
df	3	3				
p	<0.0001	<0.0001				

Means followed by same lower (upper) case letters in the same row (column) are not significantly different at $p < 0.05$ (Wilcoxon rank sum test and two-sample *t* test)

Proportion of females varied according to the rearing temperature. There was no significant difference in the sex ratio at 15°C ($\chi^2 = 0.09$, $df = 1$, $p = 0.757$) and at 25°C ($\chi^2 = 0.29$, $df = 1$, $p = 0.59$). However, this was statistically different at 20°C ($\chi^2 = 5.68$, $df = 1$, p = 0.017) and 30°C ($\chi^2 = 9.8765$, $df = 1$, $p = 0.001$) where it was male biased (Table 6.6).

Table 6.6: Effect of constant temperatures on *Apanteles hemara* sex ratio when reared on *Spoladea recurvalis*

Temperatures	Sex ratio (%)		χ^2	*df*	*p*
	Male	Female			
15°C	55.56^{a}	44.44^{a}	0.09	1	0.757
20°C	60.87^{a}	39.13^{b}	5.68	1	0.017
25°C	46.77^{a}	53.23^{a}	0.29	1	0.59
30°C	62.96^{a}	37.04^{b}	9.8765	1	0.001

Means followed by different letters in the same row were significantly different by chi-square test, $p < .05$.

6.3.5 Female realized fecundity and survival

The realized fecundity of *A. hemara* was influenced by temperature and showed significant differences between the two tested temperatures ($W = 117$, $df = 1$, $p = 0.001$). The mean daily realized fecundity in terms of the number of produced cocoons was 8.15±1.03 at 20°C and 2.41±0.2 at 25°C per female. No pre-oviposition period was observed. At 20°C, the mean oviposition period was 12.20±5.89 and at 25°C, it was 11.61±5.41. The post-oviposition was longer at 20°C than at 25°C (Figures 6.1; 6.2).

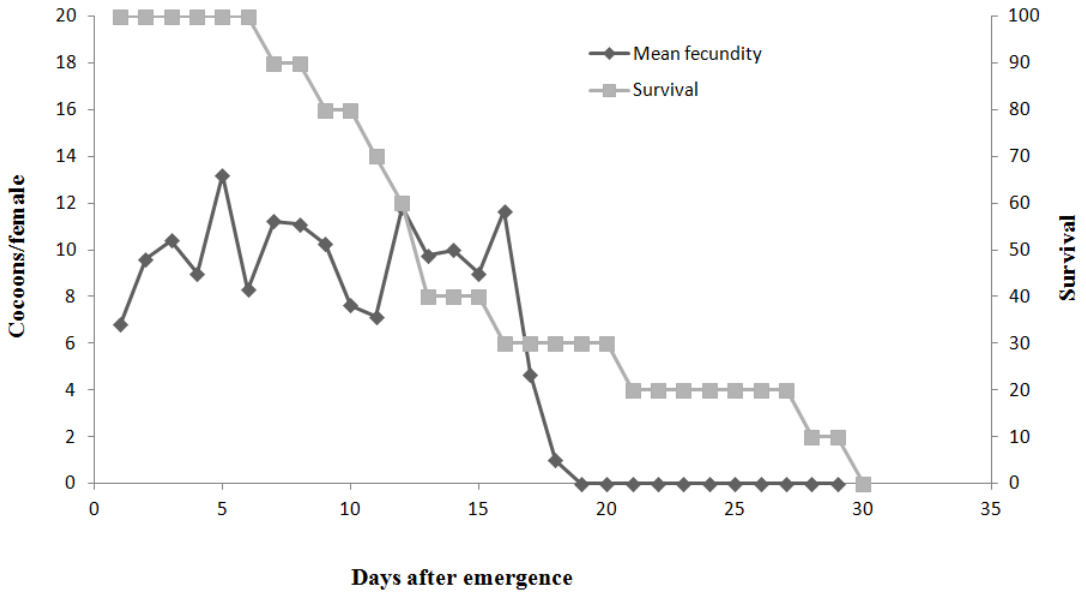

Figure 6.1: Age-specific reproduction and survival of adult females of *Apanteles hemara* reared on *Spoladea recurvalis* at 20°C, 60-70% relative humidity and 12: 12 h (light: dark) photoperiod

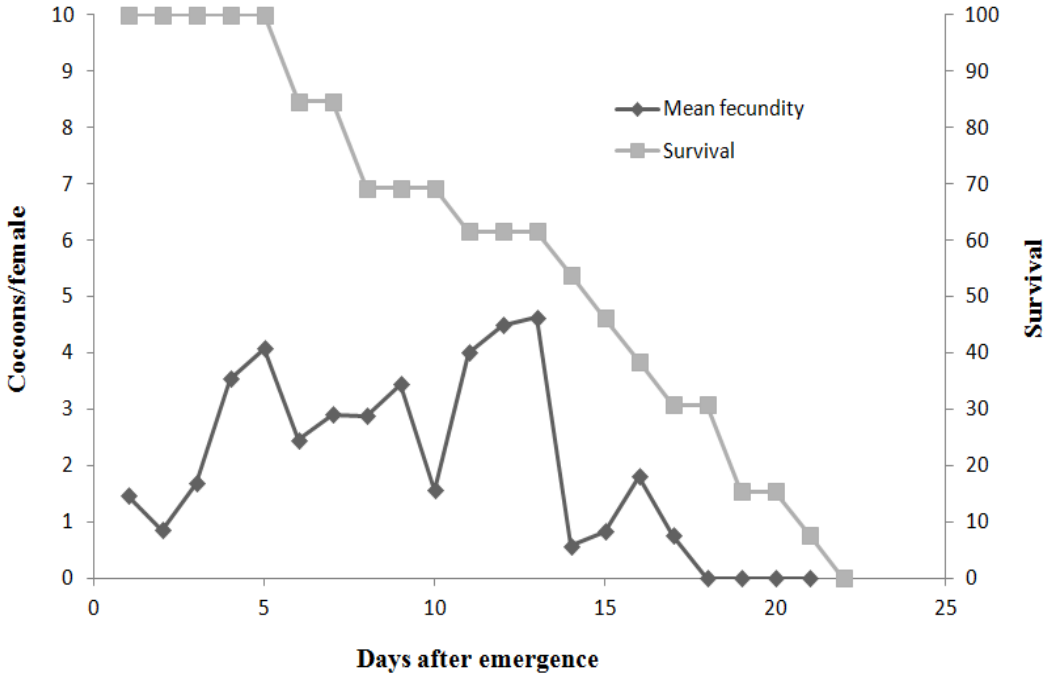

Figure 6.2: Age-specific reproduction and survival of adult females of *Apanteles hemara* reared on *Spoladea recurvalis* at 25°C, 60-70% relative humidity and 12 : 12 h (light : dark) photoperiod

6.4 Discussion

In biological control programmes, detailed information concerning thermal requirements and thresholds is useful for selecting natural enemies that are best adapted to conditions favouring target pests (Jervis, 1996). To the best of our knowledge, no data on the development of *A. hemara* on *S. recurvalis* at different temperatures are available. Results in the current study clearly showed that the development and the survival of *A. hemara* on *S. recurvalis* are affected by temperature. Previous studies have shown the effect of temperatures on the development of parasitoids (Eliopoulos & Stathas, 2003; Kalaitzaki *et al.*, 2007; Malina & Praslicka, 2008; Mawela *et al.*, 2013). Insects, being poikilothermic, are particularly sensitive to their environmental temperature. Similar results were obtained by Cardona & Oatman (1975) who reported that *Apanteles subandinus*

Blanchard (Hymenoptera: Braconidae) completes its development from egg to adult at temperatures of 15.5 - 32°C but not at low temperatures of 11.2°C. Effect of temperature on insects is explained by its interference with the metabolism, respiration, nervous system, endocrine system and heat shock proteins capacity (Neven, 2000). At the lower extreme temperature, a delay in development occurs due to suboptimal feeding; but as the temperature increases, it is accompanied with an increase in developmental rate up to a lethal limit, where the rate of metabolism decreases (Van Steenis, 1994).

Although studies reported a significant difference in total developmental time of females and males parasitoids (Urbaneja *et al.*, 1999; Hohmann & Luck, 2000; Ferreira de Almeida *et al.*, 2002; Luo *et al.*, 2015; Pala, 2016), in the current study, the trend was not always the case at all temperatures. Studies supporting the case where there is no difference in the total developmental time of females and males parasitoids are also many. Kalaitzaki *et al.* (2007) reported that the total development time of *Pnigalio pectinicornis* (Hymenoptera: Eulophidae) males on *Phyllocnistis citrella* (Lepidoptera: Gracillariidae) was not significantly different from the total development time of females at all tested temperatures on sweet orange and mandarin. The same way, Bari *et al.* (2015) found that unlike other trichogrammatid egg parasitoids, female *Trichogramma zahiri* (Hymenoptera: Trichogrammatidae) did not take significantly longer time than males to reach adulthood when reared on *Dicladispa armigera* (Chrysomelidae: Coleoptera) at different temperatures. Hypothesis formulated in case of sexual dimorphisms in development time was that to achieve a larger size (usually females have a higher fitness than males), female parasitoid larvae must extend their period of larval development in growing hosts, allowing them to accrue more resources than male offspring but at the cost of extending their development time (Mackauer *et al.*, 1997).

Selection of mathematical models that suitably describe the relationship between temperatures and the developmental rate is essential. Linear functions fitted well the effect of temperature on the development rate for all *A. hemara* immature stages (egg-larval and pupal) and the total developmental time, especially for temperature

between 10 and 30°C. However, for higher temperatures, Lactin-1 model gave good results and allowed the calculation of the maximum temperature threshold. Although the coefficient of determination of Taylor model was low, the parameters estimates are highly significant and give the value of optimum development temperature of the parasitoid at 31.76 ± 0.31 and 29.58 ± 0.25°C for egg-larval and pupal stage respectively. This estimated value sounds reasonable based on the larval mortality, the parasitism rate and the adult emergence rate obtained at 30°C. The lower developmental threshold of the immature stage of *S. recurvalis* reported in the literature is 10.4°C (Lee *et al.*, 2013) while the one of *Apanteles hemara* obtained during this study is 10.30°C showing the similarity of this parameter between the two species. However, the lethal (upper) temperature for *S. recurvalis* is 48.80°C (Lee *et al.*, 2013) and the one of *A. hemara* is 35.00°C meaning *A. hemara* will be unable to control *S. recurvalis* in location where the ambient temperature is above 35°C, though the possibility of an outbreak above that temperature is low; *Spoladea recurvalis* optimal temperature range for growth is 25.0 -30.0°C (Lee *et al.*, 2013).

The linear model predicted the sum of effective temperatures (K) at 106.38, 75.76 and 185.18 DD for the egg-larval stage, pupal stage and total development respectively. The emergence, development, and seasonal abundance of species in given habitats are strongly related to their thermal summation and thermal thresholds. Therefore, with those values and knowing the climatic conditions in a given location, one can predict the number of generation per year or season of *A. hemara*, bearing in mind that other factors such as relative humidity and the availability of the host can also influence the number of generation.

These experiments showed that temperature affected adult longevity. Contrary to a constant decrease in adult parasitoid longevity as per the increase of the temperature usually reported (Lysyk, 2001; Uçkan & Erginin, 2003; Gonçalves *et al.*, 2014; Luo *et al.*, 2015), these results showed a particularity of 30.0°C where the longevity experienced an increase, confirming the suitability of that temperature to *A. hemara*. The extension of adult longevity at 15°C is due to the reduction or inactivity in biological processes whereas at 30°C is due to the comfort of that condition to its

survival. Similar results were obtained by Castillo *et al.* (2006) but for the immature stages of *Quadrastichus haitiensis* (Gahan) (Hymenoptera: Eulophidae), an endoparasitoid of *Diaprepes abbreviatus* L. (Coleoptera: Curculionidae), where a constant decrease in total development time was observed from 20 to 30°C and an increase at 33°C.

The sex ratio was male-biased at 20°C and 30°C whereas it was balanced at 15°C and 25°C. Sex ratio is an important parameter when considering biological control agents. A female-biased sex ratio is sought as females are the ones responsible for attacking the pests through host feeding or oviposition (Chow & Heinz, 2005). However, in *A. hemara* reared on *S. recurvalis* that advantageous parameter is not met, especially at 30°C where other parameters aforementioned are favourable. In a study carried by Othim *et al.* (2017) at 25 ± 2°C and 60 ± 10% RH, they obtained female-biased sex ratio in a proportion of 59.09% whereas in this study we obtained 53.23 % with no significant difference. The difference obtained in both results might be due to the fluctuation of temperature of 2°C in their study whereas in an environmental chamber as in the present study, the fluctuation was only by 0.5°C.

There was a significant difference in the daily realized fecundity registered at 20°C and 25°C with 20°C recording the highest fecundity. This result can be explained by the higher mortality registered at the larval stage at 25°C as compared to 20°C and most likely also by the higher number of eggs deposited at 20°C. The number of ovipositing days could not be a reason since there is roughly only one day difference in the number of ovipositing days at the two temperatures. The extended longevity of the female at 20°C is translated into the post-oviposition period, meaning that *A. hemara* lays most of their eggs in the first two weeks following their emergence. Şengonca & Peters (1993) showed that *Apanteles rubecula* Marsh. (Hymenoptera: Braconidae) oviposition period lasts about 17 days which is similar to the results obtained in this study. Also, no pre-oviposition was observed during this experiment on *A. hemara*. Absence of pre-oviposition period was reported in some species of *Apanteles* such as *Apanteles dignus* Muesebeck (Hymenoptera: Braconidae), a primary parasite of the tomato pinworm (Cardona & Oatman, 1971), *Apanteles*

machaeralis Wilkinson (Hymenoptera: Braconidae) a parasite of *Diaphania indica* (Saunders) (Lepidoptera: Pyralidae) (Peter & David, 1990), *Apanteles subandinus* Blanchard (Hymenoptera: Braconidae) (Cardona & Oatman, 1975) and *A. rubecula* (Şengonca & Peters, 1993).

This study is the first to report the effect of temperature on *A. hemara*, an important parasitoid of amaranth lepidopteran defoliator, *S. recurvalis*. In summary, the study showed that temperature has a strong significant effect on development rate, mortality, sex ratio, fecundity and longevity of *A. hemara*. This parasitoid has an optimal development at 31°C which gives an indication of the optimal conditions for mass rearing. For this study to be complete, the fecundity should be studied at the other remaining temperatures (15 and 30 °C). This study has contributed to a deeper understanding of the biology of *A. hemara* and has formed a basis for future phenology modelling studies.

CHAPTER SEVEN

GENETIC TAXONOMY OF *Spoladea recurvalis* POPULATIONS FROM EAST AFRICA AND THEIR IMPLICATION FOR BIOLOGICAL CONTROL

Abstract

Spoladea recurvalis is one of the most destructive pests of African indigenous vegetables especially amaranth. Although the pest outbreaks occur in East Africa with heavy damages, its genetic structure has never been studied. In the scope of developing effective bio-ecological control strategies, this study was designed to understand and establish baseline information on the population structure of *S. recurvalis* from Kenya and Tanzania in relation to populations from other countries. *Spoladea recurvalis* genomic DNA samples from Kenya and Tanzania were analyzed and compared based on cytochrome C oxidase I (COI) subunit gene. The gene sequence analysis revealed A-T rich sequences in nucleotide composition, which is consistent with mtDNA of insect species. Molecular diversity indices showed overall high haplotypes diversity (Hd = 0.82182), but low nucleotide divergence (PiT = 0.00366) between haplotypes, a finding that is typical for migratory species. Forty-six haplotypes were found in overall samples, whereas 24 and 29 haplotypes were identified in Kenya and Tanzania respectively. Comparison of Kenyan and Tanzanian populations showed high homogeneity translated by the low value of F_{ST} (0.0153). This resemblance was also confirmed by the existence of high gene flow among the two populations (N_m= 16.15). The negative and significant Tajima's D test, Fu's FS test as well as Fu and Li's D* statistics suggested recent population growth in both populations. Phylogenetic analysis showed weak phylogenetic segregation of the analyzed samples. This study confirmed the species identity of studied *S. recurvalis* populations and therefore represents an asset for successful biological control in terms of biological agents' exchange such as parasitoids. The implications of the current findings for developing integrated pest

management strategies against the pest in the different agro-ecological zones are therefore discussed.

Keywords: Amaranth leaf webber, beet webworm, bio-ecology, COI gene, gene flow, phylogenetic.

7.1 Introduction

Spoladea recurvalis F. (Lepidoptera: Crambidae) commonly known as the beet webworm or amaranth leaf webber is a key pest of vegetables especially amaranth and spinach not only in East Africa (Kahuthia-Gathu, 2011; Othim *et al.*, 2017), but also worldwide. It is known to attack other plants such as *Trianthema monogyna*, *T. postulacastrum*, silverbeet, beetroot, beans etc. (Walker & Anderson, 1940; Pande, 1969; Bailey, 2007). The caterpillars of *S. recruvalis* can completely destroy amaranth fields during outbreaks. Currently, no safe and cost-effective method exists to manage this pest efficiently. The International Centre of Insect Physiology and Ecology (*icipe*) and its partners are currently investigating the possibility of developing an integrated pest management (IPM) technique against the pest in the African indigenous vegetable farming systems. *Spoladea recurvalis* are encountered in the three agro-ecological zones of Kenya: high altitude (>1,800 m a.s.l), mid-altitude (1,000 - 1,800 m a.s.l) (Othim *et al.*, 2018a; Agbodzavu et al. unpublished data) and in low altitude (< 1,000 m a.s.l) (Mureithi *et al.*, 2015). However, their incidence and damage severity vary according to the agro-ecological zones. The pest is also found in Tanzania (Mureithi *et al.*, 2015; Othim *et al.*, 2018b).

Insects do adapt to multiple variables and factors within an environment, not necessarily in terms of genetic or evolutionary response, but in terms of response plasticity at the behavioural or physiological level (Young, 2012). However, variation in the environment can maintain genetic variation within a single population (Futuyma & Peterson, 1985). It is well known that if different populations inhabit different niches, they often lead to different species or different 'biotypes' / 'ecological races' (Nei, 2013). The niche-variation hypothesis states that some genetic variations and variation of morphological characters can be correlated with

and determined by the type of foods and the habitats used by a population (Soule & Stewart, 1970). No studies have so far been carried out to assess potential behavioural, physiological and or morphological differences in *S. recurvalis* populations in Kenya and Tanzania. We hypothesize that being in different niches/habitats could lead to a certain level of genetic variations and probably to a speciation. Polyphagous or widely distributed species do not share the same life traits (biology, ecology, impact on yield losses) according to the host plants and regions. This suggests that ecologically differentiated population of such phytophagous species could also be genetically different, a situation which can lead to the formation of races and possibly to cryptic species (Sezonlin, 2006).

Spoladea recurvalis is reported to be a migratory species (Goater, 1986; Miyahara, 1990). Therefore, if no natural barrier exists, frequent interbreeding of different ‘‘regions’ populations’’ will increase the gene flow and as consequence, no significant divergence in form of genetic, behaviour or physiology is expected. Referring to *Busseoala fusca* (Fuller) (Lepidoptera: Noctuidae), Ndemah *et al.* (2001) suggested that geographical barriers (mountain ranges and forests) may allow for the development of distinct geographical borer races that vary in their climatic requirements. In addition, many authors have also argued that sympatric speciation is common as a direct consequence of adaptation to a specific host, termed as ‘‘host shifting’’ (Berlocher & Feder, 2002; Drès & Mallet, 2002).

Population genetics tools have been successfully applied to insect pest management studies characterizing gene flow among populations (Alstad & Andow, 1995; Medina *et al.*, 2010) using different markers namely the cytochrome oxidase I and II (COI, COII), the 16S and 12S subunits of rDNA, the 18S and 28S subunits of rRNA and the first and second internal transcribed spacer regions (ITS 1 and ITS 2) (Caterino *et al.*, 2000; Gariepy *et al.*, 2007). Failure of some biological control programs could be attributed to the misunderstanding of the presence of genetic difference among targeted pest as well as their associated natural enemies as pointed out by Sezonlin, (2006). For instance in the control of the tomato red spider mite *Tetranychus evansi* Baker and Pritchard (Acarida: Phytoseiidae, Tetranychidae), the

South American population of the predatory mite *Phytoseiulus longipes* Evans (Acarida: Phytoseiidae) was found efficient while the South African population of the same predator failed to control the pest (De Moraes & McMurtry, 1985; Furtado *et al.*, 2007). It has also been reported that various populations of the fall armyworm, *Spodoptera frugiperda* J.E. Smith (Lepidoptera: Noctuidae) differ in their response to insecticides (Pashley, 1993). Similarly, studies on the European corn borer, *Ostrinia nubilalis* (Hübner) (Lepidoptera: Pyralidae) and codling moth, *Cydia pomonella* L. (Lepidoptera, Tortricidae) demonstrated differential production of and response to sex pheromone between different population of the same species, affecting significantly the wide use of pheromone traps in the pests' monitoring and control (Duménil *et al.*, 2014). Integrated pest management (IPM) programs should, therefore, integrate a clear understanding of the genetic difference of target pest species in order to achieve accurate management packages with wider geographical application potentials. The objectives of this study were, therefore (i) to confirm the taxonomic identity of *S. recurvalis* collected from different locations in Kenya and Tanzania and (ii) to infer on phylogenetic relationships between African populations of *S. recurvalis* with American, Asian and European populations.

7.2 Material and method

7.2.1 Sampling site and preservation of *Spoladea recurvalis*

Spoladea recurvalis samples were collected by picking larvae from amaranth plants or catching directly the adults using a sweep net in farmers' fields in Kenya and Tanzania (Figure 7.1 and Table 7.1). The sampling surveys were carried out between December 2016 and January 2017. Five locations were sampled in Kenya while four were sampled in Tanzania. Information regarding date of samples collection, geographic coordinates of sampling points and host plants were recorded using a GARMIN eTrex 12 portable Global Positioning System (GPS) gadget (Garmin International, Inc. Kansas, U.S.A) (Table 8.1). A minimum of 50 *S. recurvalis* adults and/or larvae were sampled from each location. In the field, collected adults were kept in cages (40 x 40 x 45 cm) while the larvae were maintained on amaranth leaves

in plastic boxes (15 cm × 7 cm × 5 cm) and taken to the laboratory. Larvae were maintained on the leaves until adulthood as described by Othim *et al.* (2017). Adults were placed in absolute alcohol (99%) molecular grade while still alive before DNA extraction (Sezonlin *et al.*, 2012). All insect samples were collected from

Amaranthus dubius Mart. ex Thell except at Kitengela site, Kenya (Table 7.1) where the insects were taken from a wild unidentified species of amaranth (*Amaranthus* sp.).

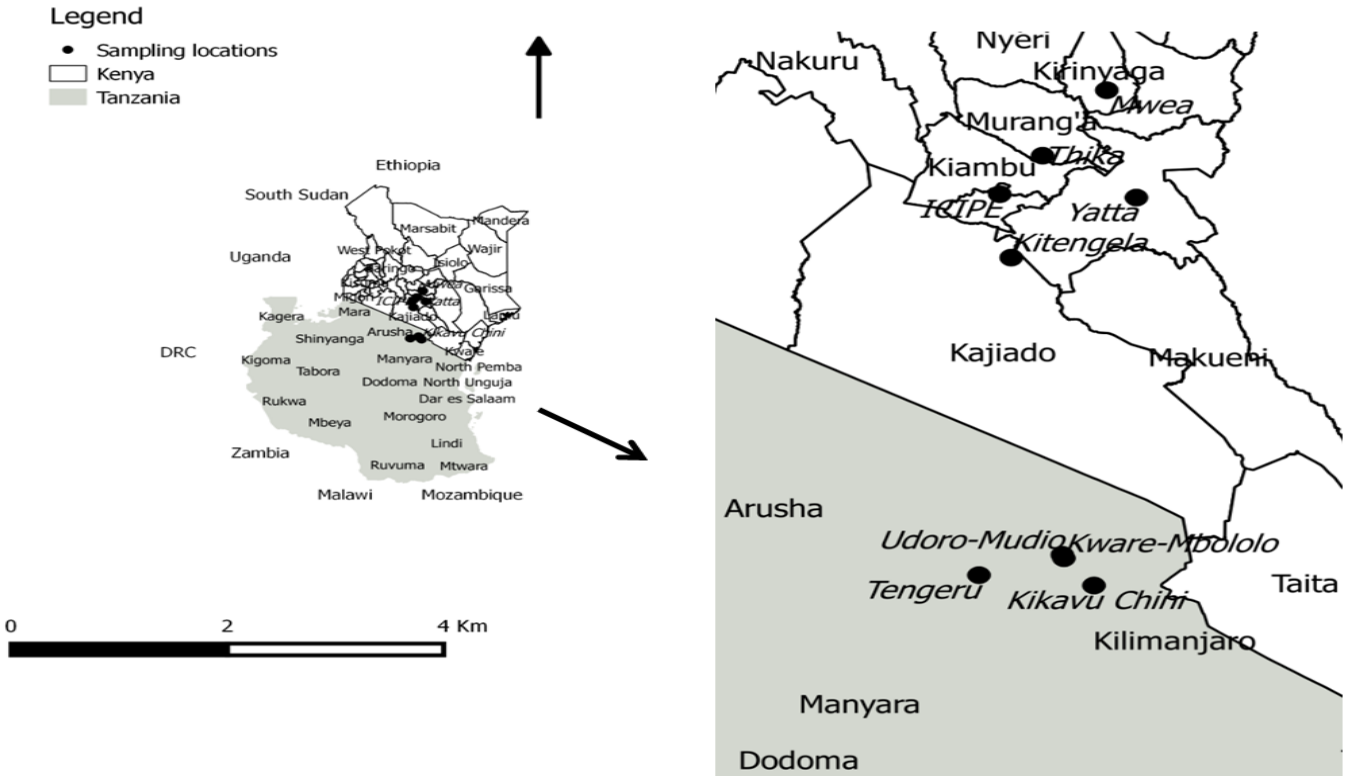

Figure 7.1: Map of localities where *Spoladea recurvalis* were sampled from Kenya and Tanzania

Table 7.1: Sampling localities, host plants, date of collection and number of *Spoladea recurvalis* individuals sequenced

Country	Locations	Geographic coordinates	Altitude (m asl)	Host plants	Date of collection	Number of insects sequenced
Kenya	Mwea	00° 37.854'S 037° 21.070'E	1197	*Amaranthus dubius*	06/12/16	15
	Yatta	01° 14.455'S 037° 28.622'E	1173	*Amaranthus dubius*	08/12/16	15
	Kitengela	01° 34.914'S 036° 56.696'E	1621	*Amaranthus* sp. (wild amaranth).	12/12/16	13
	Thika	01° 00.161'S 037° 04.715'E	1508	*Amaranthus dubius*	14/12/16	15
	icipe	01° 13.302'S 36° 53.788'E	1602	*Amaranthus dubius*	04/01/17	14
Tanzania	Tengeru	03° 23.145' S 36°48.488'E	1216	*Amaranthus dubius*	16/02/17	15
	Kware-Mbololo	03° 17.374'S 037° 10.144'E	1024	*Amaranthus dubius*	11/01/17	15
	Kikavu Chini	03°26.678'S 037° 17.813E	738	*Amaranthus dubius*	11/01/17	15
	Udoro-Mudio	03°16.218'S 037° 09.744'E	1120	*Amaranthus dubius*	11/01/17	14

7.2.2 DNA extraction from collected samples

Before DNA extraction, digital photographs of the adult moths (ventral, dorsal and lateral views) were taken using a calibrated Leica microscope EZ4D connected to a

laptop computer (Hewlett-Packard Company, Cupertino California). The photographs were to be used if the DNA sequence of the samples matched with a species different from *S. recurvalis* during an online search using BLAST hit results. Surface sterilization was then done by dipping the insect in 70 % ethanol for 30-40 seconds, in 3% sodium hypochlorite (NaClO) for 1 min and rinsed in distilled water 3 times. The insect was then placed in an Eppendorf tube and stored at -80 °C in a freezer awaiting DNA extraction. Total genomic DNA was extracted by crushing the whole adult insect and using the Isolate II Genomic DNA kit (Bioline, GmbH, Germany) as per manufacturer's instructions.

The extracted DNA was stored at -80°C prior to PCR amplification. The DNA was quantified using the NanoDrop micro-volume sample retention system (Thermo Scientific NanoDrop 2000/2000c Spectrophotometer). Any DNA sample with a concentration higher than 100 ng / µl was diluted at 10 % with PCR water before amplification.

7.2.3 DNA amplification of the samples

Polymerase Chain Reaction was performed to amplify cytochrome 1 (COI) gene of mitochondrial DNA using Lep primer pairs (5' ATTCAACCAATCATAAAGATATTGG 3' and 5' TAAACTTCTGGATGTCCAAAAAATCA 3': Lep-F1, Lep-R1 respectively). The reaction mixture consisted of 5.675 µM of PCR water, 2 µM of dNTP (dATP, dTTP, dCTP and dGTP), 0.5 µM of each primer, 0.2 µM $MgCl_2$ (25mM), 0.125 µM of Taq DNA polymerase and 1 µM of DNA template. These reactions were set up in the Master cycler Nexus gradient (Thermo Scientific) using a standard thermo-cycling conditions consisting of initial denaturation for 2 min at 95°C, followed by 35 cycles of denaturation for 30 s at 95°C, annealing at 52 °C for 40 s, and primer elongation for 1 min at 72°C followed by final step of extension for 10 min at 72°C.

7.2.4 DNA separation by electrophoresis

PCR products were separated by electrophoresis on 1 % agarose gel, immersed in 1x TAE buffer at a constant voltage of 80V (Bio-Rad model 200/2-0, Bio-Rad Laboratories, Inc., USA) for 1 hr (Plate 7.1). Six microlitres of ethidium bromide (10 µg / ml) were used for staining the PCR products for fragment size verification. About six µl of the PCR product was mixed with the loading dye loaded into the gel, alongside with 100-1200 bp molecular weight DNA ladder. Visualization and documentation of the gel were done under KETA GL imaging system trans-illuminator (Wealtec Corp, USA).

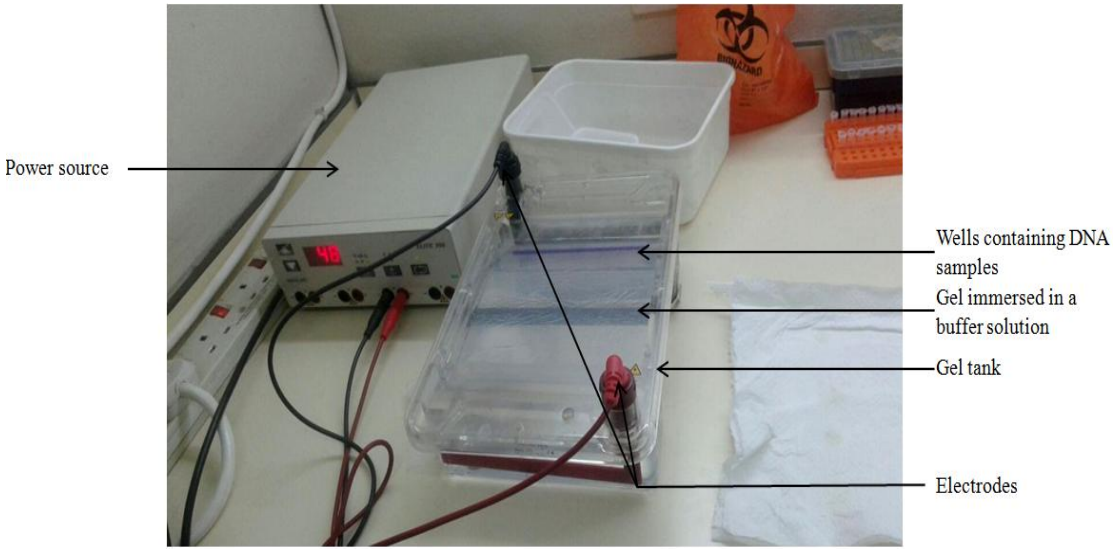

Plate 7.1: Electrophoresis on agarose gel

Photo: M. K. Agbodzavu

7.2.5 DNA purification and sequencing

The PCR products on the gel were excised and purified using PCR purification gel kit (Bioline, GmbH, Germany) according to the manufacturer's instructions. The purified PCR products were quantified for subsequent sequencing. All samples with

a DNA concentration ≥ 20 ng/ μL were sent to Macrogen Inc. in South Korea for bidirectional sequencing.

7.3 Data analyses

Sequence data were assembled and edited with Bioedit 7.2.6.1 (De Jong *et al.*, 2011). Chromatograms were manually verified to check for any ambiguous sequence. Ambiguous sequences as well as those with stop codons or gaps were discarded from further analysis. Primer ends were removed and sequences trimmed to approximate size of 640 of consensus sequences (640-652 bp) from booth reverse and forward strands. A BLAST search of GenBank sequences using the consensus sequences was conducted through the National Center of Biotechnology Information (NCBI) website (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to check for similarity (at > 90% sequence similarity with NCBI species) with already identified organisms and confirm the identity of species under study. Sequences were then aligned using Clustal X2 (Thompson *et al.*, 1997). Nucleotide composition and substitution rate were calculated using MEGA 7 (Kumar *et al.*, 2016).

To determine the level of genetic differentiation among populations, gene flow and genetic differentiation tests were conducted with DnaSP 6.10.03. The number of haplotypes, Haplotype (gene) diversity (Hd), Nucleotide diversity, Pi (π), the average number of nucleotide differences (K), genetic differentiation or genetic fixation (F_{ST}) and gene flow (Nm) values were obtained from these tests (probability obtained by the permutation test with 1,000 replicates). F_{ST} values of 0-0.05 are generally considered to indicate little genetic differentiation; where 0.05-0.25 indicate moderate genetic differentiation; and values > 0.25 represent pronounced levels of genetic differentiation (Freeland, 2005). The levels of gene flow can be categorized as Nm > 1 (high gene flow), 0.25 to 0.99 (intermediate gene flow), and Nm < 0.25 (low gene flow) (Govindaraju, 1989). Tajima's D (Tajima, 1989), Fu's Fs (Fu, 1997), as well as Fu and Li's D* statistic tests were performed to study the demographic history of *S. recurvalis* populations with the program DnaSP 6.10.03.

Pairwise nucleotide sequence divergence among populations was calculated under MEGA X (Kumar *et al.*, 2018). The pairwise nucleotide sequence divergences were used to generate principal coordinates analysis plots using GenAlEx 6.5 (Peakall & Smouse, 2006). The haplotype network of *S. recurvalis* was constructed using the phylogenetic median-joining (MJ) network algorithm using the software network 5.0. A phylogenetic tree was inferred by using the Maximum Likelihood method based on the Kimura 2-parameter model (Kimura, 1980) in MEGA X using sequences of the present study and some available in the GenBank for other countries (Table 7.2) for comparison. The reliability of the clustering pattern in the tree was evaluated using a bootstrap analysis with 1,000 replicates. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms (Saitou & Nei, 1987; Gascuel, 1997) to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value (Tamura & Nei, 1993). The tree was drawn to scale, with branch lengths measured in the number of substitutions per site. *Hymenia perspectalis* (Lepidoptera: Crambidae) sequence retrieved from the GenBank was used as an out-group (Table 7.2). The analysis involved 129 nucleotide sequences.

Table 7.2: Accession numbers and country/location of provenance

Accession codes	Country	Location
KY816497.1	China	Not specified
KJ657578.1	Florida	Not specified
KX054779.1	French Polynesia	Tahiti island, Mahina
HQ952588.1	Australia	Queensland, Bucasia
KX863087.1	Pakistan	Khyber Pakhtunkhwa, Dargai, Malakand
KR936327.1	Canada	Ontario, Lambton Co., Port Franks
KX047380.1	Greece	Epirus, Ioannina, Pindos Mts
JQ540156.1	Costa Rica	Area de Conservacion

		Guanacaste,
		Sector Horizontes, Vado Esperanza
KF492140.1	Japan	Shizuoka, Fujinomiya, Nonaka
KJ940214.1	India	Bahowal
HQ952585.1 *Hymenia perspectalis*, outgroup	Australia	New South Wales, Ebenezer

7.4 Results

7.4.1 Analysis of COI gene sequences

The COI sequences of the collected specimens identified as *S. recurvalis* were all between 98–100% matches with identified conspecific specimens in the BOLD database. After removing the primers used in the PCRs, the effective COI sequences consisted of 640 bp (Plate 7.2). After alignment, the number of nucleotide's sites including gaps was 677. The number of variable (polymorphic) sites (S) was 57 when considering all sequences whereas it was 41 and 35 for Kenya and Tanzania populations respectively (Table 7.3). The sequence conservation (similarity) rate (C) was 0.854.

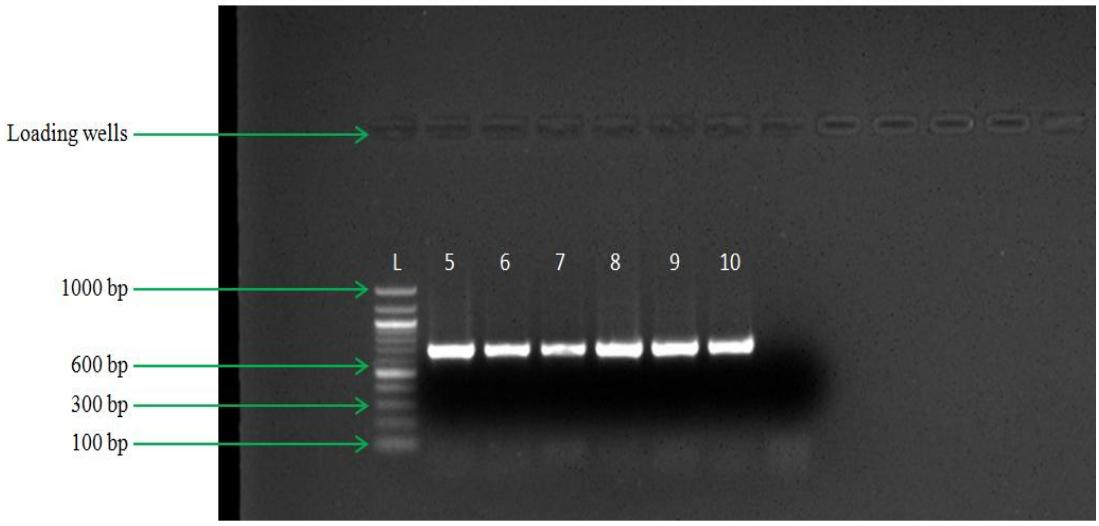

Plate 7.2: Sample of PCR products of COI gene of *Spoladea recurvalis* using Lep-F1 and Lep-R1 primers

L: ladder; Number 5-10 represent different samples from Kitengela

Photo: M. K. Agbodzavu

The nucleotide frequencies observed for the combined Kenyan and Tanzanian processed sequences were 30.47 %, 38.70 %, 15.74 %, and 15.09 % for A, T, C and G respectively, which was not different from the frequencies observed when considering separately Kenyan (A, T, C and G: 30.33 %, 38.73%, 15.63%, and 15.31 % respectively) and Tanzanian (A, T, C and G: 30.52 %, 38.61 %, 15.84 %, and 15.03 % respectively) populations (Table 7.3). In addition, the gene sequence analysis revealed a nucleotide composition richer in A-T sequences than C-G for all the samples, indicating consistency with mtDNA of insect species. Further, transitional and transversional substitutions are summarised in Table 7.4. The transitions were more frequent than transversions with an estimated value of transitions/transversions = 2.05 for combined samples, while it was 1.78 and 1.44 for Kenya and Tanzanian populations respectively when considered separately (Table 7.4). For instance, the transitions rates from A to G, and G to A were 11.5994 and 22.9775 respectively, while the transversions rates of A to T and T to A showed lower values of 6.2827 and 4.9199 respectively, in Kenyan population. Similar trend of the transitions and transversion rates was also obtained in Tanzanian population (Table 7.4).

A total of 24 and 29 haplotypes were recorded in Kenya and Tanzania, respectively. 46 haplotypes were obtained when all sequences were considered together. The haplotype diversity (Hd), the average number of nucleotide differences (Kt) and the nucleotide diversity (Pi JC) were 0.84699, 2.01585 and 0.00334 for Kenyan and 0.79261, 2.41792 and 0.00401 for Tanzania respectively (Table 7.3). Generally, high values of haplotype diversity and number of nucleotide differences were recorded except for the nucleotide diversity indices where low values were obtained.

Table 7.3: Nucleotide frequencies and genetic diversity indices of Kenyan and Tanzanian populations of *Spoladea recurvalis*

	Nucleotide frequencies (%)				Number of Haplotypes	Haplotype diversity (Hd)	Number of nucleotide differences (Kt)	Nucleotide diversity (Pi JC)	Number of variable (polymorphic) sites (S)
Populations	A	T	C	G					
Combined populations	30.47	38.7	15.74	15.09	46	0.82182	2.2186	0.00366	57
Kenyan population	30.33	38.73	15.63	15.31	24	0.84699	2.01585	0.00334	41
Tanzanian population	30.52	38.61	15.84	15.03	29	0.79261	2.41792	0.00401	35

Table 7.4: Maximum Likelihood Estimate of Substitution Matrix between nucleotides within studied populations

Population	From\To	A	T	C	G
Both populations combined	A	-	*5.6891*	*2.3136*	**12.0098**
	T	*4.4793*	-	**9.9274**	*2.2183*
	C	*4.4793*	**24.4111**	-	*2.2183*
	G	**24.251**	*5.6891*	*2.3136*	-
Kenyan population	A	-	*6.2827*	*2.5353*	**11.5994**
	T	*4.9199*	-	**9.4822**	*2.4836*
	C	*4.9199*	**23.4981**	-	*2.4836*
	G	**22.9775**	*6.2827*	*2.5353*	-
Tanzanian population	A	-	*7.1773*	*2.9455*	**10.7915**
	T	*5.6745*	-	**8.76**	*2.7934*
	C	*5.6745*	**21.3451**	-	*2.7934*
	G	**21.922**	*7.1773*	*2.9455*	-

Each entry is the probability of substitution (r) from one base (row) to another base (column). Substitution pattern and rates were estimated under the Tamura-Nei (1993) model. Rates of different transitional substitutions are shown in bold, and those of transversional substitutions are shown in italics. For simplicity, the sum of r values is made equal to 100. For estimating ML values, a tree topology was automatically computed. The maximum Log likelihood for this computation was -1636.307. The analysis involved 118 nucleotide sequences.

7.4.2 Population genetic structure

When considering Kenyan and Tanzanian samples as a single population, the value of Tajima's D statistic was significantly negative (D = -2.26684, P < 0.01) revealing a population size expansion. Additionally, the significantly negative value of Fu's Fs statistic (Fs = -93.921, P = 0.0001) as well as Fu and Li's D* statistic (D* = -

5.33613, P < 0.02) confirmed a recent population expansion in both countries. Similarly, Tajima's D (D = -1.89212, P < 0.05), Fu's (Fs = -33.743, P = 0.0001) as well as Fu and Li's D* (D* = -2.90490, P < 0.05) statistics obtained for Kenya and Tajima's D (D = -2.00168, P < 0.05), Fu's Fs (Fs =-34.126, P = 0.0001) and Fu and Li's D* (D* = -5.25563, P < 0.02) statistics recorded for Tanzania showed the same trend.

The results obtained showed that Kenyan and Tanzanian populations were highly similar and had a similar genetic pattern as per the low value of F_{ST} (0.015240). High gene flow between the two populations was noted (N_m= 16.15). The in-between populations mean genetic distances (0.005 ± 0.001) and the pairwise genetic distance between sampling locations recorded low values (Table 7.5). For example, the in-between genetic distance value of samples from Thika, Kenya and Tengeru, Tanzania was 0.005; similar low value was also obtained when considering samples from Yatta, Kenya and Kware Mbolo, Tanzania (Table 7.5). The pairwise genetic distance matrix between *S. recurvalis* populations from Kenya, Tanzania and from other countries available in the Genbank is presented in Table 8.6. The results showed that the genetic distance between Kenyan sequences and sequences available in the Genbank in one hand, and between Tanzanian sequences and sequences available in the Genbank on another hand varied from 0.0009-0.0112 and from 0.0010-0.0099 respectively (Table 7.6). The results of the principal coordinate analysis (PCoA) indicated that the two axes explained 90.01 % of the variation (the first axis 74.51 % and the second axis 15.50 %) (Figure 7.2). The PCoA separated all analyzed samples into three distinct clusters, where the first cluster was composed of sequences from Canada, Costa Rica, Florida, Greece, India, Japan, Kenya, Pakistan, and Tanzania. The second cluster was comprised of sequences from Australia, China and French Polynesia; and the third one was represented by the out-group *H. perspectalis* included in the study (Figure 7.2). It is worth noting that though Australia, China, and French Polynesia sequences were grouped in a different cluster on the PCoA (Figure 7.2), the genetic divergence between them to Kenyan and Tanzanian samples ranged from 0.22 to 1.11 % (Table 7.6) which was quite small to

conclude in any significant genetic divergence. These results, therefore, showed the genetic homogeneity of all *S. recurvalis* samples.

Table 7.5: Pairwise genetic distance between sampling locations

Samples	Kenya *icipe*	Kenya Kitengela	Kenya Mwea	Kenya Thika	Kenya Yatta	Tanzania Kikavu Chini	Tanzania Kware Mbolo	Tanzania Tengeru	Tanzania Udoro Mudio
Kenya *icipe*	0.000								
Kenya Kitengela	0.006	0.000							
Kenya Mwea	0.006	0.005	0.000						
Kenya Thika	0.006	0.005	0.005	0.000					
Kenya Yatta	0.006	0.005	0.005	0.005	0.000				
Tanzania Kikavu Chini	0.005	0.005	0.004	0.004	0.005	0.000			
Tanzania Kware Mbolo	0.006	0.006	0.005	0.005	0.005	0.005	0.000		
Tanzania Tengeru	0.006	0.006	0.005	0.005	0.005	0.005	0.006	0.000	
Tanzania Udoro Mudio	0.007	0.006	0.006	0.006	0.006	0.005	0.006	0.006	0.000

Table 7.6: Estimate of evolutionary divergence between *Spoladea recurvalis* populations from Kenya, Tanzania and from other countries available in the GenBank

Samples	KX054779.1 French Polynesia	HQ952588.1 Australia	KF492140.1 Japan	KJ940214.1 India	KJ657578.1 Florida	KY816497.1 China	Kenya	Tanzania	KR936327.1 Canada	KX863087.1 Pakistan	KX047380.1 Greece	JQ540156.1 Costa Rica	HQ952585 *Hymenia perspectalis*
KX054779.1French_Polynesia	0.0000												
HQ952588.1_Australia	0.0018	0.0000											
KF492140.1 Japan	0.00 91	0.0109	0.0000										
KJ940214.1 India	0.0127	0.0146	0.0036	0.0000									
KJ657578.1 Florida	0.0091	0.0109	0.0000	0.0036	0.0000								
KY816497.1 China	0.0073	0.0091	0.0018	0.0054	0.0018	0.0000							
Kenya	0.0093	0.0112	0.0009	0.0046	0.0009	0.0022	0.0000						
Tanzania	0.0099	0.0117	0.0010	0.0047	0.0010	0.0028	0.0019	0.0000					
KR936327.1 Canada	0.0091	0.0109	0.0000	0.0036	0.0000	0.0018	0.0009	0.0010	0.0000				
KX863087.1 Pakistan	0.0109	0.0128	0.0018	0.0054	0.0018	0.0036	0.0028	0.0029	0.0018	0.0000			
KX047380.1 Greece	0.0109	0.0128	0.0018	0.0054	0.0018	0.0036	0.0026	0.0027	0.0018	0.0036	0.0000		
JQ540156.1 Costa Rica	0.0128	0.0146	0.0036	0.0072	0.0036	0.0054	0.0044	0.0045	0.0036	0.0054	0.0018	0.0000	
HQ952585 *Hymenia perspectalis*	0.0818	0.0838	0.0838	0.0878	0.0838	0.0818	0.0836	0.0842	0.0838	0.0858	0.0818	0.0838	0.0000

The number of base substitutions per site from averaging over all sequence pairs between groups is shown. Standard error estimate(s) are shown above the diagonal and were obtained by a bootstrap procedure (500 replicates). Analyses were conducted using the Kimura 2-parameter model. The analysis involved 129 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There was a total of 555 positions in the final dataset. Evolutionary analyses were conducted in MEGA X.

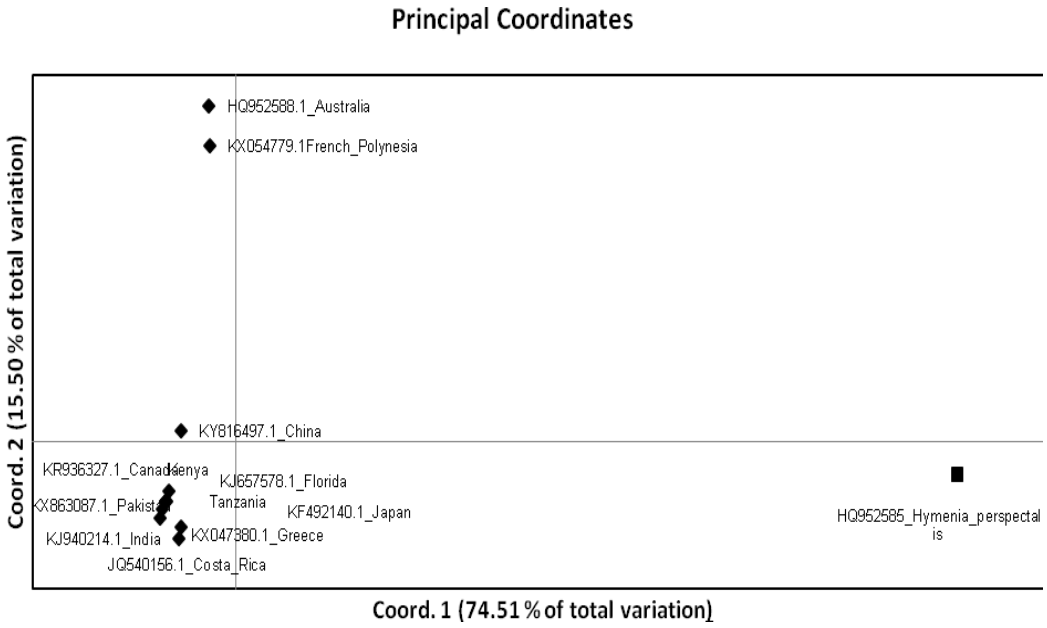

Figure 7.2: Plot of principal coordinates analysis (PCoA) via the pairwise genetic distance matrix

The haplotype network (Figure 7.3) showed a star-like pattern with the most common haplotypes in the star's center while the haplotype H 31 representing the outgroup *H. perspectalis* was completely distant from others. The most common haplotype represented more than half (55.81%) of all analyzed sequences (Figure 7.3).

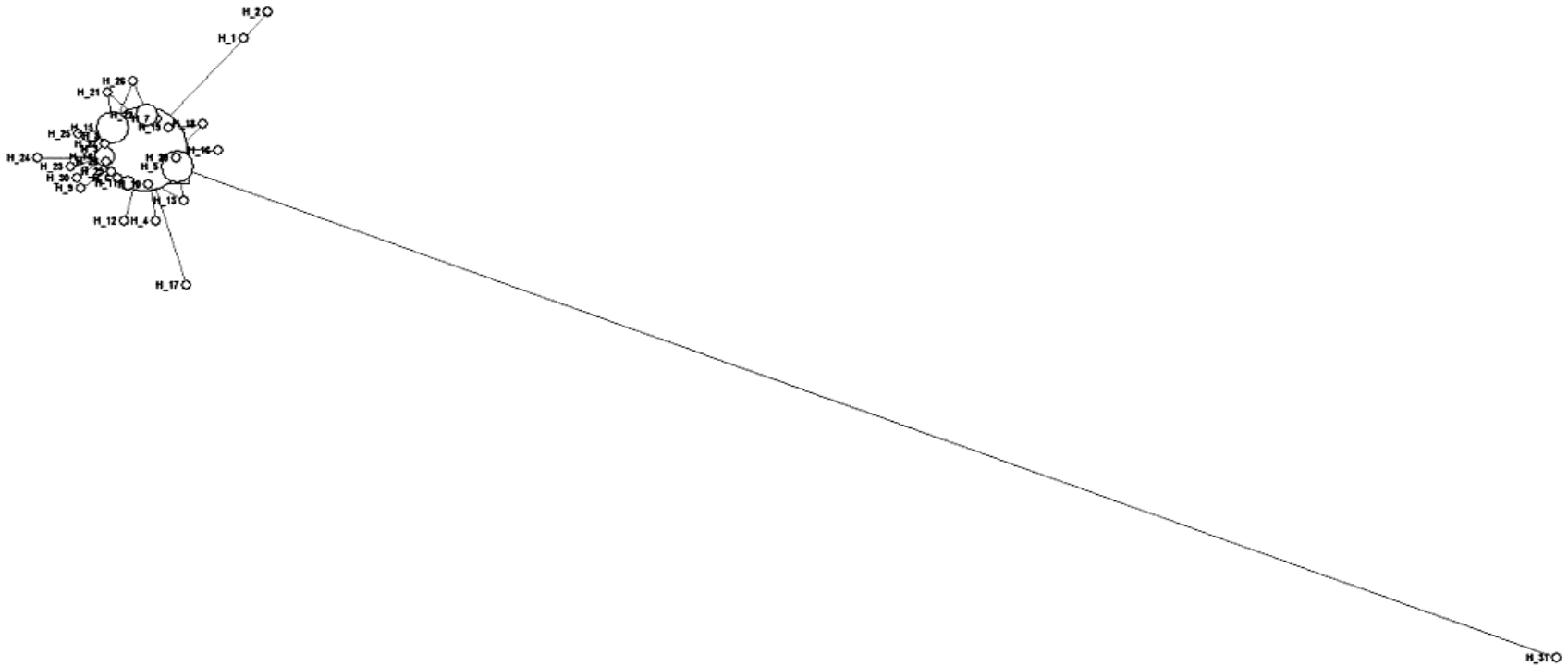

Figure 7.3: Median-joining haplotype network of *Spoladea recurvalis* samples from Kenya, Tanzania, and Gen-Bank (including the out-group) based on COI gene.

Each circle represents a unique haplotype connected by a line to those that differ by one or more base pairs. The size of the pie chart is proportional to the frequency of sequences forming the haplotype

7.4.3 Evolutionary relationships of *Spoladea recurvalis* specimens from Kenya, Tanzania and those available from other countries in the Gen-Bank

The generated tree with the highest log likelihood (-1279.52) is shown in Figure 7.4. The percentage of trees in which the associated taxa clustered together is shown next to the branches. The phylogenetic tree was paraphyletic with three distinct branches. The first branch grouped samples from Canada, China, Costa Rica, Florida, Greece, India, Japan, Kenya, Pakistan and Tanzania together. The second branch clustered samples from French Polynesia, Australia and one sample from Tanzania, while the third one represented the out-group *H. perspectalis*. However, considering the length of the branches which were very small (less than 0.010), a synonym of very slight variability, all studied samples can, therefore, be considered as genetically homogenous population except the out-group, as indicated above by previous analyses outputs (Figure 7.4).

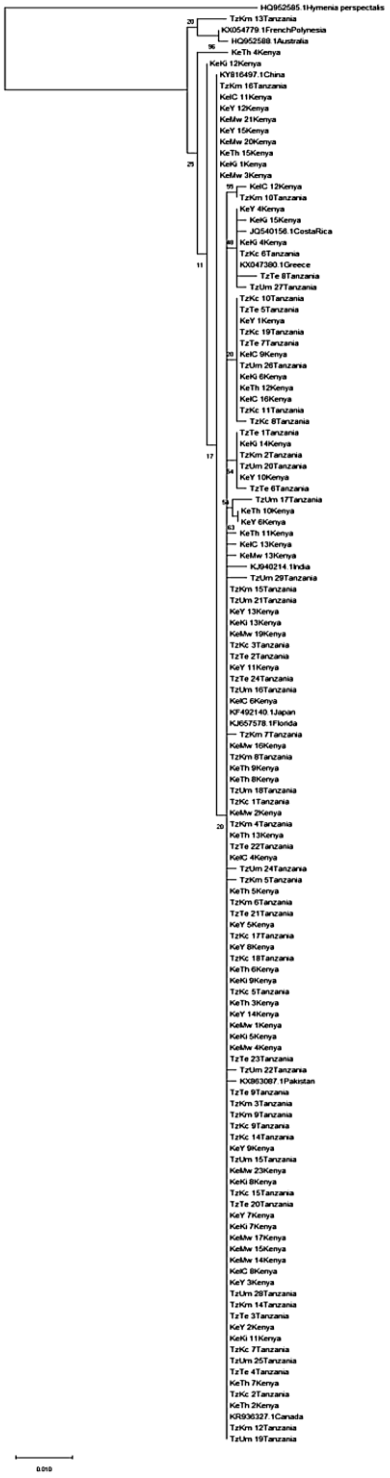

Figure 7.4: Maximum Likelihood tree showing evolutionary relationships between *Spoladea recurvalis* **samples from the current study and retrieved from the GenBank**

7.5 Discussion

This is the first study analyzing the genetic taxonomy of the beet webworm *S. recurvalis* populations from Africa and specifically in Kenya and Tanzania. There is, therefore, no genomic data available in the Genbank for African populations of *S. recurvalis*, and the current study comes in handy to fill this gap. Information regarding genetic diversity, genetic structure and gene flow are key factors when developing pest management strategies (Roderick, 1996; Estoup & Guillemaud, 2010) since the responses or behaviour of the targeted pests could be influenced by genetic and/or environmental variations. In this study, *S. recurvalis* showed A-T rich sequences nucleotide composition, which is a pattern that has been repeatedly seen in the mtDNA of most insect species (Simon *et al.*, 1994). Shayanmehr & Yoosefi-Lafooraki (2016) while studying the genetic diversity of rice stem borer *Chilo suppressalis* Walker (Lepidoptera: Crambidae) found the same trend. Bajpai & Tewari (2010), Qin *et al.* (2016) and Sun *et al.* (2015) also reported A-T rich sequences nucleotide composition of flesh flies (Sarcophagidae: Diptera), *Bactrocera correcta* (Bezzi) (Diptera: Tephritidae) and *Oedaleus infernalis* (Saussure) (Orthoptera: Acridoidea) respectively.

The results of the current study also revealed high values of haplotype diversity but low nucleotide diversity among the studied populations indicating only slight differences between haplotypes. Similarly high haplotype diversity values have been reported in other Lepidoptera species, such as *Aglais urticae* (L.) **(**Lepidoptera: Nymphalidae) (Vandewoestijne *et al.*, 2004) and the invasive horse-chestnut leaf miner *Cameraria ohridella* Deschka & Dimic **(**Lepidoptera: Gracillariidae) (Valade *et al.*, 2009), which are all migratory species like *S. recurvalis*. Reasons explaining this fact could be a population which underwent a bottleneck followed by an expansion. A population bottleneck according to Franks *et al.* (2011) may result in the loss of genetic diversity. An association of one common haplotype with others in low frequencies is a pattern frequently attributed to populations that have undergone recent range expansion (Slatkin & Hudson, 1991) as showed in the haplotype network results. Geographic isolation by distance or natural barriers is one of the factors reported to shape genetic structuring of populations. Despite the fact that a

correlation between the genetic distances and the geographic distances was not calculated in this study (because low genetic diversity was recorded), being a migratory species, *S. recurvalis* have the ability to overcome the geographic isolation by flight, therefore allowing an interbreeding and a high gene flow among the two studied populations. There are studies evidencing that *S. recurvalis* is a long-distance migrating insect. In Japan, it is thought to be one of the lepidopterous insects that migrate overseas from subtropical and tropical regions, together with *Cnaphalocrocis medinalis* (Lepidoptera: Crambidae) during the rainy season (Yamada *et al.*, 1979; Miyahara, 1997). *Spoladea recurvalis* has also been known to invade, through a transoceanic migration, New Zealand from Australia, together with other Lepidoptera (Fox, 1978). Gene flow is one of the main factors used to estimate population genetic structure. Current results demonstrated a high gene flow among both populations supporting their low genetic structure. It was reported by Llewellyn *et al.* (2003) that the little spatial genetic structure observed in the grain aphid (*Sitobion avenae* (F.) (Hemiptera: Aphididae) in Britain was due to its high migratory ability. Therefore, sufficient gene flow between populations as a consequence of insects migration can therefore slow down or prevent the process of geographic differentiation, and leave a signature of little population structure over large areas (Millar & Libby, 1991; De Jong *et al.*, 2011). Wongsa *et al.* (2017) supported the same concept while studying the genetic structure of *Aphis craccivora* Koch (Hemiptera: Aphididae) from Thailand.

The fixation indices between populations are among the common parameters used to measure the degree of genetic differentiation (Willing *et al.*, 2012). In this study, the obtained F_{ST} values showed negligible differentiation when comparing inter or intra-populations variabilities of the two countries, which was also confirmed by the pairwise genetic divergences comparison of sampling locations.

As stipulated above, a bottleneck and recent demographic expansion were markedly evidenced by Tajima's D, as well as Fu and Li's D* and Fu's Fs neutrality tests in *S. recurvalis*. According to Fu & Li (1993), negative significant values of those statistics indicate an excess of recently derived haplotypes and suggesting that either population expansion or background selection has occurred. Prijović *et al.* (2014) in

their study concluded that negative values of Tajima's D and Fu's Fs indicated an excess of low frequency polymorphisms in the populations, which could imply recent population expansion.

Phylogenetic analysis revealed a weak phylogeographic structuring among the geographic populations, indicating their close relationship (Wongsa *et al.*, 2017). It appeared that there was no clear separation of Kenya populations and Tanzania populations in one hand and on the other hand with samples retrieved from the GenBank. Here also, the lack of notable phylogeographic structure can be explained by rapid range expansion and migration.

Considering these results, we conclude that the populations of *S. recurvalis* sampled in Kenya and Tanzania, as well as data available in the GenBank for Australia, Canada, China, Costa Rica, Florida, French Polynesia, Greece, India, Japan, and Pakistan, constitute a single species basing on COI gene. This represents an important finding that may have implications for future biological control programs as well as for other IPM components in the way that having a single species and not a complex of cryptic species increases the chance for the released strains of the biological control agent as well as biopesticides and attractants to be successful. Moreover, it will facilitate the exchange of biological control agents among regions with a high chance of success. Further studies are therefore warranted to assess these findings under integrated *S. recurvalis* management approaches.

CHAPTER EIGHT

GENERAL DISCUSSION, CONCLUSIONS, RECOMMENDATIONS AND LIMITATIONS

8.1 General discussion

Amaranth has become an essential component of the farming systems and the diet of many African people. However, the stability of its production in East Africa is limited due to damage by lepidopteran defoliator pests, among other factors. Amaranth lepidopteran defoliators management has relied on indiscriminate use of synthetic chemical insecticides, which have proved inefficient (Clarke-Harris & Fleischer, 2003). The indiscriminate and frequent uses of these chemicals have resulted in the development of resistance in the lepidopterans (Mosallanejad & Smagghe, 2009; Ahmad & Arif, 2010; Che *et al.*, 2013). Moreover, the issues of human health and environmental risks including the elimination of natural enemies, have emerged as key problems resulting from indiscriminate use of chemical pesticides (Ruberson *et al.*, 1994; Özkara *et al.*, 2016). Therefore, there is an urgent need to identify alternative control methods to the chemical application, which are ecologically acceptable and compatible with other management options within an overall Integrated Pest Management strategy.

Field experiments showed that there was a diversity of lepidopteran species feeding on amaranth crop. The most important was *S. recurvalis*, *S. exigua* and *S. littoralis*. However, their relative importance was dependent on the agro-ecological zone. In high altitude areas, *S. exigua* was the predominant species, while in the mid-altitude it was *S. recurvalis*. In studies carried by Mureithi *et al.* (2015) and Othim *et al.* (2018a) in Central Kenya, the presence of four species, *S. recurvalis*, *Spodoptera* sp., *Choristoneura* sp. and *Spilosoma* sp. was reported as lepidopteran species attacking amaranth, with *S. recurvalis* and *S. littoralis* being the most abundant. These results confirm the fact that amaranth is attacked by a complex of lepidopteran pests amongst which *S. recurvalis* and *Spodoptera* species are predominant. In the view of implementing an integrated management approach of these defoliators, the focus should not target one species but a complex. Outbreaks of *S. recurvalis* were not

consistent throughout the five seasons of study though there is always a peak of abundance from December 2015 to February 2016. Another peak was also observed in April-June in farmers' fields but was not captured in the experimental field studies. Local conditions such as rainfall, temperature and relative humidity as well as the availability of alternative host plants might play an essential role in the build-up of the population. During this study only two alternative host plants were recorded for *S. recurvalis*: *Achyranthes aspera* which is a wild host and *Spinacia oleracea* a cultivated host. It is reported that alternative hosts play an important role in maintaining the population of *S. recurvalis* all year round awaiting favourable conditions to migrate to the amaranth (Bhattacherjee & Ramdas Menon, 1964). Nevertheless, the infestation of amaranth field can also be due to migratory individuals since *S. recurvalis* is a long-distance migrating insect (Yamada *et al.*, 1979; Miyahara, 1997). Presence of *S. recurvalis* on *S. oleracea* which is also an important vegetable in East African population diet, gives more insight into the necessity of managing this pest. Four parasitoid species were found associated with each group of defoliators: *C. icipe, C. ater, C. curvimaculatus, C. luteum* were associated to leafworms while *A. hemara*, *A. tricolor*, *Phanerotoma* sp. and *S. testacea* were associated to leaf webbers. This demonstrates the richness of the local fauna in biological control agents. Conservation or augmentative biological control could be a promising option for the management of these lepidopteran defoliators. Farmers therefore, need to be educated on the need for preserving them by changing their farming practices. Results from this study showed the limitation of using Phenylacetaldehyde and *S. recurvalis* pheromone developed in India conditions as a means of monitoring and managing lepidopteran species of interest. More research such as isolating and identifying a suitable and effective attractant need then to be carried. *Spodoptera exigua* pheromone produced by the same company as for *S. recurvalis* pheromone was efficient in trapping adult moths; however, it was not effective in reducing the damage levels. Experiment regarding the minimum number of traps per unit surface to obtain satisfactory results need to be carried out.

Performance studies carried out on the newly discovered *Cotesia* species, *C. icipe* in laboratory conditions showed that it attacks only *Spodoptera* species. The parasitoid was effective on both species of *Spodoptera* tested and holds excellent potentials in

controlling other *Spodoptera* species that cause havoc in various farming systems. *Spoladea recurvalis*, *U. ferrugalis* and *H. bipunctalis* all belonging to Crambid family were not attacked. Other suitable hosts for *C. icipe* might exist, but in the scope of this study, the screened hosts were the ones found on amaranth. Parasitism rate obtained on *S. littoralis* was higher than the one recorded on *S. exigua* in the same conditions. The reason for this could be the rearing host which was *S. littoralis*. *Cotesia icipe* individuals used in the experiment were, therefore, accustomed to *S. littoralis* cues. It has been shown that oviposition may be a matter of experience as demonstrated by Samson-Boshuizen *et al.* (1973) and that a female parasitoid with a wide host range often prefers a host species from which she has been reared (Thorpe & Jones, 1937). It is, however, important to note that this associative learning can vanish in the absence of continued experiences as pointed out by Geervliet *et al.* (1998) and Papaj & Vet (1990). *Cotesia* species have been intensively used in agricultural pest management with successful results (Overholt *et al.*, 1997; Gillespie *et al.*, 1999; Cameron *et al.*, 2006; Herlihy & Driesche, 2013). The discovery and performance of this species make it a good candidate for efficient management of *Spodoptera* species in an augmentative/conservation biological approach.

Temperature-dependency of *A. hemara* development, survival and reproduction were reported in this study. Results showed that a temperature lower than 10°C and higher than 35°C as being lethal to the development of *A. hemara*. Using a modelling approach to determine the minimum and maximal thresholds, the optimal temperature and the thermal constant and how the temperature affects other biological traits of an insect is a widely adopted methodology. This approach has been intensively used to map the current and future distribution of species and determine their establishment index (Kroschel *et al.*, 2013; Ngowi *et al.*, 2017). These results provide baseline information for such studies. Knowledge of *A. hemara* thermal requirements could also be used to optimize mass production. The high realized fecundity (in terms of the number of produced cocoon) obtained proves that *A. hemara* is an efficient biocontrol agent to be considered in the management program of *S. recurvalis*. This parasitoid combined with *C. icipe* could significantly reduce damages due to the preponderant amaranth lepidopteran defoliators' complex and will constitute a significant component of IPM strategy.

Genetic taxonomy studies based on COI gene carried out on Kenyan, and Tanzanian populations of *S. recurvalis* showed the absence of genetic structure. This study is of most importance since genetic divergence in targeted pest can be a reason for the failure of a biological control program. There are many studies highlighting the implication of having different populations of a pest or a biological agent on the outcome of the biological control program (De Moraes & McMurtry, 1985; Pashley, 1993; Furtado *et al.*, 2007). I also found that Kenya and Tanzania DNA sequences, as well as data available in the GenBank for Australia, Canada, China, Costa Rica, Florida, French Polynesia, Greece, India, Japan, and Pakistan, constitute a single closely related population. This fact represents an important finding that may have implications for future biological control programs as well as for other IPM components in the way that having a genetically homogenous pest population increases the chance for the released strains of the biological control agent as well as bio-pesticides and attractants to be successful. Moreover, it will facilitate the exchange of biological control agents among regions with a high chance of success.

8.2 Conclusions

1. Diversity of amaranth lepidopteran defoliators in Kenya is influenced by agro-ecological zones. For example, *S. recurvalis* is more abundant in mid-altitude while *S. exigua* is the major one in high altitude; *S. littoralis* is equally distributed in both agro-ecological zones.
2. Attractants tested in the current study did not reduce the level of the damage due to lepidopteran feedings.
3. There are eight indigenous parasitoids species on amaranth in Kenya namely *C. icipe, C. ater, C. curvimaculatus, C. luteum, A. hemara, A. tricolor, Phanerotoma* sp. and *S. testacea. Apanteles hemara* is the main parasitoid on the main pest species *S. recurvalis.*
4. *Cotesia icipe* (first identified and described in the present study), and *A. hemara* represent two good candidates for biological control program.
5. *Cotesia icipe* oviposits and successfully develops in Noctuidae species *S. littoralis* and *S. exigua* but not on the Crambidae species *H. bipunctalis*, *S. recurvalis* and *U. ferrugalis*.

6. Development, survival and reproduction of the parasitoid *A. hemara* on *S. recurvalis* are strongly affected by temperature. *Apanteles hemara* could not develop at temperatures below 10 and above 35°C, and the optimum temperature is 32°C.
7. Populations of *S. recurvalis* from Kenya and Tanzania do not form a complex of cryptic species but belong to same species based on COI gene.

8.3 Recommendations

1. Management of amaranth lepidopteran defoliators must be focused on the complex of the three major lepidopteran species *S. recurvalis*, *S. exigua* and *S. littoralis*.
2. An efficient attractant for *S. recurvalis* needs to be developed.
3. *Cotesia icipe* and *A. hemara* need to be tested in field trials for their ability to reduce damages due to amaranth lepidopteran defoliators and should represent the main parasitoids to be produced by biological agents producing companies.
4. Further studies for continuous field evaluation of *S. recurvalis* outbreaks are necessary to well establish factors triggering their sudden and high populations appearance.
5. For the newly discovered and promising parasitoid *C. icipe*, it is important to determine the effect of host larval instar on the parasitism rate and the effect of different temperatures and humidity regimes on its life table parameters. It will also be good to test whether the rearing host of the parasitoid will affect the tested host acceptability and the suitability.
6. Since *C. icipe* is attacking the two *Spodoptera* species recovered on amaranth, I recommend assessing its acceptability and suitability on other *Spodoptera* species such as *S. frugiperda* and *S eridania* which is a new invasive species recorded in different Sub-Saharan African countries.
7. Studies need to be carried to assess the performance of all indigenous parasitoids and their potential to be used in an augmentative biological control program.

8. Genetic taxonomy studies of *S. recurvalis* should further be pursued using a different highly variable marker to ascertain results obtained in this study.
9. National agricultural research institute should take over and promote conservation and augmentative biological controls in amaranth producing areas.

8.4 Limitations

1. Difficulties to have a continuous laboratory colony of the three important lepidopteran defoliators delayed experiments in the laboratory and prevented us from being able to carry the study on the reproduction of *A. hemara* at all set temperatures. In fact, *S. recurvalis* was found to be extremely difficult to maintain under laboratory conditions for more than three continuous generations.

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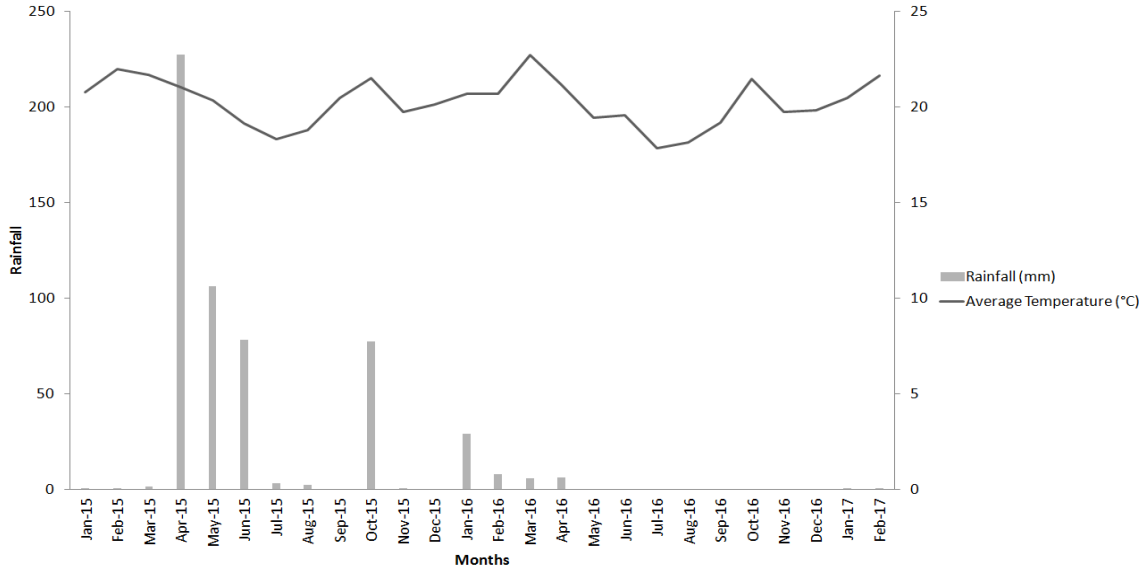
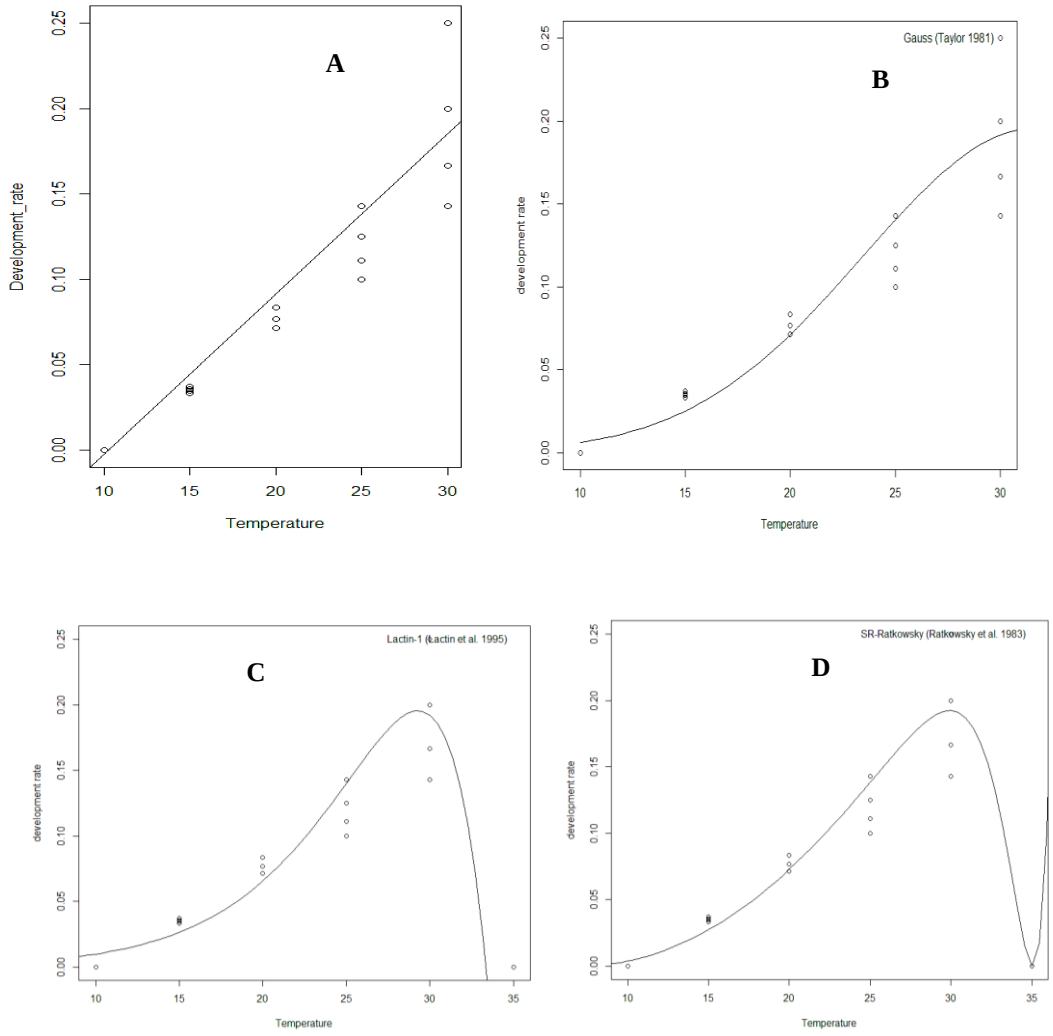
APPENDICES

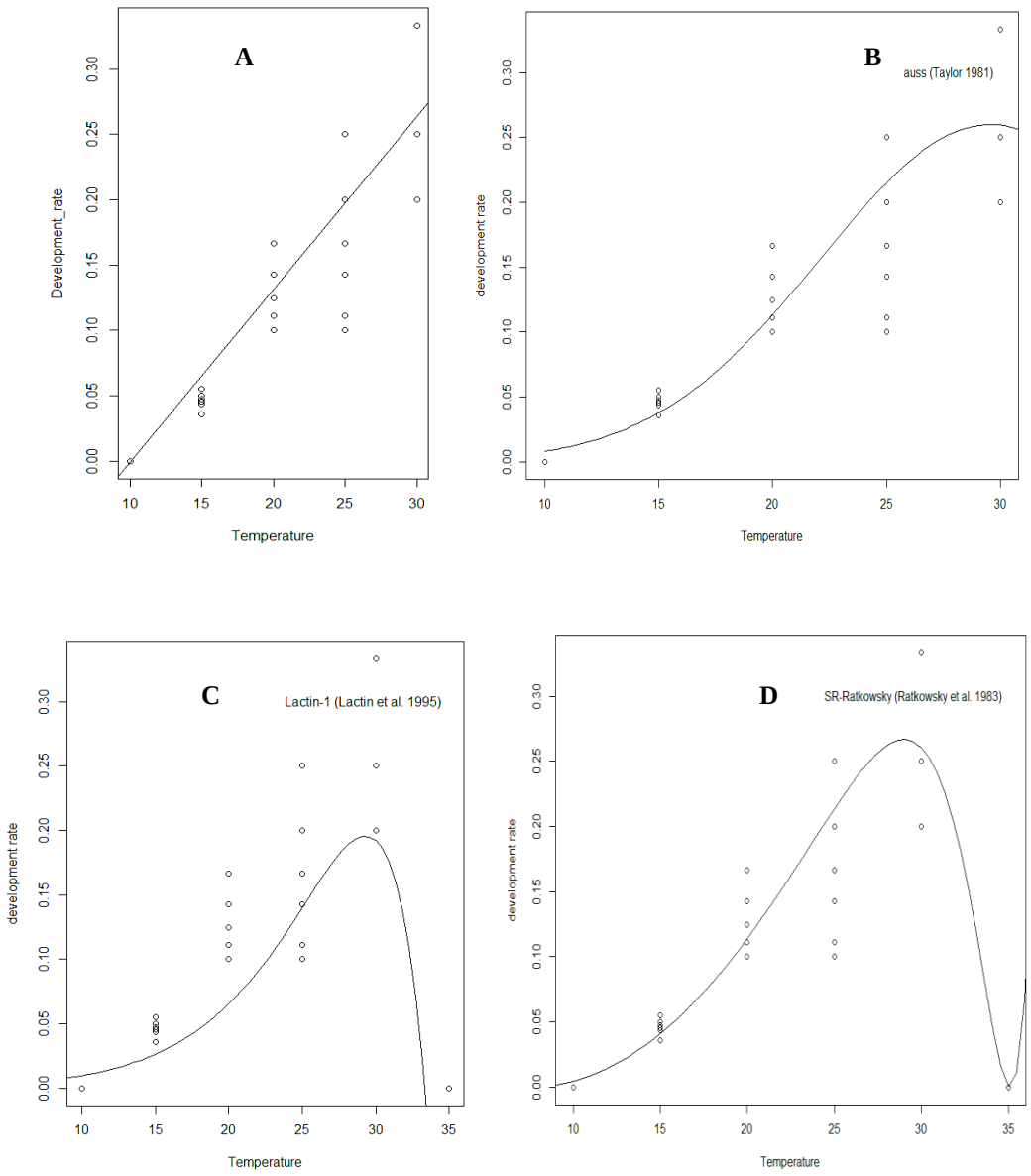
Appendix I: Rainfall and temperatures as recorded in Thika



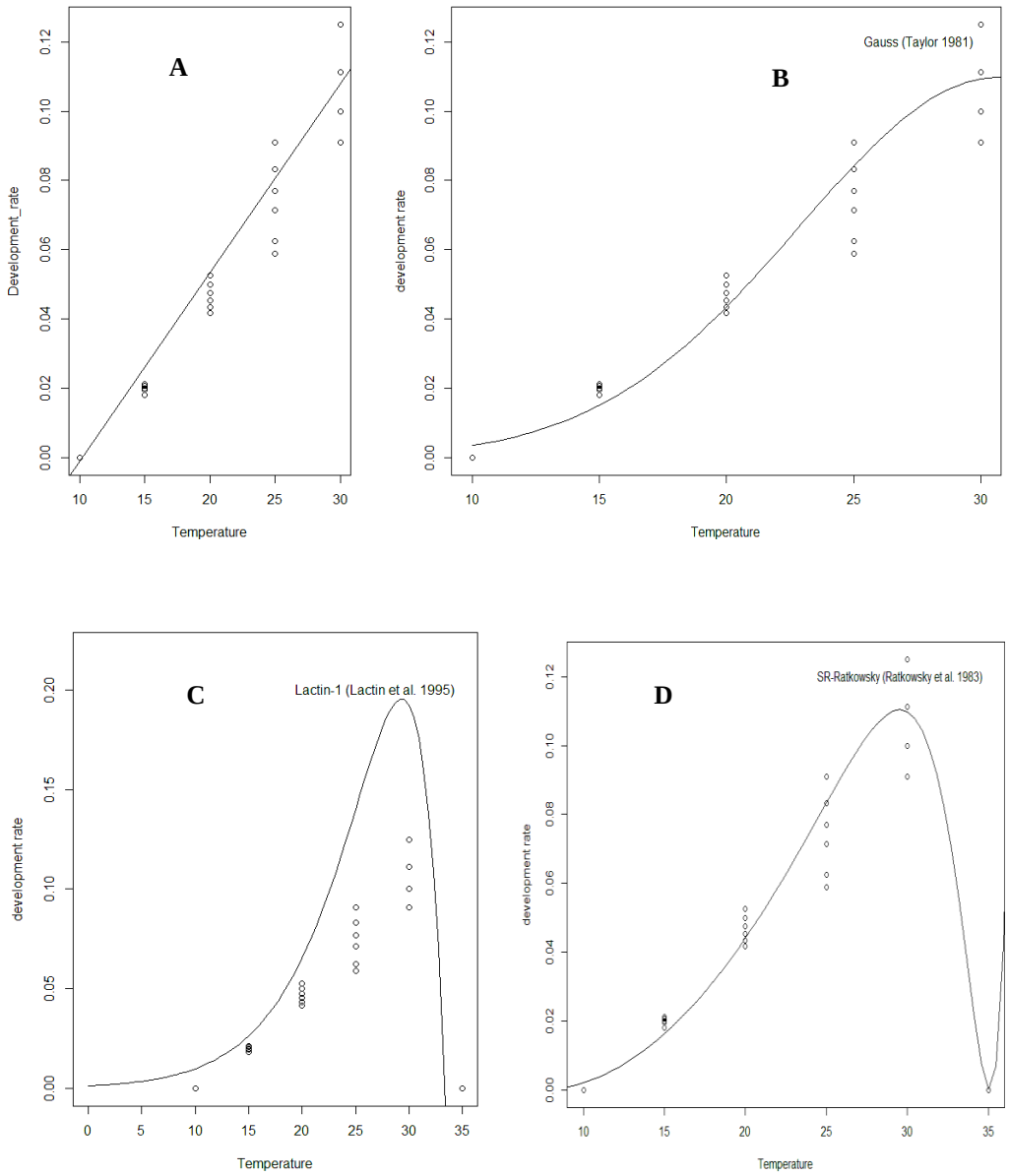
Appendix II: Fitted models of development rate (1/development duration) for the larval stage of *Apanteles hemara*



Linear (A), Taylor (B), Lactin-1 (C) and Ratkowsky (D) fitted models of development rate (1/development duration) for the larval stage of *Apanteles hemara*



Linear (A) Taylor (B), Lactin-1 (C) and Ratkowsky (D) fitted models of development rate (1/development duration) for pupal stage of *Apanteles hemara*



Linear (A) Taylor (B), Lactin-1 (C) and Ratkowsky (D) fitted models of development rate (1/development duration) for total developmental time of *Apanteles hemara*

Appendix III: Presentations and publications from this thesis

Publications

Agbodzavu, M. K., Lagat, Z. O., Gikungu, M., Rwomushana, I., Ekesi, S., & Fiaboe, K.-K. M. (2018). Acceptability and suitability of *Spodoptera exigua* (Hübner) for *Cotesia icipe* Fernandez-Triana & Fiaboe on amaranth. Journal of Applied Entomology. 142:716-724. https://doi.org/10.1111/jen.12525

Agbodzavu, M. K., Lagat, Z. O., Gikungu, M., Rwomushana, I., Ekesi, S., & Fiaboe, K.-K. M. (2018). Performance of the Newly Identified Endoparasitoid *Cotesia icipe* Fernandez-Triana & Fiaboe on *Spodoptera littoralis* (Boisduval). Journal of Applied Entomology. 142:646-653. https://doi.org/10.1111/jen.12514

Presentations

Agbodzavu, M. K, Lagat, Z.O., Gikungu, M., Rwomushana, I., Ekesi, S. & Fiaboe, K. K. M. (2017). Performance and host range of a new species of *Cotesia* sp. on key amaranth lepidopteran defoliators. Presentation: 22nd Meeting and Conference of the African Association of Insect Scientists, 23rd 26th October 2017, Wad Medani, Sudan. Oral presentation.

Agbodzavu, M. K., Lagat, Z. O., Gikungu, M., Ekesi, S. & Fiaboe, K. K. M. (2017). Population dynamics and Efficacy of different Attractants on Amaranth Lepidopteran pests at High and Mid altitude in Kenya. African Indigenous Vegetables-IPM end of project Workshop. 12th 14th December 2017. Arusha, Tanzania. Oral presentation.

Agbodzavu, M. K., Lagat, Z. O., Gikungu, M., Ekesi, S. & Fiaboe, K. K. M. (2017). Genetic diversity of S*poladea recurvalis* (lepidoptera: crambidae) populations from East Africa and their implication to biological control. African Indigenous Vegetables-IPM end of project Workshop. 12th 14th December 2017. Arusha, Tanzania. Oral presentation.