BIOCHEMICAL AND MOLECULAR ASPECTS OF HOST RECOGNITION AND ACCEPTANCE BY *COTESIA* SPP. POPULATIONS IN COASTAL AND WESTERN KENYA

BY

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DECLARATION

I, Gladys Bosibori Bichang'a, duly declare that this thesis is my original work and has not been presented for a degree or any award in any other University.

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DEDICATION

To Enock, Kelvin, Alvin, Gloria and Emmanuel; No matter how much of your time I spend away from you; the God who has healed us along the way still promises our family a blessing.

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ABBREVIATIONS AND ACRONYMS

1D gel	One-dimensional
ACN	Acetonitrile
ANR	Project BIOINV4I
BCA	Bicinchoninic acid
BLAST	Basic local alignment search tool
BLASTx	Search protein database using translated nucleotide querry
cDNA	Complementary DNA
CID	Collision-Induced Dissociation
CrV1	Ceratocystis resinifera virus 1
Cs Inland	*Cotesia sesamiae* - Inland
Cs-Coast	*Cotesia sesamiae*- coast
(CsBV),	*Cotesia. sesamiae* bracovirus
CNRS	*Centre National de la Recherche Scientifique* (National Center for Scientific Research, France
DNA	Deoxyribonucleic acid
DT	Drought Tolerant
DTT	Dithiothreitol
EST	Expressed sequence tag
FAO	Food and Agriculture Organization (United Nations)
GE	General Electric Healthcare Company
GC-MS	Gas Chromatography - Mass Spectrometry
HCl	Hydrochloric acid
H_2O	Water
HPLC	High-performance liquid chromatography
IPCC	Intergovernmental Panel On Climate Change
ICIPE	International Centre of Insect Physiology and Ecology
Kit	Kitale
kDa	Kilodalton
LC-MS/MS	Liquid Chromatography - Mass Spectrometry
LTQ	Linear Trap Quadrupole (mass spectrometer)

Mbsa	Mombasa
MLN	Maize Lethal Necrosis
MWCO	Molecular Weight Cut-Off
MOPS	(3-(N-morpholino) propanesulfonic acid) buffer
NGS	Next generation sequencing technologies
OECD/FAO	Organisation for Economic Co-operation and Development (OECD) and the Food and Agriculture Organization (FAO) of the United Nations
PAGE	Polyacrylamide gel electrophoresis
PDV	Polydnavirus
PBS	Phosphate-buffered saline
QTL	Quantitative Trait Locus
TFA	Trifluoroacetic acid
USA	United States of America
VOCs	The volatile organic compounds
RADseq	Restriction site Associated DNA sequencing
S1 and S2	Supplementary table 1 and 2
SNP	Single Nucleotide Polymorphism
RH	Relative humidity
LD	Light /Dark

DISCLAIMER

This thesis consists of chapters that have been prepared as a stand alone paper already published or manuscripts for different scientific journals. Consequently, unavoidable overlaps and /or repetitions may occur.

ABSTRACT

Lepidopteran stemborers are among the major constraints to maize production in Africa due to the crop losses they cause. *Cotesia* spp. are one of the key parasitoids that have been used in biological control of cereal stemborers. For example, in eastern Africa, the braconid larval endoparasitoid, *Cotesia flavipes* Cameron was introduced in a classical biocontrol programme for the control of the invasive stemborer *Chilo partellus* (Swinhoe): (Lepidoptera: Crambidae). Although the plant volatiles play a key role in the parasitoids for location of their hosts feeding on plants, studies have indicated that the host identification process for acceptance occurs mainly during contact between the parasitoid and its host where host products related to feeding activities including fecal pellets and oral secretions, play a crucial role in determining the suitability of the stemborer and to induce the host recognition and acceptance.

For a better and efficient biological control management systems, this study sought to unravel the origin, identity and the chemical variability of the contact chemical(s) involved in host recognition and acceptance, of suitable stem borer hosts by the *Cotesia* species/population present in Kenya. It also entailed to identify the candidate genes involved in host recognition and acceptance by *Cotesia* species.

Using stemborer host and non-hosts of *C. flavipes*, this study demonstrated the oral secretions of the larvae that harbour the active chemical(s) that mediate host acceptance for oviposition by *C. flavipes.*Through the integration of behavioural observations, biochemical and proteomics approaches, the active compound of the oral secretions was identified as an α-amylase. Using synthetized α-amylases from *Drosophila melanogaster* (an insect model for which syntheses of active and inactive α-amylases are available), it was observed that the conformation of the enzyme rather than its catalytic site as well as its substrate and its degradation product is responsible for host acceptance and oviposition mediation of *C. flavipes* females. The present work also investigated whether the variations in this enzyme could explain specific host recognition in different host-parasitoid associations. Different species and populations of the *C. flavipes* complex specialized on graminaceous lepidopteran stemborers were used. Electrophoresis of α-amylase and enzyme specific amylolytic test (formation of specific enzyme -substrate complex) revealed different isoforms that mediate oviposition acceptance and preference of the parasitoid for a specific host. Because of the presence of two populations of *Cotesia sesamiae* in Kenya, viz, Cs-Coast and Cs-Inland, with contrasted level of acceptance of *Busseola fusca* (Lepidoptera: Noctuidae) host, advantage of this was exploited in order to determine the candidate genes involved in host acceptance by the parasitoids. A genetic analysis approach of crosses between these two populations was thus initiated and confirmed that their acceptance towards *B. fusca* for oviposition is heritable. In conclusion the discovery presented in this thesis opens new avenue to investigate evolutionary processes at play in host specialization in the species-rich *Cotesia* genus.

CHAPTER ONE

1.0 INTRODUCTION

Maize (*Zea mays* L. [Poaceae]) is a staple crop for many individuals in Africa. It is cultivated by subsistence farmers mainly for human consumption while the surplus is used as animal fodder (Minja, 1990; Kfir *et al*., 2002). Despite its importance, many countries in sub-Saharan Africa have remained net importers of maize for almost more than three decades. This has been mainly attributed to a rapidly expanding population and stagnating yields over the years. In spite of this, it is forecasted that by the year 2027, the global demand for maize will have grown by 16% o.OECD/FAO (2018).In order to deal with the surging demand, newer and better methods of crop production need to be developed while reinforcing the existing ones to better manage the complex of problems facing maize farmers as well dealing with the perennial demands of maize in the tropical Africa (FAO, 2002).

Maize crop production in Africa is faced by a number of challenges and constraints such as pests and diseases, deterioration of soil nutrients, climate changes and poor infrastructure that have led to the reduction in crop yields (Jones and Philip 2003; Sanchez, 2002; Godfrey *et al.*, 2010). Among the various insect pests of maize and sorghum, lepidopteran stemborers of the Crambidae *Chilo partellus* (Swinhoe), the Pyralidae *Eldana saccharina* (Walker) and the Noctuidae *Busseola fusca* (Fuller) and *Sesamia calamistis* (Hampson) are economic important pests of maize and sorghum in the sub-Saharan Africa region (Kfir *et al*., 2002, Polaszek 1998; Overholt *et al*., 2001; Le Ru *et al*., 2006). Most of these pest species are indigenous to Africa, except *C.*

partellus, which was accidentally introduced from Asia to the continent in the 1930s (Kfir *et al*., 2002).

Several control methods have been researched, tested and implemented to alleviate the problem caused by insect pests on maize crop. These methods include among others: control by use chemicals, cultural practices, host plant resistance as well as the use of biological control agents (Kfir *et al*., 2002). However, biological control methods have been demonstrated to be more reliable, cost effective and environmentally friendly as compared to the other stem borer control options (Lundborg, 1999). One of the many strategies of biological control of pests relie on the use of natural enemies which include microbial pathogens, parasitoids, nematodes and predators. In parasitoids family, the egg, larval and pupal parasitoids are the most abundant and widespread in the East Africa region (Bonholf *et al.,* 1997). The larval parasitoid, *Cotesia* species is one of the most diverse genera of the subfamily Microgastrinae (Hymenoptera, Braconidae), with almost 300 species having already been described and probably over 1,000 species thought to exist world-wide. Many *Cotesia* species exhibit generalist behaviours though careful ecological studies have revealed a hidden complexity with an assemblage of populations having more limited range of host. They are frequently and widely used in a number of biological control programs (Kaiser *et al.,* 2017).

In Kenya, *Cotesia flavipes* Cameron (Hymenoptera: Braconidae) from Pakistan (Asia) was introduced in a classical biocontrol program in 1993 by *icipe* in the coastal region for the control of *C. partellus* an invasive exotic stem borer of maize and sorghum in

Eastern and Southern African lowlands (Overholt *et al*., 1994a, b; Overholt *et al*., 1997). Due to its successful history in its aboriginal home in Asia (Overholt *et al*., 1994a), *C. flavipes* was considered as the best candidate to complement the action of the predominant indigenous larval endoparasitoid *Cotesia sesamiae* (Cameron) (Hymenoptera: Braconidae) linked with the indigenous borer species such as the noctuids *S. calamistis* and *B. fusca* (Mohyuddin & Greathead, 1970; Overholt *et al*., 1994a, b; Zhou *et al*., 2001; Songa *et al*., 2002). The species successfully established itself within four years of its release in the coastal region and later spread to other regions of the country (Anderson *et al*., 2006). The percentage parasitism due to the activity of this parasitoid was established between 1995 and 2004 and indicated that, since the introduction of parasitoids, the average annual parasitism increased linearly (Songa *et al*., 2002; Zhou *et al*., 2001; Anderson *et al*., 2006; Omwega *et al*., 2006). At the coast, the parasitoid reduced the *C. partellus* densities by 57%, while maize yields increased by 10-15% (Zhou *et al*., 2001). Following its successful parasitism and stability in Kenya and western Tanzania (Omwega *et al*., 1995; 1997), the endoparasitoid was adopted as a biological control method in eleven other countries of Eastern and Southern Africa though become established in only ten of these countries (Omwega *et al*., 2006). However, as demonstrated by Jiang *et al*. (2006) parasitism by this endoparasitoid is still on the increase indicating that the pest-parasitoid system is not yet at equilibrium.

The ability of natural enemies to locate, accept, parasitize and successfully develop in their hosts is crucial for the success of parasitoids used in biological control programs (Godfray, 1994). In order for successful parasitism to occur, a sequence of events

including host habitat location, host location, host acceptance and host suitability must occur in proper succession (Vinson, 1976). During foraging, parasitoids rely on volatile chemical cues to guide them to the specific host habitat and eventually to their host stemborer pest. In long-range host location studies, both *C. flavipes* and *C. sesamiae* have been shown to become attracted to the stem borer-infested plants regardless of the plant or the borer species. However, the parasitoids lack the ability to detect from a long distance whether the plant infested by the stemborer is suitable for their development or not (Obonyo *et al*., 2008). Most recently, it has been shown that during the host feeding process, contact between the parasitoid and its host is very crucial, and oral secretions from the host play a key role during the first encounter for such evaluation by the parasitoid (Obonyo, 2009). However, the chemical identity and the origin of these compounds (whether from plant tissues or stemborer larvae feeding) are yet to be established.

In Kenya, two populations of *Cotesia sesamiae* are present at different geographic areas, namely *C. sesamiae* coast *(*Cs-Coast) and *C. sesamiae* inland *(*Cs-Inland), and are exhibiting contrasted acceptance toward *B. fusca*. The use of these two parasitoid populations within the same species might help to determine the candidate genes involved in host acceptance by this parasitoid species using genetic analysis of crosses between these two populations. This will also aid in explaining the molecular basis of specific host recognition and acceptance in *C. sesamiae*.

Therefore, for a better and efficient biological control management systems, the present study sought to unravel the origin, identity and the chemical variability of the

compounds involved in host recognition and acceptance, of suitable stem borer hosts by the *Cotesia* species/population present in Kenya. This study also aimed to identify the candidate genes involved in encoding proteins/chemicals that are important for host acceptance by *Cotesia* species. This information is necessary in finding the evolutionary relationship to host acceptance for oviposition in *Cotesia* spp and it will open a new route of investigation in host parasitoids interactions as well as explaining the varied observations in biological control programs when the endoparasitoid is used.

1.1 Problem statement

One of the biggest challenges facing people in sub-Sahara Africa is the production of sufficient food to feed the rapidly growing population. FAO has predicted that by the year 2050 there will be a 70% increase in the demand for food (FAO, 2017). Damage of maize by lepidopteran stemborer pests is one of the greatest obstacles to increased or sustained maize production in the region. Several methods have been researched,tested and implemented for the control of stemborers. These methods include among others: control by use of chemicals, cultural practices, host plant resistance as well as the use of biological control agents. However, most of these methods have been faced with various challenges. Biological control methods have been demonstrated to be more reliable, cost effective and environmental friendly as compared to the other stem borer control options. One of the many biological pest control strategies is based on using the natural enemies of insects which include among others nematodes, parasitoids, microbial pathogens and predators. In eastern Africa, the braconid larval endoparasitoid, *C. flavipes* was introduced in a classical

biocontrol program for the control of the larvae *C. partellus* to complement the action of the predominant indigenous larval endoparasitoid, *C. sesamiae.*

In Kenya, two *C. sesamiae* populations (Cs-Coast and Cs-Inland) exist. These two *Cotesia* populations are host–specific differing in their degree of host acceptance and development in the stem borer *B. fusca*. In contrast to the coastal population (Cs-Coast), the inland population of *C. sesamiae* (Cs-Inland) accepts *B. fusca* host for oviposition and is able to complete its development in the host. However, both species have been shown to readily accept and develop in *S. calamistis*, the main host of *C. sesamiae* in the coastal Kenya (Ngi-Song *et al*., 1998). The variability in acceptance behaviour between Cs-Inland and Cs-Coast populations of *C. sesamiae* on *B. fusca* larvae have been well described by Gitau (2006) and has been reported to be triggered by the recognition of a chemical compound present on the surface *B. fusca* larvae (Obonyo, 2009). Moreover, it has also been reported that *C. sesamiae* was unable to parasitize population of *Sesamia nonagrioides* (Lefebvre) (Lepidoptera: Noctuidae) from the eastern as compared to the western Kenyan population (Obonyo, 2005) similarly, a new species of *Cotesia*, *Cotesia typhae* Fernandez-Triana sp. n., has been recently identified to be specifically associated with *S. nonagrioides* (Kaiser *et al.*, 2017). All these varied results in such biological control system implies that there is existence of possible variations of the chemicals involved in host recognition and acceptance by these endoparasitoids. Besides, such variations in host acceptance by these endoparasitoids can also arise from the genetic variations that influence the encoding of different chemicals.

Therefore, for better and efficient biological control management systems, the present study sought to unravel the origin, identity and the chemical variability of the compounds involved in host recognition and acceptance mechanisms by the *Cotesia* species/population present in Kenya. This study also aimed to identify the candidate genes involved in encoding the proteins/chemicals that are important for host acceptance by *Cotesia* parasitoid species. This information is necessary in determining the evolutionary relationship to host acceptance for oviposition in *Cotesia* parasitoid species and it will open a new route of investigation in host parasitoids interactions as well as explaining the varied results observed in biological control programs when the endoparasitoid are used.

1.2 Justification and significance of the research

For biological pest control strategy to be reliable and effective, proper insight into the foraging behavior of the pests‘s natural enemies is required. Chemicals play a major role in almost every behavioural step during host searching and acceptance by parasitoids (Vinson, 1985; Godfray, 1994; Bénédet *et al.,* 1999). The information conveyed by chemicals often elicit a series of behavioral responses to parasitoids in their host recognition and selection process. The volatile organic compounds (VOCs) and the non-volatiles are two major types of chemicals involved in the process of parasitoid host recognition. Although the volatile chemicals play a significant role to parasitoid in the location of their host feeding on plants, host identification process for acceptance occurs mainly during contact between the parasitoids and its host where host products related to feeding activities i.e. fecal pellets and oral secretions plays a crucial role. The non-volatile chemicals involved in host recognition and acceptance

by endoparasitoids are frequently non-protein chemicals e.g. phenols (Thompston *et al*., 1983), sugars (Hassel, 1968), sesquiterpens (Elzen *et al*., 1984) and those, which have been demonstrated as proteins, have been described mainly from silk cocoons (Gauthier *et al*., 2004). Apart from a 30 kDa proteins identified in the frass, the haemolymph and the entire larvae of a noctuid species *Heliothis virescens* (Lepidoptera: Noctuidae)(Nettle & Burks, 1975), there are very few examples in the literature that demonstrate the involvement of proteins from the host in contact host recognition and acceptance by the endoparasitoids. Moreover, none of them have been clearly identified. Thus if the active compounds involved in host recognition and acceptance mechanisms in *Cotesia* parasitoid species are identified and their structure elucidated, an understanding of the mechanisms involved in host recognition and acceptance in *Cotesia* species can be conceptualised. This will be an important step in the investigation of the host parasitoid interactions and hence form a basis for the provision of an evolutionary solution to host acceptance for oviposition in *Cotesia* parasitoid species. The information obtained will provide an important baseline information useful in improving biological pest control prorammes in maize fields in Africa.

1.3 General Objectives

This study's main objective was to determine the chemical and molecular basis of host recognition and acceptance by *Cotesia* spp. found in the coastal and western parts of Kenya. The study was therefore conducted along three main lines of objective and is reported as separate chapters of the thesis as a whole.

1.3.1Specific objectives

1. To determine the source and identity of the chemical(s)/kairomone(s) involved in host recognition and acceptance by *C. flavipes* parasitoids;

2. To evaluate the variability of the chemical(s)/kairomone(s) involved in host recognition and acceptance according to stemborer species/populations and *Cotesia* species/populations;

3. To determine the candidate genes involved in host recognition and acceptance by *C. sesamiae* parasitoids.

1.4 Research questions

This study was based on the following research questions:

1. What is the source and identity of the chemical signals present in stemborer that mediate host recognition and acceptance by the associated parasitoids?
2. Do these chemicals vary according to the stemborer species/populations and the *Cotesia* species/population association?
3. What are the candidate genes involved in host acceptance by *Cotesia* parasitoid species?

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Introduction

Maize, *Zea mays* L. (Poaceae) is Africa's largest cereal crop, with cultivation particularly important in both East and Southern Africa (Kfir *et al.*, 2002; Seyoum *et al.*, 2017). Historically, maize was first domesticated in southern Mexico and grown in the form of wild grass called teosinte. Its potential as a major food crop was recognized and through systematic selection of certain varieties for desired traits it was possible to improve the crop over time. This process resulted in a gradual transformation of teosinte into the present maize form. Maize is a versatile crop that grows under all kinds of conditions of edaphic, altitude and fertility, explaining its global adaptability and its many varieties (Ouma *et al*., 2002). It is used as a staple food source especially in Latin America and Africa. However, due to the associated low prices and worldwide distribution, it has become the most important raw material for several industrial processes as well as being used as animal feeds (OECD/FAO 2016). Maize use analysis reports have indicated that the crop is mainly used as animal fodder, human consumption and a number of industrial uses (James, 2003). According to the OECD/FAO (2016), the trend for global cereal demand is expected to increase with maize surpassing the demand of wheat and rice.

In Kenya, maize is the main food and cash crop for millions of people in the predominantly mixed crop-livestock farming system, which accounts for about 40% of the total agricultural land (Kfir *et al.*, 2002). It is grown as an income-generating

crop in nearly all agro-ecological areas, including marginalized areas on large and small-scale farms. The Kenyan, rift valley region accounts for more than 51% of the total maize production. This is followed by Nyanza and Western Kenya respectively, while Nairobi and Notheastern areas accounts for less than 0.1% (DT Maize, 2015). Despite its enormous importance for the country's food security and economic well-being, there has been no significant improvement in maize productivity over the years. Regionally, Kenya's per capita maize consumption is estimated at 103 kg/person/year, compared to 73 kg/person/year for Tanzania, 52 kg/person/year for Ethiopia, and 31/person/year kg for Uganda (DT Maize, 2015). In addition, maize production in Kenya has not kept pace with population growth leading to severe food insecurity (Hassan *et al*., 1998; Pingali & Pandey, 2001; FAO, 2017). Kenya's average national maize yield is about 1.5 tons per ha, while there is potential to increase yield to more than 6 tons per ha through increased use of improved seeds, fertilizers and good crop husbandry. Low maize production is also associated with various constraints including unreliable rainfall, land degradation, infestation of pests, poor infrastructure, marketing and policy bottlenecks (ICIPE, 2000).

Most recently, repeated outbreak of Maize Lethal Necrosis (MLN) and the fast spreading fall armyworm (*Spodoptera frugiperda*) are among the major constraints to maize production in Kenya (Sage-el & Gitonga, 2018), As a result, the 2016/17 "short-rains" maize production was 70% below the average of the previous five years, with a near-total failure having been reported in the coastal areas (FAO, 2017).

Facing the prediction of ongoing human population growth, there is need to increase food production. The effect of stem boring larvae pests is one of the greatest obstacles for increasing the production of maize in East Africa, especially Kenya (Overholt *et al*., 1996; Kfir *et al.*, 2002; FAO 2017). Among the Lepidoptera stem borers, *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) and *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) are considered the most harmful pest of maize (Kfir *et al.*, 2002). These pests seriously limit potentially attainable yield by infesting the crops throughout its growth and development stages (Overholt *et al*., 1996). On everage stem borers destroy 20-40% of Africa's maize harvest and as much as 80% in heavy infestations (Gatsby Charitable Foundation, 2005). Reducing losses caused by stem borers through improved management strategies would hence significantly increase maize production and result in improved nutrition as well as the prize of maize (Gurr *et al.*, 2004).

2.2 Lepidoptera cereal stem borers

Lepidoptera stem borers are made up of a number of moth species distributed worldwide. These lepidoptera stem borers are generally considered to be the most important group of insect pests in many parts of the world that attack maize, sorghum and sugarcane. Several major lepidopteran species have been identified in eastern and southern Africa (Overholt *et al*., 2001; Le Ru *et al*., 2006). These pests lay their eggs at night on the leaves of young host plants. Thereafter, the young larvae initially feed on young leaves while older larvae bore into the plant stems and/or the cob **(Figure 2.1)**. Stem borers also attack other cereal crops such as sorghum, millet and sugarcane (Kfir *et al.*, 2002).

A **B** **C**

Figure 2.1 Damages caused by stemborers on maize plants (A) on young plants (the center of the plant is drying and is refered to as dead-heart); (B) inside the stem and (C) inside the cob (source: *icipe* stem borer project).

2.3 Classification of lepidopteran cereal stem borers

According to Le Ru *et al.* (2006) and Ong'amo *et al*. (2006), cereal stem borers are classified into five families: Noctuidae, Crambidae, Pyralidae, Tortricidae and Cossidae. In Kenya for example, a total of 61 stem borer species belonging to families Noctuidae (25), Crambidae (14), Pyralidae (9), Tortricidae (11) and Cossidae (2) are reported to have been recovered from 42 wild plant species. However, two noctuid species, *B. fusca*, *Sesamia calamistis* (Hampson) and two crambids, *C*. *partellus* and *Chilo orichalcociliellus* Strand are the four main borer species found associated with maize plants in Kenya (Ong'amo *et al*., 2006). Except for *C. partellu*s, all the maize stem borer pests are believed to be indigenous. Probably before 1930 when it was first found in Malawi, *Chilo partellus* invaded Africa from Asia (Overholt *et al*., 1996). Since its coming to Africa, *C*. *partellus* has spread to all countries in Eastern and

southern Africa and has often become the most damaging stem borer of maize and sorghum especially in warmer lowland areas (Ong'amo *et al*., 2016).

2.4 The main lepidopteran cereal stem borers of the Sub-Saharan Africa

Busseola fusca is distributed widely throughout sub-Saharan Africa with the population in East and Southern Africa differing in environmental adaptability to those exhibited by the West African populations. In the continent's eastern and southern part, *B. fusca* occurs mostly in mid- and high- altitude areas (>600m) where it is often the most serious pest of maize. However, low altitude areas were also reported to harbour some (Overholt *et al.,* 2001; Le Ru *et al*., 2006; Ong'amo *et al.,* 2006). *Busseola fusca* is oligophagous species mostly feeding on maize as well as cultivated and wild sorghum (Calatayud *et al*., 2014). On the other hand, the African pink stalk borer, *S. calamistis*, is mainly found in the sub-Saharan Africa and some of the Indian Ocean islands commonly occurring in wetter localities at all altitudes (Tams & Bowden, 1953). This species is more polyphagous than *B. fusca* and infests maize, sorghum, pearl millet, wheat, rice and sugarcane.

Sesamia nonagrioides is a polyphagous insect living on maize, sorghum, millet, rice, sugar cane, wild grasses and *Typha* plant species. The pest is widely distributed, having been reported in several European and African countries including France, Greece, Italy, Portugal, Sardinia, Spain, Burundi, Canary Islands, Cape Verde Islands, Ghana, Ivory Coast, Kenya, Madeira, Mali, Morocco, Nigeria, Rwanda, Sudan, Tanzania, Togo, Uganda and Congo (Velasco *et al*., 2007; Moyal *et al*., 2011). In Kenya, this species is mainly found on wild habitats including the *Cyperus* and *Typha*

plant species (Glazer, 2013) and is hence not economically considered as a pest of maize.

Another stemborer species, *C. partellus* or the spotted stem borer is native to Asia where it is a pest of maize and sorghum. It was first reported in Malawi in the 1930s and spread in the 1950s to most East Africa countries. Since then, it is widespread throughout eastern and southern Africa (Kfir *et al*., 2002) and several West African countries (Overholt *et al*., 2000). *Chilo partellus* is considered to be the most important stem borer species in most low to medium elevated areas of eastern and southern Africa. All these stem borer species develop in about 30 to 40 days; laying eggs after mating and developing through 5 instar stages **(See Figure 2.2).**

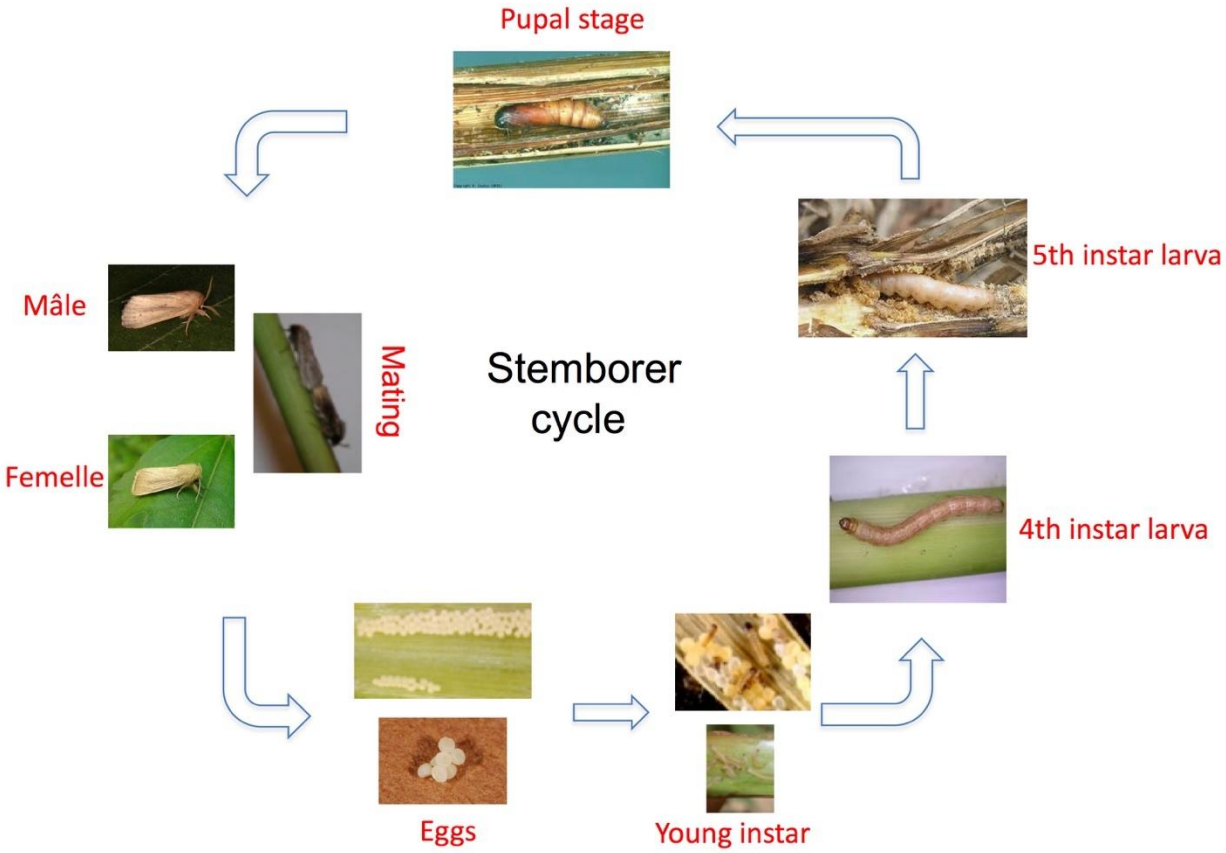

Figure 2.2 General scheme of stem borers' life cycle showing different stages of development.

2.5 Control of maize stem borers

There are various approaches and methods that have been researched, tested and implemented to alleviate the problem of Lepidoptera stem borer and their associated losses. These include among others: control by use of chemicals, cultural practices, host plant resistance as well as use of biological control agents (Kfir *et al.*, 2002).

2.5.1 Chemical control methods

The primary method of stem borer control in Africa is the use of chemical pesticides. This has led to a dramatic increase in crop yields since World War II (Hegge, 2007). Pesticides are used primarily on cash crops, such as cotton, cut flowers and in the peri-urban horticulture (Schwab *et al*., 1995; Hegge, 2007). However, long-term insect pest control has not been achieved; rather, the consequences have been several problems including deleterious impacts on human health and environment, especially in developing countries (Hegge, 2007; Murray 2019). Most recent surveys for example, those conducted in Ethiopia shows that majoriy of the farmers do not use chemicals insecticides to control stemborers due to lack of capital, unavailability and lack of knowledge (Muluken *et al*., 2016). Although sometimes pesticides are freely provided by the donors or readily available but most subsistence farmers in Africa do not apply. There is also an inadequate public awareness on the dangers of pesticides in nutritional imbalance and reduction in the quality of agricultural product in developing countries and compared to developed countries (Bo Hou & Linhai Wu, 2010).

2.5.2 Cultural control methods

Cultural control practices have been found to be the most promising, relevant and economical cereal stemborers control methods used by the majority of African farmers. Farmers practice crop rotation, intercropping, manipulation of planting dates, disposal of crop residues (field sanitation) and post-harvesting as common control strategies in cultural control method (Muluken *et al.*, 2016; Kfir *et al*., 2002; Polaszek, 1998). However most cultural control methods are labour intensive and

some farmers are not able to implement the practice as required (Van den Berg *et al.*, 1998; Obonyo, 2009). Some of the cultural control practices that are widely used to control stemborer pests include:

2.5.2.1 Field sanitation

Field sanitation includes practices such as destroying crop residues (Kfir et al., 2002). This consists of eliminatating the maize residues after crop harvesting. This practice kills the diapausing stemborer larvae remaining inside the residues and thus prevents the carry-over of populations to the next cropping season. Practices in field sanitation help to reduce borer populations by burying the pest deep in the soil or breaking the stems and exposing the caterpillars to natural enemies and to adverse weather. Burning crop residues is also an effective way of killing stem borer larvae. However, this can cause problems in farms where the organic soil content is low and soil erosion is severe, since crop residues are in many cases the only organic matter added to the soil as a source of fertilizer, particularly in smallholder farms (Kfir et al., 2002). Moreover, destruction of crop residues should be avoided, especially in areas where such residues are used as fencing and building materials, as fuel, or animal bedding. Partial residue burning is recommended in such cases while the leaves are dried, but the stalks are still green. The heat generated from the burning leaves kills up to 95% of stem borer caterpillars inside the stems, while at the same time healing the stalks, improving their quality as building materials and making them more resistant to termite attack. Kfir et al. (2002) recommended the use of crop residues as fodder and silage. Nevertheless, farmers in a particular region need

to cooperate effectively with these cultural measures, as moths from untreated fields can infest crops around them.

2.5.2.2 Improving soil fertility

In order to manage African stemborers, it is important to preserve soil fertility as this will increase the efficiency of nitrogen use in maize production. As per the studies carried out in Cameroon, applying nitrogen to soil improves the nutritional status of maize thereby increasing its tolerance to the African maize stem borer attack (Chabi-Olaye *et al*., 2008). It has been shown, however, that if nitrogen is applied at rates above the required, the biomass of the plants increases at the cost of the yield. Innovations to restore soil for agricultural productivity include but are not limited to cereal-legume rotations, farm manure use, and green manure cover crops. The cultivation and rotation of legumes is remarkably effective in enhancing the nitrogen availability in the soil (Chabi-Olaye *et al*., 2005; Obonyo, 2009).

2.5.2.3 Rotation of crops

Rotation sequences of maize-legume improve the supply of nitrogen in the soil and the nutritional status of maize as compared to monocropping (Chabi-Olaye et al., 2005; Obonyo, 2009). The use of short-lived fallows with leguminous cover crops and grain legumes is useful in reducing yield losses in subsequent crops due to borers. For example, according to Chabi-Olaye et al. (2005), a study conducted in rotation of maize fields with leguminous crops in Cameroon showed an improved nitrogen supply in the soil and an increased maize yield. In the same way, improved plant nutritional status leads to increased attacks by the African stem borer at the beginning

periods of the plant development, together with improved plant life, resulting at long last in a net advantage for the plant and grain yield (Chabi-Olaye et al., 2005).

2.5.2.4 Intercropping and habitat management

In sub-Sahara Africa, the significance of plant biodiversity in maize agroecosystems has been percieved to diminish borer maize infestation (Chabi-Olaye *et al.*, 2006). According to Chabi-Olaye *et al*. (2005) and Chabi-Olaye *et al*. (2006), maize intercropped with non-host crops (e.g. cassava and grain legumes) diminished stem borer harm altogether and yielded higher than monocropped maize fields. Some studies conducted in Kenya suggested that intercropping maize or potentially sorghum with cowpeas reduces African stem borer damage (Amoako-Atta *et al*., 1983; Seshu Reddy & Masyanga, 1988). Likewise, field trials in Eritrea have demonstrated that sorghum intercropped with haricot beans, cowpea, desmodium and *Dolichos lablab* have much lower dead-heart harm as compared to pure stand sorghum (Songa *et al.*, 2007).

Vandermeer (1989) recorded three potential mechanisms responsible for the decreased pest infestation in mixed cropping systems: (a) the disruptive yield theory, where a second non-have plant species disrupts the ability of the pest to locate its proper host plant species; this can be because of both diminished chemical and signals cues ; (b) the trap crop hypothesis in which a second non- host plant species pulls in the pest away from its primary host; (c) the natural enemy theory in which the intercropping situation draws in a greater number of predators and parasitoids than the monocrop along these lines reducing the pest on the primary host plant.

According to Infonet Biovision (2011), when molasses grass (*Melinis minutiflora*), a non-host for stem borers is intercropped with maize, there is reduction in the infestation of stem borers on maize significantly. It has also been observed that, in such fields, there is a considerable increment in parasitism on stem borers by *Cotesia sesamiae* wasps. This is due to the fact that molasses grass produces volatile compounds, which attracts the parasitic wasp but repels the stem borers. The molasses grass is also an effective cover crop, providing good animal feed. Greenleaf desmodium (*Desmodium intortum*) repels egg-laying stem borer moths, when intercropped with maize, at the same time suppressing and eliminating striga (infonet Biovision, 2011).

2.5.3 Biological control methods

Biological control, the oldest form of managing insect pests is defined as the pest management strategy in which the manipulation of natural enemies leads to the reduction of a pest population. Different from natural control, biological control, or bio-control, heavily relies on natural enemies of pests for their control (Pedigo, 1996; Hegge, 2007). In biological control, a natural enemy feeds or prey on the host to extend its own population at the expense of the pest population. Natural enemies are living organisms, which kill or weaken insects thereby reducing their numbers (Orr & Suh, 1998; Hegge, 2007). Insects' natural enemies are diverse and include insects themselves, other invertebrates, vertebrates, nematodes and microorganisms. These natural enemies have been effectively divided into parasites, parasitoids, predators or pathogens (Pedigo, 1996).

Parasitoids are a group of insects that parasitizes other insects or arthropods at any host stage. A parasitoid is only parasitic in its immature stage. The free-living adult parasitoids lay their eggs inside the host or attach them outside (Godfray, 1994). Parasitoids are the most effective agents in biological control because of their good survival abilities: they only need one (or less) host for development, survive on low host populations and their narrow host range leads to a good numerical response to host density. Nevertheless some problems are reported to be associated with parasitoids in biological control for several reasons which includes (i) weather and other forces reduce the parasitoid host searching capacity, (ii) only the females parasitoids are the best searchers and often lay few eggs, (iii) synchronization between parasitoids and hosts' life cycles is often difficult. Parasitoids occur in eighty-six families belonging to the Coleoptera, Diptera, Hymenoptera, Lepidoptera, Neuroptera and Strepsiptera. The most important of these parasitoids include the small parasitic wasps in the families of Ichoneumonidae, Braconidae and Chalcidoidae (Pedigo, 1996).

In Kenya, the koinobiont (parasitoids that allow host to continue to grow in size after parasitism) larval endoparasitoid (feeding from inside) *Cotesia flavipes* Cameron (Hymenoptera: Braconidae) was released in 1993 by *icipe* in the coastal region for the control of *C. partellus* an invasive exotic stem borer; of maize and sorghum in Eastern and Southern African lowlands (Overholt *et al*., 1994a, b; Overholt *et al*., 1997). Due to its successful history in its aboriginal home in Asia (Overholt *et al*., 1994a), *C. flavipes* was choosen as the best candidate to complement the activity of *C. sesamiae*

which was linked initially with indigenous borer species such as the noctuids *S. calamistis* and *B. fusca* (Mohyuddin & Greathead, 1971; Overholt *et al*., 1994a, 1994b; Zhou *et al*., 2001; Songa *et al*., 2002). After four years of its release, the parasitoid successfully established at the coastal region and later spread to other areas of the country. The percentange parasitism of this parasitoid was established between 1995 and 2004, and it was found out that the average annual parasitism increased linearly since the time of its introduction (Zhou *et al*., 2001; Songa *et al*., 2002; Omwega *et al*., 2006). At the Kenya coast, *C. flavipes* reduced the *C. partellus* population densities by 57% while at the same time maize yields increased by 10-15% (Zhou *et al*., 2001). Following its success in Kenya and western Tanzania (Omwega *et al*., 1995; 1997), eleven other countries in Eastern and Southern Africa adopted this control method and the parasitoids were released and become established in ten of these countries (Omwega *et al*., 2006). However, as shown by Jiang *et al*. (2006) parasitism on this parasitoid is still on the increase indicating that the pest-parasitoid system is not yet at equilibrium.

2.6 The *Cotesia* parasitoids species

The *Cotesia* species are parasitoids wasps that are natural enemies of lepidopterous stem borer larvae which some of them are pests of cereal crops such as maize and sugarcane (Annette, 1994). The *Cotesia* complex is hence economically important biological control agents. In Kenya, Lepidoptera stem borer are ranked as the most important group of pests attacking maize (Kfir *et al*., 2002). Among insect parasitoids, *Cotesia* is one of the most diverse genera of the subfamily Microgastrinae (Hymenoptera, Braconidae), with almost 300 species already described and probably

over 1,000 species existing world-wide. Many *Cotesia* species may appear generalists but careful ecological studies may reveal a hidden complexity with an assemblage of populations having a more restricted host range (Kaiser *et al.*, 2017). There are three (and probably four) species of *Cotesia* that exist in Kenya (Mailafiya *et al*., 2009): *Cotesia flavipes* Cameron, *Cotesia sesamiae* (Cameron) (Hymenoptera: Braconidae) and a newly identified species *Cotesia typhae* Fernandez-Triana sp. n. (Kaiser *et al*., 2017).

2.6.1 *Cotesia flavipes* as a biological control agent of maize stemborer in the coastal region of Kenya

The braconid larval parasitoid *C. flavipes* (**Figure 2.3 A**) is used as a biological control agent against the crambid *C. partellus* a serious pest of cereal crop in east and southern Africa (Overholt *et al.*, 1994). This braconid species from Pakistan (Asia) was introduced into Africa as classical biological control agent to control the exotic maize stem borer, *C. partellus*. This parasitoid was released in the coastal region of Kenya in 1993 (Overholt *et al*., 1994), where it reduced *C. partellus* population densities by over 50% (Zhou *et al*., 2001; Jiang *et al*., 2006).

Obonyo *et al*. (2008) assessed the interactions of *C. flavipes* with non target lepidopteran stem borer species in Africa. The study showed that *C. flavipes* has a high specificity and a higher searching efficiency for its aboriginal host, *C. partellus* attacking more larvae than *C. sesamiae* and with minimal non-target harm. This parasitoid has also been shown to attack and develop successfully in several other stem borer species such as *C. orichalcociliellus* and *S. calamistis*. In contrast, the

parasitoid does not attack the noctuid *B. fusca* in which *C. flavipes* eggs becomes encapsulated (Ngi-Song *et al*., 1995; Obonyo *et al.,* 2010a).

During the presentation of a parasitoid into another environmental condition, data concerning the life span, time of maximum offspring creation effect of temperature on survival, developmental time and its versatility/adaptability to different states of the host or elective hosts and the host condition are of extreme importance (Jackson *et al*., 1976). Studies on the impact of temperature on development, life span and population growth of *C. flavipes* and *C. sesamiae* show that the advancement of both *Cotesia* species from oviposition, cocoon formation and adult emergence is inversely related to temperature (Mbapila & Overholt, 2001)

Gifford &Mann (1967) and later by Mohyuddin (1970) studied and recorded the biology of *C. flavipes*. The female adult, which lives only for few days, is a small wasp of about 3-4 mm in length and lays between 15-65 eggs into the host larva. The eggs hatch after 3 days within the stem borer larva and develop through three instars while feeding on the larval hemolymph. The period between the egg-larval is about 10-15 days at 25^{o}C, 50- 80% relative humidity (RH), and 12:12 (L:D) hr photoperiod. When the lavae is mature, the last instars of this parasitoid chew through the stem borer larval integument and immediately spin a cocoon and pupate killing the host. The adult parasitoids emerge 6 days later under the same conditions aforementioned usually in the morning hours of the day and soon afterwards mating begins (Smith *et al*., 1993; Obonyo *et al*., 2009).

2.6.2 *Cotesia sesamiae* as a biological control agent of maize stemborer in Africa

Cotesia sesamiae (**Figure 2.3B**), is one of the most important native parasitoids of stem borers in many countries of sub-Saharan Africa. This is an indigenous gregarious larval endoparasitoid that attacks mid to late instars of the stem borer larvae (Bonhof *et al*., 1997, Kaiser *et al*., 2015). Although the parasitoid is distributed widely in Africa, not all local population of the species appear to be equally effective in controlling stem borer pests (Mohyuddin, 1990; Polaszek & Walker, 1991). *Cotesia sesamiae* attacks several stem borer species including *S. calamistis*, *B. fusca*, *C. partellus* and *C. orichalcociliellus* (Mohyuddin, 1971; Polaszek & Walker, 1991). Within the continent, there has been an interest in redistributing the populations of *C. sesamiae* for a biological control of stem borers (Schulthess *et al*., 1997, Rousse1 & Gupta, 2013). For example, the introduction of a Kenyan population of *C. sesamiae* into West Africa is thought to have improved the biological control of stem borer in the region, (Schulthess *et al.,* 1997; Mochiah *et al*., 2002a). More recently, *C. sesamiae* has successfully been introduced in Cameroon (ANR project BIOINV4I) and its full impact assessed (Ndema *et al*., 2012; Kaiser *et al*., 2017). *Cotesia sesamiae* is the main larval parasitoid attacking *B. fusca* (Overholt *et al.,* 1994, Getu *et al.,* 2003) in Kenya in regions of elevations higher than 600 m above sea level (Nye, 1960; Harris & Nwanze, 1992).

Even though *C. sesamiae* is the most abundant larval endoparasite in Africa (Mohyuddin & Greathead, 1970; Polaszek & Walker, 1991), it has not managed to efficiently supresss the *C. partellus* population in Kenya (Overholt *et al*., 1994). There

are two biotypes of *C. sesamiae* in Kenya that differ in their ability to parasitize *B. fusca* (Ngi-Song *et al*., 1995, 1998); *C. sesamiae inland* (Cs. inland) from western Kenya and *C. sesamiae* coast (Cs-coast) from Mombasa (coastal Kenya). Whereas Cs. Inland population is virulent and completely develops in *B. fusca* (Mochiah *et al*., 2002b), Cs-coast population is regarded as avirulent and not able to completely develop in *B. fusca* and its oviposited eggs get encapsulated in *B. fusca* larvae (Ngi-Song *et al*., 1995; Mochiah *et al*., 2002b). This encapsulation mechanism, where oviposited eggs are melanised (undergo a chemical change) in *B. fusca* (Ngi-Song *et al*., 1995; Ngi-Song *et al*., 1998; Mochiah *et al*., 2002b), reduces the efficiency of the parasite especially in regions where the predominant pest species is the unsuitable host (Ngi-Song *et al*., 1995; Obonyo *et al*., 2008). Conversely, both biotypes develop effectively in *S. calamistis* larvae (Ngi-Song *et al*., 1995; Mochiah *et al*., 2002b).

2.6.2.1 Mechanism of parasitism by *Cotesia sesamiae*

The variation in parasitism in *B. fusca* by *C. sesamiae* is linked to two mechanisms: the first mechanism involves the expression of CrV1 polydnavirus (PDV) genes in the wasp whose presence is asymptomatic but causes major physiological disturbances in the host larvae resulting in the expression of several viral genes. This leads to developmental arrest prior to metamorphosis and suppression of the immune system (Beckage & Gelman, 2004; Gitau *et al.*, 2007; Kaiser *et al*., 2017). The other mechanism involves the co-injection of the wasps' venom from the accessory glands as well as other factors from their eggs (Asgari *et al*., 2003) which causes severe immunosuppresion as revealed by Caryx fluid experiment of Gitau *et al*., (2006).

A **B**

Figure 2.3 Females of *Cotesia flavipes* (A) and *Cotesia sesamiae* (B) (Source:http://www.uky.edu/~mjshar0/genera/Cotesia/cotesia.html).

2.6.3 *Cotesia typhae*: a potential biological control agent of maize stem borers.

A new population directly associated with *C. sesamiae* has recently been observed parasitizing African *S. nonagrioides*. This parasitoid exhibits a strict ecological specialization on *S. nonagroides* host in Kenya (Kaiser *et al*., 2017). *Cotesia typhae* was first considered as host race of *Cotesia sesamiae* Cameron (Hymenoptera, Braconidae) (Branca *et al.,* 2011; Kaiser *et al.,* 2017). It has been suggested that this species could be a potential candidate for the biological control of the Mediterranean maize stem borer, *Sesamia nonagrioides* (Lefebvre 1827) (Lepidoptera: Noctuidae)

Figure 2.4 *Cotesia typhae* female living in *Typha* plant species from Makindu-Eastern Kenya (Source:https://doi.org/10.3897/zookeys.682.13016 accessed on 20/2/2018).

2.7 Factors influencing the efficacy of exotic parasitoids

The suitability of indigenous stem borer species and their host plants are the major factors affecting the efficacy of exotic parasitoids (Hailemichael *et al*., 2008). Studies conducted by Hailemichael *et al*. (2008) on *C. sesamiae* (Cameron) (Hymenoptera: Braconidae) and by Jiang *et al*. (2004) on *C. flavipes* showed that depending on the *Cotesia* species, parasitized stem borer larvae feed and continue growing at the same rate as unparasitized ones. In addition, their growth rate is greatly influenced by ambient temperature and host age (Jiang *et al*., 2004) and by allelochemicals present in the host diet (Cortesero *et al*., 2000; Sznajder & Harvey, 2003; Ode, 2006). These intimate parasitoid-host relationships expose young parasitoid life stages to the host's immune system (Godfray, 1994; Pennachio & Strand, 2006). Nevertheless, due to their inability to metabolize plant secondary compounds present in their hosts (Quicke, 1997); the parasitoids are more susceptible to these compounds as compared to their phytophagous hosts. For example, survival of *C. flavipes* was shown to be lower with prolonged developmental time when the host, *C. partellus,* fed on wild as compared to cultivated crops (Sétamou *et al*., 2005).

Moreover, the ability of the parasitoids to locate, accept, parasitize and successfully develop in their hosts is crucial for the success of parasitoids used in biological control programs (Godfray, 1994). In order for successful parasitism to occur, the following sequence of event must occur: host habitat location, host location, host acceptance and host suitability (Vinson, 1976; 1985; Godfray, 1994). During foraging, parasitoids rely on volatile chemical cues (infochemicals) that guide them to the specific host habitat and eventually to the host stem borer (Vinson, 1976; Godfray, 1994). The ability to perceive infochemicals is an important factor in host location, selection, evaluation, actual handling and eventual parasitism (Dicke & Vet, 1999). For example, in olfactometric studies *C. flavipes* females are reported to prefer odours from stem borer-infested plants over those from their uninfested counterparts (Potting *et al*., 1993; Ngi-Song *et al*., 1996; Jembere *et al*., 2003; Obonyo *et al*., 2008).

For biological control method to be a reliable and effective, insight is needed into the foraging behaviour of candidate natural enemies. Host location and attack is a key determinant of the efficiency of a given parasitoid population; thus, variability in host-location or host-selection can be a major source of inconsistent results in biological control with parasitoids (Godfray, 1994). It has been demonstrated that *C. flavipes* and *C. sesamiae* are remotely attracted to stemborer infested plants regardless of the species (herbivore or host plant) used (Potting *et al.,* 1993; Ngi-Song *et al.*, 1996). Moreover, the wasps could not discriminate between host plants infested by *C. partellus*, *C. orichalcociliellus*, *B. fusca* or *S. calamistis* implying that the parasitoids cannot remotely detect the suitability of stem borer species in the plants. Therefore, it seems that the emitted volatiles do not convey much information as pertains to the

damaging herbivore species (Ngi-Song & Overholt, 1997). It hence appears that discrimination of hosts by the parasitoids occurs at "short-range" rather than "long-range", that is, once the parasitoid has made contact with the herbivore larvae.

2.8 Host recognition and acceptance by the parasitoids

Parasitoid searching behavior can be divided into three different steps: host habitat location, host location and host acceptance, which are completed with the oviposition in or on the host (Vinson, 1976; Godfray, 1994). Host location in a complex environment filled with different plants and animal species is a complex task. Predatory and parasitic insects have specialized nervous systems that allows them to use a variety of cues to find and target their hosts. Cues can be physical such as colour, sound, shape and size as well as chemical (infochemicals/semiochemicals) that may be useful for long or short-range attraction to the prey (Hatano, 2008; Obonyo *et al*., 2010a). Thus, during foraging, parasitoids use volatile chemical cues, to guide them to specific host habitat and to eventually locate the host (Vinson, 1975). General host/prey selection by the parasitoid is a stepwise process consistent with Vinson framework.

2.8.1 Behavioral steps of parasitism by the parasitioids

Host finding is a process of locating the parasitoid. It consists of: (i) localising the habitat where the host is present and (ii) localising the plant where the host (stemborer) feeds on. After finding the host, the parasitoids need to accept the host for parasitism to occur or reject the host altogether (Godfray, 1994).

2.8.1.1 Host habitat location by parasitoids

Host habitat location by parasitoids consists of finding the plant where the parasitoid's host is feeding on (Godfray, 1994). In host habitat location process, parasitoids perceive by olfaction plant volatiles or herbivore pheromones to assist them orient towards the habitat where the host is localised. However, these habitat stimuli do not convey sufficient and reliable information on the suitability of a host species but are mere indicators of the presence of a herbivore (Vet, 1990). As a result, *C. sesamiae* and *C. flavipes* may often be attracted to plants harboring unsuitable stemborer species (Potting *et al*., 1993; Ngi-Song *et al*., 1996; Obonyo *et al*., 2008).

2.8.1.2 Host location by parasitoids

Host location by the parasitoids consists of locating the plant where the host is feeding upon. In this process, parasitoids perceive and orientate towards the plants where their hosts are located from a distance by responding to stimuli originating from the the host microhabitat, food plant or its byproducts (Smith *et al*., 1993, Obonyo *et al*., 2010a). These stimuli can also directly or indirectly be associated with the presence of the host or can arise from the host itself (Godfray, 1994; Vinson, 1976; Vet & Dicke, 1992). Generally, perception of infochemicals plays a very important role in host location, selection, evaluation, actual handling and eventual parasitism (Dicke &Vet, 1999)

2.8.1.3 Host acceptance by parasitoids

This is the final step in host selection process by parasitoids according to Vinson (1976) terminology, and includes the proper act of oviposition in the parasitoid's host

(Steiddle & Van Loon, 2002). This final step has been divided into host recognition and host acceptance (Michaud & Mackauer, 1994; Muratori *et al.,* 2006). A host may be recognized visually or by antennal contact with chemical cues. Finally host acceptance depends on the assessment of host quality made during ovipositor probing, but the host may be rejected either after recognition or ovipositor probing.

It has been demonstrated that host acceptance and oviposition by two congeneric wasps, *C. flavipes* and *C. sesamiae*, in the laboratory are induced by contact with host frass and other host products as well as chemicals emenating from the larval body surface (Ngi–Song & Overholt, 1997). The role of contact cues in host recognition and acceptance by braconid larvae endoparasitoid *C. sesamiae* and *C. flavipes* has been studied (Obonyo *et al*., 2010a) and involve use of different chemical cues for acceptance and oviposition in stemborer larvae. It has been reported that the stemborers, *B. fusca* and *C. partellus* share the same chemical cues to induce oviposition by *C. flavipes* (Obonyo *et al*., 2010a).

The behavioral sequences of host recognition and acceptance of *C. sesamiae* and *C. flavipes* have been well identified (Obonyo *et al*., 2011). The two parasitoid wasps were shown to exhibit similar hierarchial behavioral events in detecting both the volatile and contact stimuli from their host. The antennae and particulary the distal antennomeres appear to be important for host recognition, while both antennae and tarsi are involved in host acceptance for oviposition (Obonyo *et al*., 2011).

2.8.1.4 Host rejection by parasitoids

Parasitoids may reject a specific host after perceiving that the host is unsuitable due to several conditions which includes; presence of internal marking pheromones when the host is already parasitized and is physiologically unsuitable, lack of the necessary cues that indicates its suitability or due to its chemical combination of amino acids and inorganic ions as compared to heomplymph composition which is perceived to be the true host (Vinson, 1985; Godfray, 1994).

Rejection of hosts has not been reported for *C. flavipes* and *C. sesamiae* and especially given the fact that both parasitoids can still oviposit on the unsuitable host such as *B. fusca* (Ngi-Song *et al*., 1995; Gitau, 2006 Obonyo *et al*., 2008;). A good example was in the noctuid *S. nonagrioides* (Eastern-Kenya biotype) where the parasitoid eggs were not found after *C. flavipes* probed and stung the larvae with the ovipositor. It was clear whether the parasitoids rejected the stemborers (failed to lay eggs) after perceiving their unsuitability (Obonyo, 2005). It has also been found that the ovipositor of this parasitoids has sensilla that function solely for the purpose of host examination and discrimination (Obonyo *et al.*, 2011).

2.9 Chemical perception by parasitoids during host recognition and acceptance.

The antennae, tarsi and the ovipositor (**Figure 2.5**) are considered to be the most important sensory organs for the perception of chemical cues during habitat/host location, host selection and acceptance (Godfray, 1994). These organs habour sensilla involved in mechanical or chemical perception. On the basis of morphological and ultrastructural characteristics, these sensilla are broadly categorized into three main

groups; (i) mechano-receptors, which are non-porous inverteb and with one neuronolfactory, (ii) olfacto-receptors, which are multiporous and inverted with several neurons, and (iii) gustato-receptors, which have uniporus tip and frequently associated with a mechanoreceptor neuron (Zacharuk, 1985, Obonyo *et al*., 2011).

Van Baaren (1994) showed that female parasitoids use their antennae as the primary sensory organ for host external examination. For example, for braconid parasitoids, gustatory sensilla are useful for host examination prior to oviposition, whereas olfactory organs are useful for long-range host location (Canale & Raspi, 2000; Obonyo, 2005; Obonyo *et al*., 2008; Obonyo *et al*., 2011). The role of antennal, tarsi and sensila chaetica in the perception of contact semiochemical has also been studied in an egg parasitoid *Trissolcus brochymenae* (Ashmead) (Hymonptera: Scelionidae), whereby in the behavioral assay, the female wasp displayed an intense searching behavior in an open arena treated with host extract (Lacovone *et al.,* 2016). Uniporous gustatory sensila chaetica are particulary suited for electrophysiological recording (Lacovone *et al*., 2016). The external morphology of the parasitoid antennomeres differs from one family to the other. For example, it is simpler and more uniform for braconids as compared to chalcids (Canale & Raspi, 2000). Similarly, the parasitoid the tarsi (pretarsis) are important in host location and reception of the host vibration signals (Meyhofer *et al*., 1997). For example, the tarsi of *Opius concolar* (Szepligeti) (Hymenoptera: Braconidae) has been found to posses both mechano- and chemo-receptors believed to be important in host detection and reception of the associated vibration signals.

The parasitoid ovipositor is an organ which is mainly involved in the host discrimination and is primarily composed of a sting and the gasostyli. The sting is an organ, which is inserted into the host and is usually enveloped in a pair of valve whereas the gonostylus is surrounded by the sting (Hermann & Douglas, 1976). The sting is covered by compariform sensilla whose function is in chemoreception, that is, to detect oviposition stimulo mechanoreceptors sensitive to tactile stimulations (Greany *et al.*, 1977). It may act as deterrant factor associated with suitable and unsuitable host respectively. The gonostyli have abundant trichoidea sensilla, which are believed to be stimulated during the pre-stinging or pre-oviposition probing (Harmann & Douglas, 1976; Obonyo *et al*., 2010).

For *C. flavipes* and *C. sesamiae*, it is believed that the antennae and possibly the leg (Smith *et al*., 1993; Ngi-Song & Overholt, 1997; Obonyo *et al*., 2010a & b) are involved in host examination and recognition. Previuosly it was noted that when the parasitoids draws closer to the host caterpillar the rate of antennating and walking increased and soon stings the larvae. On the other hand, if the host larvae is washed (in distilled water), the female wasp often walks several times over it without showing any sign of increased searching behavior (Ngi-Song & Overholt, 1997; Obonyo *et al*., 2010a & b). Therefore, these observations indicate clearly that females of *C. flavipes* and *C. Sesamia* use their antennae for host recognition and both their antennae and tarsi for final acceptance of a host for oviposition, in which tactile and contact chemoreception stimuli from the host seems to play a role in the decision to oviposit.

Generally for endoparasitoids, the cue for oviposition is detected while the ovipositor is inside the host (Vinson, 1985). Parasitoids have also been observed to frequently insert their ovipositors into a host without laying eggs. This is because their ovipositors are usually covered in sensilla that may be used in perceiving the suitability of the host (Godfray *et al*., 1994).

Electron microscopic observations made by Obonyo *et al*., (2011), observed that from both parasitoids, there were four types of sensilla on the three terminal antennomeres that performed different roles. These were (i) non-porous sensilla trichodea likely to be involved in mechanoreception, (ii) uniporous sensilla chaetica with porous tips that have gustatory functions, (iii) multiporous sensilla placodea, which are likely to have olfactory function, and (iv)sensilla coeloconica known to have thermo-hygroreceptive function. The tarsi of both parasitoids possess a few uniporous sensilla chaetica with porous tips, which are believed to have gustatory functions.

Cotesia sesamiae *Cotesia flavipes*

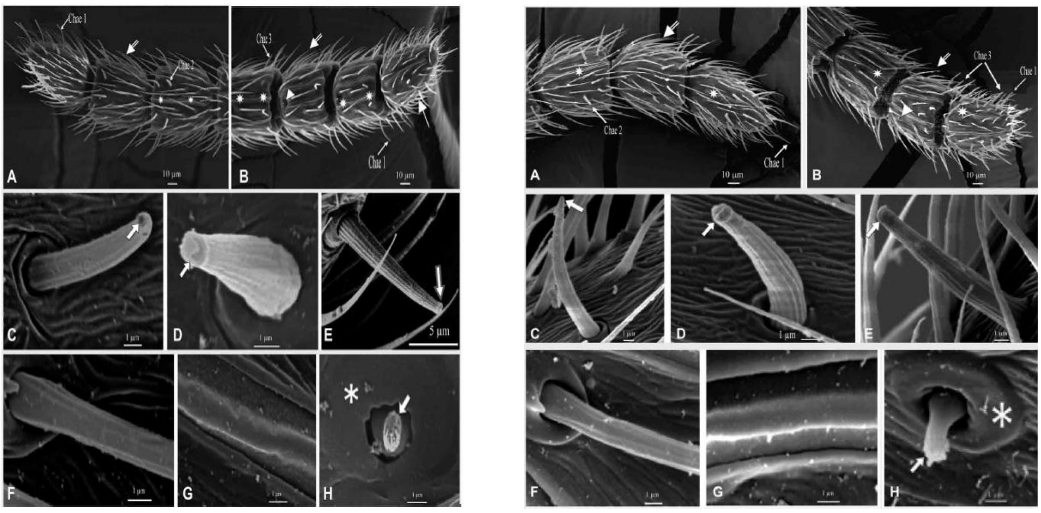

Figure 2.5 Gustatory sensilla at the tip of the parasitoid's antennae involved in host recognition. Distal antennomeres of adult females of *Cotesia sesamiae* (left) and *Cotesia flavipes* (right) (A, dorsal view and B, ventral view) observed by scanning electron microscopy, showing distributions of sensilla chaetica (arrows indicating sensilla chaetica type 1, 2 and 3 denoted Chae 1, 2 and 3, respectively), sensilla trichoidea (double-stemmed arrow), sensilla placodea (asterisks) and sensilla coeloconica (arrowhead). C, Sensillum chaeticum type 1 with a single pore on the tip (arrow). Sensilla chaetica type 2 and 3 which each tip forming a flap with a slit (arrows) (D and E). F, The basis of a sensillum trichoideum with a grooved cuticular surface and the absence pores. G, Portion of a placodeum sensillum with a sponge-like surface. H, Sensillum coeloconicum with a bulb-like terminal (arrow) surrounded by a doughnut-shaped ring (asterisk) (from Obonyo *et al.*, 2011).

2.10 Semiochemicals involved in insect's communication

Chemical communication plays an important and essential role in the survival of insects, which enable them to appraise immediate environment through modification of their behavior.

Semiochemicals are chemical substances that convey specific chemical messages from one individual to another, envoking behavioral or physiological responses that are adaptive to one or both of the organisms involved (Vet & Dicke, 1992; El Shafie & Faleiro, 2017). Semiochemicals determines insects's life situation such as feeding (host or food source), mating, egg laying /oviposition avoiding competition, escaping natural enemies, and overcoming natural defense systems of their hosts (El Shafie & Faleiro, 2017). Semiochemicals are biologically active at very low concentrations in the environment, thus their chemical characterization is quite complicated. Semiochemicals are classified, not with respect to their chemical properties or source, but rather according to their ecological roles, effect or function. Thus, this should always be taken into account since the same molecule could have dual properties: act as a pheromone for one insect species and as a kairomone or allomone for another species (El Shafie & Faleiro, 2017; Dicke & Sabelis, 1988; Nordlund & Lewis, 1976; Vet & Dicke, 1992).

Semiochemicals are divided into two broad groups: pheromones that mediate interactions among individuals of the same species (intraspecific reactions) and allelochemicals that mediate interactions among individuals of different species (interspecific interactions). According to the behavioral response studies, pheromones

are further subdivided into primer pheromones that have long-term physiological changes and releaser pheromones that elicit short-term or immediate behavioral response. On the other hand, allelochemicals are further divided into kairomones that mediate interactions favoring the recipient and allomones that favor the emitter. Synomones are semiochemicals that favour both the emitter and the recipient, while apneumones, are substances, produced by nonliving material that elicit favorable behavioral response to the receiving organism but harmful to a second organism found on the nonliving material (El Shafie and Faleiro, 2017). Schematic diagram showing the classification of semiochemicals is shown in **Figure 2.6.**

Semiochemicals have been shown to arise from many sources including host food, the host itself, host by-products, associated organisms, or interactions among sources (Vinson, 1988; Godfray, 1994). In different parasitoid–host systems, the source and function of various semiochemicals have been determined and in some of these systems chemical cues have been also identified as reviewed by Rutledge, (1996) and experimentally used for rearing or improving parasitism (Jones *et al*., 1973; Lilley *et al.*, 1994). It is important to elucidate the chemicals utilized in each step of parasitoid foraging behaviour because it offers an understanding of plant-insect interactions as well as providing tools for improvements in biological control (Hare & Morgan, 1997). Although crop protection based on semiochemicals is not yet widely used, this method has several advantages over the conventional use of insectsides. In this study, emphasis was placed on use of kairomones since they are generally involved in host recognition and acceptance by the parasitoids.

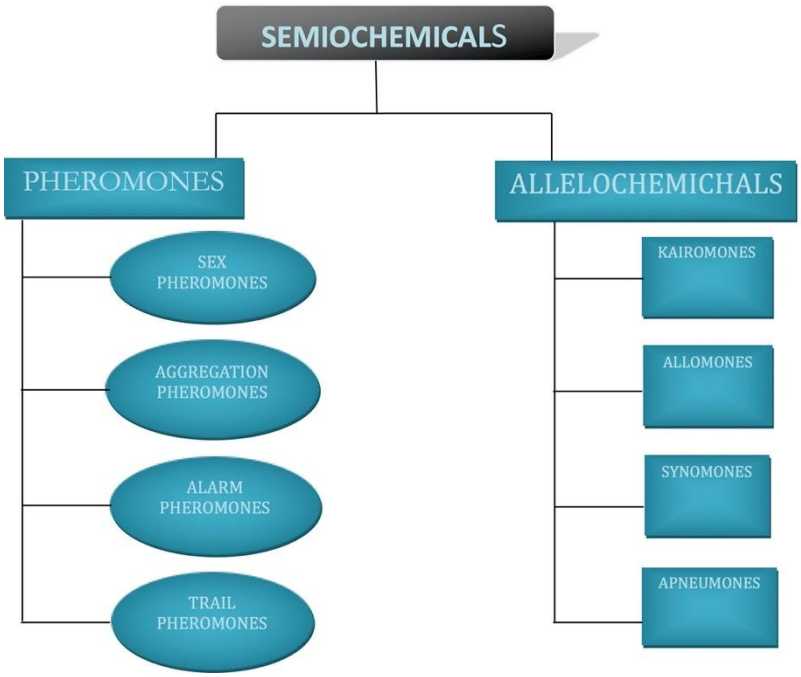

Figure 2.6 Schematic diagram showing the classification of semiochemicals based on their effect and role in specific interactions (source: http://dx.org/10.5772/66463).

2.10.1 Kairomones involved in host recognition by parasitoids

Kairomones are semiochemical emitted by an organism, which mediates interspecific interaction in a way that benefits an individual of another species, which receives it; without benefitting the emitter (Grasswitz & Jones, 2002; El Shafie & Faleiro, 2017). Kairomones are used by foraging parasitoids in the host parasitoid relationship starting from habitat preference up to host recognition. This process is categorized into three stages: habitat location, host location and host acceptance and oviposition (Rutledge, 1996). Specificity is important for parasitoids to seek the correct host species and of particular development stage. This appears to more appropriately relate to the host selection process (Conti *et al*., 2004).

Kairomones are host-derived semiochemicals that include but are not limited to: aldehydes, alcohols, sulfur-containing compounds, esters, terpenes, alkanes, heterocyclic aromatic compounds, proteins, amino acids, triglycerides and salts (**Table 2.1**). Rutledge *et al.* (1996) as cited by El Shafie & Faleiro (2017) stated that semiochemicals identified in the habitat-location step are likely to emanate from the host-plant of the host insect, while in host-location, acceptance and oviposition steps, semiochemicals are predominantly accrue from the host. The importance of kairomones increases with decreasing distance from the host, thus kairomones because of their original source appears to be most important in determining the differential attraction of parasitoids towards their specific host among the blend of infochemicals available for foraging parasitoids. However, under circumstances where different herbivorers are present, the use of kairomones from the herbivore and its by-product becomes increasingly important in mediating the attraction of the parasitoid towards its target host at the target stage (Reddy *et al*., 2002; Mizutani, 2006).

In several studies, chemicals responsible for envoking a particular response to kairomone source have been identified and have been reported to include aldehydes, esters, isothiocynates, sulfides, nitriles, uranoids, and dieserpenoids. Sometimes, the kairomone source contains a mixture of chemicals but only one or few of them in specific ratio in the blend can be effectively proven to mediate specific responses in the parasitoid (Reddy *et al*., 2002; Mizutani, 2006). It has also been proposed by Rani *et al*. (2007) that compounds that have a high number of carbon atoms might act as

contact stimulants, whereas those with less than 10 carbon atoms are more volatile and might attract parasitoids to the near vicinity of the host.

The importance of kairomones for the parasitoids to locate their target herbivore has been reviewed (Afsheen *et al*., 2008). Parasitoids exploit either volatile (Harris & Todd, 1980; Vet *et al*., 1991; Hardie *et al*., 1994; Feener *et al*., 1996) or non-volatile contact kairomones (Dmoch *et al*., 1985; Hare *et al*., 1993; Hare, 1996; Meiners & Hilker, 1997) as cues for locating their specific hosts. Parasitoids are able to differentiate between host species (host and non-host) (Alborn *et al*., 1995) by use of emissions of species-specific kairomones from the herbivore host (Alborn *et al*., 1995; Meiners *et al*., 2000; Conti *et al*., 2004). These emissions inform the parasitoids of the presence, identity, availability and suitability of the host. In addition, several other studies have reported that host kairomones from a specific herbivore stage for example, egg, larvae, pupa and adult (Zaki *et al*., 1998) or by-product such as the frass, exuvial, mandibular gland secretion, defense secretion among others of its specific host (Rojas *et al*., 2006) are the most reliable information for the respective parasitoids (see **Table 2.1**).

Various analytical methods have been used in the isolation and identification of kairomones in a number of biological matrices. Among the kairomones found in the literature (**Table 2.1**), volatile kairomones are essentially identified using the GC-MS methods while the LC-MS techniques are used for the identification of hydrosolubles kairomones. For aminoacids and proteins based kairomones presented in this study,

technique related to protein biochemistry such as electrophoresis, western blot, LC-MS-MS and enzyme bioassays were used for their identification.

Although parasitoids use other cues such as non-chemical information source to locate their target host in the target stage, parasitoid get attracted differently based on the specificity of the source of the kairomones. However, the chemical identity of host kairomones exploited by *Cotesia* species is not known, therefore, the identification of active chemical constituents of the kairomones, its source as well as its mechanisms of actions that determine their specificity for the different types of *Cotesia* species will be elucidated in this study. The findings may in future be exploited in design of better herbivore pest management programmes.

Table 2.1 Kairomones involved in the attraction of varius parasitoids and their possible source following literature search.

References	**Compounds /Kairomone**	**Source**	**Host stage**	**Parasitoid species**	**Host species**
Ananthakrishnan *et al.*, (1	hexatriacontane pentacosane	Moth scale	Eggs	*Trichogramma* *Chilonis*	*Heliothis armigera*
Bénédet *et al.* (1999)	glycopolypeptides	silk cocoon	Pupae	*Diadromus subtilicornis*	*Acrolepiopsis assectella*
Burks & Nettles (1978)	Chemical not named	cuticle of the larvae	Larvae	**Eucelatoria bryani**	**Heliothis armigera**
Boo & Yang (2000)	*Z*11-16:Ac	sex pheromone	Eggs	*Trichogramma* *chilonis*	*Helicoverpa assulta*
Calatayud *et al*. (2001)	*O*-caffeoylserine	host cover	Larval	*Acerophagus coccois,* *Aenasius vexans*	*Phenacoccus herreni*
Colazza *et al.* (2007)	*n*-nonadecane	adult tarsi	Eggs	*Trissolcus basalis*	*Nezara viridula*

		and scutella			
DeLury *et al.* (1999)	heptanal, octanal, nonanal, decanal undecan-2-one, dodecanal, pentadecan-2-one, (*Z*)-6-pentadecen-2-one, (*Z*)-9-hexadecenal, (*Z*)-6-hept 3,7,11-trimethyl-2E,6E, 10-dodecatrien-l-ol acetate	Scales	eggs	*Ascogaster quadridentata*	*Cydia pomonella*
Elzen *et al*. (1984)	Sesquiterpens	plant (cotton)	Plant	*Campoletis sonorensis*	**Heliothis armigera**
Fatouros *et al.* (2005)	benzyl cyanide	anti-aphrodisiac	Eggs	*Trichogramma brassicae*	*Pieris brassicae*
Gauthier *et al.* (2004)	Polypeptides	silk cocoon	Pupae	*Diadromus subtilicornis*	*Acrolepiopsis*

					assectella
Hassel (1968)	sucrose, fructose	plant (Fagaceae)	Egg	*Cyzenis albicans*	*Operophtera brumata*
Hare *et al.* (1993) Millar & Hare (1993)	*O*-caffeoyltyrosine	host cover	Larvae	*Aphytis melinus*	*Aonidiella aurantii*
Hilker *et al.* (2000)	acetate and propionate of (2S,3R,7R)-3,7-dimethyl -2-tridecanol (2S,3S,7S)-3, 7-dimethyt-2-pentadecyl aceta	sex pheromone	eggs	*Chrysonotomyia ruforum*	*Diprion pini*
Jones *et al.* (1971)	Methylhentriacontane	frass, saliva, haemolymph	Larvae	*Microplitis demolitor*	*Heliothis virescens*
Jones *et al.* (1973)	docosane, tricosane	wing scales	Eggs	*Trichogramma evanescen*	*Heliothis zea*

Kuwahara *et al*. (1983)	2-palmitoyl- and 2-oleoyl-cyclohexane- l, 3-dione	Frass	Larvae	*Venturia canescens*	*Plodia interpunctella*
Lewis *et al.* (1982)	(*Z*)-9-Hexadecenal	sex pheromone	Eggs	*Trichogramma pretiosum*	*Heliothis zea*
Lou *et al.* (1999)	palm oil	adult, nymph	Eggs	*Anagrus nilaparvata*e	*Nilaparvata lugens*
Lou & Cheng (2001)	palm oil	adult, nymph	Eggs	*Anagrus nilaparvata*e	*Sogatella furcifera*
Mattiacci *et al.* (1993)	α^{β} - unsaturated aldehyde, (*E*)-2-decenal	defensive metathoracic gland	Eggs	*Trissolcus basalis*	*Nezara viridula*
Mizutani (2006)	(*E*)-2-hexenyl (*Z*)-3-hexenoate (*E*2HZ3H)	aggregation pheromone	eggs	*Ooencyrtus nezarae* \	*Riptortus clavatus*

Ma *et al*. (1992)	11 free amino acids including serine and glutamic Acid	frass, oral secretion	Larvae	*Eriborus argenteopilosus*	*Ostrinia nubilalis*
Mudd & Corbet (1982) Mudd *et al.* (1984)	2-acylcyclohexane-1-3-dione	mandibular glands	Larvae	*Nemeritis canescens*	*Ephestia kuehniella*
Nemoto *et al*. (1987)	2-palmitoyl- 2-stearoylcyclohexane-1,3-dic	Frass	Larvae	*Venturia canescens*	*Cadra cautella, Plodia interpunctella*
Nettles & Burks (1975)	protein (30 kD)	frass, haemolymph	Entire larvae, pupae, Emerged adults	*Archytas marmoratus*	*Heliothis virescens*
Nordlund & Lewis (1985)	13-methylhentriacontane	larval frass	Larvae	*Microplitis demolitor*	*Heliothis zea*
Ramachandran *et al.* (199	3-octanone and guaiacol	larval frass	Larvae	*Microplitis demolitor*	*Pseudoplusia inchudens*

Reddy *et al.* (2002)	*Z*11-16Ald, *Z*11-16:Ac and *Z*11-16:OH (1:1:0.01 ratio), *Z*11-16:Ac alone, *Z*11-16:Ac and *Z*11-16:Ald (1:1 ratio)	sex pheromone	eggs	*Trichogramma chilonis*	*Plutella xylostella*
Reddy *et al.* (2002)	allyl isothiocyanate	larval frass	Eggs	*Trichogramma chilonis*	*Plutella xylostella*
Roux *et al*. (2007)	Lipids	larval cuticle	Larvae	*Cotesia plutellae*	*Plutella xylostella*
Rani *et al.* (2007)	long chain alkanes and alkenes like docosane, tetracosane, pentacosane and eicosane	adult extracts	Eggs	*Trichogramma japonicum*	*Scripophaga incertulas*

Renou *et al.* (1992) .	heneicosane, tricosane, pentacosane, heptacosane, nonacosane, ethyl and palmitic acid palmitate	Egg extract	Eggs	*Trichogramma brassicae*	*Ostrinia nubilalis, Mamestra brassicae*
Strand *et al.* (1989)	2-acylcyclohexane-1-3-dione	Mandibular glands	Larvae	*Bracon hebetor*	*Ephestia*, phytisiine mot
Steidle & Ruther (2000)	Alpha-tocopherol, beta-tocopherol beta-tocotrien cholesterol, ergostenol and beta-sitosterol	Feces	Larvae	*Lariophagus distinguendu*	*Sitophilus granaries*
Shu *et al.* (1990)	mixture of 11,15-, 13,17-and 15,19-dimethylnonatriacontan	moth scale	Eggs	*Trichogramma nubilale*	*Ostrinia nubilalis*
Silva *et al.* (2006)	methyl 2,6,10-trimethyltridecanoate	male sexual pheromone	Eggs	*Telenomus podisi*	*Euschistus heros*

Takabayashi & Takahashi (1989-	2, 5-dialkyltetrahydrofuran	Frass	Larvae	*Apanteles kariyai*	*Pseudaletia separata*
Thompson *et al.* (1983)	phenols, alcohols	Frass	Plant (sugarcane) sugars	*Lixophaga diatreae*	*Diatraea saccharalis*
Vinson *et al.* (1975)	methyl hen-, di-,tritriacontane	mandibular glands	Larvae	*Cardiochiles nigriceps*	*Heliothis virescens*
Vinson *et al.* (1975)	mixture of three long chain hydrocarbons (11-methyl-hentriacontane, 16-methyl-dotriacontane and 13-methyl-hentriacontane)	mandibular glands	Larvae	*Cardiochiles nigriceps*	*Heliothis virescens*
Weseloh (1977)	sericin or fibrinogen likeprote	silk producing glands	Larvae Pupae	*Cotesia marginiventris*	*Limantria dispar*

2.11 Genetic adaptation in parasitoids

Genetic adaptation can occur rapidly at ecological scale (Thompson, 1998). Yet, models to predict the response of ecosystem communities and ecosystem services to global changes do not consider rapid adaptation as an ongoing process. This is likely due to the difficulties to acquire data on such processes and to test models linking gene and population levels. Parasitoid-host interaction provides a unique system to link individual traits to population levels. Due to the simple key events determining the issue of the interactions, parasitoid-host systems formed historically the first basics of theoretical modeling in ecology (Godfray & Shimada, 1999). However, apart from few studies on the genetics of host parasitoid immune interaction in *Drosophila*-parasitoid systems (Dupas *et al*., 2003), the link between genes and the traits determining the interactions remains unclear. Similarly, the link between genetic and molecular variation is rarely studied (Dupas *et al.*, 2008; Branca *et al*., 2011a, 2011b) and particularly no study on the parasitoid's genes involved in host acceptance.

New technologies, and in particular the next generation sequencing technologies (NGS) (Stapley *et al*., 2010) can provide large amount of markers to understand molecular aspects of adaptation and among them those involved in host acceptance. New approaches in demogenetic analysis (i.e. estimation of demography models from genetic data) may allow to infer, from these new molecular markers, historical processes of adaptation operating in nature (Beaumont, 2010). These opportunities can facilitate the estimation of adaptation parameters for the prediction of population responses to environmental changes.

2.12 Heritable genetic variations in parasitoids

Introduced parasitoids need to adapt rapidly to their new environment (Henry *et al*., 2010), while in the long-term biocontrol sustainability relies on its ability to co-evolve with host resistance and not to switch to non-target hosts. Parasitoids need to kill or impede the host reproduction success. Therefore, traits affecting the issue of the interaction are under strong selection. Such critical traits include immunological and physiological response of larval stages (Pennacchio & Strand, 2006) and adult behaviour (Fellowes *et al*., 1997). At each step of host-parasitoid interaction, the host can resist and the parasitoid can counteract host resistance.

Heritable genetic variations have been described in several host-parasitoid systems for behavioural and physiological (virulence) parasitoid traits. Host specific virulence mechanisms, adapted to the local host community, have been identified in phylogenetically distant host-parasitoid associations, including Diptera, Homoptera and Lepidoptera hosts, and Hymenoptera Braconidae and Figitidae parasitoids (Kraaijeveld *et al*., 1998; Dupas *et al*., 2003; Branca *et al*., 2011a). Several studies have shown that the parasitoids from these groups are rapidly able to genetically adapt to new hosts by experimental selection (Kraaijeveld *et al*., 2001; Henry *et al*., 2010; Dion *et al*., 2011) and lose their adaptations to their original host due to trade-off (Dupas & Boscaro, 1999; Kraaijeveld *et al*., 2001; Antolin *et al*., 2006 ; Henry *et al*., 2008).

Genetic variation linked to behavioural factors involved in parasitoid success has been observed in various families (Althoff & Thompson, 2001; Wang *et al*., 2003; Dubuffet *et al*., 2006; Kaiser *et al*., 2009; . The variability of other fitness traits, such as sex-ratio, has large environmental component and its additive genetic component has far less been studied, even if it has been shown by experimental selection (Parker & Orzak, 1985). QTL for sex ratio have recently been discovered in *Nasonia vitripennis* (Pannebaker *et al*., 2011).

CHAPTER THREE

3.0 CHEMICALS ASSOCIATED WITH HOST RECOGNITION AND ACCEPTANCE BY THE BRACONID PARASITOID *COTESIA FLAVIPES*

3.1 Introduction

The ability of parasitoids to successfully utilize cues that allow the host's habitat location and to discriminate between suitable and nonsuitable hosts is vital for their efficiency in the field (Wajnberg *et al.,* 2008; Wajnberg & Colazza, 2013). During location of hosts, parasitoids typically exploit both long and short-range stimuli emanating from the host habitat (Vinson, 1975, 1976; Godfray, 1994), followed by stimuli that are directly associated with the host and its products (Vinson, 1976; 1985; Vet & Dicke, 1992; Godfray, 1994). The host's habitat location is often mediated by plant volatile organic compounds (plant VOCs) resulting from the elicitation of plant defense metabolites produced constitutively in response to insect salivary enzymes produced when feeding on the plant (Turlings *et al.,* 1990; Heil, 2008; Erb *et al.,* 2011; Dicke, 2016). However, VOCs do not often convey reliable information on the suitability of the caterpillar species but rather act as indicators of the presence of herbivores (Ngi-Song & Overholt, 1997; Obonyo *et al.,* 2008). It is only when approaching the host that reliable information on the host's identity is perceived via contact-chemoreception by the parasitoid. It has been reported that such information is obtained from of fecal pellets and oral secretions produced as a result of host`s feeding activities (Ngi-Song & Overholt, 1997; Obonyo *et al.,*

2010a & b; see also Kaiser *et al.*, [2017a] for a recent review). To ensure that they will parasitize a suitable host, the parasitoids need to be able to discriminate between different species and particularly between hosts and non-hosts. Parasitoids have generally been classified as generalists able to parasitize a wide range of herbivorous hosts while others such as many endoparasitoids that are restricted to parasitize only one or a few related host species (Harvey *et al.*, 2005). The endoparasitoids *Cotesia* sppare among the most diverse genera involved in parasitism of several insect species (Kaiser *et al.*, 2017b).. Although many *Cotesia* species possess generalists feeding behaviour careful ecological studies indicates a hidden complexity with many population assemblages on the genus exhibiting restricted host ranges (Kaiser *et al.*, 2017b).

In sub-Saharan Africa, lepidopteran stemborers of the Crambidae, Pyralidae and Noctuidae families are economically important pests of maize and sorghum (Harris, 1990; Polaszek, 1998; Kfir *et al.*, 2002). The most cited and economically important species are the crambid *Chilo partellus* (Swinhoe), the noctuids *Busseola fusca* (Fuller) *Sesamia calamistis* Hampson, and the pyralid *Eldana saccharina* (Walker) (Polaszek, 1998). With the exception of *C. partellus*, which was accidentally introduced from Asia into Africa before the 1930s (Kfir, 1992), the other species are indigenous to Africa. During the early 1990s, the International Centre of Insect Physiology and Ecology (*icipe*) initiated a project on the biological control of *C. partellus* with the introduction of *Cotesia* into Kenya from Asia. *Cotesia flavipes* parasitizes the larvae of more than 30 Lepidoptera species including *C. partellus*,

Chilo suppressalis (Walker), *S. calamistis* and *Spodoptera mauritiana* Boisduval (Boisduval) (Lepidoptera: Noctuidae) (https://www.cabi.org/isc/datasheet/5951). The parasitoid was first released in the coastal area in 1993 (Overholt *et al.*, 1994), where it is reported to have reduced *C. partellus* population densities by over 50% (Zhou *et al.*, 2001; Jiang *et al.*, 2006). However, its presence and efficiency to parasitize stemborers appear variable according to the location and the type of the crop cultivated (Cugala *et al.*, 2001). It is therefore important to understand the relationships between this parasitoid species and its host for a better and all-inclusive biological control programme (Bichang'a, 2013).

The present study thus sought to evaluate first the role of stemborers fecal pellets and oral secretions in mediating host acceptance for oviposition in *C. Flavipes parasitoid*. Behavioural assay and biochemical approaches were used to identify the active chemical compounds present in parasitoid host products.

3.2. Materials and methods

3.2.1 Insects

Parasitoids: *Cotesia flavipes* adults were obtained from laboratory-reared colonies established at the International Centre of Insect Physiology and Ecology (*Icipe*), Nairobi, Kenya. The colony originated from individuals collected in the field in the coastal region of Kenya in 1998. Field-collected *C. flavipes* were added twice a year to rejuvenate the colony. The parasitoid was reared on *C. partellus* larvae according to Overholt *et al.* (1994). Parasitoid cocoons were kept in a Perspex cage (30 cm x 30

cm x 30 cm) until their emergence. Adult parasitoids were fed on a 20% honey/water solution, put under artificial light and left for 24 h to mate. In the behavioral bioassays, only 1-day-old naïve (i.e. without oviposition experience), mated females were used. Experimental conditions were maintained at 25 ± 2 °C, 50–80% relative humidity (RH), and a 12:12 h (L:D) photoperiod (Overholt *et al.*, 1994).

Stemborers: The natural host *C. partellus*, the suitable new association host *S. calamistis* as well as the non-hosts *B. fusca* and *S. nonagrioides* were used in this study as described by Obonyo *et al.* (2008). The larvae of *C. partellus* and *S. calamistis* were collected from maize grown in coastal regions, *B. fusca* from maize grown in Western (Kitale), while *S. nonagrioides* from *Typha domingensis* in Makindu (Eastern part of Kenya). The larvae of *C. partellus* were reared on artificial diet as described by Ochieng *et al.* (1985) whereas larvae of the other species were reared on their respective artificial diets as described by Onyango and Ochieng'-Odero (1994). For each host species, feral stemborer larvae from their respective region were added twice a year to rejuvenate the colonies.

It was previously demonstrated that the acceptance of host larvae for oviposition by *C. flavipes* is enhanced when the host larvae are fed on maize stems for 24h prior to exposure to parasitism by parasitoids (Mohyuddin *et al.*, 1981; Inayatullah, 1983; Van Leerdam *et al.*, 1985; Potting *et al.*, 1993; Overholt *et al.*, 1994). Therefore, for isolation of the semiochemicals produced during feeding of larvae of suitable and

unsuitable borer species that could be involved in host acceptance by *C. flavipes*, larvae previously fed for 24h on maize stems were used.

3.2.2 Collection of maize stems extracts and fecal pellets of *Chilo partellus*

The purpose of this study was to collect and confirm that the active compound(s) mediating *C. partellus* acceptance came from the host's products related to feeding activities and not from the host's food. In this context, a piece of maize stem was crushed using a mortar and pestle and the resulting extract collected and directly used for behavioural bioassays. Thereafter, fresh fecal pellets collected from larvae previously fed for 24h on maize stems were also directly used for behavioural assays. The remaining samples were kept at -80°C for further use.

3.2.3 Collection of oral secretions from both host and non-host stemborer larvae

Since the oral secretions in section 3.2.2 above were found to harbour the active compound(s) mediating host acceptance (see results section, **Table 3.1**), the remainder of the study focused on larval oral secretions. For the collection of oral secretions, a single larva held with soft forceps was squeezed behind the head and a capillary tube used to collect the oral secretions (**Figure 3.1**), which were put into and Eppendorf tube placed on ice. The process was repeated with at least 100 larvae. The volume of oral secretions obtained was estimated by weighing. The oral secretion samples were either used directly in behavioural bioassays or preserved at -

80°C for later use. Different types of oral secretions which included the followings were used:

i. Oral secretions from larvae of *C. partellus*, *S. calamistis*, *B. fusca*, and *S. nonagrioides* previously fed for 24h on maize stems to confirm the involvement of oral secretions in the specificity of host-parasitoid associations;

ii. Oral secretions of *C. partellus* previously fed 24h on stems of *Pennisetum purpureum* Schumach. (Poaceae), which is often found in the surroundings of maize fields, as well as previously fed on the artificial diet as described by Ochieng *et al.* (1985) to test if the active compounds depended on the host plant or the type of food;

iii. The oral secretions from larvae starved for 48h to verify if production of these semiochemicals were induced by larval feeding;

iv. In a previous study, it was hypothesized that the semiochemicals from oral secretions involved in host recognition by *C. flavipes* might include enzymes or thermo-labile proteins (Obonyo *et al.*, 2010b). Therefore, the oral secretions from larvae fed on maize stems and previously treated with proteinase K (Sigma product P2308) that destroys the proteins present in the oral secretions, were used. Hence, about 40 µl of oral secretion of larvae of *C. partellus* previously fed on maize stems was treated with 1.25 units/mg of proteinase K at 37°C for 1 hour.

Figure 3.1 Pictures showing the collection process of the oral secretions from single stemborer larvae.

3.2.4 Behavioural bioassays

In previous studies, it had been reported that the parasitic wasps exhibit host recognition and acceptance by antennating the surface of the host body followed by at least one stinging attempt indicating acceptance of a caterpillar as a host for oviposition (Obonyo *et al.*, 2010a; 2010b). Therefore, these two behavioural steps (**Figure 3.2**) were used as indicators of the parasitoid host acceptance in the presence of *C. partellus* fresh fecal pellets, different plant extracts and oral secretions (i.e. plant stem extract and different types of oral secretions, as well as electrophoretic bands obtained in the next section, known proteins and other compounds). The sample to be tested was placed at the centre of an 8 cm diameter Petri dish arena and presented to a single female wasp. For each replication, about 0.01 g of fresh fecal

pellet or about 0.5 to 1 µl of the extract to be tested were applied on a 2 mm cotton wool ball. A single female wasp was then introduced into the arena near the fresh fecal pellet or the cotton wool ball and were both covered with a transparent circular Perpex lid (3 cm diameter, 1 cm height) to prevent the parasitoid from flying off.

The behaviour of the wasp in the petri dish was then monitored for a maximum of 120 s. For each wasp, the number of both antennation and stinging attempts were recorded. The percentage of positive responses was calculated from 10 or 30 wasps tested per electrophoretic band or per the type of sample, respectively. The wasp, the cotton wool ball with applied extracts, and the arena were replaced after each wasp had been tested. All behavioural experiments were carried out in a room at 26 ± 1 °C between 10h00 to 14h00 with a constant source of light to maintain an optimal temperature for the behavioural activities of the female wasps.

A B

Figure 3.2 Host recognition and acceptance by parasitic wasps: by antennation (i.e. antennal drumming) of the host surface (A) followed by at least one stinging attempt (B), these two behavioural steps were used as indicators for host acceptance by the parasitoid.

3.2.5 Characterisation of stemborers oral secretions using non-denaturing polyacrylamide gel electrophoresis

To ensure recovery of proteins in their native state for behavioural assays, identification as well as accurate determination of their molecular weight, 500µl of the oral secretions from *C. partellus* were first centrifuged at a maximum speed of 14,000 ×g for 5 minutes in order to remove any debris such as undigested food materials. This was followed by desalting and concentrating the samples using Amicon® Ultra-0.5 centrifugal filter devices (Merck Millipore). The samples were then quantified using the Pierce BCA protein assay Kit (Thermo Scientific No. 23227) based on bicinchoninic acid assay (Smith *et al.*, 1985). All the measurements were carried out using Eppendorf-Biospectrometer fluorescence machine (SN 667).

Electrophoresis was then conducted under non-denaturing conditions (native PAGE electrophoresis, Ornstein-Davis discontinuous buffer system) according to Chrambach & Jovin (1983) and Niepmann & Zheng (2006). The gels were cast in two sections using the Bio Rad Mini-PROTEAN® Electrophoresis System and Hoefer™ Mini Vertical Electrophoresis Systems (Fisher Sci.com). A stacking gel (4%T, 2.7%C, 0.125M Tris-Cl pH 6.8) was cast on top of a resolving gel of (7.5%, T4.4%C, 0.125M Tris-Cl pH 6.8). Electrophoresis was conducted (running buffer: 0.025M Tris, 0.192M glycine pH 8.3) immediately after loading the samples at a constant voltage of 150V and current of 25mA for 1-2hr in a cold room. The pH discontinuity between the two sections of the gel were designed to regulate the effective mobility of the glycine ions from the cathode chamber. The concentration of all the four buffers were derived from electrochemical considerations based on the properties of the regurgitant evaluated earlier on. The correct gel concentration for the regurgitant (untested protein mixture) was predicted by analysing the protein mixture in gels.

The porosity of the resolving gel was empirically determined to match the mobility of the protein in the sample. The amount of 40% acrylamide/bis acrylamide stock solution (37.5:1) needed to make monomer solution at the desired % T (7%) was calculated as follows. $V_{resolver}$ = (2/3) (% $T_{resolver}$), in ml. Whereas the amount of water used for the resolving gel was calculated as : V_{water} = (15 - $V_{resolver}$), in ml. At the end of the run, gels were immediately removed and stained for 30 min in a

staining solution consisting of 0.2 % Coomassie Brilliant Blue R250. The gels were then destained with a solution of methanol, glacial acetic acid and water at the ratio of 4:1:5. The stained proteins were compared to a molecular mass standard (Sigma Aldrich) containing albumin from bovine serum (Sigma A8654, 132 kDa), urease from jack bean (Sigma U7752, 272 and 545 kDa), α lactalbumin from bovine milk (Sigma L4385, 14.2 kDa) and albumin from chicken egg white (Sigma A8529, 45 kDa).

3.2.6 Isolation of oral secretion protein bands from the poylacrylamide gel

Proteins in their native form were required for the subsequent experiments including the bioassay towards the parasitoids to test for the behavior and their subsequent identification**.** For the isolation of electrophoretic bands, the protein bands were manually excised from the gel before staining process following the method of Kurien and Scofield (2012) with some modifications. The excised gel fragments containing the protein of interest were frozen overnight at -80°C. Each frozen gel fragment was ground using a mortar into fine powder under liquid nitrogen and the resulting gel powder transferred to the upper chamber of the Costar® column (centrifuge tube filters, Costar lot No. 22304012 Corning incorporated, NY 14831-USA). The protein trapped in the gel powder was eluted using native elution buffer 0.25M Tris HCl buffer pH 6.8, or normal saline depending on subsequent application. After 10 min of centrifugation at 13000 ×g, 300 to 350 µl of the filtrate was recovered and stored for further concentration and desalting. A second elution was performed with fresh elution buffer and a filtrate of approximately 250-300 µl

was collected and combined with the previous one. Each protein eluted was concentrated 25-30 × folds using an Amicon centrifugal device equipped with 30K MWCO omega membrane. The concentrated protein eluents were assayed for protein content based on the Pierce BCA protein assay Kit. For each protein eluent, the purity and elution efficiency were checked by native PAGE electrophoresis. Proteins in the gel were Coomassie-stained as described above. All the 7 major bands revealed in the oral secretion of maize-fed *C. partellus* (see **Figure 3.5**) were separated and purified as previously described for use in behavioural assays..

3.2.7 Identification of purified proteins from the stemborers oral secretions

The gel purified protein eluent inducing parasitoids' host recognition and oviposition were identified using LC-MS/MS (Liquid chromatography–mass spectrometry).\The protein eluents were first denatured in Laemmli buffer and then concentrated using a short electrophoretic migration, which also allowed the removal of any contaminants that could interfere with the trypsic digestion. Electrophoretic bands were excised and the gel pieces were washed in successive baths consisting of 50mM ammonium bicarbonate and acetonitrile. Proteins were then reduced by 10 mM of 1.4 dithiothreitol (DDT) and alkylated with 55mM of iodoacetamide to block the sulfide bonds between the cysteine residues. After rinsing the protein to remove DTT and iodoacetamide residues, the protein samples were hydrolyzed by the addition of 0.125µg trypsin for 7 hours. After hydrolysis, the resulting peptides were extracted from the gel pieces with 50% acetonitrile acidified with 0.5% of trifluoroacetic acid (TFA). After complete speed vac drying, peptides were resuspended in a solution of

2% acetonitrile, 0.05% formic acid and 0.05% trifluoroacetic acid. Peptide mixes were then analyzed by LC-MS/MS using a nanoRSCL (thermoFinnigan) coupled to LTQ Orbitrap Discovery (Thermo). The samples were then loaded onto a PepMap100C18 trap column for 5min with 2% acetonitrile (ACN), 0.08% TFA qsp H2O. Two buffers systems were used to elute the peptides: 2%ACN and 0.1% formic acid in water (buffer A); 98% ACN and 0.1%formic acid in water (buffer B). Peptide separation was performed using a linear gradient from 4% to 38 % of buffer B in 15min. The nanoHPLC was connected to the mass spectrometer using a nano electrospray interface (non-coated capillary probe 10µ I.d. New objective). Peptide ions were then analyzed using Thermo Xcalibur (version 2.0.7) using the following data dependant steps: (1) full MS scan with a 300 to 1400 m/z range in the Orbitrap with a resolution of 15,000; (2) fragmentation by CID in the linear trap with a normalized energy at 35%. Step 2 was repeated for the three most intense ions with a minimum intensity of 500 with dynamic exclusion set to 30 seconds.

Raw files were converted to the mzxml format using msconvert (3.0.9576 http://proteowizard.sourceforge.net/tools.shtml) while database search was performed using X!tandem JACKHAMMER (Craig & Beavis 2004). Tolerance was set at 10 ppm for precursor ions and 0.5 Th for fragment ions. Cys-carboxyamidomethylation was set to static modification. Methionine oxidation, Nter acetylation of proteins, glutamine Nter deamidation and glutamic acid Nter water loss were set to variable modifications. Three databases were used: the *Spodoptera frugiperda* (Smith) EST database (http://www.ncbi.nlm.nih.gov/nucest version 2015,

translated in the six reading frames and filtered to a minimum of 80 amino acids; 392,538 entries); the *Zea mays* database (from maizegdb, version v5a; 136,770 entries) and a standard contaminant database (55 entries). Identified peptides were filtered using X!tandemPipeline v3.3.4 (Langella *et al.,* 2016) with the following criteria: peptide E-value less than 0.03, minimum 2 peptides per protein, protein E-value less than 10^{-4}. Unassigned spectra were subjected to *de novo* identification using denovopipeline v1.5.1 (http://pappso.inra.fr/bioinfo/denovopipeline/), that allows the selection of unassigned spectra of good quality and their submission to pepnovo (v2010117, Frank 2005). Spectrum quality score was set to 0.2 and pepnovo score to 70. *De novo* sequences were then aligned to the same databases as for X!Tandem search using Fasts.v36.06 (Mackey *et al.,* 2002). Proteins with a homology score of less than 10^{-4} were validated. The biological and analytical reproducibility were addressed by quantitative western blot (see next section).

Identified EST sequences obtained from digested peptides were submitted to a BLAST procedure (BLASTX, NCBI). The resulting protein was characterized by the name, the source and the molecular weight and an E-value/log E-value coverage. In order to calculate the coverage per cent of a peptide, the EST sequence was translated into a protein sequence using the Expasy Translate tool (http:// www.expasy.org/tools/dna.html).

Using a bioinformatic approach, the protein sequence was searched against the protein database to identify homology. Significance of any similarity hits in the

public databases was determined by combination of the expected value (should be below 10^{-4}) bit score and manual inspection. The multiple sequence alignments were generated using MUSCLE in SeaView version 4 (Gouy *et al*., 2010) and exported in Phylip format. The best substitution model for the alignment was determined using Jmodel test (Posada, 2008). The workflow of the protein identification and characterization using LC-MS/MS data is given in **Figure 3.3**.

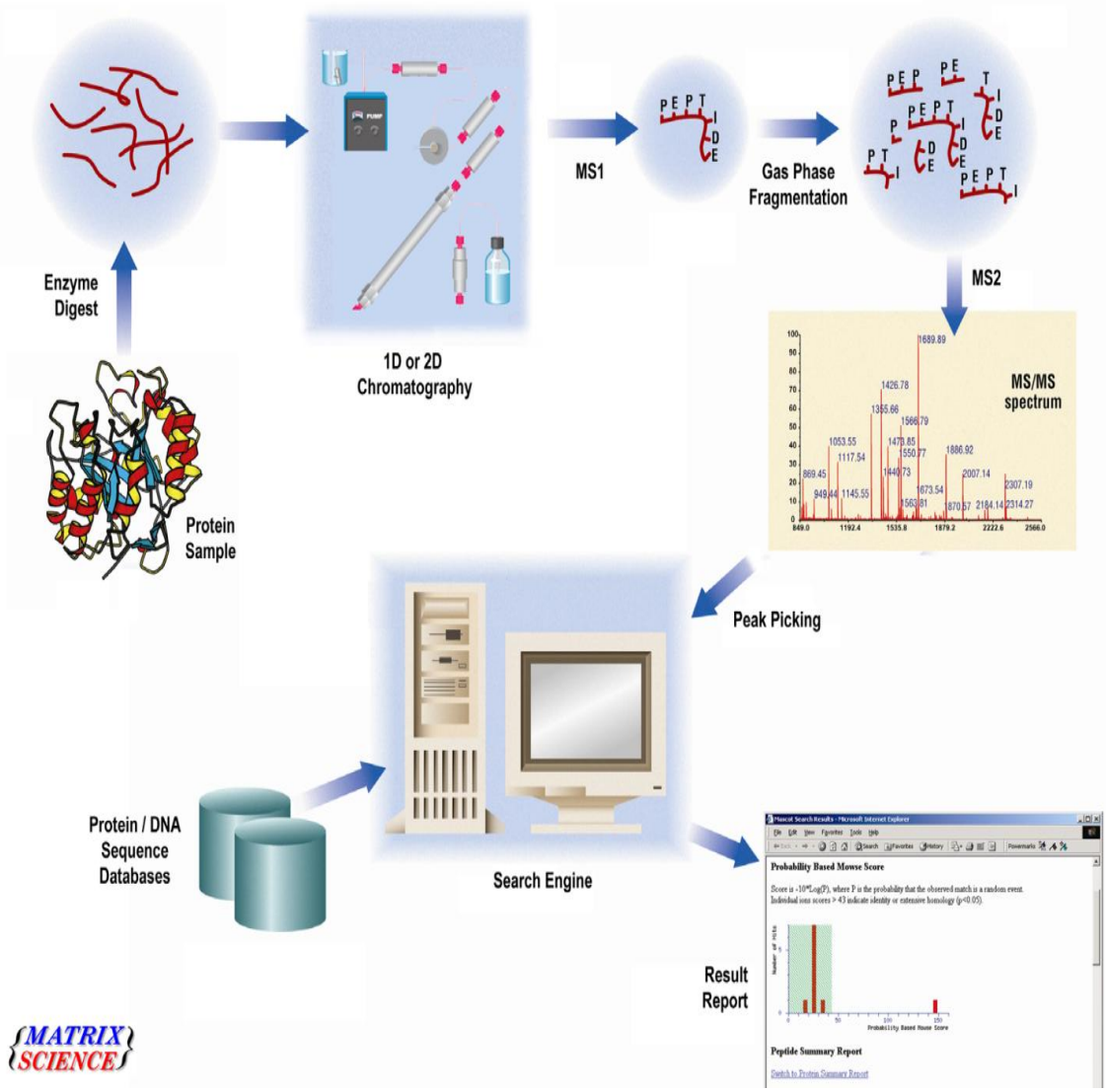

Figure 3.3 Experimental workflow for protein identification and characterization using LC-MS/MS (Source: Matrix Science © 2007-2014). 3.2.8

3.2.8 Western blot analysis of the protein eluent inducing parasitoid oviposition

In order to confirm that the proteins purified and identified were indeed α-amylases, a western blot using an antibody specific to *Drosophila melanogaster* Meigen

(Diptera: Drosophilidae) α-amylase was performed (See the schematic methodology of Western blotting procedure, **Figure 3.4**). Ten microliters of each heat denatured protein sample (of about 500 ng/µl) were loaded on a NuPAGE 4-12% Bis-Tris Gel (Invitrogen) and electrophoresis conducted for one hour at 200 volts in MOPS buffer. The proteins were then transferred to an iBlot Gel Transfer Nitrocellulose membrane (Invitrogen) using the iBlot Gel Transfer Device (Invitrogen). The membrane was washed in 1X PBS for 20 minutes, after which it was incubated for 90 minutes in a milk solution (1X PBS, 0.1% Tween, 5% milk) in order to saturate the membrane with proteins. The membrane was then incubated with the primary anti *Drosophila melanogaster* α-amylase antibody (gift from Dr B. Lemaitre) according to Chng *et al.* (2014), it was diluted 1000-fold in a solution of 1X PBS, 0.1% Tween, 1% milk) for several hours. After this step, the membrane was washed six times in 1X PBS, 0.1% Tween before incubating with the secondary antibody (Anti guinea pig IgG Peroxidase, Sigma A7289), 1000-fold diluted in a solution of 1X PBS, 0.1% Tween, 1% milk, for one hour. The membrane was then washed 3 times in 1X PBS, 0.1% Tween. The peroxidase activity was detected with Amersham ECL Prime Western Blotting Detection Reagent (GE Healthcare) and recorded on an Odyssey FC imager.

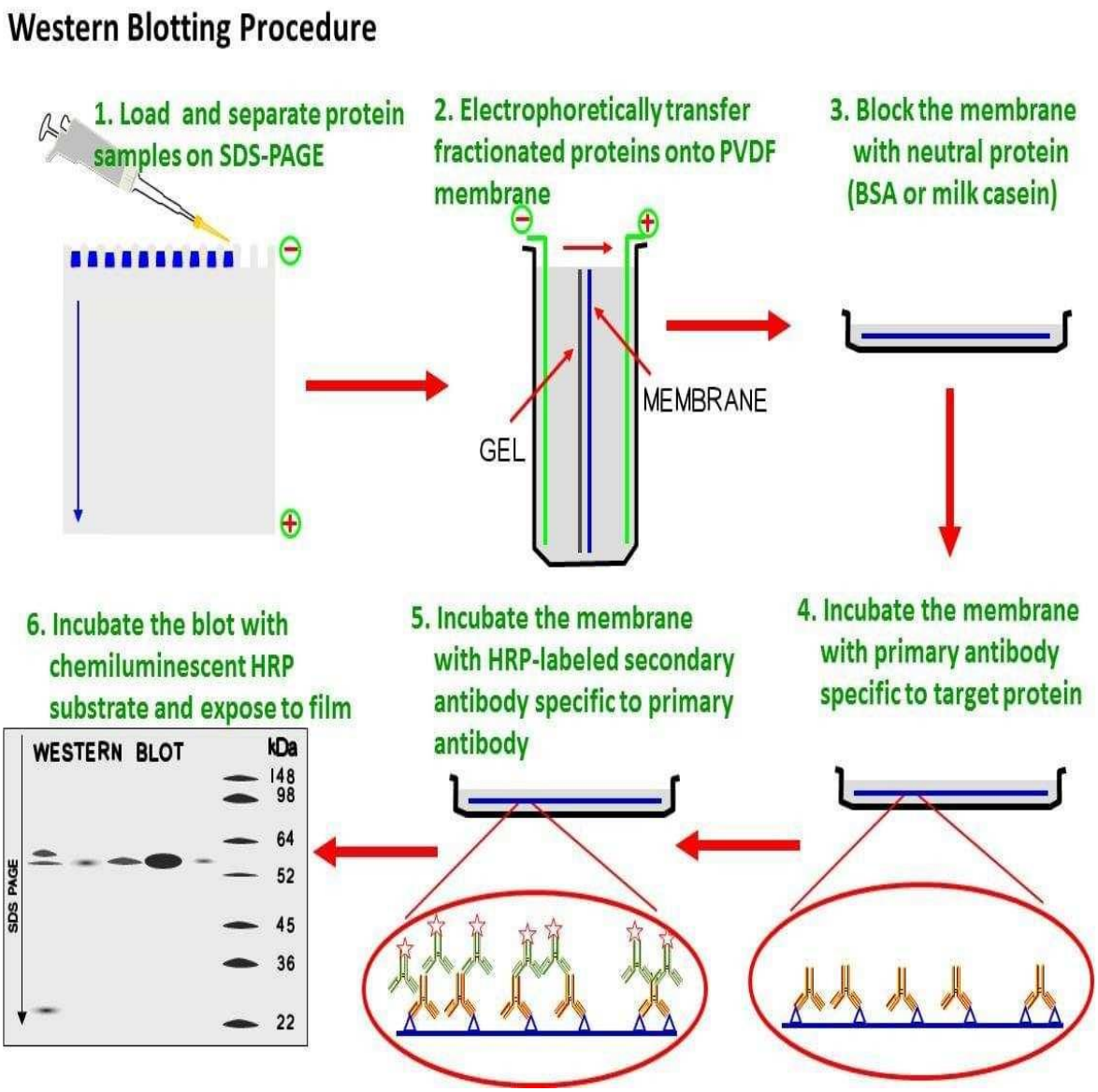

Figure 3.4 Schematic experimental workflow for western blotting procedure (source: https://www.creativebiomart.net/resource/principle-protocol-western-blot-protocol-351.htm).

3.2.9 Determination of the sources of assayed α-amylases

To confirm the identity of the protein (α-amylase) mediating host acceptance and oviposition by *C. flavipes*, synthetic α-amylases from different organisms commercially available or from those found in our mother laboratory at Gif-sur-Yvette were used. The following were the sources of various α-amylases used for the assays; the micro-organism, *Aspergillus oryzae* (Ahlburg) E. Cohn, the insects, *Drosophila melanogaster* and *Chilo suppressalis* (Walker), and the pig as a mammal (porcine pancreas). The α-amylases from *A. oryzae* and porcine pancreas were obtained commercially from Sigma No A9857 and A3176, respectively. The α-amylase from *D. melanogaster* was produced on the yeast *Pichia pastoris* (Guillierm) Phaff, as described by Commin *et al.,* (2013). The α-amylase of *C. suppressalis* was also produced in *P. pastoris*; the coding sequence of the *C. suppressalis* amylase gene 108827 was synthetized (Eurofins MWG), with replacement of the signal peptide by the one of *D. melanogaster* amylase **(Appendix 1).** An amylase from *C. suppressalis* was assayed because its genome was available, as opposed to the one of *C. partellus*. In addition, to check if the behavioural activities of *C. flavipes* triggered by α-amylase was due to the structural conformation and/or the catalytic activity, an inactive α-amylase with no change in its structural conformation synthesized at Gif-sur-Yvette was used. An inactivated α-amylase of *D. melanogaster* was obtained by a single replacement of the crucial catalytic residue Aspartate 186 by an asparagine, which does not change the structural conformation (Aghajari *et al.,* 2002). Another inactive α-amylase, named

amyrel, with an alteration in its structural conformation differing by 42% from the classical α-amylase protein of *D. melanogaster* was also synthesized at Gif-sur-Yvette (Da Lage *et al.,* 1998). A colorimetric activity test (Infinity Amylase Reagent, Thermo Fisher) was used to confirm that these inactive α-amylases of *D. melanogaster* had no catalytic activity.

As controls, the following extracts were also used in behavioral bioassays: the buffer used to solubilize the α-amylases; the glycogen (at 17g/L) used to purify the synthesized α-amylases of *D. melanogaster* and *C. suppressalis*; the corn starch (at 17g/L); the inactivated α-amylase of *D. melanogaster* and amyrel; and the degradation product of the α-amylase, i.e. maltose (at 34g/L).

3.2.10 Statistical analysis

The Marascuilo's procedure, i.e. a pairwise comparison after Pearson's Chi-square test to test the overall significance differences, was used to separate the proportions of wasps that exhibited positive responses (i.e. antennation + stinging attempts) (Marascuilo 1966).

3.3 Results

The oral secretions of larvae fed on maize stems mediated a significantly higher response (i.e. antennation and stinging attempt) of *C. flavipes* than the fecal pellets, whereas no behavioural response was observed with maize stem extracts **(Table 3. 1).**

Table 3.1 Behavioural responses of the parasitoid *Cotesia flavipes* to maize stem extracts, fresh fecal pellets and oral secretions of *Chilo partellus* larvae fed on *Zea mays*.

Type of sample	Antennation + stinging attempt (%*, n=30)
Maize stem juice	0 (0) a
Fresh fecal pellets	40.0 (12)b
Oral secretions	93.3 (28)c

***** After Pearson's Chi-squared test (Chi-square = 21.773; df = 2; p < 0.0001), percentages with different letter are significant at 5% level according to the Marascuilo's procedure (multiple proportions comparison).

The percentages of individuals that exhibited antennation and stinging attempt are given followed in parenthesis by their total number over thirty individuals tested.

The strongest response by *C. flavipes* was obtained on oral secretions of *C. partellus* followed by those of *S. calamistis*, and the non-hosts *B. fusca* and *S. nonagrioides* were used **(Tables 3.2**).

Table 3.2 Behavioural response of *Cotesia flavipes* to oral secretions of host and non-host larvae fed on *Zea mays.*

Oral secretions of different species	Antennation + stinging attempt

	(%*, n=30)
Chilo partellus (host)	90.0 (27)c
Sesamia calamistis (host)	46.7 (14)b
Busseola fusca (non-host)	16.7 (5)a
Sesamia nonagrioides (non-host)	13.3 (4)a

* After Pearson's Chi-squared test (Chi-square = 15.348; df = 3; p = 0.001542), percentages with different letter are significant at 5% level according to the Marascuilo's procedure (multiple proportions comparison).

The percentages of individuals that exhibited antennation and stinging attempt are given followed in parenthesis by their total numberover thirty individuals were tested.

The oral secretions from *C. partellus* larvae previously fed on *P. purpureum* triggered similar number of responses as those from maize-fed larvae **(Table 3.3).** By contrast, oral secretions of larvae fed on artificial diet did not elicit any behavioural activity. Likewise, the oral secretions from larvae starved for 48h as well as those from larvae fed on maize stems treated with proteinase K did not elicit any behavioural response.

Table 3.3 Behavioural response of *Cotesia flavipes* parasitoid to oral secretions of *Chilo partellus* stemborer larvae.

Type of sample	**Antennation + stinging attempt (%*, n=30)**
Larvae fed on *Zea mays* stems	90.0 (27)c
Larvae fed on *Pennisetum purpureum* stems	86.7 (26)c
Larvae fed on artificial diet	0 (0)a
Starved larvae	0 (0)a
Larvae fed on maize stems treated by proteinase K	0 (0)a

* After Pearson's Chi-squared test (chi-square = 57.14; df = 4; $p < 0.0001$), percentages with different letter are significant at 5% level according to the Marascuilo's procedure (multiple proportions comparison).

The percentages of individuals that exhibited antennation and stinging attempt are given followed in parenthesis by their total number, over thirty individuals were tested.

The electrophoretic analyses of the active oral secretions (larvae fed on maize) yielded more intense electrophoretic bands (i.e. higher quantities of proteins) than those of the inactive oral secretions (**Figure 3.5**). In a one-dimension gel electrophoresis under non-denaturing conditions, the oral secretion of larvae fed on maize stems showed seven major electrophoretic bands (**Figure 3.5**). Each major

band was manually excised from the gel, (**Figure 3.6**) and further used to test for the behavioural responses as shown in (**Table 3.2.).**

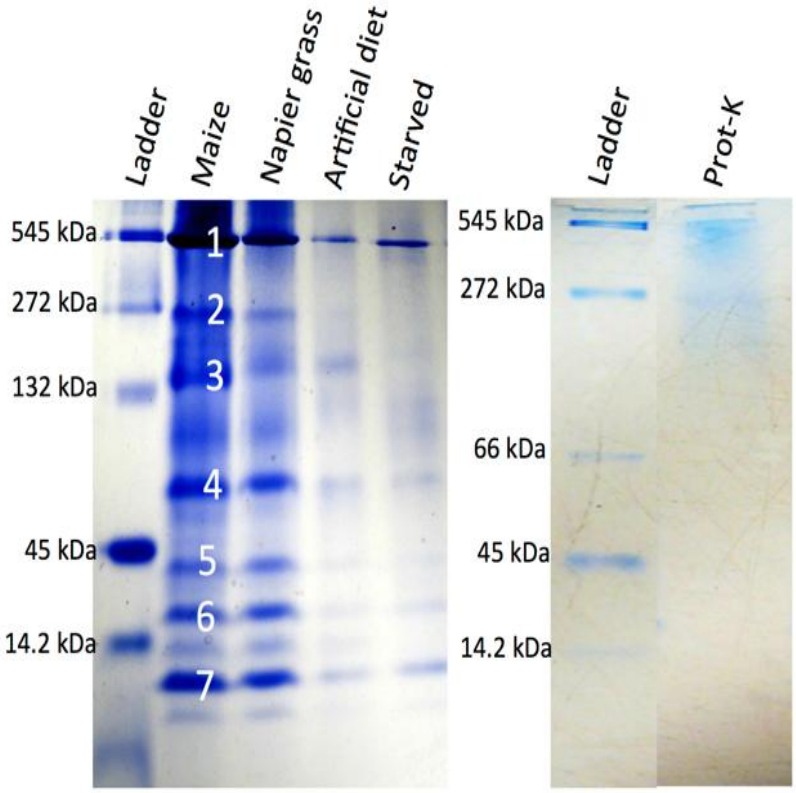

Figure 3.5 Native gel electrophoretic analysis of oral extracts of *Chilo partellus* larvae previously fed on different plant diets.

Protein samples in the stemborer larval extracts were separated by 1D gel, 7% native Onstein-Davis discontinuous (Tris-glycine) PAGE before Coomassie staining. A comparison of the proteins extracted from oral secretions of *Chilo partellus* larvae fed on different diets are presented in **Figure 3.5**: ladder: Sigma molecular weight markers; In lane 1: proteins from oral secretions of *Chilo partellus* larvae fed on *Zea mays* stems (Maize) (each main electrophoretic band [noted 1 to 7 on the gel] were individually extracted from the gel (see **Figure 3.6**) under non-denaturing conditions and tested towards *Cotesia flavipes* (see **Table 3.4**)); lane 2: proteins from oral secretions of *Chilo partellus* larvae fed on *Pennisetum purpureum* stems (Napier grass); lane 3: proteins from oral secretions of *Chilo partellus* larvae fed on artificial diet (Artificial diet); lane 4: proteins from oral secretions of *Chilo partellus*

(Starved). For each lane, 15µl of the oral secretion was loaded after concentrating and before quantification of the samples (Bio Rad Mini-PROTEAN® Electrophoresis System). No band was obtained after proteinase K treatment (Prot-K).

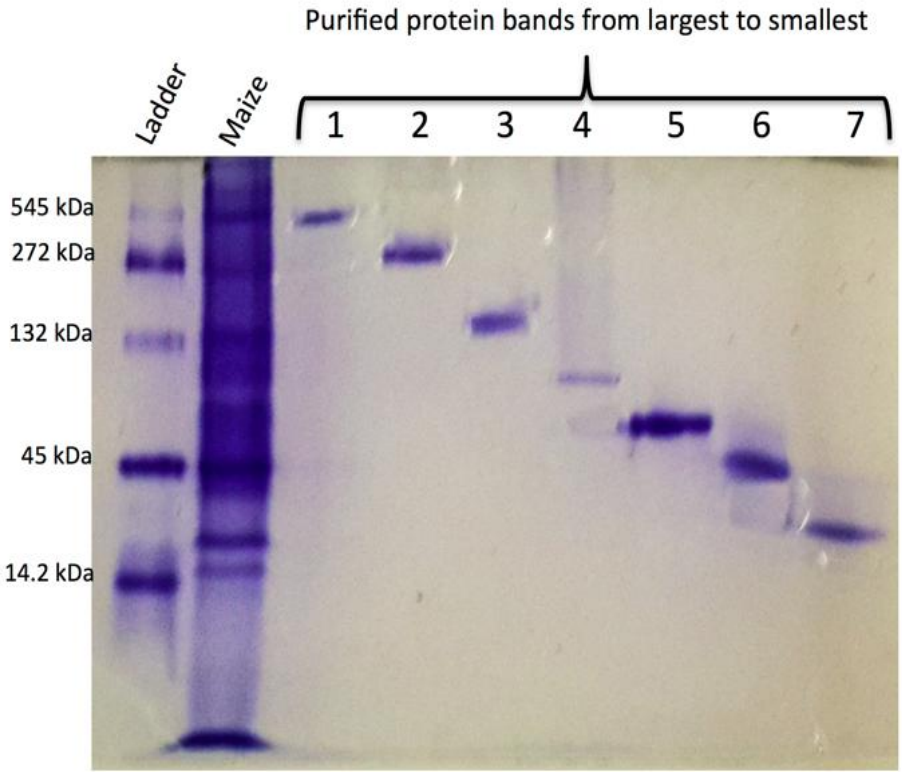

Figure 3.6 Analysis of *Chilo partellus* larval oral secretions in a native gel system. Individual protein bands were purified from the gel of maize-fed *Chilo partellus* larval oral secretion.Protein samples were separated by 1D gel, 7% native Onstein-Davis discontinuous (Tris-glycine) PAGE before Coomassie staining. Individual protein band purified from the gel of regurgitant of *Chilo partellus* fed on maize. Lanes: 1 molecular weight marker (sigma Aldrich), 2 regurgitants from *Chilo partellus* fed on maize (Maize); lanes 1-7 bands purified and tested for activity against *Cotesia flavipes* (Hoefer™ Mini Vertical Electrophoresis Systems (Fisher Sci.com) (see Table 3.4).

Out of the seven protein bands generated on the native gel system, only two bands elicited activity. Band no 4 (≈ 50 kDa), triggered 90% response in all of the parasitoids tested (**Table 3.4**). This protein band was extracted and was subjected to further analysis and identification.

Table 3.4 Behavioral response of *Cotesia flavipes* parasitoid to the components of the seven main electrophoretic bands obtained from the oral secretions of *Chilo partellus* larva.

Band tested	Antennation + stinging attempt (%*, n=10)
1	0 (0)a
2	0 (0)a
3	30 (3)a
4	90 (9)b
5	0 (0)a
6	0 (0)a
7	0 (0)a

* After Pearson's Chi-squared test (Chi-square = 25.61; df = 6; p = 0.00026), percentages with different letter are significant at 5% level according to the Marascuilo's procedure (multiple proportions comparison).

The percentages of individuals that exhibited antennation and stinging attempt are given followed in parenthesis by their total number, over thirty individuals tested.

In order to identify the active protein band that induced the highest behavioural response, proteins from band No 4 were digested and the resulting peptide mixture analyzed by liquid chromatography-mass spectrometry. Database search allowed the identification of two distinct maize proteins with 5 and 2 peptide sequences respectively, while *de novo* sequencing allowed the identification of 22 peptides that matched the accession gi|295290041|gb|FP379314.1|FP379314| of the *S. frugiperda* database of mid gut cDNA sequences (**Figure 3.7**). The protein sequence blasted significantly with α-amylase superfamilies (**Figure 3.8**).

>gi|295290041|gb|FP379314.1|FP379314|Frame3 FP379314 *Spodoptera frugiperda* cDNA library, induced midguts *Spodoptera frugiperda* cDNA clone Sf2M05200-5-1, mRNA sequence

VIVHGVISVRMFRLILCLAAVTLALAYKNPHYASGRTTMVHLFEWKWDDIA
RECETFLG
PRGYGGIQISPPNENLAIWSRQRPWWERYQPISYRLVTRSGNEQQFANMVR
KCNDAGVRIYVDAIINHMTGTWNENTGTGGSTADFGNWGYPGVPYGRND
FNWPHCVIQGHDYGCCADRVRNCELSGLKDLNQGNEYVRQQIVNYMNHLI
NLGVAGFRIDAAKHMWPGDLRVIYDRLHNLNTAHGFPSGARPYIYQEVIDL
GGEIISRDEY

Figure 3.7 Best de novo protein sequence associated with EST specific to *Spodoptera frugiperda* database (see appendix 2).

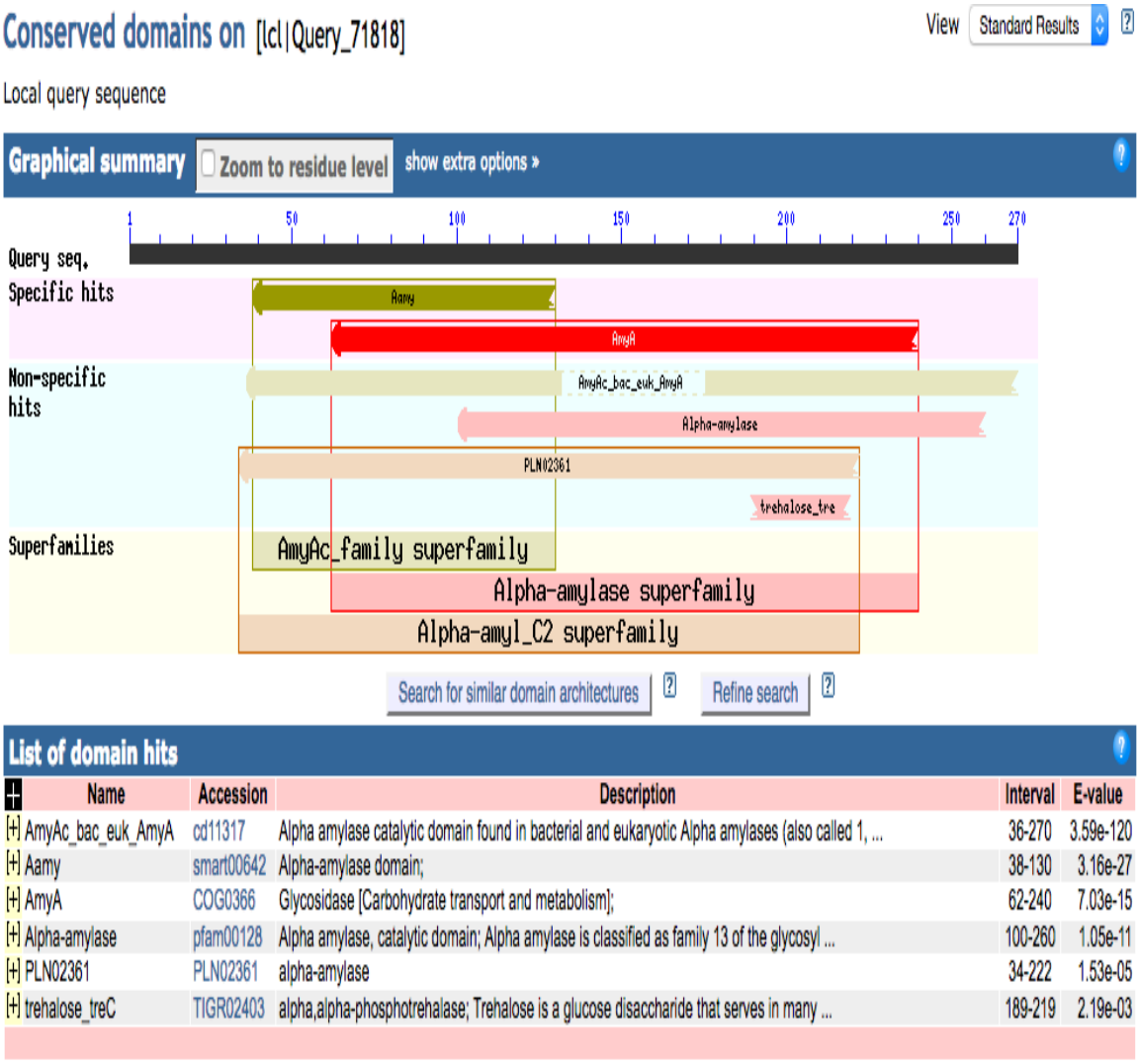

Figure 3.8 The pre-computed domain annotation for the best de novo protein sequence as **indicated in Figure 3.7).**

The protein data bank of the BLAST ® online software (https://blast.ncbi.nlm.nih.gov/Blast.cgi) was used. The section circled in red provides the functional label that has been assigned to the subfamily domain (Marchler-Bauer *et al.*, 2017).

A blast search performed on the protein sequence (**Figure 3.7)** confirmed that the above best *de novo* sequence encodes homology of α-amylase gene. The data (NCBI blast software) demonstrated a high homology with α-amylase of other lepidopteran species sharing 99% amino acid identity. Other data given by NCBI blast revealed identity of at least 80% between α-amylase of lepidopteran larvae indicating their high homology. The highest probability score was for *Mamestra configurata* α-amylase E-value of -174 accession number AEA76309.1 followed by that *Bombyx mori* α- amylase E -value of -168 accession number NP001166624.1 as shown in **Figure 3.9**. The least expected values, the number of matches expected by chance between the query sequence and random or unrelated database sequence was (e value -153), value that is below 10^{-4} and the identities are above 85% showing the high relatedness of the identified protein to α-amylase in the public database.

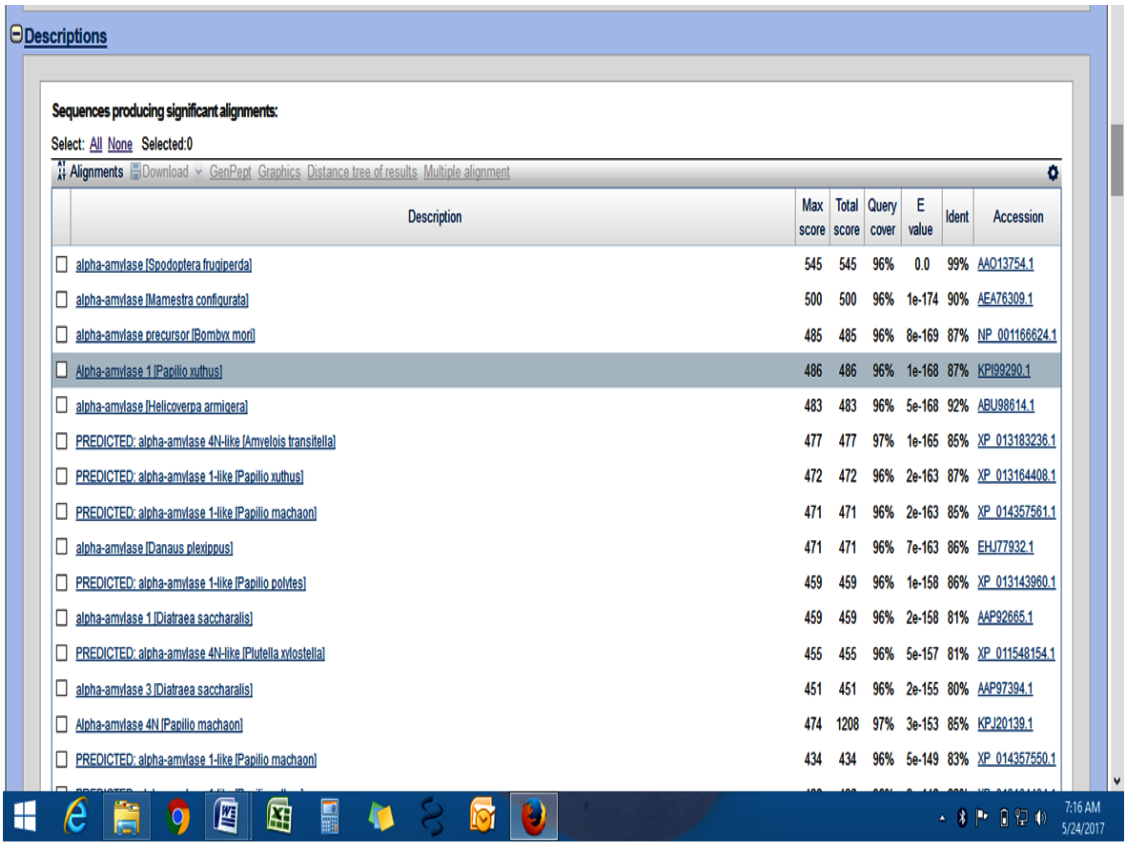

Figure 3.9 Homology search of the protein sequence of *Chilo partellus* best de novo sequence, which was submitted to the blast search (NCBI).

Protein homology was considered to be significant when the alignment involved most of the sequence length or a significant proportion of identities (**Figure 3.10**). The α-amylase sequence of *C. partellus* appears to be well conserved within the chosen lepidopteran species, *Mamestra configurata* and *Helicoverpa armigera* (Lepidoptera: Noctuidae). In the A terminal region only few amino acid residues at position, 21, 22, and 23 are different between the *C. partellus* best *de novo* protein sequence and that of. *M configurata* and *H. armigera*. Since the α-amylases of most lepidopteran species are directly related to each other, it is likely that their similarities at most positions are due to their relatedness.

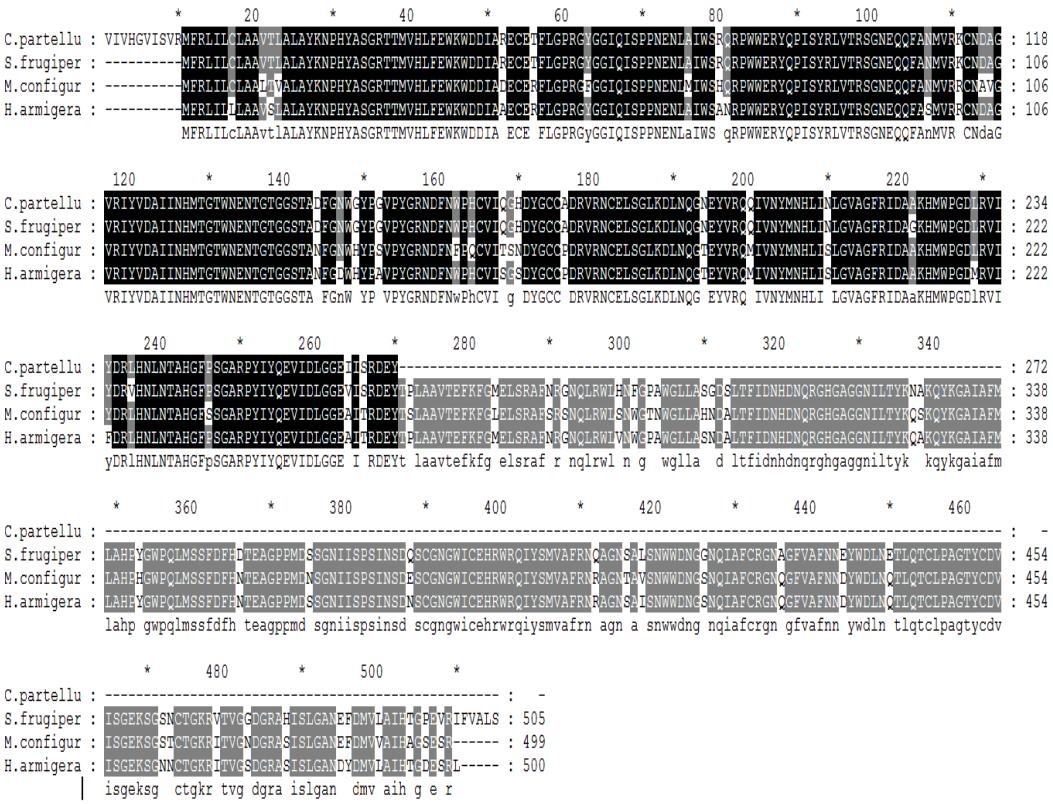

Figure 3.10 Pair-wise protein sequence alignment of the best de novo protein sequence of *Chilo partellus* associated with EST specific to *Spodoptera frugiperda* database sequence with other two lepidopteran α-amylase sequences from the public database. Identical and conserved amino acid sequences are shown in black.

The confirmation of α-amylase assigned as electrophoretic band 4 was done by western blot analysis (**Figure 3.11**). The anti-α-amylase of *D. melanogaster* linked mostly with this band (band no 4) (≈ 50 kDa) of the oral secretion of *C. partellus* and with that extracted from the gel.

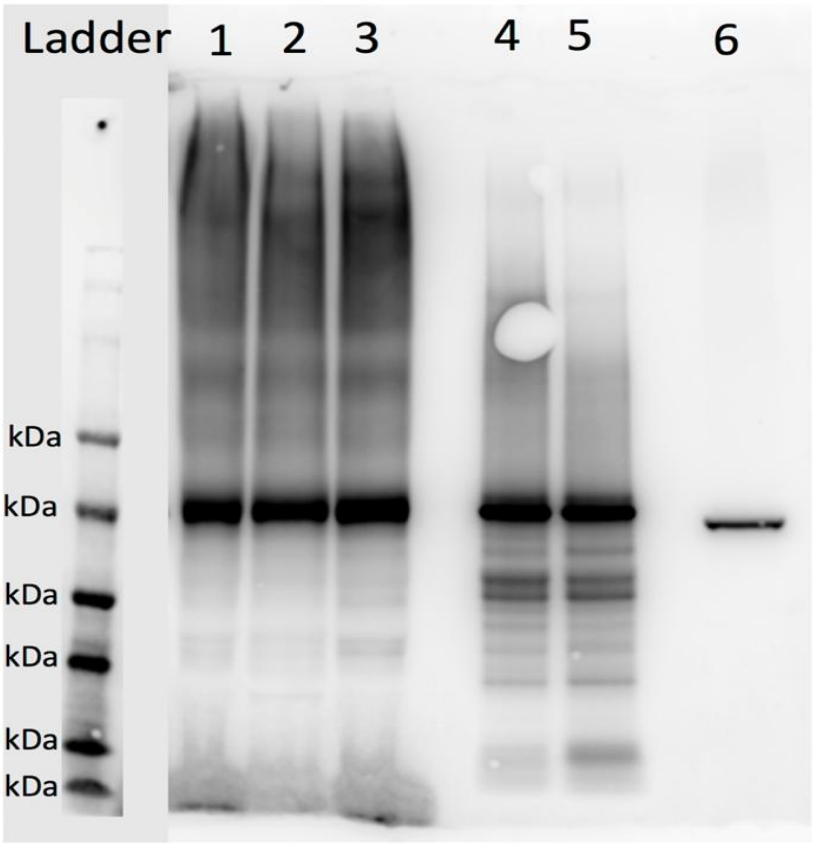

Figure 3.11 Western blot analysis using a *Drosophila melanogaster* α-amylase-specific antibody. Ladder: molecular weight markers (pre-stained SeeBlue Plus2, Thermo Fischer); 1, 2 and 3: oral secretions from *Chilo partellus* larvae fed on maize stems; 4 and 5: band n°4 of Figure 3.6 which was extracted from the gel and used for Western Blot analysis; 6: α-amylase from *Drosophila melanogaster*.

The activity elicited by different α-amylases from different origins confirmed the involvement of this enzyme in *C. flavipes* antennation and stinging attempts **(Table 3.5**). In contrast, the control protein BSA, for example, did not induce any

behavioural response in the wasp, and neither did amyrel a stereoisomer (differ in structural conformation) in contrast to the classical α-amylase protein of *D. melanogaster*. In addition, the buffer solution, the glycogen, the corn starch as well as the maltose used in the experiment did not induce any behavioural response in the wasp. The α-amylases from *D. melanogaster* and *C. suppressalis* induced the highest behavioural responses in *C. flavipes* antennation and stinging attempts (**Table 3.5**). To determine whether the behavioural activity of *C. flavipes* triggered by α-amylase arose from the structural conformation and/or the catalytic activity, an inactivated α-amylase from *D. melanogaster* without any structural conformation was used. Suprisingly this inactivated α-amylase did still induce behavioural responses indicating that the conformation rather than the catalytic activity of α-amylase is responsible for the host acceptance process by *C. flavipes*.

Table 3.5 Behavioural responses of *Cotesia flavipes* parasitoid to different proteins (at 300-500 ng/µl) as well as to buffer, maltose, glycogen and corn starch.

Proteins tested	Antennation + stinging attempt (%*, n=30)
α-amylase from *Aspergillus oryzae*	40 (12)bc
α -amylase from pig	20 (6)b
α -amylase from *Drosophila melanogaster*	63.3 (19)c
α -amylase from *Chilo suppressalis*	46.7 (14)bc
Inactive α-amylase from *Drosophila melanogaster*	43.3 (13)bc
Amyrel	0a
BSA	0a
Buffer	0a
Maltose	0a
Glycogen	0a
Corn starch	0a

* After Pearson's Chi-squared test (Chi-square = 71.92; df = 1; $p < 0.0001$), percentages with different letter are significant at 5% level according to the Marascuilo's procedure (multiple proportions comparison).

The percentages of individuals that exhibited antennation and stinging attempt are given followed in parenthesis by their total number over thirty individuals were tested.

3.4 Discussion

The findings show that the oral secretions of the larvae of lepidopteran stem borers harbour active compound(s) that mediate host acceptance for oviposition in *C. Flavipes* parasitoid. These secretions allow *C. flavipes* females to discriminate among hosts and non-host larvae. The most active compound isolated from the oral secretion from the larvae of the natural host *C. partellus* was identified as the protein α-amylase. Polypeptides and proteins have previously been reported as chemical signals in the host selection process of hymenopteran parasitoids (Weseloh 1977; Bénédet *et al.,* 1999; Gauthier *et al.,* 2004). However, their identity has not yet been elucidated. The use of synthesized α-amylases in this study aided in the determination of the identity of the active proteins present in the oral secretion of *C. partellus* that mediates host acceptance for oviposition. The α-amylases that induced behavioural responses of *C. flavipes* possessed a similar molecular weight as those of *D. melanogaster* (51 kDa), *C. suppressalis* (≈ 50 kDa), *A. oryzae* (51 kDa), and pig (50 kDa, suggesting that the size of the active protein is important. However, a different protein such as BSA with a similar molecular weight but which was used as the control did not induce any behavioral response indicating that the conformation of the protein rather than its weight is important in host acceptance for oviposition of

the parasitoid. In fact, amyrel, an inactive α-amylase of *D. melanogaster*, and a stereoisomer of the the classical α-amylase protein of *D. melanogaster*, did not induce any behavioural response in *C. flavipes* in contrast to the inactive α-amylase but a stereoisomer of the active α-amylase form of *D. melanogaster*, exhibited activity. This indicates that it is the conformation of the α-amylase rather than its catalytic site that induces this behavioural activity. It is therefore prudent to suggest that *C. flavipes* can perceive this protein through its sensorial equipment, antennae or tarsi (Obonyo *et al.,* 2011).

However, the results presented herein showed that the *C. flavipes* parasitoid response to α-amylase is not a host specific since similar responses by the parasitoid were also observed when α-amylases from *D. melanogaster*, *A. oryzae*, *C. suppressalis* and in a lowest response to α-amylase from pigs were used. This is probably due to the fact that *C. flavipes* is a generalist, parasitizing larvae of more than 30 Lepidoptera species including *C. suppressalis* (https://www.cabi.org/isc/datasheet/5951). It may also be explained that, in its natural habitat, *C. flavipes* has no chance of exposure to the α-amylases of *D. melanogaster*, *A. oryzae* or pigs clearly explaining why the amylases are not utilised by the parasitoid. In addition, the response of *C. flavipes* females to α-amylase is not binomial (yes or no) but gradual according to the source of the enzyme and, in this study, the rate of response was strongest in insects such as *D. melanogaster* and *C. suppressalis.* Consequently, the parasitoid`s response to α-amylase should allow them to discriminate between hosts, being more intensely favourable towards their natural hosts as compared to their non-hosts. The amino

acid sequences of various α-amylases of animals show high variability at the protein level (Da Lage *et al.*, 2002). This diversity of α-amylase proteins may have adaptive or functional significance in their diverse utilisation by insects. For example, the stemborers use their own forms of α-amylase proteins for feeding process. Thus, it may be suggested that the different variations of α-amylase proteins in the stemborer pests may be linked to the different host plants on which they feed upon and the parasitoids exploit this variation in the structure of α-amylase to discriminate stemborer hosts in different habitats where the parasitoids inhabit. In this study, a strong behavioral response of *C. flavipes* to the oral secretions of its natural host *C. partellus* was observed.

On the other hand, an intermediate response to the oral secretions of *S. calamistis* and similarly weak responses to the oral secretions of the non-hosts were also observed. These multiple results might be positively correlated with the different α-amylases present in these stemborer pests. In addition, the paraistoid *Cotesia flavipes* complex is composed of four species, namely *C. chilonis* (Matsumura), *C. flavipes* Cameron, *C. nonagriae* (Olliff) and *C. sesamiae* (Cameron). All these species are gregarious endoparasitoids of a few families of lepidopteran stem borers including the Crambidae, Pyralidae, and Noctuidae that attack plants in the Poales family (Poaceae, Typhaceae and Cyperaceae) (Kaiser *et al.*, 2017b). A large diversity of *Cotesia* spp. with a strong host specificity, particularly on *Busseola* spp. and *Chilo* spp. has already been described (Mailafiya *et al.*, 2009). Consequently, the parasitoid`s response to host kairomone such as α-amylase should allow them to

discriminate among hosts. In this context, this study suggests that α-amylases from oral secretions of the caterpillar hosts are good candidates for determining an evolutionary solution to host acceptance for oviposition by the *C. flavipes* complex. However, additional studies are needed to demonstrate whether this protein is responsible for the specific host-parasitoid association in the *Cotesia flavipes* species-group of parasitoids.

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CHAPTER FOUR

4.0 DETERMINATION OF HOST SPECIFIC VARIABILITY OF THE KAIROMONE INVOLVED IN HOST RECOGNITION AND ACCEPTANCE BY DIFFERENT *COTESIA* PARASITOID SPECIES/ POPULATIONS

4.1 Introduction

Parasitoids comprise the major biological control agents of insects pest (Pimentel *et al.,* 1992; Tilman *et al.,* 2001; Lazarovitz *et al.,* 2007; Godfray *et al.,* 2010). Among them, the parasitoids in the order of Hymenoptera, contains the most diversified species with approximately 50,000 species reported to exist, in contrast to only 15,000 species in Diptera and approximately 3000 in other orders having been reported (Quicke, 1997). To reproduce successfully, the parasitoids need to overcome both the behavioural and physiological defenses of their hosts (Kaiser *et al.,* 2017a). These host's defenses which co-evolved with the parasitoids may, but not necessarily, be linked to host range changes and the appearance of host races within different parasitoid species (Kaiser *et al.,* 2017a). These underlying mechanisms provide insight in evolutionary biology and may be important in the improved selection of parasitoids for bio-control programme.

The ability of parasitoids to efficiently utilize cues from their habitat as well as to distinguish suitable from nonsuitable hosts determines their field efficiency of

parasitism (Wajnberg *et al.,* 2008; Wajnberg & Colazza, 2013). During hosts location, parasitoids initially use long (i.e. at a distance) and short-range chemicals that emanate from host habitat and subsequently, those that are directly present on the host and its feeding products (Wajnberg *et al.,* 2008; Wajnberg & Colazza, 2013). However, long-range chemicals from the parasitoid's habitat do not generally provide sufficient and reliable information on the suitability host's status (Vet, 1999). In contrast, host associated chemicals as well as chemicals from feeding products are directly used during host-contact evaluation by the parasitoids. These chemicals generally allow the parasitoids to assess both the quality and status of the herbivore (Lewis & Martin, 1990; Vinson, 1991; Godfray, 1994; Wajnberg *et al.,* 2008; Wajnberg & Colazza, 2013). Moreover, the structure and quantity of these semiochemicals, which have been reported to vary according to host's species, developmental stage, size, condition and diet greatly, influence host acceptance and selection by the parasitoids (Vinson, 1991; Röse *et al.,* 1997; Wajnberg *et al.,* 2008; Wajnberg & Colazza, 2013).

Among the parasitoids, *Cotesia* spp. has been reported as the most diverse genera in the Braconidae family (Kaiser *et al.,* 2017a). Although it has been suggested that *Cotesia* species may appear to have broad host ranges, recent ecological studies have revealed a hidden complexity consisting of an assemblage of populations with more restricted host ranges (Branca *et al.,* 2011; Kaiser *et al.,* 2017b). Whereas recent studies have revealed that variations in virulence genes accounts for differences in host range and in the degree of specialization towards a host (Gauthier

et al., 2018), little information is available as pertains to the variations in functions involved in specific host recognition and acceptance.

The *Cotesia flavipes* species-monophyletic group is composed of small wasps of four sister species which include *C. chilonis* (Matsumura), *C. flavipes* Cameron, *C. nonagriae* (Olliff), and *C. sesamiae* (Cameron). All these species are gregarious endoparasitoids of crambid, pyralid and noctuid stem borers that feed on Poaceae, Typhaceae and Cyperaceae plant species (Kaiser *et al.,* 2017b). After mating, these small wasps frequently lay several eggs into the caterpillar host body., These parasitoids use a domesticated virus called bracovirus (PolyDNA virus) to inhibit the immune response of the caterpillars once the gain entry into their hosts. The bracoviruses are located in the wasp ovaries and are integrated in the genome of the wasp; and they are injected into the caterpillar together with the eggs during the parasitism process (see Kaiser et al. [2017a] for review).

Cotesia flavipes Cameron is wide-spread in Asia and was introduced into Africa to control the invasive Asian crambid *Chilo partellus* Swinhoe (Overholt *et al.,* 1994a & b). The parasitoid parasitizes the larvae of more than 30 Lepidoptera species including the crambids *C. partellus* and *Chilo suppressalis* (Walker) as well as the African noctuid *Sesamia calamistis* Hampson (https://www.cabi.org/isc/datasheet/5951). The *C. flavipes* population brought into Africa for classical biological control was already specific to *C. partellus* in Asia (Muihead *et al.,* 2012).

A related parasitoid, *Cotesia sesamiae* is widespread in Sub-Saharan Africa and is regarded as the major and common parasitoid of *Busseola fusca* and *S. calamistis*. However, it has been reported that *C. sesamiae* parasitism success depends greatly on the host species and parasitoids populations (Mochiah *et al.,* 2002; Gitau *et al.,* 2010). Two important factors have been reported to contribute to the differences and hence the performance of *C. sesamiae* populations on stem borer pests across Africa. These factors have been identified to include the symbiotic polyDNA viruses which are responsible for the differences in virulence of *C. sesamiae* population on *B. fusca* (Gitau *et al.,* 2010) and the bacteria *Wolbachia* which has been associated with cytoplasmic incompatibilities between populations of *C. sesamiae* populations (Mochiah *et al.,* 2002). The Kenyan *C. sesamiae* species have been categorised based on their inhabited regions, as the coastal and inland populations. In contrast to the *C. sesamiae* population from Mombasa - coastal (*Cs*-Coast), the *C. sesamiae* population from Kitale – inland (*Cs*-Inland) has been reported to develop in *B. fusca*, a predominant stemborer species of the highlands. However, both parasitoid species have been reported to successfully develop in the noctuid *S. calamistis*, the main host of *C. sesamiae* population from Mombasa - coastal Kenya (Ngi-Song *et al.,* 1995). The *Cs*-Inland parasitoid population is commonly present in the wetter highland regions, where its host *B. fusca* occurs, but is absent in the dry and warmer regions where *Cs*-Coast and *C. flavipes* populations predominates (Mailafiya *et al.,* 2010; Mwalusepo *et al.,* 2015).

The genetic diversity of these *C. sesamiae* populations especially regarding their relationships with spatial, biotic and abiotic ecological factors and the importance of host forces in the evolution of diversity of parasitoid-host interactions has already been reported (Branca *et al.,* 2018). During host searching, *Cotesia sesamiae* and *C. flavipes* generally locate their hosts using plant volatile chemical cues from a distance emitted by plants infested by parasitoid's stemborer hosts. However, it has been reported that these plant volatiles do not convey reliable information regarding the host suitability, but rather provides simple indictors of the presence of herbivores on a particular plant. As a result, it has been reported that *C. sesamiae* and *C. flavipes* have wrongly been attracted to plants infested by unsuitable Lepidoptera stemborers (Potting *et al.,* 1993, 1995; Ngi-Song *et al.,* 1996; Obonyo *et al.,* 2008). Thus, it is only when approaching and touching the host that both parasitoids can properly identify their specific hosts. It has been reported that during host identification process, both parasitoids rely on specific host-produced signals, particularly from the host's oral secretions and which have been demonstrated to give reliable information on the host identity as perceived by the parasitoids tactile and contact-chemoreception (Obonyo *et al.,* 2010a & b).

It has been observed that host selection and acceptance by parasitizing parasitoid females is characterized by two behavioral steps: drumming the body of the host with the antennae (antennation) followed by at least one stinging attempt (i.e., one tentative ovipositor insertion in the host). In the previous chapter, it has been showed that α-amylase present in the oral secretions of *C. partellus* larvae mediates these

common behavioural responses of *C. flavipes* (Bichang'a *et al.*, 2018). However, little is known about the molecular variations in α-amylase that could account for host-range differences between parasitoid species. Therefore in this chapter, the study investigated whether α-amylase can be involved in host recognition and selection by *Cotesia* parasitoids in relation with their respective stemborer hosts. In this context, the two populations of *C. sesamiae* living in Kenya with their respective hosts *B. fusca* and *S. calamistis* as well as *C. typhae* Fernandez-Triana sp., a new species of *Cotesia* that was recently described and parasitizes *Sesamia nonagrioides* (Lefèbvre) (Lepidoptera, Noctuidae) (Kaiser *et al*., 2017a), and the introduced *C. flavipes* and its old association host *C. partellus* were used.

4.2 Materials and methods

4.2.1 Insect rearing

Females of *C. flavipes*, the inland and coastal populations of *C. sesamiae* (thereafter named *Cs*-Inland and Cs-Coast, respectively), as well as *C. typhae* were sourced from laboratory-reared colonies established and maintained at *icipe*, Nairobi, Kenya. The *Cotesia flavipes* colony was initially obtained in 2005 from *C. partellus* larvae collected in maize fields in Mombasa, coastal Kenya. On the other hand, the *Cs*-Inland colony was initially obtained in 2006 from *B. fusca* larvae from infested maize fields in Kitale, Western Kenya while the *Cs*-Coast was initially obtained in 2007 from *S. calamistis* larvae from infested maize fields in Mombasa (coastal Kenya). *Cotesia typhae* colony was initially obtained in 2013 from *S. nonagrioides* larvae infesting *Cyperus dives* at Kobodo in the vicinity of the Victoria Lake, Kenya.

All the four *Cotesia* species, viz, *C. flavipes*, *Cs*-Inland, *Cs*-Coast and *C. typhae* were continuously reared on larvae of *C. partellus*, *B. fusca*, *S. calamistis* and *S. nonagrioides,* respectively, as previously described by Overholt *et al.* (1994). Twice a year, all colonies were rejuvenated by adding field-collected individual parasitoids from the respective regions.

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

Different host species with varying suitability according to the *Cotesia* species or strains were used in the study (**Table 4.1).** Old host association (= natural host) was defined according to both the origin of the parasitoid and the host **(Table 4.1).** For example, *C. partellus* is considered as an old host association due to its evolutionary close association with its Asian aboriginal host (Overholt *et al.,* 1994b) and was parasitizing this host before its introduction into Africa, whereas the African *S. calamistis* is considered as a new association.

The parasitoid's hosts, *Chilo partellus and S. calamistis* were initially collected from maize fields in coastal regions of Kenya, *B. fusca from maize fields in* Western

Kenya (Kitale), while *S. nonagrioides* were initially collected from *Typha domingensis* in Makindu, in the eastern part of Kenya. The larvae of *C. partellus* were continuously reared at *icipe* on artificial diets of Ochieng *et al.,* (1985) whereas the larvae of the other species used in the study were reared on the artificial diet of Onyango & Ochieng'-Odero (1994). Twice a year, all host's colonies were rejuvenated by addition of field-collected stemborer larvae (**Table 4.1).**

Table 4.1 Suitability of lepidopteran stem borer species to different *Cotesia* parasitoid species and strains based on field observations and literature search.

	Chilo partellus	***Busseola fusca***	***Sesamia calamistis***	***Sesamia nonagrioides***
Cotesia flavipes	o	w	New	non
Cotesia sesamiae				
Cs-Inland	w	o	O	non
Cs-Coast	new	w	O	non
Cotesia typhae	non	non	W	o

Different codes are used to indicate the level of host suitability, whereby non = non-host, w = "weak" host association, new = new host association, o = old host association.

4.2.2 Collection of oral secretions from the parasitoid's stemborer host larvae

The α-amylase enzyme was isolated from third and fourth instar larvae previously fed for 24h on maize stems as was similarly done in the previous chapter. In this

experiment, each larva was squeezed behind the head using soft forceps to collect its oral secretion into a cool capillary tube, placed directly on ice. This was repeated for at least 100-200 larvae per species in order to obtain sufficient volume of oral secretions (about 500-800 µl per species), estimated by weighting. All samples were preserved at -80°C before use.

4.2.3 Purification of the α-amylase sample extracts from stemborers

The stemborer's oral secretions were first centrifuged at 11,000 ×g for 5 minutes to remove the undetected debris (frass and undigested food materials). About 600-800 µl of supernatant was transferred to a clean tube and the proteins present in the supernatant salt precipitated. During the precipitation, ammonium sulphate salt was added gradually to the supernatant to a final salt saturation of 90% and precipitated overnight at 4°C. The proteins were subsequently pelleted by centrifugation at 12,000 ×g for 1 hour at 4 °C and were then resuspended in Hepes-NaCl buffer (Hepes 20 mM, NaCl 20 mM, $CaCl_2$ 1 mM, pH 7.5) and dialyzed (MWCO 12-14000 Da) overnight at 4 °C in the same buffer.

The protein, α-amylase present in the sample was then purified using the glycogen-amylase complex precipitation method as initially described by Loyter and Schramm (1962) but with some modifications. Briefly, ice-cold absolute ethanol (2/3 v/v) was added dropwise to the dialyzed samples placed on ice and mixed for 40 minutes at 4 °C. This mixture was centrifuged at 20,000 rpm for 30 minutes at 4°C to pellet the nucleic acids. To the supernatant, glycogen (Sigma Aldrich) was added to a final

concentration of 2.4 mg/ml per sample and mixed for 20 minutes for samples of *S. calamistis* and *S. nonagrioides*, and for 5 minutes for *B. fusca* and *C. partellus* samples, respectively at 4°C. These different timings allowed for an optimum yield of α-amylases as was observed in the previous assays. The mixtures were subsequently centrifuged for 20 minutes at 20,000 rpm at 4°C to pellet the amylase-substrate complex which was subsequently dissolved in the aforementioned Hepes-NaCl buffer. The amylase-substrate complexes were allowed to stand for 3 hours at room temperature to allow for the digestion of the glycogen in the complexes. The remaining α-amylases were dialyzed (MWCO 12-14000 Da) overnight against the same buffer and kept at -20°C for electrophoresis and bioassays.

4.2.4 Native PAGE and α-amylase zymogram

For the α-amylases of each host species, electrophoresis was conducted under non-denaturing conditions (native PAGE electrophoresis) as follows: For each host species, ten microliters of purified α-amylase were mixed separately with 10µl buffer (50 mM tris-HCl, pH 6.8, 10% glycerol (v/v) and 1% bromophenol blue) and electrophoresed in Ornstein-Davis discontinuous buffer system on a 7.5% native polyacrylamide gel at 4°C according to Schrambach and Jovin (1983) and Niepmann and Zheng (2006). After running the gel at a constant voltage of 150V and current of 25mA for 1hr and when the dye-containing sample reached the bottom of the glass, the polyacrylamide gel was stained according to Nagaraju and Abraham (1995) with minor modifications. The gel was incubated for 1 h at 37 °C in 1% soluble starch from potato (Sigma Aldrich) and 1 M $CaCl_2$, washed thoroughly with ddH_20 and

subsequently stained with 0.1% Lugol's iodine solution (I_3K) until white bands against a blue background were visible. The gel images were acquired using the myECL™ Imager (Thermo) and analyzed using myImageAnalysis™ Software (Thermo).

It was previously observed that the concentration of α-amylase present in the extract, conditioned the behavioural response of the wasp (Bichang'a *et al.*, 2018).

For each host species, the concentration of α-amylase was estimated against an electrophoretic migration calibration obtained by using an increasing concentration of between 50 and 1000µg/ml of α-amylase from *Aspergillus oryzae* (Sigma No A9857) and *D. melanogaster* produced on the yeast *Pichia pastoris* (suppl. **Figure 4.1**). However, this electrophoretic migration calibration did not provide the precise amount of α-amylase in the sample but rather a range of concentrations. Moreover, it was observed that 300-500 µg/ml was the optimal concentration range of α-amylase that could appropriately induce host recognition and acceptance for oviposition behaviour by the parasitoids (Bichang'a *et al.*, 2018). Thus, for each host species, the concentration of α-amylase used for the subsequent bioassays was adjusted to the 300-500 µg/ml.

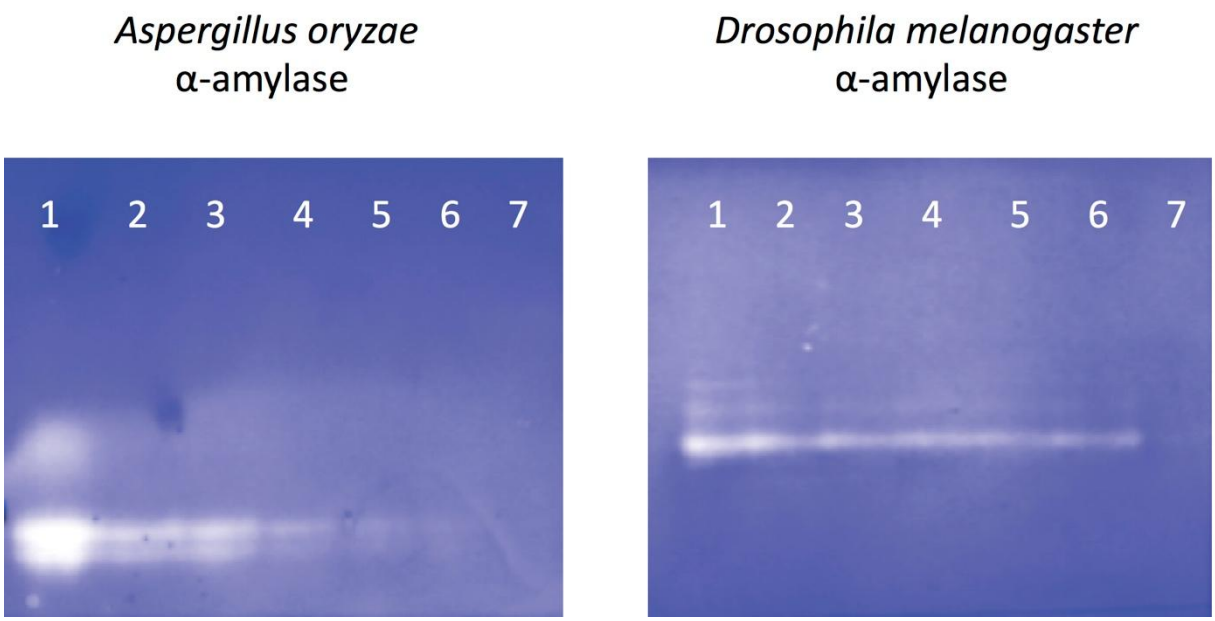

Figure 4.1 Non-denaturing gel electrophoresis of the amylolytic activity of α-amylases of *Aspergillus oryzae* and *Drosophila melanogaster* at different concentrations (1: 1000 µg/ml; 2: 500 µg/ml; 3: 300 µg/ml; 4: 100 µg/ml; 5: 50 µg/ml; 6: 25 µg/ml and 7: 0µg/ml).

4.2.5 Western blot analysis of the purified α-amylases of stemborer host species

In order to confirm for each stem borer species that the proteins purified were indeed α-amylases, after being used for all bioassays, a western blot was performed using an antibody specific to *Drosophila melanogaster* Meigen α-amylase using the similar protocol of Bichang'a *et al.* (2018). Ten microliters of each heat denatured protein sample (of about 500 ng/µl) were loaded on a NuPAGE 4-12% Bis-Tris Gel (Invitrogen) and electrophoresis conducted for one hour at 200 volts in MOPS buffer.

The proteins were then transferred to an iBlot Gel Transfer Nitrocellulose membrane (Invitrogen) using the iBlot Gel Transfer Device (Invitrogen). The membrane was washed in 1X PBS for 20 minutes, after which it was incubated for 90 minutes in a milk solution (1X PBS, 0.1% Tween, 5% milk) in order to saturate the membrane with proteins. The membrane was then incubated with the primary anti *Drosophila melanogaster* α-amylase antibody (gift from Dr. B. Lemaitre) according to Chng *et al*. (2014), it was diluted 1000-fold in a solution of 1X PBS, 0.1% Tween, 1% milk) for several hours. After this step, the membrane was washed six times in 1X PBS, 0.1% Tween before incubating with the secondary antibody (Anti guinea pig IgG Peroxidase, Sigma A7289), 1000-fold diluted in a solution of 1X PBS, 0.1% Tween, 1% milk, for one hour. The membrane was then washed 3 times in 1X PBS, 0.1% Tween. The peroxidase activity was detected using Amersham ECL Prime Western Blotting Detection Reagent (GE Healthcare) and recorded on an Odyssey FC imager.

4.2.6 Behavioural bioassays of Cotesia species towards α-amylases from different sources

Similar to the previous chapter, the two behavioural steps (antennation + stinging attempt) as described by Obonyo *et al.* (2010a & b) were used to determine the host acceptance by *Cotesia* females for oviposition of the parasitoids. To test the behavioural activities, approximately 300-500 µg/ml of α-amylases (the minimal concentration reported to mediate a positive response of *C. flavipes* [Bichang'a *et al.*, 2018]) were placed on small cotton wools and presented to female parasitoids. A small piece of cotton wool was rolled into a spherical shape of around 2 mm in

diameter and placed at the centre of a Petri dish of 8 cm diameter without cover. About 0.5 to 1 µl of α-amylase was then deposited on the cotton wool ball. A single female wasp was introduced near the cotton wool and both were covered with a transparent circular Perpex lid (3 cm diameter, 1 cm height) to prevent the parasitoid from flying off and to allow for observations.

The behaviour of the parasitoid in the Petri dish was monitored for a maximum of 120 s. For each female, both antennation and stinging attempts were recorded. The percentage of positive responses (i.e. antennation + stinging) was calculated from 30 females tested per the type of α-amylase used in the bioassay. After each observation, the experimental females, the cotton wool ball with tested α-amylase and the arena were replaced for the new set of experiments.

As according to Obonyo *et al.* (2010a), all the behavioural experiments were carried out in a room at 26 ± 1°C between 10h00 to 14h00 with a constant source of light to maintain an optimal temperature for the behavioural activities of the female parasitoids.

4.2.7 Statistical analysis

For each bioassay, the Marascuilo's procedure, i.e. a pairwise comparison after Pearson's Chi-square test to test the overall significance differences, was used to separate the proportions of wasps that exhibited positive responses (i.e. antennation + stinging attempts) (Marascuilo 1966).

4.3 Results

The α-amylase exhibited species-specific electrophoretic migrations showing different number of isoforms using the Lugol test (**Figure 4.2**). The α-amylase of *C. partellus* exhibited mostly 1 band whereas α-amylase of *B. fusca* appeared to have two main different isoforms while that of *S. calamistis* exhibited two thick well visible isoforms, three thinner bands between and three faint bands, which migrated much faster than the others. α-mylase of *S. nonagrioides* had three thick groups of isoforms, one thin band and a pair of well visible thin bands with faster migration. The western blot analysis for *S. nonagrioides*, *S. calamistis* and *B. fusca* confirmed that these were α-amylase proteins (**Figure 4.3**). The non-denaturing gels stained using iodine showed white bands corresponding to different active amylases (**Figure 4.2)**. The proteins were separated based on the differences in their electric charge, which differed mostly between (Lys and Arg) and (Asp and Glu) amino acids. To link these active amylases with their respective genes was not evident since a single gene may exhibit two bands if the two alleles differ in charge. Moreover, if there are more than two bands, one band can result as simply two active copies. In contrast, in the SDS-PAGE (denaturing) used for Western blot, all those proteins bands migrate to the same position indicating that they have the same molecular weight. This is the reason why a single labeled band was observed after Western blot analysis **(Figure 4.3)**. Thefore the different proteins revealed in **Figure 4.2** are due to both diffences in electric charge and molecular weight (as well as conformation, shape...). Nevertheless, since they all had similar molecular weight according to the Western

blot results, the different proteins revealed in **Figure 4.2** are only due to the differences in electric charges (electromorphs). However, no band was revealed for *C. partellus* after Western blot analysis **(Figure 4.3)** although α-amylase activity was seen **(Figure 4.2)**. This is probably due to a very low amount of protein sample of the *C. partellus* used for western blot analyses as compared to the other species. The limit of protein detection was therefore attained for this sample type by western blot.

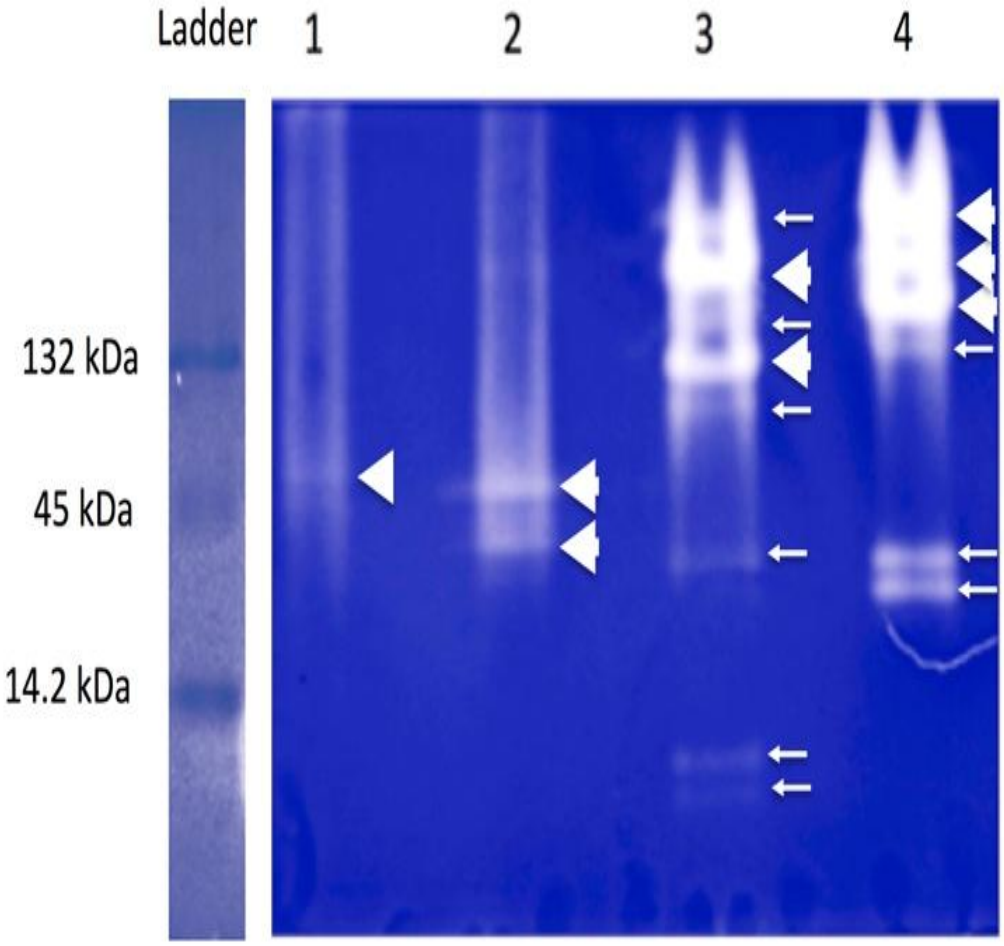

Figure 4.2 Non-denaturing gel electrophoresis of the amylolytic activity of the purified α-amylases from the oral secretions of different stemborer larvae of *Chilo partellus* (1), *Busseola fusca* (2), *Sesamia calamistis* (3) and *Sesamia nonagrioides* (4). The arrows highlight the main isoforms obtained for each species.

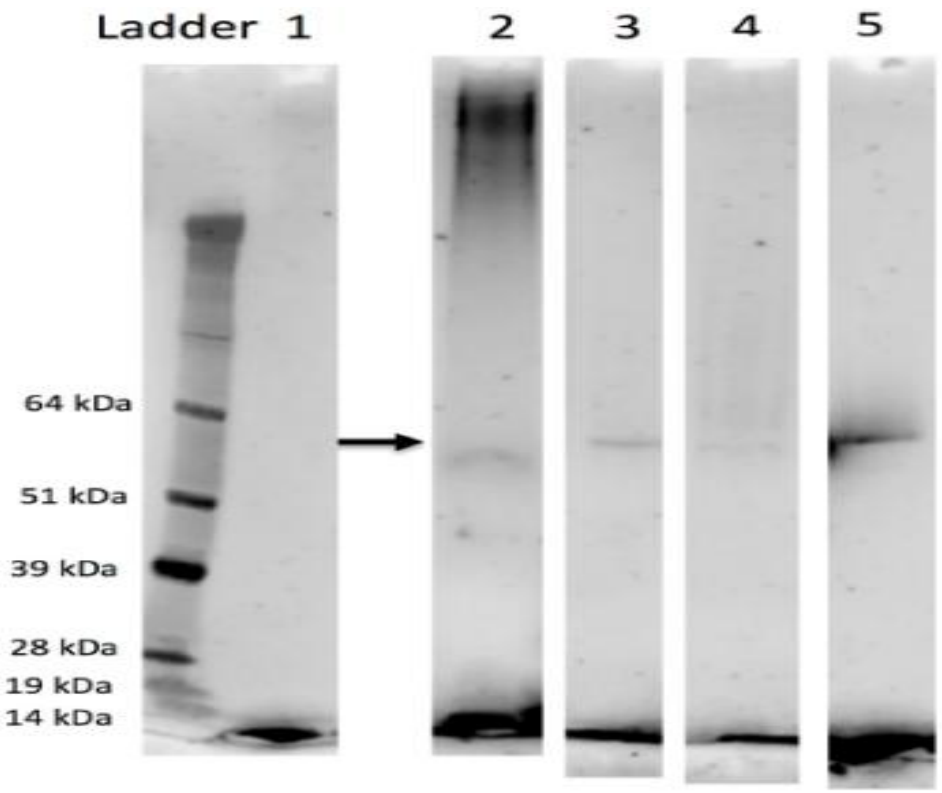

Figure 4.3 Results of western blot analysis using a *Drosophila melanogaster* α-amylase-specific antibody towards the purified α-amylases from the oral secretions of different stemborer larvae of *Chilo partellus* (1), *Busseola fusca* (2), *Sesamia calamistis* (3) and *Sesamia nonagrioides* (4). Ladder: molecular weight markers (pre-stained SeeBlue Plus2, Thermo Fischer). 5: α-amylase from *Drosophila melanogaster*.

Each female parasitoid species or strains used in this study exhibited different behaviour according to the origin of the extracted α-amylase (*C. flavipes*: χ^2 =13.43; df=3, *P*=0.0038; *Cs*-Inland: χ^2=27.548; df=3, *P*<0.0001; *Cs*-Coast: χ^2=8.2458; df=3

and P=0.04119 and *C. typhae:* χ^2=15.239; df=3 and P=0.001623) (**Figure 4.4**). For *C. flavipes* females, α-amylases derived from larvae of the old association host *C. partellus* and the new association host *S. calamistis* induced the highest positive responses. These were closely followed by those from *B. fusca,* whereas those from *S. nonagrioides* larvae did not induce any behaviourial response (**Figure 4.4**). The, α-amylases from the preferred host *B. fusca* females from *Cs*-Inland population induced the highest positive response, followed by those from the suitable *S. calamistis* whereas those from the unsuitable hosts *C. partellus* and *S. nonagrioides* did not induce any response (**Figure 4.4**). For the *Cs*-Coast females, α-amylases from the suitable new association host *C. partellus* and the natural host *S. calamistis* induced higher responses than those from the unsuitable *B. fusca* and *S. nonagrioides* (**Figure 4.4**). For the more specific *Cotesia* species, α-amylase from the suitable host *S. nonagrioides* induced a higher response than those from the unsuitable species (**Figure 4.4**).

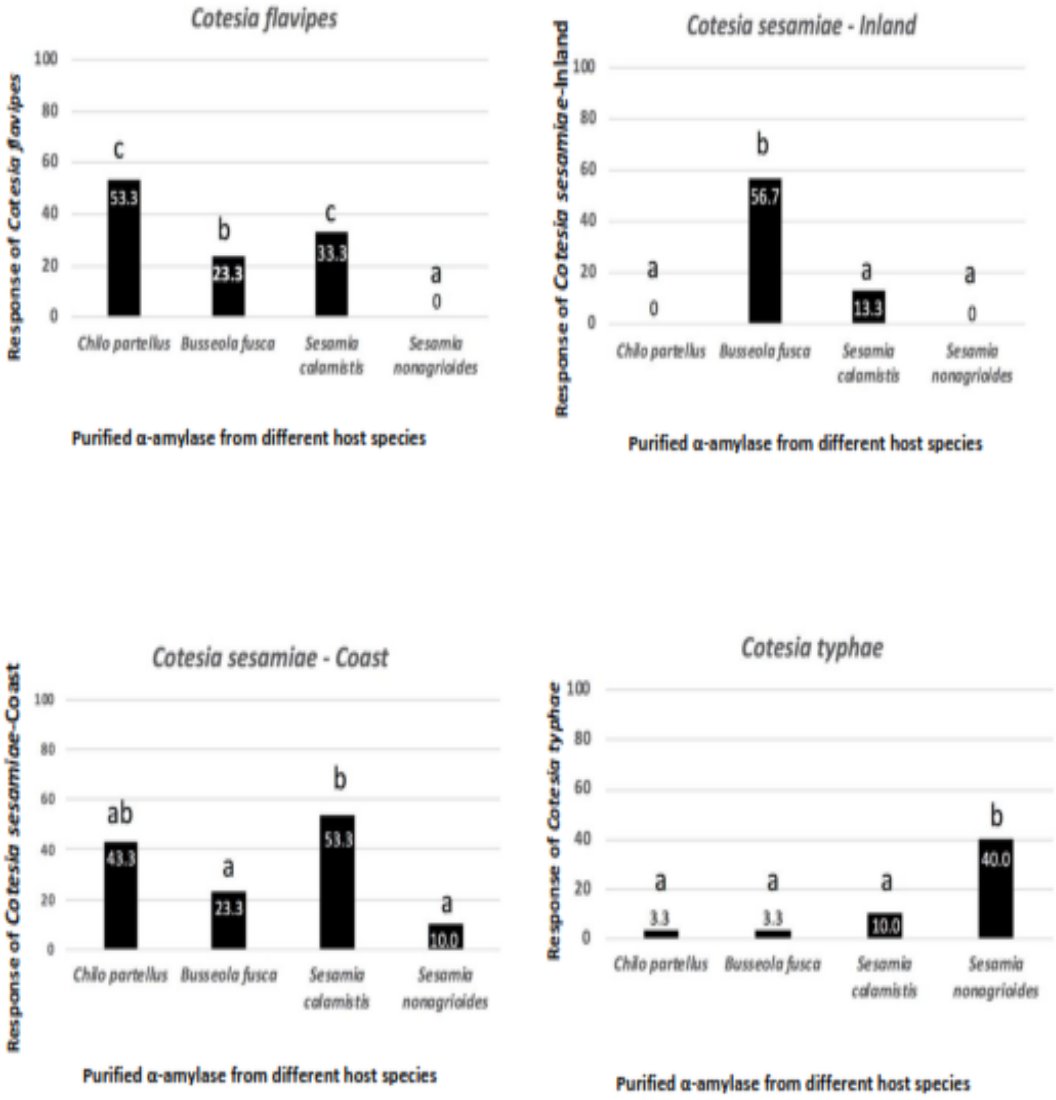

Figure 4.4 Behavioral responses of *Cotesia flavipes*, *Cotesia sesamiae*-Inland, *Cotesia sesamiae*-Coast, and *Cotesia typhae* females parasitoids towards purified α-amylase from different stemborer host species.

The percentages of females (n=30) that exhibited antennation and stinging attempt are given for each bar. After Pearson's Chi-squared test, bars headed with different letter are significant at 5% level according to the Marascuilo's procedure (multiple proportions comparison).

In summary, there was a positive relationship between the α-amylases of the stemborer larvae and the behavioural response exhibited by each of the female parasitoid species or population (**Figure 4.4**). Moreover, this relationship corresponds well with the level of host suitability in each combination of host stem borer species – parasitoid species/population (**Table 4.1**).

4.4 DISCUSSION

This study revealed that the response of female *Cotesia* to the α-amylase from larval stemborers parasitoids hosts' oral secretions depended on both host and parasitoid species or population. A strong positive relationship was demonstrated between the level of parasitoid response to oral secretions and host preference/suitability. Highest parasitoid responses to their hosts extracts were observed with the proteins extracted from the old association hosts (i.e. most suitable host), whereas protein secretions from unsuitable species triggered little or no response. Variations in α-amylases among host species thus explains the specific host recognition and acceptance behavior exhibited by the parasitoids studied.

It has been reported that Lepidopteran stemborers in Africa exhibit high ecological and genetic diversity (Le Ru *et al.,* 2006a; 2006b), characterized by a high number of closely related plant-specific species (Le Ru *et al.,* 2006a; 2006b; Moolman *et al.,* 2014; Ong'amo *et al.,* 2014; Goftishu *et al.,* 2018). Correspondingly, Mailafiya *et al.,* (2009) reported a high host diversity of the *Cotesia* spp. particularly among *Busseola* spp. and *Chilo* spp., which also revealed a strong host-parasitoid specificity. This suggests that the chemical(s) involved in host recognition and acceptance by these parasitoids are specific to the host species involved, as verified in the present study. Although, the response of female parasitoid to α-amylase is not binomially distributed (yes or no), it is evident that α-amylase from the parasitoids's stemborer natural hosts are more potent compared to that of the unsuitable/unnatural hosts. Besides, some behavioural responses still occurred with α-amylases of unsuitable hosts indicating that the *Cotesia* parasitoids are able to parasitize stemborer species that are not original/natural hosts. Nevertheless, due to the different geographical distribution of their respective hosts the probability of encounter between *B. fusca* with *C. flavipes* and *Cs*-coast as well as between *C. partellus* with *Cs*-Inland is very limited: *B. fusca* is mostly present in the highlands whereas *C. partellus* is mostly present in the lowlands (Mailafiya *et al.,* 2010; Mwalusepo *et al.,* 2015). Such ecological patterns of the host-parasitoid associations suggest that their preference for the α-amylase according to specific host may be explained to arise from environmental adaptations (even recent adaptation, cf. for *C. flavipes* towards *S. calamistis*) to local hosts. Such similar adaptations have been

already been shown for the virulence function of *C. sesamiae* populations (Dupas *et al.,* 2008; Gauthier *et al.,* 2018).

Alpha-amylases are important classes of digestive enzymes used by the insects to hydrolyse starch to oligosaccharides in various plant tissues. Thus, these enzymes have been reported to play a critical role in energy acquisition by insects for survival (Franco *et al.,* 2000). They have been identified in most insect orders such as Orthoptera, Hemiptera, Heteroptera, Hymenoptera, Diptera, Lepidoptera and Coleoptera (Kaur *et al.,* 2014). In Lepidoptera, several α-amylase genes have been reported to occur (Pytelkova *et al.,* 2009; Özgür *et al.,* 2009; Da Lage *et al.,* 2011) explaining the likely existence of isoenzyme forms. In the present study, the gut extracted α-amylases existed as different isoforms that exhibited species-specific migration patterns in electrophoresis. However, since migration distance of an enzyme isoform depends on the molecule's electric charge, it is thus not obvious whether different bands in this study represented allelic variations or duplicate gene copies. However, in species such as the two species of *Sesamia* it is likely that the electrophoretically well separated groups of bands, reflected different gene copies. It can thus be hypothesized that individuals stemborer species, can express different isoforms of the α–amylase enzyme. To confirm this hypothesis, it would be necessary to look at the α -amylase expressed in one individual. Up to now, only one α-amylase gene sequence has been identified in *S. nonagrioides* (actually a cDNA; Da Lage J.-L., unpublished study), but, given that most Lepidoptera with published genomes harbor several α -amylase genes (Da Lage [2018] for a review), it is quite

likely that this is the case in *S. nonagrioides*. Several α-amylase gene copies are expressed in a species close to *C. partellus*, *C. suppressalis*; and three α -amylase gene copies in *Ephestia kuhniella* (Pytelkova *et al*., 2009). Nevertheless, all these studies indicated that the stemborer insect pest studied express multiple α–amylase forms at the same time. This clearly suggests that no individual variation in α-amylase gene expression occurs within the same species making the process of gene expression species-specific. It was previously demonstrated that the two *Sesamia* species have different ranges of host plants (Le Ru *et al.,* 2006a & b). It can thus be suggested that the genes coding for digestive enzymes like α-amylase may have evolved under different selective pressures. Similarly, it has been reported that tridimensional amylase structures may vary according to the species or even to the isoform if significant sequence differences such as presence or absence of some disulfide bonds, or particular loops exists (Da Lage *et al.,* 2002). Such structural differences might be discriminated by the sensory equipment of the parasitoid wasp possibility explaining the host specific variations that were exhibited between the different stem borer species in relation to their respective parasitoid species/populations used in this study.

It has been shown that the conformation of the α-amylase rather than its catalytic activity induces the parasitoid responses (i.e. antennation + stinging attempts) in *C. flavipes* (Bichang'a *et al.,* 2018). Therefore, the existence of different α-amylase isoforms specific to each stem borer species as is illustrated in **Figure 4.2**

corroborates the variable behavioural responses obtained in relation to the host-parasitoid association.

The question arises of how the parasitoids access host α-amylase in nature. Lepidopteran stemborers larvae spend most of their life feeding inside the plant stems. Prior to entering in the feeding tunnel of the host larvae, the wasp initially makes a contact with the fecal pellets of the stemborer larvae pushed outside of the stem by the feeding larvae. These pellets act as a marker of the status of the feeding larva inside the stem tunnel as a host or non-host (Obonyo *et al.,* 2010b) and whether they are actively feeding or not. It is most probable that these fecal pellets already contain some parasitoid oviposition stimulatory compounds since it has been reported that those pellets also induce oviposition (Bichanga *et al.,* 2018). However, the definitive host recognition and acceptance of the host for oviposition by the parasitoid only occurs during contact with the stemborer host body (Obonyo *et al.,* 2010a; 2010b). It can thus be hypothesized that it is during this final step of host evaluation that the parasitoid can confirm the identity of the host larva by detection of the same stimulatory compounds previously present in the fecal pellets and on deposited on the larval surface due to its feeding activity. These stimulatory compounds need to provide a quick and an appropriate information to the parasitoid on the suitability of the larva (both host and health status) since it has been reported that host larvae often attack the wasps inside the tunnel, causing a 50% mortality risk (Takasu and Overholt, 1997). The high selection pressure exerted due to the high mortality during oviposition should favour wasps that are able to recognize their

hosts with minimal injury risks (Ward, 1992). In this context, the parasitoid response to α-amylase needs to be specific to the host involved. In addition, this supposes that the parasitoids can perceive the α-amylase through their sensorial equipment.

Obonyo *et al.* (2010a) observed that female parasitoids use the tip of their antennae to recognize and accept their host larvae for oviposition. Thus, the presence of specific sensilla, sensilla chaetica known to have gustatory functions in insects have been identified on the last antennal segment of the female parasitoids (Obonyo *et al.*, 2011) and have been shown to able to detect the α-amylase (Mailhan, 2016). However, this is contrary to the recent findings in the study by Tolassy (2018), suggesting that other sensilla from other sensorial organs, such as from the tarsi, might be involved.

However, there is no physiological evidence to suggest that the studied parasitoid can detect the α-amylase since gustation in insects is known to be influenced generally by small compounds such as sugars, free amino acids, water-soluble alkaloids (see Thiéry *et al.* [2013] for review). Nevertheless, it is well known that hymenopterans are able to detect large molecules such as long chain (more than 60 carbons) cuticular hydrocarbons (Cvačka *et al.*, 2006; Blomquist and Bagnères, 2010) and that non-volatile long-chain hydrocarbons can be detected by olfactory sensilla (Ozaki *et al.*, 2005; 2012). Hence it is not possible to rule out the detection of α-amylases by specialized olfactory sensilla present on *Cotesia* spp. antennae.

In conclusion, this study shows that α-amylase is a key protein involved in host recognition and acceptance for oviposition by the parasitoid species of the *C. flavipes* complex. However, the specific variations are involved in determining the specificity of host-parasitoid association. These findings open new routes for future investigation of the evolutionary processes at play in Lepidoptera stem borers-*Cotesia* and their interactions.

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CHAPTER FIVE

5.0 TESTING THE HERITABILITY OF HOST ACCEPTANCE IN *COTESIA SESAMIAE PARASITOID*: PRELIMINARY STUDIES FOR THE DETERMINATION OF THE CANDIDATE GENES INVOLVED IN HOST ACCEPTANCE

5.1 Introduction

Parasitoids are naturally considered as the major and most important biological control agents involved in many insect species' mortality (Hawkins, 1994). Several parasitoid species have been described with most species reported to develop cryptic host races (Dupas & Boscaro 1999; Antolin *et al.*, 2006; Branca *et al.*, 2011). Thus, an understanding of the emergence of various parasitoid host races and their genetic potential for adaptation to new hosts is of critical importance to the study of the adaptation to ecological and climate changes and prediction of parasitoid non-target effects. Although previous work on parasitoids have mainly focused on the study of behavioural, physiological and molecular interactions and their link with theoretical population biology models; little information is however available on the possible parasitoid adaptation in classical biological control programmes. Thus development of novel genomic approaches for the elucidation of parasitoid adaptive process is essential in the management of some of the most damaging families of lepidopteran

stem borers pest families including the Noctuidae, Pyralidae and Crambidae (Kfir *et al.,* 2002).

Yield losses due to stemborer pests in sub-Saharan Africa is reported to vary between 10-70% and have been projected to increase in future due to changes in stem borer distribution (Zhou *et al.,* 2001; Assefa *et al,* 2009) as a result of climate changes (IPPC, 2007; Mwalusepo *et al.,* 2015) and possible pest resistance to some currently applied pest management options. For example, the indigenous noctuid pest *Busseola fusca* (Fuller), which is widely distributed throughout Sub-Saharan Africa, is documented to have developed some resistance to the Bt-maize that was recently introduced in South Africa (see Tabashnik *et al.* [2009]).

Wasps of the genus *Cotesia* are widely recognized for their parasitic efficiency to prevent the outbreak of various crop pests and as such, have been used successfully in several biological control programs (Kaiser *et al.,* 2017). In Sub-Saharan Africa, the most widespread parasitoid, *Cotesia sesamiae* Cameron (Hymenoptera: Braconidae), wasp has been used in the biological control of the noctuid Lepidoptera stemborer *Busseola fusca* (Fuller) a major stemborer pest of maize and sorghum crops in East Africa (Kfir 1995; Kfir *et al.* 2002). *Cotesia sesamiae* is a stenophagous parasitoid that successfully parasitizes diverse host species (Ngi-Song *et al.* 1995; Branca *et al.* 2011). However, different population of this parasitoid species have been shown to exhibit variable degree of parasitism successes on different hosts (Mochiah *et al.* 2002; Gitau *et al.,* 2010). For example, a *C. sesamiae*

population collected from Mombasa - coastal Kenya has been reported to be selective in their host choice. The species is regarded to be avirulent towards *B. fusca* and thus unable to develop on the stem borer species. In contrast, *C. sesamiae* population from Kitale – inland Kenya, is regarded to be virulent towards *B. fusca* and hence has been shown to successfully develop in this specific host. However, both parasitoid populations have been reported to develop in *Sesamia calamistis* Hampson (Lepidoptera: Noctuidae), the main host of *C. sesamiae* present in the coastal Kenya (Ngi-Song *et al.*, 1995).

These developmental differences of the two populations of *C. sesamiae* in *B. fusca* host have been associated to variations in the CrV1 gene of *C. sesamiae* bracovirus (CsBV), a polydnavirus (Dupas *et al.,* 2008; Gitau *et al.*, 2007; Branca *et al.*, 2011). This gene has been shown to contribute to immune suppression of the host by active de-structuration of the cytoskeleton of host immune cells (Asgari *et al.* 1997). These polydnaviruse genes integrated into the genome of braconid wasps are generally contributing to their adaptive radiations (Whitfield 2002; Dupuy *et al.* 2006) and are thus good candidate genes for adaptation of *C. sesamiae* to *B. fusca*. Another possible factor that could be used to explain their differences in *S. sesamiae* geographic distribution is the possible involvement of *Wolbachia,* a widespread bacterium that infects the majority of insect species. It has been demonstrated that *Wolbachia* bacteria induce reproductive incompatibilities among insect species (Werren 1997; Hilgenboecker *et al.,* 2008). Several *Wolbachia* strains have been identified in *C. sesamiae* expressing cytoplasmic incompatibilities between

populations of the parasitoid and thus have been postulated to be responsible for the reproductive isolation exhibited among *C. sesamiae* populations (Mochiah *et al.,* 2002; Gitau *et al.,* 2007; Gounou *et al.,* 2008). In Kenya, four *Wolbachia* variants have been identified in *C. sesamiae*, with the *Bwest* variant infecting *C. sesamiae* parasitoids that attack *B. fusca* from western Kenya while the *A*, *Beast* and the *A+Beast* (bi-infection) variants that have been shown to infect *S. calamistis* found in the eastern part of the Kenyan coastal regions. Infection of parasitoids by *Wolbachia* may reduce the performance of the parasitoid especially when crosses between infected males and uninfected females occur, particularly in hybrid zones, where bidirectional incompatibility is expressed. Therefore, as a reproductive isolation agent, *Wolbachia* has been suggested as a major contributing factor of parasitoid host specialization and local adaptation (Branca *et al.,* 2009). This phenomenon of differences in host acceptance by these two Kenyan populations of *C. sesamiae* towards *B. fusca* has already been documented (Gitau *et al.,* 2010).

Thus in this context, genetic studies that involve use of these two *Sesamiae* populations could be useful in the identification of the candidate genes involved in host acceptance by the parasitoid species. Such genetic variations that influence behavioural factors important in parasitoid's success has been observed in various parasitoid families (Althoff & Thompson, 2001; Kaiser *et al.,* 2009; Dubuffet *et al*., 2006; Wang *et al*., 2003). Therefore, the work in this chapter sought to initiate preliminary studies that were geared towards the determination of the candidate genes involved in host acceptance through cross-mating the two *C. sesamiae*

populations in the laboratory to provide a proof for the heritability of host acceptance in the resulting progenies.

5.2 Materials and methods

5.2.1 Insects

Females of both virulent and avirulent *C. sesamiae* strains towards *B. fusca* were obtained from laboratory-reared colonies in *icipe* mass-rearing unit. The virulent species, thereafter named Kitale (Kit) *C. sesamiae* strain was obtained from *B. fusca* larvae collected from maize fields in Kitale, Western Kenya, in 2006, while the avirulent *C. sesamiae* strain thereafter named Mombasa (Mbsa), was obtained from *S. calamistis* larvae collected from maize fields in the coastal region of Kenya in 2007. These two parasitoid lines have different *Wolbachia* infection status: The Kitale line was infected with *Wolbachia* WCsesB1 strain while the Mombasa line was infected with two strains of *Wolbachia,* WCsesA and WCsesB2. Twice a year, both colonies were rejuvenated by adding other field collected parasitoids of the same populations. The wasps of both strains were continuously reared on larvae of *S. calamistis* as previously described by Overholt *et al.* (1994). Parasitoid cocoons were kept in Perspex cages (30 x 30 x 30 cm) until emergence.

The adult parasitoids were fed on a 20% honey–water solution imbibed in a cotton wool pad and kept under artificial light for 24 h to mate. In all experiments, only 1-day-old females, putatively mated and unexperienced to oviposition were used. The

experiments were carried out at 25 ± 2 °C, 50–80% RH, and a 12:12 h (L: D) photoperiod.

The stemborer species, *B. fusca* and *S. calamistis*, were continuously reared on artificial diet as previously described by Onyango & Ochieng'-Odero (1994). For each species, several stemborer larvae were added three times yearly to rejuvenate the colonies. Fourth larval instars were introduced into jars (10 x 20 cm), each containing pieces of maize stem, and left for 48 h to feed and produce frass to facilitate parasitoid wasp host acceptance for parasitism experiments.

5.2.2 Cross-mating of *Cotesia sesamiae* parasitoid populations of Mombasa and Kitale

Since infection by *Wolbachia* does not allow cross-mating between individuals of *C. sesamiae* populations, the gravid females of each aforementioned parasitoid lines were reared on larvae of *S. calamistis* previously fed on artificial diet of Onyango & Ochieng'-Odero (1994) enriched with 2000 mg/L rifampicin (Dedeine *et al.,* 2001) to obtain *Wolbachia*-free parasitoids colonies (named cured lines). This process was repeated for three generations of female wasps resulting in the generation of cured colonies of Mombasa (Mbsa) and Kitale (Kit) *C. sesamiae*. To confirm the absence of *Wolbachia* infections, in both *C. sesamiae* populations used in the cross-mating experiments, individuals were tested using PCR technique on *ftsZ* and *wsp* genes as described by Ngi-Song & Mochiah (2001). DNA was first extracted from about 50

individuals (a mixture of males and females) from individuals of each population previously stored in 99% ethanol.

Cross experiment tests were conducted between Mbsa and Kit *C. sesamiae* cured lines to assess the mating incompatibilities due to the presence of different *Wolbachia* types. Individual parasitoids were allowed to emerge singly by separating single cocoon from each cocoons mass. Finally, individual male and female parasitoids from each colony (i.e. Kit *C. sesamiae* cured as well as Mbsa cured) were used for cross-mating experiments.

F0 parents from inbred lines (i.e. females MBsa x Males Kit) were crossed, and the resulting F1 resulting females (females MK) were crossed to the recombinant males KM. Then, the successive progenies were put together to be reared up to F8 progeny. It was expected that at F8, the most mixed genome compared to the previous generations (i.e. one half from Kitale and one half from Mombasa) would be generated as was earlier reported by Stephane Dupas (EGCE, CNRS/IRD, Gif-sur-Yvette, France) following genetic simulation experiments/analyses. In each generation, the female progenies were allowed to oviposit on *S. calamistis*, a host susceptible to both initial parents (**Figure 5.1**). Only males of F8 population were crossed with the original parental females (either MBsa or Kit) since males are haploids and transmit their entire genome (exhibit recombination information events). It was also estimated that female parasitoids from parental lines were 100% homozygous due to inbreeding. The resulting daughters from the cross between one

recombinant male and one inbred female of the parental line are genetically identical and they are called clonal sib ship.

In order to get a large amount of DNA for subsequent RAD-tag molecular analyses, the resulting crossed female progenies (called clonal sib ships) were used directly for phenotypic evaluations (= acceptance towards *B. fusca*) and parasitism without being mated in order to give males only (unmated females gives males only). Since the RAD-tag analysis requires an amount of DNA that is not present in a single individual, advantage of parthenogenetic and gregarious reproduction of *C. sesamiaeparasitoid* was exploited in order to generate large numbers of genetically near-identical female sib ships (Dupas et al., 1998; Pannebakker et al., 2011).

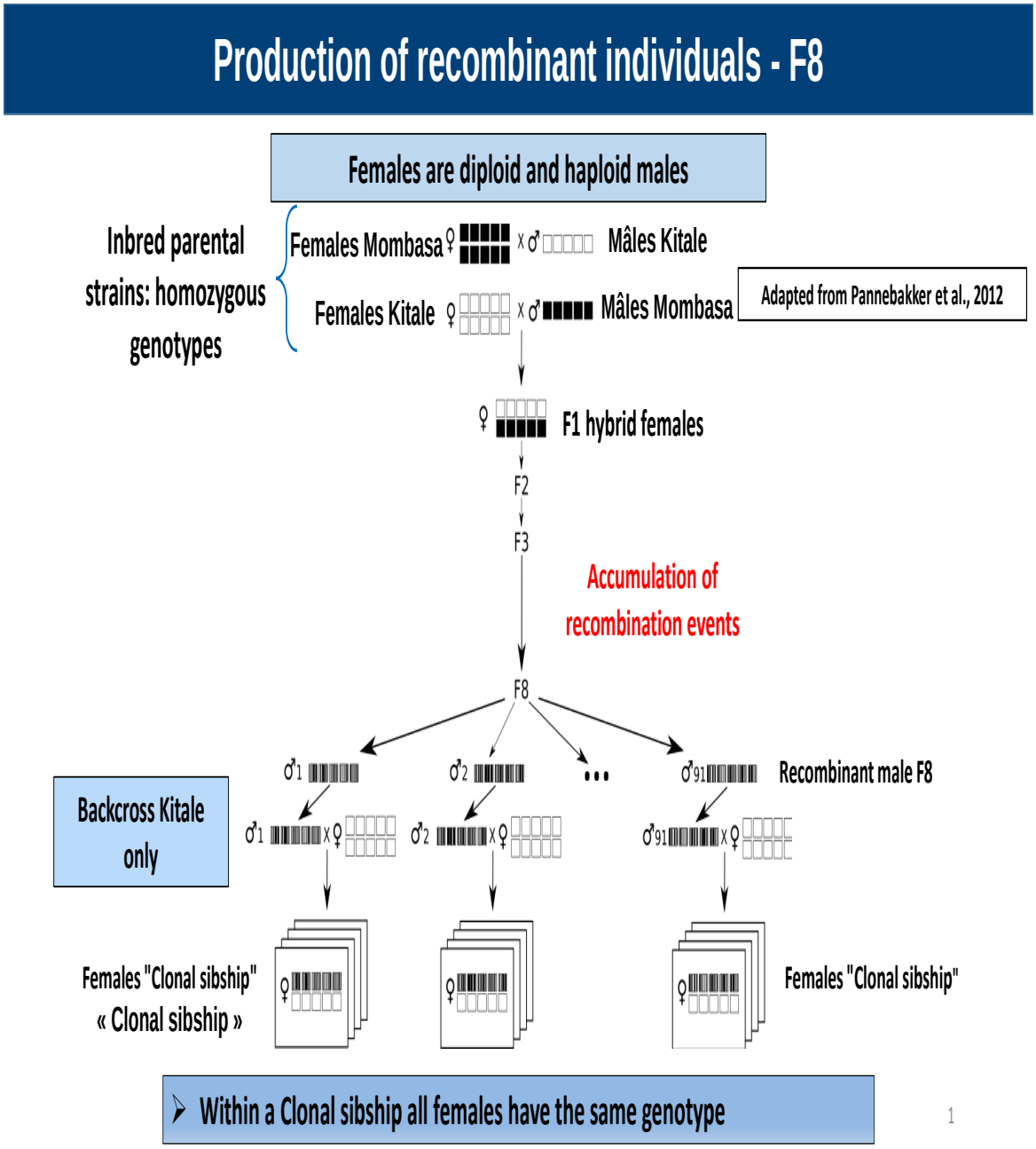


Figure 5.1 Scheme of the different crosses of *Cotesia sesamiae* Mombasa and kitale developed from F1 to F8 generations.

In total, 1597 females were produced and phenotyped for *Busseola fusca* acceptance: 730 from F8 Kitale backcross and 867 from F8 Mombasa backcross F0 parental lines as well as F8 females from clonal sib ships, were individually used to parasitize *B. fusca* larvae prior to being preserved for molecular analysis. For each female parasitoid, their acceptance towards a *B. fusca* larva for oviposition was observed and noted.

5.2.3 Marker analysis: RAD-tag genotyping and analysis

Phenotypic characterization (host acceptance) of the progeny was linked to genotype in a QTL (Quantitative Trait Loci) analysis approach to generate genotypes using a RADseq (Restriction site Associated DNA sequencing) strategy. The F8 clonal sib ships and parental strains were genotyped to determine the molecular variations and to search for variations linked to the phenotypes. Briefly, for each clonal sib ship, high quality DNA was digested using the restriction enzyme *PstI*; the cutting site was ligated with an adaptor specific to each sample, prior to mixing of the samples and to random shearing and prior to ligation with a second adaptor. These libraries were then PCR amplified based on adaptor sequences so that only sequences with a restriction sitewere represented and sequenced by Illumina. Each samplewas specifically tagged. This method was performed by an external company (CNRS Plateforme Imagif), and it gave a whole genome representation of restriction sites flanking regions and allowed SNP (Single Nucleotide Polymorphism) discovery in these sequences between populations. The genotype of individuals within clonal sib ship backcrosses was almost the same and corresponded to that obtained from a

classic backcross (one parental genome and one recombined genome). Due to 99% homozygosity of F0 parental females and to the genotyping of the parental strains, the recombinant male genotype was deduced.

5.3 RESULTS

From the results of parental lines phenotyping experiments, in total 265 females for each parental line were tested for *B. fusca* larvae acceptance: 81 females from Kitale and 184 females from Mombasa. The Mombasa phenotype (M) parasitoids did not frequently oviposit on *B. fusca* host unlike the Kitale phenotype (K) parasitoids that readily accepted to oviposit on *B. fusca* (**Figure 5.2**).

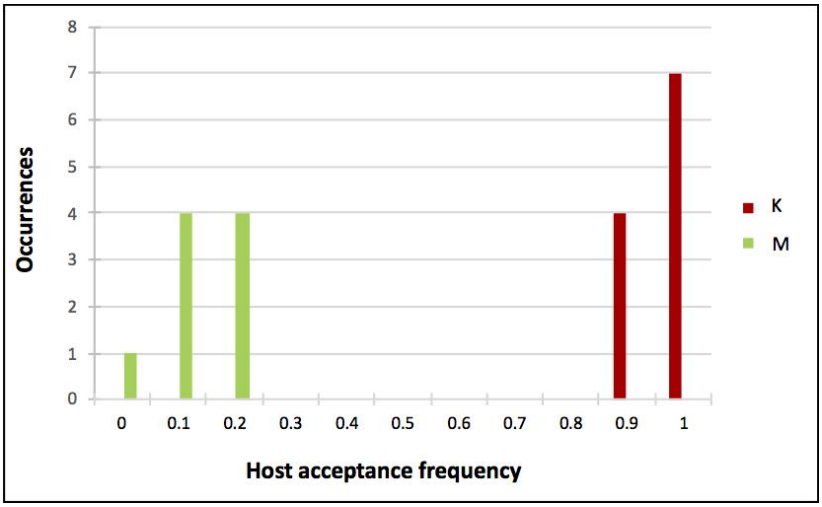


Figure 5.2 Phenotyping experiments of the parental lines Kitale (K) and Mombasa (M) before crosses. The number of female parasitoid observed (occurrences) that showed host acceptance (highest host acceptance frequencies) or not (lowest host acceptance frequencies) towards *B. fusca* by Mbs females (in green) and Kit females (in Red) of *Cotesia sesamiae*.

From the results of F8 females from clonal sib ships phenotyping experiments, 1597 females were produced and phenotyped for *Busseola fusca* acceptance: 730 from F8 Kitale backcross and 867 from F8 Mombasa backcross. The results indicated that the backcross with a female Kitale (i.e. KMxK of Figure 5.3) gave higher acceptance rates than in the other direction of backcross (i.e. KMxM of Figure 5.3). However, the acceptance rate was more variable than the parental lines.

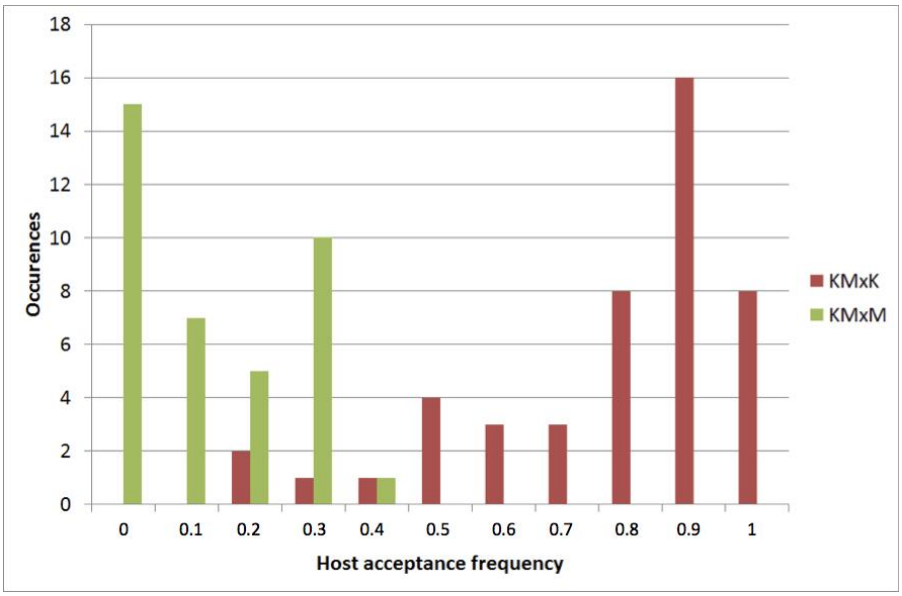


Figure 5.3 Phenotyping experiments (=host acceptance behavioural observations) of the resulting F8 females of KM x K and KM x M from clonal sib ships (KMxK: KM males x females K; KMxM: KM males x females M). The number of female parasitoid observed (occurrences) that showed host acceptance (highest host acceptance frequencies) or not (lowest host acceptance frequencies) towards *B. fusca* by KMxM females (in green) and KMxK females (in Red) of *Cotesia sesamiae*.

The results on phenotyping experiments (=acceptance tests towards *Busseola fusca* for oviposition) of both parental lines and F8 females from clonal sib ships indicated that acceptance towards *B. fusca* for oviposition, although is heritable, it is not a character of all or nothing but rather variable with a higher rate of acceptance for backcrosses with a female Kitale than backcrosses with a female Mombasa.

5.4 DISCUSSION

This chapter presentedpreliminary experiments to link phenotypic characterization (host acceptance) of the progeny to genotype in a QTL (Quantitative Trait Loci) analysis approach to generate genotypes using a RADseq (Restriction site Associated DNA sequencing) strategy.

The results of a preliminary RADseq analysis showed that about 1,500 variable markers were generated using the two Cotesia strains. These variable markers will in the future enable the grouping of the loci into several linkage groups, corresponding to chromosomes and then to organize the loci along each chromosome (order and genetic distances between following loci). Therefore, genotype information from RADseq will eventually be used to detect chromosomal regions involved in phenotype, i.e. along the chromosome. This is important because it will aid in checking the presence of a gene or groups of genes that determines the phenotypic variations hence giving genotypic characterization and allelic variations associated with phenotype.

CHAPTER SIX

6.0 GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

6.1 General Discussion

The aim of this study was to identify the chemical(s) involved in host recognition and acceptance by three *Cotesia* species present in Kenya parasitizing Lepidoptera stemborers as well as to initiate a study on the identification of candidate genes involved in host recognition and acceptance by the two parasitoids species, *C. flavipes* and *C. sesamiae.*

Using an integrated behavioural observations, biochemical and proteomic approaches as illustrated in chapter three of this thesis, it was demonstrated that female parasitoids of *C. flavipes* recognize their host for oviposition in response to a protein, α-amylase, present in the oral secretions of the larvae of their host, *Chilo partellus*. Although polypeptides and proteins have previously been reported as mediating semiochemicals in the host utilization process by hymenopteran parasitoids (Weseloh, 1977; Bénédet *et al.,* 1999; Gauthier *et al.,* 2004), the definitive identification of such proteins or polypeptides had not been achieved before this study. The identity of this active protein was confirmed using purified α-amylase synthetized from *Drosophila melanogaster*. However, the study demonstrated that

the conformation of the enzyme rather than its catalytic site is responsible for mediating the host utilization activity.

This study also showed that the *C. flavipes* parasitoid response to α-amylase is not a host explicit response since the parasitoid was also sensitive to other similar α-amylases from different species including *D. melanogaster, A. oryzae, C. suppressalis* though was less sensitive to pig associated α-amylase. This is presumably due to the fact that *C. flavipes* is a generalist parasitoid, that parasitize larvae of more than 30 Lepidoptera species including *C. suppressalis* (https://www.cabi.org/isc/datasheet/5951). However, in its natural habitat, *C. flavipes* has no chance to be exposed to the α-amylases of *D. melanogaster, A. oryzae* or pigs. Moreover, the sensitivity of *C. flavipes* females to α-amylase is not binomial (mutually exclusive) (yes or no), but is gradual depending on the origin of the enzyme and, in my examination; it was strongest in insect species such as, *D. melanogaster* and *C. suppressalis*. It can thus be postulated that the parasitoid's response to α-amylase enables them to discriminate between hosts. This aspect is more profoundly enhanced, in the presence of natural hosts as compared to the non-host stemborer species.

The amino acid sequences of animal-associated α-amylases demonstrates high variations at the protein level (Da Lage *et al.,* 2002) and thus have been thought to confer adaptive or functional importance in the diversity of host utilization by insects. The stemborers utilize their own α-amylase proteins for feeding process;

subsequently, the species-specific variation in α-amylase proteins might be associated with the distinctive host plants on which the stemborer hosts feeds upon. However, it is possible that the parasitoids exploit these variations in α-amylase proteins produced by different parasitoid hosts to discriminate among different stemborer hosts in various habitats where the parasitoids live. In this study, *C. flavipes* exhibited a strong behavioural response to the oral secretions of its natural host *C. partellus,* weakly responded to the oral secretion of *S. calamistis* but exhibited a very weak response to the oral secretion of their non-hosts. This diversified differences in behavioural responses by the parasitoids can be explained by the existence of α-amylase isoforms present in specific parasitoid hosts.

Furthermore, the *Cotesia flavipes* complex consists of four species, in particular *C. chilonis* (Matsumura), *C. flavipes* Cameron, *C. nonagriae* (Olliff) and *C. sesamiae* (Cameron), all gregarious endoparasitoids of diverse families of lepidopteran stem borers (Crambidae, Pyralidae, and Noctuidae) of Poales (Poaceae, Typhaceae and Cyperaceae) (Kaiser *et al.,* 2017b). In Kenya, there is a strong host-parasitoid specificity particularly with *Busseola* spp. and *Chilo* spp. stemborers (Mailafiya *et al.,* 2009). Therefore, the parasitoid's response to host kairomone, especially the α-amylase, ought to enable the parasitoids to discriminate among hosts. In this specific circumstance, the results of this study propose that α-amylases from caterpillar hosts oral secretions is an important and a good candidate for the determination of an evolutionary solution to host acceptance for oviposition in *C. flavipes* complex.

These findings thus open new avenues for investigations in hosts-parasitoids interactions.

It was however, unclear whether host recognition and selection processes mediated by the α-amylase protein was important in explaining the host-parasitoid specific association. Thus, by using different species and populations of *Cotesia*, as explained in chapter four of this thesis, it was demonstrated that the α-amylase secreted by respective parasitoid hosts exhibited different number and size of the enzyme isoforms; and that the female parasitoids of each parasitoid's species and/or population preferred to oviposit in response to the α-amylase isoforms of the respective host. This clearly implicates the role of the α-amylase in the specific host-parasitoid association. Investigations in this study further uncovered that the response/sensitivity of female *Cotesia* to the α-amylase from larval oral secretions relied upon both host and parasitoid species or population, with a strong relationship between the level of response and host suitability. Most outstanding responses were seen with the old affiliated host proteins (i.e. most suitable host), while protein of unsuitable species induced practically little or zero response. Thus, variations of host α-amylase between host species would in this manner permit host recognition and acceptance by the parasitoids.

α-Amylases are among the most important classes of digestive enzymes used by the insects to hydrolyse starch to oligosaccharides in various plant tissues and play a critical role in insect survival by providing energy (Franco *et al.*, 2000). These

enzymes have been identified in most insect orders such as Orthoptera, Hemiptera, Heteroptera, Hymenoptera, Diptera, Lepidoptera and Coleoptera (Kaur *et al.,* 2014). In Lepidoptera, several α-amylase genes commonly occur (Pytelkova *et al.,* 2009; Özgür *et al.,* 2009; Da Lage *et al.,* 2011). In this study the same enzyme had different isoforms in the non denaturing gel electrophoresis that exhibited species-specific migration patterns. Since isoform migration distance relies upon the molecule electric charge, it is not evident whether different bands represent allelic variation or duplicated gene copies. Nonetheless, in species that demonstrated very well separated groups of bands, for example, the two species of *Sesamia, S. calamistis* and *S. nonagrioides,* it is highly possible of the existence of different gene copies. Therefore, the existence of different α-amylase isoforms specific to each stem borer species corroborates the variable behavioural responses demonstrated in this study in relation to the host-parasitoid association.

Lepidopteran stemborers in Africa present high environmental and genetic diversity (Le Ru *et al.,* 2006a; 2006b), portrayed by a high number of firmly related plant-specific species (Le Ru *et al.,* 2006a; 2006b; Moolman *et al.,* 2014; Ong'amo *et al.,* 2014; Goftishu *et al.,* 2018). Likewise, Mailafiya *et al.,* (2009) found a high assorted diversity of the *Cotesia* spp. especially among *Busseola* spp. and *Chilo* spp., which in a likewise manner revealed a strong host-parasitoid specificity. This implies that the chemical(s) associated with host recognition and acceptance by these parasitoids must be specific to the host species involved, as confirmed in the present investigation. In spite of the fact that, the response of parasitoid females to α-amylase

is not binomial/mutually exclusive (yes or no) becoming more intense with the α-amylase of its natural host, some behavioural responses still occurred when parasitoids were presented with α-amylases of unsuitable hosts. Considering all scenarios constant, the likelihood of encounter between *B. fusca* with *C. flavipes* and Cs-coast as well as between *C. partellus* with Cs-Inland is very low due to the different geographical distribution of their respective hosts: *B. fusca* is generally present in the highlands whereas *C. partellus* is mostly present in the lowlands (Mailafiya *et al.,* 2010; Mwalusepo *et al.,* 2015). Such ecological patterns of the host-parasitoid associations suggest that their preference for the host specific α-amylase results from adaptation (even recent adaptation, of *C. flavipes* towards *S. calamistis*) to local hosts, as shown for the virulence function for *C. sesamiae* populations (Dupas *et al.,* 2008; Gauthier *et al.,* 2018).

Subsequent to studying and identifying the specific chemical mediating host recognition and acceptance in *C. flavipes* complex and *C. sesamiae*, the molecular basis of specific host recognition in these parasitoids remained largely unknown. However due to the existence of two populations of *C. sesamiae* in Kenya, viz, Cs-Coast and Cs-Inland, with contrasted level of acceptance of *B. fusca* host, advantage of this was exploited in order to determine the candidate genes involved in host acceptance by the parasitoids. A genetic analysis approach of crosses between these two populations was thus initiated. These two populations were crossed up to F8 in experiments which confirmed that their acceptance for *B. fusca* for oviposition is heritable. However, phenotyping experiments (=acceptance tests towards *B. fusca* for

oviposition) of both parental lines and F8 females from clonal sib ships indicated that acceptance towards *B. fusca* for oviposition is not a character of all or nothing but rather variable with a higher rate of acceptance for backcrosses with a female Kitale than backcrosses with a female Mombasa.

6.2 Conclusions

The present findings show that the oral secretions of the larvae of lepidopteran stem borers harbour active compound(s) that mediate host acceptance for oviposition in *C. flavipes.* These secretions allow the female parasitoid species used in this study to discriminate among hosts and non-host larvae. α-Amylase was the most active compound identified from the parasitoid's host oral secretions. This is a key protein for host acceptance and oviposition by three species of the *C. flavipes* complex, and its conformation variation is largely involved in the specificity of host-parasitoid association of these three species constituting a good candidate for determining an evolutionary solution to host acceptance for oviposition in those parasitoids.

6.3 Recommendations

This study shows for the first time in the literature that α-amylases from the oral secretion of larvae of stemborers are involved in host recognition and acceptance by different species of parasitoid belonging to *Cotesia flavipes* complex. However, since their isoform electrophoretic migration distance depends on the molecule electric charge, it is not obvious whether different bands represent allelic variation or are duplicated individuals can express different isoforms of the α-amylase. To confirm this, it will

gene copies. It can thus be hypothesized that within stemborer species, be necessary to isolate and characterise the α-amylase expressed by the individual stemborer hosts.

In addition, this α-amylase protein might be perceived through the sensorial equipment of the parasitoid, antennae or tarsi via gustation. Obonyo *et al.* (2011) showed the presence of specific sensilla, sensilla chaetica on the last antennal segment of the female parasitoids and they are known to possess gustatory functions in insects. These sensorial organs have been shown to electrophysiologically detect (cf gustation) the α-amylase (Mailhan, 2016). However, this detection have not yet been confirmed (Tolassy, 2018), suggesting that other sensilla from other sensorial organs, such as the tarsi, might be involved in such detection. Therefore, there is still a need to determine electrophysiologically whether the parasitoids can detect the α-amylase protein via gustation process and also to identify the sensorial organs involved in such detection.

After showing that the acceptance towards *B. fusca* for oviposition is heritable, the identification of candidate genes involved in such host acceptance was initiated through RAD Seq analyses. These analyses will in the future link the genetic map and the QTLs generated to the analyses of the sensorial phenotypic traits associated to host recognition and acceptance behavior towards *B. fusca*. A preliminary analysis showed that there are about 1,500 variable markers in the two Cotesia strains used in the study. In the future, these markers can be organised along a genetic map and a QTL analysis can then be performed as per the developed honey-bee protocol

(Mougel *et al*., 2012). Mapping of marker sequences linked to the obtained QTL for the identification of candidate genes involved in host acceptance will be possible since reference genomes have already been determined for the two parental populations (Unpublished data).

REFERENCES

Afsheen, S., Wang, X., Li, R., Zhu, C.-S. & Lou, Y.-G. (2008). Differential attraction of parasitoids in relation to specificity of kairomones from herbivores and their by-products. *Insect Science* 15, 381–397.

Aghajari N, Roth M, Haser R. (2002). Crystallographic evidence of a transglycosylation reaction: ternary complexes of a psychrophilic α-amylase. *Biochemistry* 41, 4273-4280.

Alborn, H.T., Lewis, W.J.& Tumlinson, J.H. (1995). Host specific recognition kairomone for the parasitoid Microplitis croceipes (Cresson). *Journal of Chemical Ecology* 21, 1697-1708.

Althoff D.M. & Thompson J.N. (2001). Geographic structure on the searching behavior of a specialist parasitoid: combining molecular and behavioral approaches. *Journal of Evolutionary Biology* 14, 406-417.

Amoako-Atta, B., Omolo, E.O. & Kidega, E.K. (1983). Influence of maize, cowpea and sorghum intercropping systems on stem-/pod-borer infestations. *International Journal of Tropical Insect Science* 4, 47-57.

Ananthakrishnan, T.N., Senrayan, R., Murugesan, S. & Annadurai, R.S. (1991). Kairomones of *Heliothis armigera*and *Corcyra cephalonica* and their influence on the parasitic potential of *Trichogramma chilonis* (Trichogrammatidae: Hymenoptera). *Journal of Biosciences* 16, 111-119.

Annette, K.W. (1994). Species of Microgastrinae (Hymenoptera: Braconidae) parasitizing lepidopterous cereal stem borers in Africa. *Bulletin of Entomological Research* 84, 421-434.

Antolin M. F., Bjorkstein T. A. & Vaughn T. (2006). Host-related fitness trade-offs in a presumed generalist parasitoid, *Diaeretiella rapae* (Hymenoptera: Aphidiidae). *Ecological Entomology* 31, 242–254.

Asgari, S., Zhang, G. & Schmidt, O. (2003). Polydnavirus particle proteins with similarities to molecular chaperones, heat-shock protein 70 and calreticulin. *Journal of General Virology* 84, 1165-1171.

Assefa Y., Van den Berg J., Mitchell A., Le Ru B.P. & Conlong D.E. (2009). Record of *Eldana saccharina* Walker (Lep, Pyralidae) in inland South Africa and its genetic relationship with the coastal Population. *Journal of Applied Entomology* 133, 440-455.

Barbosa, P., Kemper, J., Gross, P. & Martinat, P. (1990). Influence of dietary nicotine and colony source of *Manduca sexta* (Lepidoptera: Sphingidae) on its suitability as a host of *Cotesia congregata* (Hymenoptera: Braconidae). *Entomophaga* 35, 223-231.

Beaumont M. A. (2010). Approximate Bayesian Computation in Evolution and Ecology. *Annual Review of Ecology, Evolution, and Systematics* 41, 379-406.

Beckage, N.E. & Gelman, D.B. (2004). Wasp parasitoid disruption of host development: implications for new biologically based strategies for insect control. *Annual Review of Entomology* 49, 299-330.

Bénédet F, Bigot Y, Renault S, Pouzat J, Thibout E. (1999). Polypeptides of *Acrolepiopsis assectella* cocoon (Lepidoptera: Yponomeutoidea): an external host-acceptance kairomone for the parasitoid *Diadromus pulchellus* (Hymenoptera Ichneumonidae). *Journal of Insect Physiology* 45, 375-384.

Benedet, F., Bigot, Y., Renault, S., Pouzat, J. & Thibout, E. (1999). Polypeptides of *Acrolepiopsis assectella* cocoon (Lepidoptera: Yponomeutoidea): an external host-acceptance kairomone for the parasitoid *Diadromus pulchellus* (Hymenoptera Ichneumonidae). *Journal of Insect Physiology* 45, 375-384

Bichang'a, G., Da Lage, J.L., Capdevielle-Dulac, C., Zivy, M., Balliau, T., Sambai, K., Le Ru, B., Kaiser, L., Juma, G., Maina, E.N.M. & Calatayud, P.A. (2018). α-Amylase mediates host acceptance in the braconid parasitoid Cotesia flavipes. *Journal of Chemical Ecology* 44, 1030-1039.

Bichang'a G. (2013). Identification of kairomones mediating host recognition and acceptance by *Cotesia* spp. in Kenya. Dissertation, University of Nairobi

Blomquist, G. J., & Bagnères, A. G. (2010). Introduction: history and overview of insect hydrocarbons. In: Blomquist, G.J. & Bagnères, A.G. 2010. Insect Hydrocarbons: Biology, Biochemistry and Chemical Ecology. Cambridge, UK: Cambridge University Press, 3–18.

Bonhof, M., Overholt, W., Van Huis, A. & Polaszek, A. (1997). Natural Enemies of Cereal Stemborers in East Africa: A Review. *Insect Science and Its Application* 17, 19-35.

Boo, K.S. & Yang, J.P. (2000). Kairomones used by *Trichogramma chilonis* to find *Helicoverpa assulta* eggs. *Journal of Chemical Ecology* 26, 359-375.

Branca, A., F. Vavre, J.-F. Silvain & Dupas, S., (2009). Maintenance of adaptive differentiation by *Wolbachia* induced bidirectional cytoplasmic incompatibility: the importance of sib-mating and genetic systems. *BMC Evolutionary Biology* 9, 185–198.

Branca A., Le Ru B. P., Vavre F., Silvain J.-F. & Dupas S. (2011a). Intraspecific specialization of the generalist parasitoid *Cotesia sesamiae* revealed by polyDNAvirus polymorphism and associated with different *Wolbachia* infection. *Molecular Ecology* 20, 959-971.

Branca A., Dupas S., & Gitau, C. W. (2011b). Maintenance of specialized parasitoid populations by polydnaviruses. In J.-M. Drezen & N. E. Beckage (Eds.), Parasitoid Viruses: Symbionts and Pathogens. Elsevier.

Branca, A., Gitau, C., & Dupas, S. (2012). Maintenance of specialized parasitoid populations by polydnaviruses. In *Parasitoid Viruses* (pp. 127-135).

Burks, M.L. & Nettles, W.C. (1978). Effects of cuticular extracts from *Heliothis virescens* and other factors on oviposition. *Environmental Entomology* 7, 897-900.

Calatayud, P.-A., Auger, J., Thibout, E., Rousset, S., Caicedo, A.M., Calatayud, S., Buschmann, H., Guillaud, J., Mandon, N. & Bellotti, A.C. (2001). Identification and synthesis of akairomone mediating host location by two parasitoid species of the cassava mealybug *Phenacoccus herreni. Journal of Chemical Ecology* 27, 2203-2217.

Calatayud, P.-A., Guénégo, H., Le Ru, B., Silvain, J.-F. & Frérot, B. (2007). Temporal patterns of emergence, calling behaviour and oviposition period of the maize stem borer, *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae). *Annales de la Société Entomologique de France* 43, 63-68.

Calatayud, P-A & Le Ru, Bruno & Van den Berg, Johnnie & Schulthess, Fritz. (2014). Ecology of the African Maize Stalk Borer, *Busseola fusca* (Lepidoptera: Noctuidae) with Special Reference to Insect-Plant Interactions. *Insects* 5, 539-563.

Canale, A. & Raspi, A. (2000). Host location and oviposition behaviour in *Opius concolor* (Hymenoptera: Braconidae). *Entomological Problems* 31, 25-32.

Chabi-Olaye, A., Nolte, C., Schulthess, F., & Borgemeister, C. (2005). Effects of grain legumes and cover crops on maize yield and plant damage by *Busseola fusca* (Fuller)(Lepidoptera: Noctuidae) in the humid forest of southern Cameroon. *Agriculture, Ecosystems & Environment* 108, 17-28.

Chabi-Olaye, A., Schulthess, F. & Borgemeister, C. (2008). Effects of Nitrogen and Potassium Combinations on Yields and Infestations of Maize by *Busseola fusca* (Lepidoptera: Noctuidae) in the Humid Forest of Cameroon. *Journal of Economic Entomology* 101, 90-98.

Chng W.A., Bou Sleiman M.S., Schüpfer F. & Lemaitre B. (2014). Transforming growth factor β/activin signaling functions as a sugar-sensing feedback loop to regulate digestive enzyme expression. *Cell Reports* 9, 336-348.

Chrambach A. & Jovin T.M. (1983). Selected buffer systems for moving boundary electrophoresis on gels at various pH values, presented in a simplified manner. *Electrophoresis* 4, 190–204.

Colazza, S. & Rosi, M.C. (2001). Difference in the searchingbehaviour of two strains of the egg parasitoid *Telenomusbusseolae* (Hymenoptera: Scelionidae). *European Journal of Entomology* 98, 47-52.

Commin C., Aumont-Nicaise M., Claisse G., Feller G. & Da Lage J.-L. (2013). Enzymatic characterization of recombinant α-amylase in the *Drosophila melanogaster* species subgroup: is there an effect of specialization on digestive enzyme? *Genes & Genetic Systems* 88, 251-259.

Conti, E., Salerno, G., Bin, F. & Vinson, S.B. (2004). The role of host semiochemicals in parasitoid specificity: a case study with *Trissolcus brochymenae* and *Trissolcus simoni* on pentatomid bugs. *Biological Control* 29, 435-444.

Cortesero, A.M., Stapel, J.O. & Lewis, W.J. (2000). Understanding and manipulating plant attributes to enhance biological control. *Biological Control* 17: 35-49.

Craig R, Beavis RC. (2004). TANDEM: matching proteins with tandem mass spectra. Bioinformatics 20, 1466-1467.

Cugala D., Overholt W.A., Santos L. & Giga D. (2001). Release of *Cotesia flavipes* Cameron for biological control of cereal stemborers in two ecological zones in Mozambique. *International Journal of Tropical Insect Science* 21, 303-310.

Cvačka, J., Jiroš, P., Šobotník, J., Hanus, R., & Svatoš, A. (2006). Analysis of insect cuticular hydrocarbons using matrix-assisted laser desorption/ionization mass spectrometry. *Journal of Chemical Ecology* 32, 409–434.

Da Lage J-L, Renard E, Chartois F, Lemeunier F, & Cariou M-L. (1998). Amyrel, a paralogous gene of the amylase gene family in *Drosophila melanogaster* and the *Sophophora subgenus*. *Proceeding of the National Academic of Science of USA* 95, 6848-6853.

Da Lage, J.-L., van Wormhoudt, A., & Cariou, M.-L. (2002). Diversity and evolution of the alpha-amylase genes in Animals. *Biologia Bratislava* 57 (suppl.11), 181-189.

Da Lage, J.-L., Maczowiak, F., & Cariou, M.-L. (2011). Phylogenetic distribution of intron positions in alpha-amylase genes of *Bilateria* suggests numerous gains and losses. *PLoS one* 6(5), e19673.

Da Lage, J.-L. (2018). The amylases of insects. *International Journal Insect Science* 10, 1-14.

Dedeine, F., F. Vavre, F. Fleury, B. Loppin, M. E. Hochberg, & M. Boulétreau. (2001). Removing symbiotic *Wolbachia* bacteria specifically inhibits oogenesis in a parasitic wasp. *Proceedings of the National Academy of Sciences of USA* 98, 6247–6252.

Delury, N.C., Gries, R., Gries, G., Judd, G.J.R. & Khaskin, G. (1999). Moth scale derived kairomones used by egg-larval parasitoid *Ascogaster quadridentata* to locate eggs of its host, *Cydia pomonella. Journal of Chemical Ecology* 25, 2419- 2431.

Dicke, M. & Sabelis, M.W. (1988). How plants obtain predatory mites as bodyguards. *Netherlands Journal of Zoology* 38, 148-165.

Dicke, M. & Vet, L.E.M. (1999). Plant-carnivore interactions: Evolutionary and Ecologicalconsequences for plant, herbivore and carnivore. In: Olff, H., Brown, V.K. & Drent, R.H. (Eds.). *Herbivores: between plant and predators*. Proceedings of 38th symposium of the British Ecological society 1997. Wageningen, The Netherlands. Black Well Science, Oxford, UK, pp. 483-520.

Dicke M. (2016). Induced plant volatiles: Plant body odours structuring ecological networks. *New Phytologist* 210, 10-12.

Dion E., Zélé F., Simon J.C. & Outreman Y. (2011). Rapid evolution of parasitoids when faced with the symbiont-mediated resistance of their hosts. *Journal of Evolutionary Biology* 24, 741–750.

Dmoch, J., Leiws, W.J., Martin, P.B. & Nordlund, D.A. (1985). Role of host-produced stimuli and learning in host selection behavior of *Cotesia (=Apanteles) marginiventris* (Cresson). *Journal of Chemical Ecology* 11, 453-463.

Doebley J, (2004). The Genetics of Maize Evolution. *Annual Review of Genetics* 38, 37–59.

DT maize (2015). A quarterly bulletin of the drought tolerant maize for Africa project. Vol. 4, No. 1, March 2015.

Dubuffet A., Rodríguez Álvarez C.I., Drezen J.M., van Alphen J.J.M. & Poirié M. (2006). Do parasitoid preferences for different host species match virulence? *Physiological Entomology* 31, 170-177.

Dupas S, Frey F., & Carton Y. (1998). A single parasitoid segregating factor controls immune suppression in Drosophila. *Journal of Heredity* 89, 306-311.

Dupas S. & Boscaro M. (1999). Geographic variation and evolution of immunosuppressive genes in a *Drosophila* parasitoid. *Ecography* 22, 284-291.

Dupas S., Carton Y. & Poirié M. (2003). The genetic dimension of the coevolution of virulence resistance in *Drosophila* - parasitoid wasp relationships. *Heredity*. 90(1), 84-89.

Dupas, S., Gitau, C. W., Branca, A., Le Ru, B. P., & Silvain J.-F. (2008). Evolution of a polydnavirus gene in relation to parasitoid–host species immune resistance. *Journal of Heredity* 99, 491-499.

El-Shafie, H.A. & Faleiro, J.R. (2017). Semiochemicals and their potential use in pest Management. Biological Control of Pest and Vector Insects. http://dx.doi.org/10.5772/66463

Elzen, G.W., Williams, H.J. & Vinson, S.B. (1984). Isolation and identification of cotton synomones mediating searching behavior by parasitoid *Campoletis sonorensis.* *Journal of Chemical Ecology* 19, 1251-1264.

Erb M., Robert C.A.M., Hibbard B.E., & Turlings T.C.J. (2011). Sequence of arrival determines plant-mediated interactions between herbivores. *Journal of Ecology* 99, 7-15.

OECD/FAO (2018), OECD-FAO Agricultural Outlook 2018-2027, OECD Publishing, Paris/Food and Agriculture Organization of the United Nations, Rome. https://doi.org/10.1787/agr_outlook-2018-en

FAO, (2002). FAOSTAT Statistical database. Agricultural data. http://www.fao.org accessed 15 September 2008.

FAO (2017). The future of food and agriculture – Trends and challenges. Rome.

FAO GIEWS (2017). Global Information and Early Warning System COUNTRY Briefing on Kenya http://www.fao.org/giews/countrybrief/country.jsp?code=KEN

Feener, D.H.Jr., Jacobs, L.F. & Schmidt, J.O. (1996). Specialized parasitoid attracted to a pheromone of ants. *Animal Behaviour* 51, 61-66.

Fellowes M.D.E., Van Alphen J.J.M. & Jervis M.A. (2005). Foraging Behaviour. Insects as natural enemies: A practical perspective (ed. M.A. Jervis). Springer.

Franco, O. L., Ridgen, D. J., Melo, F. R., Bloch, C. Jr., Silva, C.P., & Grossi-de-Sa, M. F. (2000). Activity of wheat α-amylase inhibitors towards bruchid α-amylases and structural explanation of observed specificities. *European Journal of Biochemistry* 267, 2166–2173.

Gatsby Charitable Foundation (2005). The Quiet Revolution: Push-Pull Technology and the African Farmer. Gatsby Occasional Paper, April 2005.

Gauthier N., Mandon N., Renault S., & Bénédet F. (2004). The *Acrolepiopsis assectella* silk -cocoon: kairomonal function and chemical characterization. *Journal of Insect Physiology* 50, 1065-1074.

Gauthier, J., Gayral, P., Le Ru, B. P., Jancek, S., Dupas, S., Kaiser, L., Gyapay, G., & Herniou, E. A. (2018). Genetic footprints of adaptive divergence in the bracovirus of *Cotesia sesamiae* identified by targeted resequencing. *Molecular Ecology* 27, 2109-2123.

Gifford, J.R. & Mann, G.A. (1967). Biology, rearing and trial release of *Apanteles flavipes* in the Florida everglades to control sugarcane borer. *Journal of Economic Entomology* 60, 44-47.

Gitau C.W., Dupas Stéphane, Ngi-Song A.J., Mbugi J.P., & Schulthess F. (2006a). Calyx fluid proteins of two *Cotesia sesamiae* (Cameron) (Hymenoptera: Braconidae) biotypes in Kenya : implications to biological control of the stem borer *Busseola fusca* (Fuller) (Lepidoptera : Noctuidae). *Japanese Journal of Applied Entomology and Zoology* 50, 87-99.

Gitau, A.C.W. (2006b). Geographic variation in development of *Cotesia sesamiae* (Hymenoptera: Braconidae) on *Busseola fusca* (Lepidoptera: Noctuidae) in Kenya: Co-evolutionary genetics and role of Polydnaviruses. (PP.166). Ph.D Thesis, Kenyatta University, Nairobi, Kenya.

Gitau, C. W., D. Gundersen-Rindal, M. Pedroni, P. J. Mbugi, & S. Dupas (2007). Differential expression of the CrV1 haemocyte inactivation-associated polydnavirus gene in the African maize stem borer *Busseola fusca* (Fuller) parasitized by two

biotypes of the endoparasitoid Cotesia sesamiae (Cameron). *Journal of Insect Physiology* 53, 676‑84.

Gitau, C. W, F. Schulthess, & S. Dupas. (2010). An association between host acceptance and virulence status of different populations of *Cotesia sesamiae*, a braconid larval parasitoid of lepidopteran cereal stemborers in Kenya. *Biological Control* 54, 100-106.

Glaser N., Frérot B., Leppik E., Monsempes C., Capdevielle-Dulac C., Le Ru B., Lecocq T., Harry M., Jacquin-Joly E., & Calatayud P.-A. (2014). Similar Differentiation Patterns Between PBP Expression Levels and Pheromone Component Ratios in Two Populations of *Sesamia nonagrioides*. *Journal of Chemical Ecology* 40, 923-927.

Godfray, H.C.J. (1994). Parasitoid**s:** behavioral and evolutionary ecology. Krebs J.R. & Clutton-Brock, T. (Eds.). (PP.473). *Princeton University Press*, Princeton, USA.

Godfray H.C.J. & Shimada M. (1999). Parasitoids as model organisms for ecologists. *Researches on Population Ecology* 10, 3-10.

Godfray, H. C. J., Beddington, J. R., Crute, I. R., Haddad, L., Lawrence, D., Muir, J. F., Pretty, J., Robinson, S., Thomas, S. M., & Toulmin, C. (2010). Food security: The challenge of feeding 9 billion people. *Science* 327, 812–818.

Goftishu M., Assefa Y., Niba A. & Fininsa C. (2016). Cereal stem borer management practices in subsistence farms of eastern Ethiopia, *International Journal of Pest Management* 63, 289-298.

Goftishu, M., Assefa, Y., Fininsa, C., Niba, A., Capdevielle-Dulac, C., & Le Ru, B. P. (2018). Diversity and abundance of lepidopteran stem borers and their host plants in Ethiopia. *Journal of Applied Entomology* 142, 437-449.

Gounou, S., Chabi-Olaye, A., Poehling, H.-M., & Schulthess, F., (2008). Reproductive compatibility of several East and West African *Cotesia sesamiae* (Hymenoptera: Braconidae) populations and their crosses and backcrosses using *Sesamia calamistis* (Lepidoptera: Noctuidae) as the host. *Biocontrol Science and Technology* 18, 255–266.

Gouy, M., Guindon, S., & Gascuel, O. (2010). SeaView version 4: A multiplatform graphical user interface for sequence alignment and phylogenetic tree building. *Molecular Biology and Evolution* 27, 221–224.

Grasswitz, T.R. & G.R. Jones (2002). "Chemical Ecology". Encyclopedia of life science. John Wiley & Sons, Ltd

Greany, P.D., Hawke, S.D., Carlysle, T.C. & Anthony, D.W. (1977). Sense organs in the ovipositor of *Biosteres* (*Opius*) *longicaudatus*, a parasite of the caribbean fruit fly *Anastrepha suspensa*. *Annals of the Entomological Society of America* 70, 319-321.

Groote H. De, Owuor G., Doss C., Ouma J., Muhammad L., & Danda K., (2005). "The Maize Green Revolution in Kenya Revisited," *The Electronic Journal of Agricultural and Development Economics, Food and Agriculture Organization of the United Nations*, vol. 2(1), PP.32-49.

Gurr, G.M., Wratten, S.D. & Altieri, M.A. (2004). Ecological Engineering for the pest Management: Advances in habitat manipulation for Arthropods. CABI International, Wallingford, Oxon, United Kingdom, pp. 155-156.

Hailemichael, Y., Schulthess, F., Smith Jr, J., Overholt, W. & Chabi-Olaye, A. (2008). Resource allocation and bionomic of indigenous and exotic *Cotesia* (Hymenoptera:

Braconidae) species reared on *Sesamia calamistis*. *Bulletin of Entomological Research* 98, 405-415.

Hardie, J., Hicks, A.J., Holler, C., Mann, J., Merrit, L., Nottingham, S.F., Powell, W., Wadhams, L.J., Witthinrich, J. & Wright, A.F. (1994). The response of *Praon* spp. parasitoids to aphid. Sex pheromone components in the field. *Entomologia Experimentalis et Applicata* 71, 95-99.

Harris, V.E. & Todd, J.W. (1980). Male-mediated aggregation of male, female, and 5th-instar southern green stink bugs and concomitant attraction of a tachinid parasite, *Trichopoda pennipes. Entomologia Experimentalis et Applicata* 27, 117- 126.

Harris, K.M. (1989). Recent advances in sorghum and pearl millet stemborer research. In: Nwanze, K.F. (Ed). *International Workshop on Sorghum Stemborers*, Patancheru, 1987, ICRISAT. Patancheru, India, pp. 9-16.

Harris K.M. (1990). Bioecology of sorghum stemborers. *Insect Science and Its Application* 11, 467–477.

Hare, J.D., Millar, J.G. & Luck, R.F. (1993). A caffeic acid ester mediates host selection by a parasitic wasp. *Naturwissenschaften* 80, 92-94.

Hare, J.D. (1996). Priming *Aphytis*: behavioral modification of host selection by exposure to a synthetic contact kairomone. *Entomologia Experimentalis et Applicata* 78, 263-269

Hare, J. D., & Morgan, D. J. W. (1997). Priming*Aphytis:* Behavioral Improvement of Insectary-Reared Biological Control Agents. *Biological Control* 10, 207-214.

Harvey J.A., van Nouhuys S., & Biere A. (2005). Effects of quantitative variation in allelochemicals in *Plantago lanceolata* on development of a generalist and a

specialist herbivore and their endoparasitoids. *Journal of Chemical Ecology* 31, 287-302.

Hassan, R.M., Njoroge, K., Njoro, M., Otsyulaa, R. & Laboso A. (1998). Adoption pattern and performance of improved maize development and transfer. A GIS application for research planning in Kenya. OXON (UK). CAB International and International Maize and wheat Improvement Centre (CIMMYT). pp. 107-136.

Hassell, M.P. (1968). The behavioral response of a tachinid fly (*Cyzenis albicans* Fall.) to itshost, the winter moth (*Operophtera brumata* L.). *Journal of Animal Ecology* 37, 627-639.

Hatano E., Kunert G., Bartram S., Boland W., Gershenzon J. & Weisser W.W. (2008): Do aphid colonies amplify their emission of alarm pheromone? *Journal of Chemical Ecology* 34, 1149–1152.

Hawkins B.A. (1994). Host mortality and parasitoid impact. In: *Pattern and Process in Host-Parasitoid Interactions*. Cambridge University Press.

Hegge, A. (2007). Integrated Pest Management in African Agriculture, Munich, GRIN Publishing GmbH. http://www.grin.com/en/e-book/110812/integrated-pest-management-in-african-agriculture

Heil M. (2008). Indirect defence via tritrophic interactions. *New Phytologist* 178, 41-61.

Henry, L.M., Roitberg, B.D. & Gillespie, D.R. (2008). Host-range evolution in *Aphidius* parasitoids: fidelity, virulence and fitness trade-offs on an ancestral host. *Evolution* 62, 689-699.

Henry L.M., May N., Acheampong S., Gillespie D.R. & Roitberg B.D. (2010). Host-adapted parasitoids in biological control: Does source matter? *Ecological Applications* 20, 242-250.

Hermann, H.R. & Douglas, M.E. (1976). Comparative survey of the ovipositor structures on the sting and ovipositor of hymenopterous insects. *Journal of the Georgia Entomological Society* 11, 223-239.

Hilgenboecker, K., Hammerstein, P., Schlattmann, P., Telschow, A., & Werren, J. H., (2008). How many species are infected with *Wolbachia*? – a statistical analysis of current data. *FEMS Microbiology Letters* 281, 215–220.

Hilker, M., Blaske, V., Kobs, C. & Dippel, C. (2000). Kairomonal effects of sawfly sex pheromones on egg parasitoids. *Journal of Chemical Ecology* 26, 2591-2601.

Holt R.D. & Hochberg M.E. (1997). When is biological control stable (or is it)? *Ecology*, 78, 1673-1683.

Hou, B. & Wu, L. (2010). Safety impact and farmer awareness of pesticide residues. *Food and Agricultural Immunology* 21, 191-200

ICIPE (2000). Habitat Management Strategies for Control of Stemborers and Striga Weed in Maize Based Systems. Paper Presented at ICIPE KARI-MOA-Rothamsted Collaborative Planning Workshop held at KARI-Kakamega, February, 17, 2000.

Inayatullah C. (1983). Host selection by *Apanteles flavipes* (Cameron) (Hymenoptera: Braconidae): influence of host and host plant. *J Econ Entomol* 76:1086-1087.

Infonet-Biovision. (2011). Spotted stemborer. Infonet-Biovision, http://www.infonand et-biovision.org. Accessed January 2013.

IPCC (2007). Fourth Assessment Report: *Climate Change* (AR4)

Jackson, C.G., Bryan, D.E., Neeman, E.G. & Patana, R. (1976). *Palexorista laxa*: development, longevity and production of progeny on *Heliothis* spp. *Environmental Entomology* 5, 431–434.

James, C. (2003). Global Review of commercialised Transgenic Crops: 2002 Feature: Bt - maize *ISAAA Briefs. No. 29 ISAAA Ithaca, NY* http://www.isaaa.org/ Resources/Publications accessed 13 October 2007.

Jembere, B., Ngi-Song, A.J. & Overholt, W.A. (2003). Olfactory responses of *Cotesia flavipes* (Hymenoptera: Braconidae) to target and non-target Lepidoptera and their host plants. *Biological Control* 28, 360-367.

Jiang, N., Sétamou, M., Ngi-Song, A.J. & Omwega, O.C. (2004). Performance of *Cotesia falvipes* (Hymenoptera: Braconidae) in parasitizing *Chilo partellus* (Lepidoptera: Crambidae) as affected by temperature and host stage. *Biological Control* 31, 155-164.

Jiang, N., Zhou, G., Overholt, W.A. & Schulthess, F. (2006). The synchrony of the stemborer and parasitoid populations of coastal Kenya. *Annales de la Société Entomologique de France* 42, 381–388.

Jones, R.L., Lewis, W.J., Beroza, M., Bierl, B.A. & Sparks, A.N. (1973). Host-seeking stimulants (kairomones) for the eggparasite, *Trichogramma evanescens. Environmental Entomology* 2, 593-596.

Jones, R.L., Lewis, W.J., Bowman, M.C., Beroza, M. & Beirl, B.A. (1971). Host-seeking stimulant for parasite of corn earworm: Isolation, identification, and synthesis. *Science* 17, 842-843.

Jonesa P. G and Thorntonb P. K. (2003). The potential impacts of climate change on maize productionin Africa and Latin America in 2055. *Global Environmental Change* 13, 51–59

Kaiser L., Couty A., Perez-Maluf R. (2009). Dynamic use of fruit odours to locate host larvae: individual learning, physiological state and genetic variability as adaptive mechanisms. In*: Parasitoids of Drosophila* (ed. G. Prevost). *Advances in Parasitology* 70, 67-94.

Kaiser, L., Le Ru, B. P., Kaoula, F., Paillusson, C., Capdevielle-Dulac, C., Obonyo, J. O., Herniou, E. A., Jancek, S., Branca, A., Calatayud, P.-A., Silvain, J.-F. and Dupas, S. (2015), Ongoing ecological speciation in *Cotesia sesamiae*, a biological control agent of cereal stem borers. *Evolutionary Application* 8, 807–820.

Kaiser, L., Fernandez-Triana, J., Capdevielle-Dulac, C., Chantre, C., Bodet, M., Kaoula, F., Benoist, R., Calatayud, P.-A., Dupas, S., Herniou, E.A., Jeannette, R., Obonyo, J., Silvain, J.-F., and Le Ru, B. (2017). Systematic and biology of *Cotesia typhae* sp. n. (Hymenoptera, Braconidae, Microgastrinae), a potential biological control agent against the noctuid Mediterranean corn borer, *Sesamia nonagrioides*. *ZooKeys* 682, 105-136.

Kaiser L., Ode P., van Nouhuys S., Calatayud P.-A., Colazza S., Cortesero A.-M., Thiel A., & van Baaren J. (2017a). The Plant as a Habitat for Entomophagous Insects. *Insect-Plant Interactions in a Crop Protection Perspective*, eds Sauvion, N., Calatayud, P.-A. & Thiéry, D. (Elsevier, GBR) Advances in Botanical Research series, Vol 81, pp 179-224. doi:10.1016/bs.abr.2016.09.006.

Kaiser L., Dupas S., Branca A., Herniou E.A., Clarke C.W., Capdevielle-Dulac C., Obonyo J., Benoist R., Gauthier J., Calatayud P.-A., Silvain J.-F., & Le Ru B.P. (2017b). The *Cotesia sesamiae* story: insight into host-range evolution in a Hymenoptera parasitoid and implication for its use in biological control programs. *Genetica* 145, 455-468.

Kaur, R., Kaur, N., & Gupta, A. K. (2014). Structural features, substrate specificity kinetic properties of insect α-amylase and specificity of plant α-amylase inhibitors. *Pesticide Biochemistry and Physiology* 116, 83-93.

Kfir R. (1992). Seasonal abundance of the stem borer *Chilo partellus* (Lepidoptera: Pyralidae) and its parasites on summer grain crops. *Journal of Economic Entomology* 85,518–529.

Kfir, R. (1995). Parasitoids of the African stemborer *Busseola fusca* (Lepidoptera: Noctuidae) in South Africa. *Bulletin of Entomological Research* 85, 369–377.

Kfir R., Overholt W.A., Khan Z.R., & Polaszek A. (2002). Biology and management of economically important lepidopteran cereal stemborers in Africa. *Annual Review of Entomology* 47, 701–731.

Kraaijeveld A.R., Alphen J.J.M. van & Godfray H.C.J. (1998). The coevolution of host resistance and parasitoid virulence. *Parasitology* 116, S29-S45.

Kraaijeveld A.R., Hutcheson K., Limentani E.C. & Godfray H.C. (2001). Costs of counterdefenses to host resistance in a parasitoid of *Drosophila*. *Evolution* 55, 1815-1821.

Kurien BT, & Scofield RH. (2012). Extraction of proteins from gels: A brief review. *Methods in Molecular Biology* 869, 403–405.

Kuwahara, Y., Nemoto, T., Shibuya, M., Matsuura, H. & Shiraiwa, Y. (1983). 2-palmitoyl- and 2-oleoyl-cyclohexane- l, 3-dione from feces of the Indian meal moth, *Plodia interpunctella*: kairomone components against a parasitic wasp, *Venturia canescens*. *Agricultural and Biological Chemistry* 47, 1929-1931.

Lacovone A., French A.S., Tellier F., & Cusumano, (2016). The role of contact chemoreception in the host location process of an egg parasitoid. *Journal of Insect Physiology* 92, 63-75.

Langella O., Valot B., Balliau T., Blein-Nicolas M., Bonhomme L., & Zivy M. (2016). X! TandemPipeline: a tool to manage sequence redundancy for protein inference and phosphosite identification. *Journal of Proteome Research* 16, 494-503.

Lazarovitz, G., Goettel, S., & Vincent, C. (2007). Adventures in biological control. *Biological Control: A Global Perspective: Case Studies from Around the World*, eds Vincent, C., Goettel, S., and Lazarovitz, G. (CABI, Wallingford), pp. 1-6.

Le Ru, B. P., Ong'amo, G. O., Moyal, P., Muchugu, E., Ngala, L., Musyoka, B., Abdullah, Z., Matama-Kauma, T., Lada, V. Y., Pallangyo, B., Omwega, C. O., Schulthess, F., Calatayud, P.-A., & Silvain, J.-F. (2006a). Geographic distribution and host plant ranges of East African noctuid stem borers. *Annales de la Société Entomologique de France* 42, 353–361.

Le Ru, B. P., Ong'amo, G. O., Moyal, P., Ngala, L., Musyoka, B., Abdullah, Z., Cugala, D., Defabachew, B., Haile, T. A., Matama-Kauma, T., Lada, V. Y., Negassi, B., Pallangyo, B., Ravololonandrianina, J., Sidumo, A., Omwega, C. O., Schulthess, F., Calatayud, P.-A., & Silvain, J.-F. (2006b). Diversity of lepidopteran stemborers on

monocotyledonous plants in eastern Africa and island of Madagascar and Zanzibar revisited. *Bulletin of Entomological Research* 96, 1-9.

Lenteren, J.C. van. (2006). How not to evaluate augmentative biological control. *Biological Control* 39 (2), - pp. 115 - 118.

Lewis, W.J., Nordlund, D.A., Gueldner, R.C., Teal, P.E.A. & Tumlinson, J.H. (1982). Kairomones and their use for management of entomophagous insects. *Journal of Chemical Ecology* 8, 1323-1331.

Lewis, W. J., & Martin, W. R., JR. (1990). Semiochemicals for use with parasitoids: Status and future. *Journal of Chemical Ecology* 16, 3067–3089.

Lilley, R., Hardie, J. & Wadhams, L. J. (1994). Field manipulation of *Praon* populations using semiochemicals. *Norwegian Journal of Agricultural Sciences* 16, 221–226.

Lou, Y.G., Ping, X.F. & Cheng, J. (1999). Role of kairomones in the host searching behavior of *Anagrus nilaparvatae* Pang et Wang, a parasitoid of rice planthopper. *Proceedings* of *First Asia-Pacific Conference on Chemical Ecology* (ed. J. Du), Shanghai, China. November 1-4, 1999.

Lou, Y.G. & Cheng, J. (2001). Host-recognition kairomone from *Sogatella furcifera* for the parasitoid *Anagrus nilaparvatae. Entomologia Experimentalis et Applicata* 101, 59-67.

Louda S.M., Arnett A. E., Rand T. & Russell F.L. (2003). Invasiveness of Some Biological Control Insects and Adequacy of Their Ecological Risk Assessment and Regulation. *Conservation Biology*, 17, 73-82.

Loyter, A., & Schramm, M. (1962). Charcoal-celite column. *Biochimica et Biophysica Acta* 65, 200–206.

Lundborg, G. (1999). Insect vs insect. Biological control of crop pests on the field of Africa. International Development Research Centre Ottawa. http://idrinfo.idrc.ca.archive/ReportsINTRA/pdfs/v13n3e/110878.pd

Ma, R.Z., Swedenborg, P.D. & Jones, R.L. (1992). Host-seeking behavior of *Eriborus terebrans* (Hymenoptera: Ichneumonidae) toward the European corn borer and the role of chemical stimuli. *Annals of the Entomological Society of America* 85, 72-79.

Mackey A.J., Haystead T.A., & Pearson W.R. (2002). Getting more from less algorithms for rapid protein identification with multiple short peptide sequences. *Molecular & Cellular Proteomics* 1, 139-147.

Mailafiya, D. M., Le Ru, B. P., Kairu, E. W., Calatayud, P.-A., & Dupas, S. (2009). Species diversity of lepidopteran stem borer parasitoids in cultivated and natural habitats in Kenya. *Journal of Applied Entomology* 133, 416-429.

Mailafiya, D. M., Le Ru, B. P., Kairu, E. W., Calatayud, P.-A., & Dupas, S. (2010). Geographic distribution, host range and perennation of *Cotesia sesamiae* and *Cotesia flavipes* Cameron in cultivated and natural habitats in Kenya. *Biological Control* 54, 1-8.

Mailhan, C.-M. (2016). Une α-amylase de l'hôte est-elle détectée par le système gustatif d'un Hyménoptère parasitoïde lors de ses processus de reconnaissance ? Master Report, SupAgro, Montpellier, France.

Marascuilo, L. (1966). Large-sample multiple comparisons. *Psychological Bulletin* 65, 289–299.

Marchler-Bauer, A., Bo, Y., Han, L., He, J., Lanczycki, C. J., Lu, S., Chitsaz, F., Derbyshire, M. K., Geer, R. C., Gonzales, N. R., Gwadz, M., Hurwitz, D. I., Lu, F.,

Marchler, G. H., Song, J. S., Thanki, N., Wang, Z., Yamashita, R. A., Zhang, D., Zheng, C., Geer, L. Y., … Bryant, S.H. (2016). CDD/SPARCLE: functional classification of proteins via subfamily domain architectures. *Nucleic Acids Research* 45(D1): D200-D203.

Mattiacci, L., Vinson, S.B., Williams, H.J., Aldrich, J.R. & Bin, F. (1993). A long-range attractant kairomone for egg parasitoid *Trissolcus basalis*, isolated from defensive secretion of its host, *Nezara viridula. Journal of Chemical Ecology* 19, 1167- 1181.

Meiners, T. & Hilker, M. (1997). Host location in *Oomyzus gallerucae* (Hymenoptera: Eulophidae), an egg parasitoid of the elm leaf beetle *Xanthogaleruca luteola* (Coleoptera: Chrysomelidae). *Oecologia* 112, 87-93.

Meiners, T., Westerhaus, C. & Hilker, M. (2000). Specificity of chemical cues used by a specialist egg parasitoid during host location. *Entomologia Experimentalis et Applicata* 95, 151-159.

Meyhofer, R., Casas, J. & Dorn, S. (1997). Mechano- and chemo-receptors and their possible role in host location behavior of *Sympiesis sericeicornis* (Hymenoptera: Eulophidae). *Annals of the Entomological Society of America* 90, 208-219.

Michaud, J. P. & Mackauer, M. (1994). The use of visual cues in host evaluation by aphidiid wasps. *Entomologia Experimentalis et Applicata* 70, 273-283.

Millar, J.G. & Hare, J.D. (1993). Identification and synthesis of a kairomone inducing oviposition by parasitoid *Aphytis melinus* from California red scale covers. *Journal of Chemical Ecology* 19, 1721-1736.

Millennium Ecosystem Assessment. Ecosystems and Human Well-being: Synthesis. (2005). Island Press, Washington, DC.

Minja, E.M. (1990). Management of *Chilo* spp. infesting cereals in Eastern Africa. *Insect Science and its Application* 11, 489-499.

Mizutani, N. (2006). Pheromones of male stink bugs and their attractiveness to their parasitoids. *Japanese Journal of Applied Entomology and Zoology (Japan)*.

Mochiah, M.B., Ngi-Song A.J., Overholt W.A. & Richard S. (2002a). *Wolbachia* infection in *Cotesia sesamiae* (Hymenoptera: Braconidae) causes cytoplasmic incompatibility: implications for biological control. *Biological Control* 25, 74–80.

Mochiah, M.B., Ngi-Song, A.J., Overholt, W.A. & Stouthamer, R. (2002b). Variation in encapsulation sensitivity of *Cotesia Sesamiae* biotypes to *Busseola fusca*. *Entomologia Experimentalis et Applicata* 105, 111-118.

Mohyuddin, A.I. & Greathead, D.J. (1970). Annotated list of parasites of gramineous stemborers in East Africa with a discussion of their potential in biological control. *Entomophaga* 15, 241-247.

Mohyuddin, A.I. (1971). Comparative biology and ecology of *Apanteles flavipes* and *A. sesamiae* Cameron as parasites of gramineous borers. *Bulletin of Entomological Research* 61, 33-39.

Mohyuddin, A. I., Inayatullah, C., & King, E. G. (1981). Host selection and strain occurrence in *Apanteles flavipes* (Cameron)(Hymnoptera: Braconidae) and its rearing on biological control of graminaceous stem-borers (Lepidoptera: Pyralidae). *Bulletin of Entomological Research* 71, 575-581.

Mohyuddin, A.I. (1990). Biological control of *Chilo* spp. in maize, sorghum and millet. *Insect Science and Its Application* 11, 721–732.

Moolman, J., Van den Berg, J., Conlong, D., Cugala D., Siebert S., & Le Ru, B. (2014). Species diversity and distribution of lepidopteran stem borers in South Africa and Mozambique. *Journal of Applied Entomology* 138, 52–66.

Mougel F., Solignac M., Vautrin D., Baudry E., Ogden J., Tchapla A., Schweitz H., & Gilbert H. (2012). Quantitative traits loci (QTL) involved in body colour, wing morphometry, cuticular hydrocarbons and venom components in honeybee. *Apidologie* 43, 162-181.

Moyal, P., Tokro, P., Bayram, A., Savopoulou-Soultani, M., Conti, E., Eizaguirre, M., Le Ru, B., Avand-Faghih, A., Frérot, B. & Andreadis, S. (2011). Origin and taxonomic status of the Palearctic population of the stem borer *Sesamia nonagrioides* (Lefèbvre) (Lepidoptera: Noctuidae). *Biological Journal of the Linnean Society* 103, 904–922.

Murray B.I. (2019) Challenges of Pest Management in the Twenty First Century: New Tools and Strategies to Combat Old and New Foes Alike. *Frontiers in Agronomy* 1, 2.

Mudd, A. & Corbet, S.A. (1982). Response of the ichneumonid parasite *Nemeritis canescens* to kairomones from the flour moth, *Ephestia kuehniella*. *Journal of Chemical Ecology* 8, 843-850.

Mudd, A., Walters, J.H.H. & Corbet, S.A. (1984). Relative kairomonal activities of 2-acylcyclohexane-1,3-diones in eliciting oviposition behavior from parasite *Nemeritis canescens* (Grav.). *Journal of Chemical Ecology* 10, 1597-1601.

Muirhead, K. A., Murphy, N. P., Sallam, N., Donnellan, S. C., & Austin, A. D. (2012). Phylogenetics and genetic diversity of the *Cotesia flavipes* complex of parasitoid

wasps (Hymenoptera: Braconidae), biological control agents of lepidopteran stemborers. *Molecular Phylogenetics & Evolution* 63, 904-914.

Muratori, F.R., Ralec, A., Lognay, G. & Hance, T. *(*2006). Epicuticular factors involved in host recognition for the aphid parasitoid. *Journal of Chemical Ecology* **32**, 579–593.

Mwalusepo, S., Tonnang, H. E. Z., Massawe, E. S., Okuku, G. O., Khadioli, N., Johansson, T., Calatayud, P.-A., & Le Ru, B. P. (2015). Predicting the impact of temperature change on the future distribution of maize stem borers and their natural enemies along East African mountain gradients using phenology models. *PLoS ONE* 10(6): e0130427.

Nagaraju, J. & Abraham, E. G. (1995). Purification and characterization of digestive amylase from the tasar silkworm, *Antheraea mylitta* (Lepidoptera : Saturniidae). *Comparative Biochemistry and Physiology* 110, 201–209.

Ndemah, R., Schulthess, F., Abang, A., Ghogomu, R. T., Ntonifor, N., Dupas, S. & LeRü, B. (2012), Suitability of cereal stemborers in Cameroon to Kenyan populations of the braconid larval parasitoid *Cotesia sesamiae*. *Journal of Applied Entomology* 136, 60–69.

Nemoto, T., Shibuya, M., Kuwahara, Y. & Suzuki, T. (1987). New 2-acylcyclohexane-1,3-diones: kairomone components against a parasitic wasp, *Venturia canescens*, from feces ofthe almond moth *Cadra cautella*, and the indian meal moth, *Plodia interpunctella*. *Agriculture and Biological Chemistry* 51, 1805-1810.

Nettles, W.C. & Burks, M.L. (1975). A substance from *Heliothis virescens* larvae stimulating larviposition by females of the tachinid, *Archytas marmoratus*. *Journal of Insect Physiology* 21, 965-978.

Ngi-Song, A. J., Overholt, W. A., & Ayertey, J. N. (1995). Suitability of African gramineous stemborers for development of *Cotesia flavipes* and *C. sesamiae* (Hymenoptera: Braconidae). *Environmental Entomology* 24, 978-984.

Ngi-Song, A. J., Overholt, W. A., Njagi, P. G. N., Dicke, M., Ayertey, J. N., & Lwande, W. (1996). Volatile infochemicals used in host habitat location by *Cotesia flavipes* Cameron and *Cotesia sesamiae* (Cameron) (Hymenoptera: Braconidae), larval parasitoids of stemborers on graminae. *Journal of Chemical Ecology* 22, 307–323.

Ngi-Song, A.J. & Overholt, W.A. (1997). Host location and acceptance of *Cotesia flavipes* Cameron and *Cotesia sesamiae* (Cameron) (Hymenoptera: Braconidae), Parasitoids of African stemborers: Role of frass and other cues. *Biological Control* 9, 136-142.

Ngi-Song, A.J., Overholt, W.A. & Stouthamert, R. (1998). Suitability of *Busseola fusca* and *Sesamia calamistis* (Lepidoptera: Noctuidae) for the development of two populations of *Cotesia sesamiae* (Hymenoptera: Braconidae) in Kenya. *Biological Control* 12, 208-214.

Ngi-Song, A. J., & M. B. Mochiah. (2001). Polymorphism for *Wolbachia* infections in eastern and southern African *Cotesia sesamiae* (Cameron)(Hymenoptera: Braconidae) populations. *International Journal of Tropical Insect Science* 21, 369–374.

Niepmann, M. & Zheng, J. (2006). Discontinuous native protein gel electrophoresis. *Electrophoresis* 27, 3949–3951.

Nordlund D.A. & Lewis W.J. (1976). Terminology of chemical releasing stimuli in intraspecific and interspecific interactions. *Journal of Chemical Ecology* 2, 211–220.

Nordlund, D.A. & Lewis, W.J. (1985). Response of females of the Braconid parasitoid *Microplitis demolitor* to frass of larvae of the Noctuids, *Heliothis zea* and *Trichoplusia ni* and to 13- methylhentriacontane. *Entomologia Experimentalis et Applicata* 38, 109-112.

Obonyo, M. (2005). Performance of *Cotesia flavipes* (Cameron) (Hymenoptera: Braconidae) on cereal and wild crop stemborers. MSc of in Biochemistry of Jomo Kenyatta University of Agriculture and Technology (JKUAT), Kenya, PP.52.

Obonyo M., Schulthess F., Juma G., Wanyama O., Le Ru B., & Calatayud P.-A. (2008). Location, acceptance and suitability of lepidopteran stemborers feeding on a cultivated and wild host-plant to endoparasitoid *Cotesia flavipes* Cameron (Hymenoptera: Braconidae). *Biological Control* 45, 36-47.

Obonyo, M. (2009). Basis of host recognition by the larval endoparasitoids: *Cotesia sesamiae* Cameron and *Cotesia flavipes* (Cameron) (Hymenoptera: Braconidae). PhD of Science of the North West University (Potchefstroom campus), South Africa, pp.112.

Obonyo M., Schulthess F., Le Ru B., Van Den Berg J., & Calatayud P.-A. (2010a). Host recognition and acceptance behaviour in *Cotesia sesamiae* and *C. flavipes* (Hymenoptera: Braconidae), parasitoids of gramineous stemborers in Africa. *European Journal of Entomology* 107,169–176.

Obonyo M., Schulthess F., Le Ru B., Van Den Berg J., Silvain J.-F., & Calatayud P.-A. (2010b). Importance of contact chemical cues in host recognition and acceptance by the braconid larval endoparasitoids *Cotesia sesamiae* and *Cotesia flavipes*. *Biological Control* 54, 270–275.

Obonyo M., Schulthess F., Chimtawi M., Mascarel G., Ahuya P.O., Le Ru B., Van Den Berg J., Silvain J.-F., & Calatayud P.-A. (2011). Sensilla on antennae, ovipositor and tarsi of the larval parasitoids, *Cotesia sesamiae* (Cameron 1906) and *Cotesia flavipes* Cameron 1891 (Hymenoptera: Braconidae): a comparative scanning electron microscopy study. *Annales de la Société Entomologique de France* 47, 119–127.

Ochieng, R. S., Onyango, F. O., & Bungu, M. D. O. (1985). Improvement of techniques for mass culture of *Chilo partellus* (Swinhoe). *Insect Science and Its Application* 6, 425–428.

Ode, P.J. (2006). Plant chemistry and natural enemy fitness: effects on herbivore and natural enemy interactions. *Annual Review of Entomology* 51, 163-185.

OECD/FAO (2016), OECD-FAO Agricultural Outlook 2016-2025, OECD Publishing, Paris.

OECD/FAO (2011). Agriculture outlook 2011-2020, OECD publishing and FAO. http://dx.doi.org/10.1787/agr-outlook-2011-en

Omwega, C.O., Kimani, S.W., Overholt, W.A. & Ogolv, C.K.P.O. (1995). Evidence of the establishment of *Cotesia flavipes* Cameron (Hymenoptera: Braconidae) in continental Africa. *Bulletin of Entomological Research* 85, 525-530.

Omwega, C.O., Overholt, W.A., Mbapila, J.C. & Kimani-Njogu, S.W. (1997). Establishment and dispersal of *Cotesia flavipes* Cameron (Hymenoptera:

Braconidae), an exotic endoparasitoid of *Chilo partellus* Swinhoe (Lepidoptera: Pyralidae) in northern Tanzania. *African Entomology* 5, 71-75.

Omwega, C.O., Muchugu, E., Overholt, W.A. & Shulthess, F. (2006). Release and establishment of *Cotesia flavipes* Cameron (Hymenoptera: Braconidae) an exotic parasitoid *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) in East and Southern Africa. *Annales de la Société Entomologique de France* 42, 511-517.

Ong'amo, G., Le Ru, B.P., Dupas, S., Moyal, P., Muchugu, E., Calatayud, P.-A. & Silvain, J.-F. (2006). The role of wild host plants in the abundance of lepidopteran stem borers along altitudinal gradient in Kenya. *Annales de la Société Entomologique de France* 42, 363-370.

Ong'amo, G. O., Le Gall, P., Ndemah, R., & Le Ru, B. P. (2014). Diversity and host range of lepidopteran stem borer species in Cameroon. *African Entomology* 22, 625-635.

Ong'amo, G., Khadioli, N., Le Ru, B., Mujica, N., & Carhuapoma, P. (2016). Maize stalk borer, *Busseola fusca* (Fuller 1901). In: Kroschel, J., Mujica, N., Carhuapoma, P., Sporleder, M. (eds.). Pest distribution and risk atlas for Africa. Potential global and regional distribution and abundance of agricultural and horticultural pests and associated biocontrol agents under current and future climates. Lima (Peru). International Potato Center (CIP). ISBN 978-92-9060-476-1. DOI 10.4160/9789290604761-14. pp. 182-194

Onyango, F. O., & Ochieng'-Odero, J. P. R. (1994). Continuous rearing of the maize stemborer *Busseola fusca* on an artificial diet. *Entomologia Experimentalis et Applicata* 73, 139–144.

Orr, D.B. & Suh, C.P.-C. (1998). Parasitoids and Predators. In: Rechcigl, J.E. & Rechcigl, N.A. (Eds.). *Biological and Biotechnological Control of Insect Pests*. Lewis Publishers, Boca Raton, Florida, pp. 3-34.

Ouma, J.O., Murithi, F.M., Mwangi, W., Verkuijl, H., Gethi, M. & De Groote, H. (2002). Adoption of maize seed and and fertilizer technology in Embu district Kenya. Mexico. D.F. CIMMYT Report. Pp.22.

Overholt, W. A., Ochieng, J. O., Lammers, P. M., & Ogedah, K. (1994). Rearing and field release methods for *Cotesia flavipes* Cameroon (Hymenoptera: Braconidae), a parasitoid of tropical gramineous stemborers. *Insect Science and Its Application* 15, 253–259.

Overholt, W.A., Ngi-Song, A.J., Kimani, S.K., Mbapila, J., Lammers, P. & Kioko, E. (1994a). Ecological considerations of the introduction of *Cotesia flavipes* Cameron (Hymenoptera: Braconidae) for biological control of *Chilo partellus* (Lepidoptera: Pyralidae), in Africa. *Biocontrol News and Information* 15, 19N-24N.

Overholt, W. A., Ogedah, K., and Lammers, P. M. (1994b). Distribution and sampling of *Chilo partellus* (Lepidoptera: Pyralidae) in maize and sorghum on the Kenya coast. *Bulletin of Entomological Research* 84, 367-378.

Overholt, W.A., Ngi-Song, A.J., Omwega, C.O., Kimani-Njogu, S.W., Mbapila, J, Sallam, M.N. & Ofomata, V. (1996). An ecological approach to biological control of gramineous Stemborer in Africa: The introduction and establishment of *Cotesia flavipes* Cameron (Hymenoptera: Braconidae). In: E.B. Radcliffe and W.D. Hutchison (Eds.), *Radcliffe's IPM World Textbook*, URL:http: IPM world.umn.edu/University of Minnesota, St. Paul, MN.

Overholt, W.A., Ngi-Song, A.J., Omwega, C.O., Kimani-Njogu, S.W., Mbapila, J., Sallam, M.N. & Ofomata, V. (1997). A review of the introduction and establishment of *Cotesiaflavipes* Cameron (Hymenoptera: Braconidae) in East Africa for biological control of cereal stemborers. *Insect Science and Its Application* 17, 79-88.

Overholt, W.A. (1998). Biological control. In: Polaszek A. (Ed.). *African Cereal Stemborers*: *Economic Importance, Taxonomy, Natural Enemies and Control*, CABI. Wallingford.

Overholt, W.A., Songa, J.M., Ofomate V. & Jeske R. (2000). The spread and ecological consequences of the invasion of *Chilo partellus* (Swinhoe) Lepidoptera: crambidae in Africa. http://www.icipe.org/invasive/talks/stemborer.cfm.

Overholt, W.A., Maes, K.V.N. & Goebel, F.R. (2001). Field guide to the stemborer larvae of maize, sorghum and sugarcane in Eastern and Southern Africa. ICIPE Science Press, Nairobi, Kenya.

Ozaki, M., Wada-Katsumata, A., Fujikawa, K., Iwasaki, M., Yokohari, F., Satoji, Y., Nisimura, T., & Yamaoka, R. (2005). Ant nestmate and non-nestmate discrimination by a chemosensory sensillum. *Science* 309, 311-314.

Ozaki, M., Kidokoro-Kobayashi, M., & Hiraguchi, T. (2012). Cuticular hydrocarbons sensillum for nestmate recognition in ants. In: Barth, F.G., Humphrey, J.A.C. & Srinivasan, M.V. 2012. Frontiers in Sensing: From Biology to Engineering. Wien, Austria: Springer Wien New York, 145-158.

Özgür, E., Yücel, M., & Öktem, H. A. (2009). Identification and characterization of hydrolytic enzymes from the midgut of the cotton bollworm, *Helicoverpa armigera*

Hübner (Lepidoptera: Noctuidae). *Turkish Journal of Agriculture and Forestry* 33, 285-294.

Pannebakker B., Watt R., Knott S., West S., & Shuker D. M. (2011). The quantitative genetic basis of sex ratio variation in *Nasonia vitripennis*: a QTL study. *Journal of Evolutionary Biology* 24, 12-22.

Parker E.D. & Orzack S.H. (1985). Genetic variation for the sex ratio in *Nasonia vitripennis*. *Genetics* 110, 93-105.

Pedigo, L.P., (1996). Entomology and Pest Management. Third Edition. 1999. Prentice-Hall, Englewood Cliffs, NJ, pp.691.

Pennacchio F. & Strand M.R. (2006). Evolution of developmental strategies in parasitic Hymenoptera. *Annual Review of Entomology* 51, 233–258.

Pimentel, D., Acquay, H., Biltonen, M., Rice, P., Silva, M., Nelson, J., Lipner, V., Giordano, S., Horowitz, A. & D'Amore, M. (1992). Environmental and economic costs of pesticide use. *BioScience* 42, 50–760.

Pingali, P. & Pandey. S. (2001). Meeting worlds maize needs, technological opportunities and priorities for the public sector. In: P. Pingali (Ed.), CIMMYT 1999-2000 CIMMYT, World Maize Fact and Trends. Meeting worlds maize sector. CIMMYT, MEXICO, D.F., Mexico pp. 1-24.

Polaszek, A. & Walker, A.K. (1991). The *Cotesia* species complex: Parasitoids of cereal stemborers in the tropics. *Redia* 74, 335-341.

Polaszek, A. (Ed.) (1998). African cereal stemborers; economic importance, taxonomy, natural enemies and control. CAB International, Wallingford, Oxon, UK, (pp.530).

Posada, D. (2008). jModelTest: Phylogenetic model averaging. *Molecular Biology and Evolution* 25, 1253–1256.

Potting, R.P.J., Osane-Danso, F., Overholt, W.A., & Ngi-Song, A.J. (1993). Host selection in *Cotesia flavipes* parasitoid of tropical stemborers. Proceedings Experimental and Applied Entomology, N.E.V. Amsterdam 4, 47–62.

Potting, R.P.J., Vet, L.E.M., & Dicke, M. (1995). Host microhabitat location by stemborer parasitoid *Cotesia flavipes*: the role of herbivore volatiles and locally and systemically induced plant volatiles. *Journal of Chemical Ecology* 21, 525–539.

Pytelkova, J., Hubert, J., Lepsik, M., Sobotnik, J., Sindelka, R., & Krizkova, I. (2009). Digestive α-amylases of the flour moth *Ephestia kuehniella*-adaptation to alkaline environment and plant inhibitors. *The FEBS Journal* 276, 3531-3546.

Quicke, D. L. (1997). *Parasitic wasps*. Chapman & Hall Ltd. London, UK. 470p.

Ramachandran, R., Norris, D.M., Phillips, J.K. & Phillips, T.W. (1991). Volatiles mediating plant-herbivore-natural enemy interactions: Soyabean looper frass volatiles, 3-octanone and guaiacol, as kairomones for the parasitoid *Microplitis demolitor*. *Journal of Agricultural Food Chemistry* 39, 2310-2317.

Rani, P.U., Kumari, S.I., Sriramakrishna, T. & Sudhakar, T.R. (2007). Kairomones extracted from rice yellow stem borer and their influence on egg parasitization by *Trichogramma japonicum* Ashmead. *Journal of Chemical Ecology* 33, 59-73.

Ranum, P., Peña-Rosas, J. P., & Garcia-Casal, M. N. (2014). Global maize production, utilization, and consumption. *Annals of the New York Academy of Science 1312*, 105–112.

Reddy, G.V.P., Holopainen, J.K. & Guerrero, A. (2002). Olfactory responses of *Plutella xylostella* natural enemies to host pheromone, larval frass, and green leaf cabbage volatiles. *Journal of Chemical Ecology* 28, 131-143.

Renou, M., Nagnan, P., Berthier, A. & Durier, C. (1992). Identification of compounds from the eggs of *Ostrinia nubilalis* and *Mamestra brassicae* having kairomone activity on *Trichogramma brassicae*. *Entomologia Experimentalis et Applicata* 63, 291-303.

Rojas, J.C., Castillo, A. & Virgen, A. (2006). Chemical cues used in host location by *Phymastichus coffea*, a parasitoid of coffee berry borer adults, *Hypothenemus hampei*. *Biological Control* 37, 141-147.

Röse, U. S. R., Alborn, H. T., Makranczy, G., Lewis, W. J., & Tumlinson, J. H. (1997). Host recognition by the specialist endoparasitoid *Microplitis croceipes*: Role of host and plant related volatiles. *Journal of Insect Behaviour* 10, 313–329.

Rousse, P. & Gupta A. (2013). Microgastrinae (Hymenoptera: Braconidae) of Reunion Island: a catalogue of the local species, including 18 new taxa and a key to species. *Zootaxa* 3616 (6), 501–547.

Roux, O., Gers, C., Tene-Ghomsi, J.N., Arvanitakis, L., Bordat, D. & Legal, L. (2007). Chemical characterization of contact semiochemicals for host-recognition and host-acceptance by the specialist parasitoid *Cotesia plutellae* (Kurdjumov). *Chemoecology* 17, 13-18.

Rutledge, CE. (1996). A survey of identified kairomones and synomones used by insect parasitoids to locate and accept their hosts. *Chemoecology* 7, 121–131.

Sallam, N.M., Overholt, W.A. & Kairu, E. (1999). Comparative evaluation of *Cotesia flavipes* and *C. sesamiae* (Hymenoptera: Braconidae) for the management of *Chilo partellus* (Lepidoptera: Pyralidae) in Kenya. *Bulletin of Entomological Research* 89, 185-191.

Sanchez P.A. (2002). Ecology, soil fertility and hunger in Africa. *Science* 295, 2019-2020.

Schrambach, A., & Jovin, T. M. (1983). Selected buffer systems for moving boundary electrophoresis on gels at various pH values, presented in a simplified manner. *Electrophoresis* 4, 190–204.

Schulthess, F, Bosque-Pérez, N.A, Chabi-Olaye, A, Gounou, S, Ndemah, R & Goergen, G. (1997). Exchange of natural enemies of lepidopteran cereal stem borers species between African regions. *Insect Science and Its Application* 17, 97-108.

Schulthess, F. & Ajala, S.O. (1999). Recent advances in the control of stemborers West and Central Africa. *Proceedings of WECAMAN Conference* 21st-25th April 1997, IITA Cotonou, Republic of Benin, pp. 35-52.

Schwab, A., Jager, I., Stoll, G., Gorgen R., Prexler-Schwab, S. & Altenburger, R. (1995). Pesticides in tropical Agriculture: Hazards and alternatives. *Tropics Agro-Ecology*, PAN ACTA 131, pp. 282.

Seshu Reddy, K.V. & Masyanga, A. (1988). Effects of different proportion of sorghum/cowpea intercrop rows on crop borer incidence. ICIPE 15th Annual Report 1987, Nairobi, Kenya, pp. 6–7.

Sétamou, M., Jiang, N. & Schulthess, F. (2005). Effect of host plant on the survivorship of parasitized *Chilo partellus* (Lepidoptera: Crambidae) larvae and performance of

its larval parasitoid *Cotesia flavipes* Cameron (Hymenoptera: Braconidae). *Biological Control* 32, 183-190.

Sétamou, M., Schulthess, F., Bosque-Pérez, N.A. & Thomas-Odjo, A. (1995). The effect of stem and cob borers on maize subjected to different nitrogen treatments with special reference to *Sesamia calamistis* Hampson (Lepidoptera: Noctuidae). *Entomologia Experimentalis et Applicata* 77: 205-210.

Seyoum S., Chauhan Y., Rachaputi R., Fekybelu S. & Prasanna B. (2017). Characterising production environments for maize in eastern and southern Africa using the APSIM Model Agricultural and Forest Meteorology. Elsevier

Shu, S., Swedenborg, P.D. & Jones, R.L. (1990). A kairomone for *Trichogramma nubilale* (Hymenoptera: Trichogrammatidae): Isolation, identification and synthesis. *Journal of Chemical Ecology*, 16, 521-529.

Silva, C.C., Moreas, M.C.B., Laumann, R.A. & Borges, M. (2006). Sensory response of the egg parasitold *Telenomus podisi* to stimuli from the bug *Euschistus heros. Pesquisa Agropecuaria Brasileira*, 41, 1093-1098.

Smale, S; Byerlee, D. & Jayne, T. (2011). Maize Revolutions in Sub-Saharan Africa. *World Bank Policy Research Working* Paper No. 5659.

Smith, B. H. (1993). Merging mechanism and adaptation: an ethological approach to learning and generalization. In *Insect Learning* (pp. 126-157). Springer, Boston, MA.

Smith, J.W. Jr., Weidenman, R.N. & Overholt, W.A. (1993). Parasites of lepidopteran stemborers of tropical gramineous plants. (pp.89). *ICIPE Science Press*, Nairobi, Kenya.

Solignac M., Mougel F., Vautrin D., Monnerot M. & Cornuet J.M. (2007). A third-generation microsatellite-based linkage map of the honey bee, *Apis mellifera*, and its comparison with the sequence-based physical map. *Genome Biology* 8(4), R66.

Songa, J.M., Overholt, W.A., Mueke, J.M. & Okello, R.O. (2002). Colonization of *Cotesia flavipes* (Hymenoptera: Braconidae) in stemborers in semi-arid eastern province of Kenya. *Journal Economic Entomology* 21, 289-295.

Songa, J.M., Jiang, N., Schulthess, F. & Omwega, C. (2007). The role of intercropping different cereal species in controlling lepidopteran stemborers on maize in Kenya. *Journal of Applied Entomology* 131, 40-49.

Stamatakis, A., (2014). RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30, 1312–1313.

Stapley J., Reger J., Feulner P.G.D., Smadja C., Galindo J., Ekblom R., Bennison C., Beckerman A.P. & Slate J. (2010). Adaptation genomics: the next generation. *Trends in Ecology & Evolution* 25, 705-712.

Steidle, J.L.M & Ruther, J. (2000). Chemicals used for host recognition by the granary weevil parasitoid *Lariophagus distinguendus. Journal of Chemical Ecology* 26, 2665-2675

Steidle, J.L.M., & Van Loon, J. J. A. (2002). Chemoecology of parasitoid and predator oviposition behaviour, pp. 291–317, in M. Hilker, and T. Meiners (eds.). Chemoecology of Insect Eggs and Egg Deposition Blackwell, Berlin.

Strand, M.R., Williams, H.J., Vinson, S.B. & Mudd, A. (1989). Kairomonal activities of 2-acylcyclohexane-1,3 diones produced by *Ephestia kuehniella* Zeller in eliciting

searching behavior by the parasitoid *Bracon hebetor* (Say). *Journal of Chemical Ecology* 15, 1491-1500.

Sznajder, B. & Harvey, J.A. (2003). Second and third trophic level effects of differences in plant species reflect dietary specialisation of herbivores and their endoparasitoids. *Entomologia Experimentalis et Applicata* 109, 73-82.

Tabashnik B.E., Van Rensburg J.B.J., Carriere Y. (2009). Field-evolved insect resistance to Bt crops: definition, theory, and data. *Journal of Economic Entomology* 102-6, 2011-2025.

Takabayashi, J. &Takahashi, S. (1989). Effects of fecal pellet and synthetic kairomone on host-searching and postoviposition behavior of *Apanteles kariyai*, a parasitoid of *Pseudaletia separata. Entomologia Experimentalis et Applicata* 52, 221- 227.

Takasu, K., Overholt, W. A. (1997). Aggressive behaviour of *Chilo partellus* (Swinhoe) larvae against the parasitoid *Cotesia flavipes* Cameron. *Insect Science and Its Application* 17, 131–136.

Tams, WHT. & Bowden, J. (1953)**.** A revision of the African species of Sesamia Guenée and related genera (Agrotidae- Lepidoptera). *Bulletin of Entomological Research* 43, 645– 679.

Thiéry, D., Derridj, S., Calatayud, P.-A., Nevile, M., and Marion-Poll, F. (2013). L'insecte au contact des plantes. Pp. 347-368 dans « Interactions insectes – plantes », Sauvion, N., Calatayud, P.-A., Thiéry, D. and Marion-Poll, F. (Eds.), Co-édition IRD-Quae, Paris.

Thompson, A.C., Roth, J.P. & King, E.G. (1983). Larviposition kairomone of the tachinid *Lixophaga diatraeae*. *Environmental Entomology* 12, 1312-1314.

Thompson J.N. (1998). Rapid evolution as an ecological process. *Trends in Ecology and Evolution* 13, 329-330.

Tilman, D., Fargione, J., Wolff, B., D'Antonio, C., Dobson, A., Howarth, R., Schindler, D., Schlesinger, W. H., Simberloff, D., and Swackhamer, D. (2001). Forecasting agriculturally driven global environmental change. *Science* 292, 281–284.

Tolassy, V. (2018). Validation d'une réponse gustative à l'alpha-amylase chez *Drosophila melanogaster* et *Cotesia flavipes.* Master Report, Université Paul Sabatier, Toulouse III, France.

Turlings TCJ, Tumlinson JH, Lewis WJ. (1990). Exploitation of herbivore-induced plant odors by host seeking parasitic wasps. *Science* 250, 1251-1253.

Van den Berg, J., Nur, F. & Polaszek, A. (1998). Cultural control. In: Polaszek, A. (Ed.). *African Cereal Stemborers*: *Economic Importance, Taxonomy, Natural Enemies and Control*, CABI. Wallingford, Oxon, United Kingdom, pp. 333-347.

Van Leerdam, M. B., Smith, J. W. Jr., & Fuchs, T. W. (1985). Frass-mediated, host finding behaviour of *Cotesia flavipes*, a braconid parasite of *Diatraea saccharalis* (Lepidoptera: Pyralidae). *Annals of the Entomological Society of America* 78, 647-650.

Velasco, P., Revilla, P., Monetti, L., Butron, A., Ordas, A. & Malvar, R.A. (2007). Corn borers (Lepidopotera: Noctuidae; Crambidae) in northwestern Spain: Population dynamics and distribution. *Maydica* 52, 195–203.

Vet, L.E.M. & Groenewold, A.W. (1990). Semiochemicals and learning in parasitoids. *Journal of Chemical Ecology* 16, 3119-3135.

Vet, L.E.M., Wäckers, F.L.& Dicke, M. (1991). How to hunt for hiding hosts: The reliability-detectability problem in foraging parasitoids. *Netherlands Journal of Zoology* 41, 202-213.

Vet, L.E.M. & Dicke, M. (1992). Ecology of infochemical use by natural enemies in a tritrophic context. *Annual Review of Entomology* 37, 141-172.

Vet, L. E. M. (1999). From chemical to population ecology: infochemical use in an evolutionary context. *Journal of Chemical Ecology* 25, 31–49.

Vinson, S. B. (1975). Biochemical coevolution between parasitoids and their hosts. In *Evolutionary strategies of parasitic insects and mites* (pp. 14-48). Springer, Boston, MA.

Vinson, S. B., Jones, R. L., Sonnet, P. E., Bierl, B. A., & Beroza, M. (1975). Isolation, identification and synthesis of host-seeking stimulants for Cardiochiles nigriceps, a parasitoid of tobacco budworm. *Entomologia Experimentalis et Applicata*, *18*(4), 443-450.

Vinson, S. B. (1976). Host selection by insect parasitoids. *Annual Review of Entomology*, *21*(1), 109-133.

Vinson, S.B. (1985). The behaviour of parasitoids. In: Kerkut, G.A. & Gilbert, L.I. (Eds.). *Comprehensive Insect Physiology, Biochemistry and Pharmacology*. Pergamon Press, New York, USA, pp. 417-469.

Vinson, S. B. (1991). Chemical signals used by parasitoids. *Redia* 74, 15–42.

Wajnberg, E., Bernstein, C., and Van Alphen, J. (2008). *Behavioral of ecology of insect parasitoids* (Blackwell Publishing, USA, UK, Australia).

Wajnberg, E., and Colazza, S. (2013). *Chemical ecology of insect parasitoids* (Whiley-Blackwell, A John Wiley & Sons, Ltd., Publication, UK).

Wang Q., Gu H. & Dorn S. (2003). Selection on olfactory response to semiochemicals from a plant-host complex in a parasitic wasp. *Heredity* 91, 430-435.

Ward, S. A. (1992). Environmental uncertainty and polyphagy in herbivorous insects. *Oikos* 63, 506–512.

Werren, J.H. (1997). Biology of *Wolbachia*. *Annual Review of Entomology* 42, 587-609.

Weseloh, R.M. (1977). Effects on behavior of *Apanteles melanoscelus* females caused by modifications in extraction, storage and presentation of gypsy moth silk kairomone. *Journal of Chemical Ecology* 3, 723-735.

Weseloh, R.M. (1981). Host location by parasitoids. *Semiochemicals-Their Role in Pest Control*.

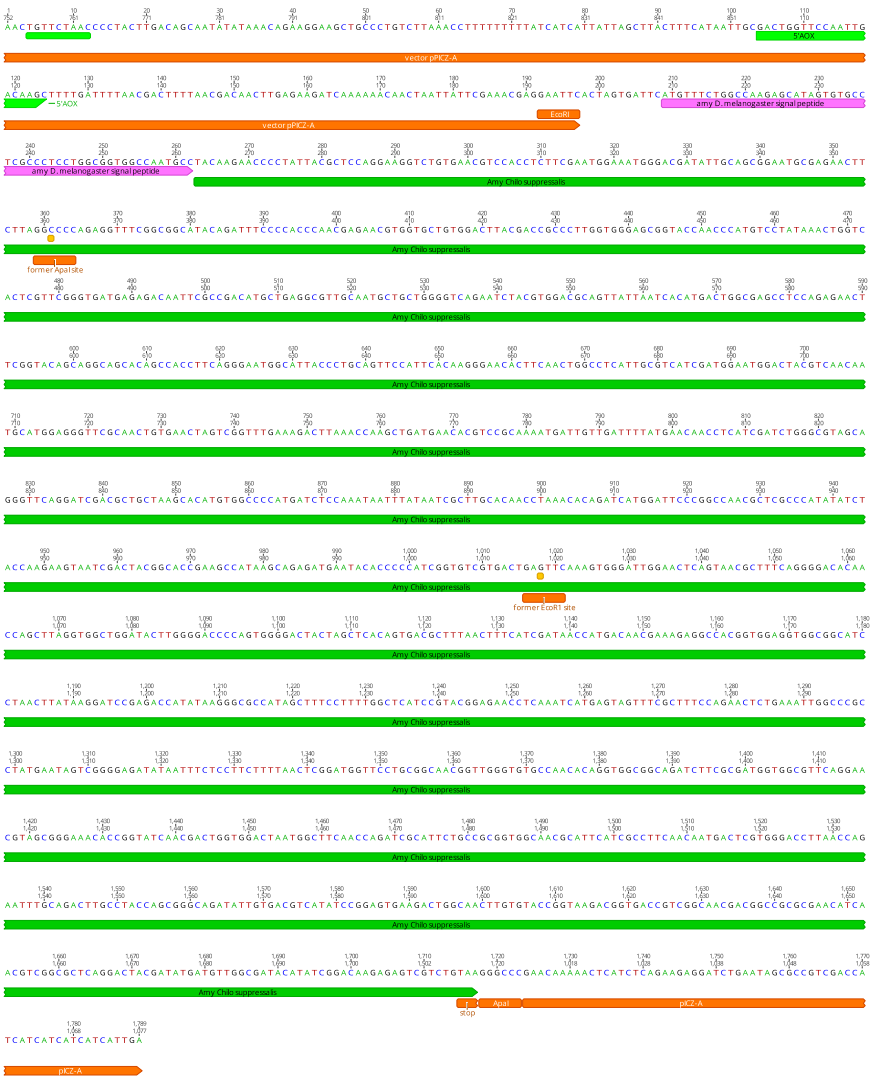
Zacharuk R.Y., (1985). Antennae and sensilla. In: Kerkut G.A. & Gilbert L.I.(Eds.).*Comprehensive Insect Physiology, Biochemistry and Pharmacology,* Pergamon Press,Oxford, UK. pp. 1-69.

Zaki, F.N., El-Saadany, G., Gomma, A. & Saleh, M. (1998). Increasing rates of parasitism of the larval parasitoid *Bracon brevicornis* (Hym., Braconidae) by using kairomones, pheromones and a supplementary food. *Journal of Applied Entomology-Zeitschrift für Angewandte Entomologie* 122, 565-567.

Zhou, G., Baumgärtner, J., & Overholt, W. A. (2001). Impact assessment of an exotic parasitoid on stemborer (Lepidoptera) population dynamics in Kenya. *Ecological Applications* 11, 1554-1562.

Zhou G., Overholt W.A. & Mochiah M. (2001a). Changes in the distribution of lepidopterans maize stemborers in Kenya from the 1950s to 1990s. *International Journal of Tropical Insect Science* 21, 395-402.

Appendix 1: A map and sequence of the Chilo suppressalis 108827 amylase gene construct in the pPICZ expression vector (Invitrogen).



The original signal peptide was replaced by that of the *Drosophila melanogaster* amylase. Two restriction sites were destroyed in the sequence to allow the use of those sites as cloning sites.

Appendix 2: Results of proteins and peptides obtained by X! Tandem and de novo protein sequencing.

Xtandem Results

Proteins

Group ID	Sub-group I	Protein ID	Description	log(E value)	Coverage	MW	Spectra	Specific	Uniques	Specific uni	Theoretical	PAI	emPAI	Sub-group	number of MS samples
a1	a1.a1	a1.a1.a1	GRMZM2G154595_P01 NP_001142	-43.1934204	15	35.20000076	5	5	7	0.714285714	4.179474679	1	1		
a2	a2.a1	a2.a1.a1	GRMZM2G374302_P01 NP_001132	-5.28232955	3	70.59999847	3	2	31	0.096774193	0.249609141	2	1		
a2	a2.a1	a2.a1.a2	GRMZM2G374302_P02 NP_001132	-5.28232955	3	70.59999847	3	2	31	0.096774193	0.249609141	2	1		

Pepides

Group ID	Peptide ID	SequenceLI	Modifs	Charge	MH+ theo	Number of s	Subgroup ic	Number of spectra
a1	pepa1a1	DDIFNINAGI		2	1318.7006	1	a1.a1	1
a1	pepa1a2	IFGVTTIDVV		2	1219.7049	1	a1.a1	1
a1	pepa1a3	NGVEEVIGIC		2	1875.934	1	a1.a1	1
a1	pepa1a4	RTQDGGTEV		2	1389.6973	1	a1.a1	1
a1	pepa1a5	TQDGGTEVV		2	1233.5962	1	a1.a1	1
a2	pepa2a1	HVVQDMVR		2	983.5095	1	a2.a1	1
a2	pepa2a2	HVVQDMVR	M6:+15.99491	2	999.5044	1	a2.a1	1
a2	pepa2a3	YSVEEDITVII		2	1537.8	1	a2.a1	1

De novo Results

Proteins

Number	Description	Evalue	Peptides	Databases	Number	Peptides
1	gi|29529004	4.8E-35	24	db57.fasta	-	0

Peptides

Number	Description									
	Scan	Sequence	Charge	MH+theo	MH+obs	DeltaMH+	N-gap	C-gap	Sequence s	Filter score
1	gi|295290041|gb|FP379314.1|FP379314|Frame3 FP379314 Spodopt									
	630	CNAVGVR	2	776.37244	775.3873	0.9851074	0	0	83.209	0.7
	782	SGNEEKmAG	3	1278.6364	1278.5962	0.040161133	0	0	122.978	0.57
	893	GVAGFR	2	606.3368	606.3355	0.001281738	0	0	77.302	0.497
	897	NLDTDHGFA	2	1348.6133	1348.6124	0.000854492	0	0	156.12	0.746
	960	GHGAGGTLA	2	1245.6589	1245.6229	0.036010742	0	0	136.486	0.933
	1033	WDDDNK	2	792.3168	792.38916	-0.07238769	0	0	77.368	0.35
	1043	YQPLSYK	2	898.4676	898.4677	-0.00012207	0	0	116.749	0.785
	1050	DLNQGNSYV	2	1380.6508	1381.6309	-0.98010254	0	0	160.103	0.974
	1060	VFLGPK	2	660.40875	660.4081	0.000671386	0	0	81.513	0.347
	1157	QVSPVNENA	2	1411.7086	1411.7566	ND	227.081	0	191.992	0.925
	1248	CELVGLR	2	991.6896	990.50604	ND	144.255	0	87.526	0.688
	1274	NCELVGLR	2	961.4774	960.4942	0.98321533	0	0	129.77	0.876
	1339	NCELVGLR	2	961.4774	960.49426	0.9831543	0	0	123.262	0.755
	1487	SDRPWWER	2	1131.5338	1131.5369	-0.00305175	0	0	80.975	0.704
	1501	PWWER	2	773.3738	773.37634	-0.00256347	0	0	73.927	0.371
	1575	TNYASGR	2	1141.4703	1141.496	ND	373.106	0	109.72	0.86
	1587	TNYASGR	2	1163.3794	1163.476	ND	395.015	0	73.893	0.332
	1688	TNSSTAASGI	2	1324.5377	1324.5327	ND	373.088	0	128.304	0.732
	1709	EHGVVAFR	3	1324.6552	1324.6163	ND	410.17	0	83.641	0.505
	1778	FVHLFEWK	2	1234.1667	1233.6079	ND	128.583	0	117.406	0.789
	1983	HMWPADLA	2	1528.7734	1528.7765	-0.00305175	0	0	155.802	0.943
	2346	VEEVMDLGG	2	1823.0653	1822.9363	ND	346.34	0	210.051	0.469
	2416	DVVmHLmTF	2	1769.8763	1769.9146	ND	636.331	0	99.877	0.35
	2464	mLDHLLDLGV	2	1899.8088	1900.0304	ND	326.988	0	171.931	0.345

Appendix 3: Abstract for chapter three work published in Journal of Chemical Ecology

α-Amylase Mediates Host Acceptance in the Braconid Parasitoid *Cotesia flavipes*

Gladys Bichang'a[1,2] · Jean-Luc Da Lage[3,4] · Claire Capdevielle-Dulac[3,4] · Michel Zivy[5] · Thierry Balliau[5] · Kevin Sambai[1] · Bruno Le Ru[1,3,4] · Laure Kaiser[3,4] · Gerald Juma[2] · Esther Njoki Mwangi Maina[2] · Paul-André Calatayud[1,3,4]

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Abstract

Foraging parasitoids use chemical signals in host recognition and selection processes. Although, the volatiles play a relevant role in the localization by parasitoids of their hosts feeding on plants, the host identification process for acceptance occurs mainly during contact between the parasitoid and its host where host products related to feeding activities, fecal pellets and oral secretions, play a crucial role. The purpose of this study was to identify the nature of the contact kairomone(s) that mediate the acceptance for oviposition of the parasitoid *Cotesia flavipes* Cameron (Hymenoptera, Braconidae), which was released in Kenya in 1993 to control the invasive crambid *Chilo partellus* (Swinhoe). Using host and non-hosts of *C. flavipes*, we showed that it is mainly the oral secretions of the larvae that harbour the active compound(s) that mediate host acceptance for oviposition by *C. flavipes*. Using an integration of behavioral observations and biochemical approaches, the active compound of the oral secretions was identified as an α-amylase. Using synthetized α-amylases from *Drosophila melanogaster* (an insect model for which syntheses of active and inactive α-amylases are available), we observed that the conformation of the enzyme rather than its catalytic site as well as its substrate and its degradation product is responsible for host acceptance and oviposition mediation of *C. flavipes* females. The results suggest that the α-amylase from oral secretions of the caterpillar host is a good candidate for an evolutionary solution to host acceptance for oviposition in *C. flavipes*.

Keywords Biological control · Pest insects · Lepidoptera stemborers · *Chilo partellus* · *Cotesia flavipes* · Kairomone · α-amylase · Host recognition · Parasitoids · Host oral secretion · Multitrophic interactions · Semiochemicals

Appendix 4: Abstract for chapter four work Published in Frontiers in Ecology and Evolution

Salivary α-Amylase of Stem Borer Hosts Determines Host Recognition and Acceptance for Oviposition by *Cotesia* spp. (Hymenoptera, Braconidae)

Gladys B. Bichang'a[1,2], Jean-Luc Da Lage[3], Kevin Sambai[1], Simon Mule[1], Bruno Le Ru[1,3], Laure Kaiser[3], Gerald Juma[2], Esther N. Maina[2] and Paul-André Calatayud[1,3*]*

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Foraging insect parasitoids use specific chemical cues to discriminate between host and non-host species. Several compounds have been identified in "host location and acceptance." However, nothing is known about the molecular variations in these compounds that could account for host-range differences between parasitoid species. In a previous study, it was shown that during the host-finding process, contact between the braconid *Cotesia flavipes* and its host is crucial, and that α-amylase of oral secretions from the host plays a key role for host acceptance and oviposition by the parasitoid. The present study sought to establish whether the variations in this enzyme could explain specific host recognition in different host-parasitoid associations. Different species and populations of the *C. flavipes* complex specialized on graminaceous lepidopteran stemborers were used. Electrophoresis of α-amylase revealed different isoforms that mediate the parasitoid's oviposition acceptance and preference for a specific host. This discovery opens up new avenues for investigating the evolutionary processes at play in chemically-mediated host specialization in the species-rich *Cotesia* genus.

Keywords: parasitic wasp, *Cotesia flavipes*, *Cotesia sesamiae*, *Cotesia typhae*, protein perception, host specificity, oviposition