Basis of host recognition by the larval endoparasitoids: *Cotesia sesamiae* Cameron and *Cotesia flavipes* (Cameron) (Hymenoptera: Braconidae)

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Maize (*Zea mays* L. [Poaceae]) an important staple crop in Africa

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Dedication

To my family, (Faith and the anonymous other)

for the love and support you accorded me all through this study.

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ABSTRACT

Host recognition behaviour of two braconid larval parasitoids *Cotesia sesamiae* and *Cotesia flavipes* was studied using suitable stemborer hosts [i.e. *Busseola fusca* for *C. sesamiae*, and *Chilo partellus* for *C. flavipes*] and one non-host [*Eldana saccharina*]. The wasps displayed similar sequences of behavioural steps when locating their hosts largely depending on their antennae for host recognition and both antennae and tarsi for final host acceptance and oviposition. Tactile and contact chemoreception stimuli from the hosts seem to play a major role in oviposition decision by the parasitoids. In addition, the external morphology and distribution pattern of sensilla present on antennae, tarsi and ovipositor of the parasitoids were examined by scanning electron and optic microscopy after staining with silver nitrate. Three sensillar types were identified on the distal antennomeres: (i) non-porous sensilla trichoidea most probably involved in mechanoreception, (ii) uniporous sensilla chaetica likely to be gustatory and, (iii) multiporous sensilla placodea likely to be olfactory. The tarsi possess a few sensilla chaetica which could be gustatory while the manubrium is likely to be used in dectection of vibrations. The distal end of the ovipositor bears numerous multiporous dome-shaped sensilla. Additionally, the ability of the wasps to discriminate between contact cues was studied. When host larvae were washed in distilled water the wasps did not insert their ovipositors. However, ovipositor insertion resumed when washed host or non-host larvae were painted with water extracts of their respective host larvae. The water extracts of the suitable hosts were more attractive to the wasps than those of non-hosts. Similarly, the frass is important in host recognition during short-range examination as those of respective hosts are more intensely antennated than of non-hosts. The parasitoids were able to discriminate the regurgitant of *E. saccharina* by not antennating the cotton wool ball of this host; while the regurgitant of *B. fusca* and *C. partellus* appeared not useful in discriminating between the two species for both parasitoid species. Further analysis suggests the presence of a protein(s) component(s) in the regurgitant possibly responsible for host recognition and oviposition by *C. flavipes*.

Keywords: Braconidae, *Busseola fusca*, *Chilo partellus*, *Cotesia flavipes*, *Cotesia sesamiae*, *Eldana saccharina*, Host recognition, Kairomones, Lepidoptera, Parasitoids, Stemborers.

UITTREKSEL

Die gasheerherkenningsgedrag van twee braconid larwale parasitoïde, *Cotesia sesamiae* en *Cotesia flavipes* is bestudeer deur gebruik te maak van geskikte stamboordergashere [i.e. *Busseola fusca* vir *C. sesamiae,* en *Chilo partellus* vir *C. flavipes*] en een nie-gasheer [*Eldana saccharina*]. Die wespes het ‘n soorgelyke volgorde van gedragstappe getoon in die gasheeropsporingsproses en het grootliks staatgemaak op hulle antennas vir gasheerherkenning, en beide die antenna en tarsi vir finale aanvaarding van die gasheer vir eierlegging. Taktiele en kontak-chemoresepsie-stimuli van die gasheer blyk ’n belangrike rol te speel in die eierleggingsbesluit van parasitoïde. Die eksterne morfologie en verspreidingspatroon van sensillae wat aanwesig is op antennas, tarsi en die ovipositor van die parasitoïd is ondersoek deur middel van skandeer-elektronmikroskopie asook optiese mikroskopie nadat dit met silwernitraat gekleur is. Drie tipes sensillae is geïdentifiseer op die distale antennomere: i) nie-porieuse sensilla trichoidea wat moontlik ‘n rol speel in meganoresepsie, (ii) uniporieuse sensilla chaetica wat moontlik ’n smaakrol vervul en, (iii) multi-porieuse sensilla placodea wat waarskynlik ’n olfaktoriese funksie het. Op die tarsi word verskeie sensilla chaetica aangetref wat ‘n smaakfunksie mag vervul terwyl die manubrium waarskynlik gebruik word vir die aanvoel van vibrasies. Die distale end van die ovipositor het verskeie multiporieuse koepelvormige sensillae. Die vermoë van wespes om te onderskei tussen kontakfaktore is bestudeer en daar is waargeneem dat op larwes wat in gedistilleerde water gewas is, geen eierboor-indringing plaasgevind het nie. Eierboor-indringing is egter weer hervat wanneer gewasde gasheerlarwes geverf is met ekstrakte wat vanaf hulle verkry is. Water-ekstrakte van die geskikte gashere was meer aanloklik vir parasiete as die van nie-gashere. Daar is ook waargeneem dat die mis van larwes ’n belangrike rol speel in gasheerherkenning gedurende kort-afstand ondersoeke en dat die gasheerlarwes meer intensief ge-antenneer word as nie-gashere. Die parasitoïde was in staat om te onderskei tussen die terugspoeging van *E. saccharina* en het nie ’n katoenballetjie van die gasheer antenneer nie terwyl die terugspoeging van *B. fusca* en *C. partellus* klaarblyklik nie bruikbaar is in die proses van onderskeiding van albei parasitoïdspesies tussen die twee gasheerpsesies nie. Verdere analises van die terugspoeging het gedui op die aanwesigheid van proteïn-komponente wat waarskynlik verantwoordelik is vir gasheerherkenning deur *C. flavipes*.

Sleutelwoorde: Braconidae, *Busseola fusca*, *Chilo partellus*, *Cotesia flavipes*, *Cotesia sesamiae*, *Eldana saccharina,* Gasheerherkenning, Kairomone, Lepidoptera, Parasitoïde, Stamruspers.

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Chapter 1: General Introduction and Literature Review

1.1 Introduction

Maize (*Zea mays* L. [Poaceae]) is an extremely important crop for millions of people in Africa mainly cultivated by subsistence farmers for human consumption while the surplus is used as animal fodder (Minja, 1990; Kfir *et al*., 2002). Since the 1980s, many countries in sub-Saharan Africa have remained net importers of maize. This is attributed to a rapidly expanding population and stagnating yields over the years (FAO, 1999). In spite of this, it is forecasted that by the year 2020, the global demand for maize will have grown by 45% of which 72% will be in developing countries while only 18% in the industrialised nations (James, 2003). In order to deal with the surging demand, new methods of production need to be sought while reinforcing the existing ones to better manage the complex of problems facing maize farmers in tropical Africa (FAO, 2002).

In the densely populated areas of eastern Africa that have a high yield potential, the crop is grown on the same plot year after year due to population pressure and land constraints. This has lead to a steady decline in soil fertility and a net reduction in yields (FEWS, 2008). For example, an estimated 1.4 million hectares of maize was under cultivation in Kenya, between 1994 and 1998, with an average annual grain production of 2.5 million tonnes. During this period, the average grain yield was approximately 1.8 tonnes per hectare (FAO, 1999) although in some areas yields often fell below 1 tonne per hectare (Grisley, 1997). In Kenya, only about 2% of arable land is farmed under irrigation systems while the rest of the farming is rainfall dependent. This over-reliance on rainfall for production poses a major hindrance to sustainable maize production because the rains are often low and unreliable (FAO, 2004). This is further aggravated by factors such as: the lack of farm inputs like seed and fertilisers, outbreak of diseases, inability to control weeds and crop losses due to damage by insect pests (Minja, 1990; Grisley, 1997; Bonhof, 2000).

Of the various insect pests attacking maize in Africa, Lepidopteran stemborers are generally considered to be geographically widespread and most destructive causing severe damage to the crop (Ingram, 1958; Youdeowei, 1989; Kfir *et al*., 2002) (Fig. 1.1). Estimates of crop losses vary greatly in different regions and agro-ecological zones. In Kenya alone, losses due to stemborer damage fluctuates around 14% on average (De Groote, 2002). Therefore, these pests present a major constraint to the increased or maintained production of maize in areas where they are abundant (Youdeowei, 1989). Due to their widespread distribution and destructive nature, stemborers have been the subject of extensive research in Africa (Calatayud *et al*., 2006).

In Africa, maize is usually grown in small plots often surrounded by land occupied by wild graminaceous plants (Fig. 1.2). For many decades, these wild plants were considered as natural hosts of stemborers attacking crops (Bowden, 1976). Recently, these plants were found to have much higher stemborer species diversity than had been reported earlier. Furthermore, very few of them were found to be hosts of economically important pest species (Le Rü *et al*., 2006a, b; Ong'amo *et al*., 2006). Cereal stemborers were classified into three families as follows: Crambidae, Pyralidae and Noctuidae (Bleszynski, 1969; Harris, 1990). There exists a complex of 12 species of stemborers from cereal crops in East Africa with the crambids *Chilo partellus* (Swinhoe) and *Chilo orichalcociliellus* (Strand), the noctuids *Busseola fusca* (Fuller) and *Sesamia calamistis* Hampson and the pyralid *Eldana saccharina* (Walker) being among the economically most important and widely distributed (Nye, 1960; Youdeowei, 1989).

1.2 Stemborer control

A wide range of methods have been researched, tested and implemented to alleviate the problem of stemborers and their associated losses. These include among others control by chemicals, cultural practices, host plant resistance as well as biological control agents (Kfir *et al*., 2002).

1.2.1 Chemical control

In Africa, pesticides are mainly used on cash crops, like cotton, cut flowers and in the peri-urban horticultural sector. However, due to inadequate public awareness on the dangers of pesticides in Africa compared to other continents, and inadequate end-user protection; the use of chemicals is often unsophisticated and abusive. For example, persistent cotton pesticides are often used on vegetables with no respect to pre-harvest intervals (Schwab *et al*., 1995). This not withstanding, the use of chemicals in stemborer control is usually recommended by national extension agencies; and research has shown that it can be effective in reducing pest densities (Mathez, 1972; Warui & Kuria, 1983). Although control using systemic insecticides is far more effective, these only provide protection against early attacks but not borers feeding in the ear (Fig. 1.3) (Sétamou *et al*., 1995; Ndemah & Schulthess, 2002). In addition, the relatively short period stemborer larvae are exposed (before tunnelling into the stems) necessitates repeated pesticide applications. This can be time consuming and expensive making chemical control impractical for the majority of resource-poor, small-scale farmers in Africa (Bonhof *et al*., 1997). Apart from being harmful to man and other non-target organisms, abuse of chemicals is a major source of environmental pollution and may eventually promote resistance among target pests if used over a long time or if the pests are exposed to sub-lethal quantities (Minja, 1990; Schwab *et al*., 1995).

Where insecticides are easily available, they are relatively cheap and sometimes provided free by donors with little application of cost-benefit calculations (Schwab *et al*., 1995). On most food crops and in most places, however, African subsistence farmers do not apply insecticides. Apart from the fact that pesticide costs are limiting, their purchase is not high on the agenda. Confronted by different risks, the farmers' strategy is not to invest in risk reduction but in the spreading of risks. This is achieved by diversifying crop types and planting different cultivars on as large an area as labour and land access can allow, in the hope of harvesting enough to survive, whatever disaster may befall. In such scenario, the only widely applied pest control practice consists of cultural control measures that are mainly concerned with the reduction of carry-over of pests from one crop cycle to the next (Neuenschwander *et al*., 2003).

1.2.2 Cultural control

Cultural control is the most relevant and economic method of stemborer control for a majority of resource-poor farmers in Africa. However, it is challenged by the inability of farmers to implement the entailed practices over and above their being labour intensive (Van den Berg *et al.*, 1998). Cultural control practices in use include: destruction of crop residues, intercropping, crop rotation, manipulation of planting dates and tillage methods (Polaszek, 1998; Kfir *et al*., 2002). Intercropping and early planting have been practised by farmers across the continent, but studies show that their impact on stemborer populations is limited (Oloo, 1989; Skovgård & Päts, 1996). Destruction of crop residues by burning can create problems in farms where the organic matter is low and soil erosion from wind and rains is severe (Van den Berg *et al*., 1998). For cultural control to be effective, the co-operation of farmers within a particular region is required because moths emerging from untreated fields can infest adjacent crops. This is an area where cultural control is severely constrained by lack of management capabilities among farmers, especially in areas where farming communities lack the support of adequate extension services (Harris, 1989). In subsistence farming systems in Africa where farmers normally intercrop cereals with other crops and lack of water is a major constraint, manipulation of sowing dates and management of plant densities is not always practical as farmers often plant after the first rains (Van den Berg *et al.*, 1998)

1.2.3 Host plant resistance

Host plant resistance is a method that aims at developing plant varieties with intrinsic resistance to pests. It has been considered to be ideal for the control of pests, posing no environmental hazard and being generally compatible with other control methods (Bosque-Perez & Schulthess, 1998). An important issue with host plant pesistance includes appropriate design of safety tests to yield meaningful results, cause effect modes of non-target harm and the acceptability of such harm (Levidow, 2003). A holistic breeding strategy which aimed at developing varieties with acceptable agronomic characteristics and yield, as well as resistance to major diseases, yielded moderate resistance to borers in West Africa (Bosque-Pérez *et al*., 1997; Schulthess & Ajala, 1999). There is need for research to develop cultivars resistant to polyphagous pests; more often, strong antibiosis is achieved at the cost of yield. Despite decades of breeding for resistance, to date no maize varieties resistant to several important stemborers is available in Africa (Kfir *et al*., 2002).

1.2.4 Biological control

1.2.4.1 What is biological control?

The use of natural enemies in controlling invasive pest species has received much attention in recent times as a potentially effective method of pest control. As such, biological control has become relatively successful especially because of natural enemy specificity on target organisms (Godfray, 1994). A compelling motivation for adoption of biological control is a potential permanent return to ecological conditions similar to those seen prior to the arrival of the invasive pest and a reduced ongoing expenditure on pesticides, labour and specialised equipment (Hoddle, 2004). Hoddle (2004) skirts the essential ecological issue: predicting the magnitude of outcome of new interactions in a new environment; this is because the exotic species can cause a decrease of native parasitoids through competition for food (Elliot *et al*., 1996) and can also feed on native non-target organisms (Louda *et al*., 2003). Biological introductions have also disrupted key ecological functions in many systems, with far reaching implications for economic activities supported by those systems (Heywood, 1995). However, intentionally introduced species are likely to establish in the environment since they are selected for their ability to survive where they are introduced (Lonsdale, 1994; Smith *et al*., 1999). Due to the potential risks associated with biological introductions, it is necessary to elucidate whether the target species is actually a pest. Also evaluate the effect of natural enemies on non-target organisms before massive release followed by elaborate post-releasing monitoring.

1.2.4.2 Biological control of cereal stemborers

There has been renewed interest in the use of biological control agents to reduce stemborer population densities including ants, spiders and earwigs, believed to cause a high mortality of stemborer eggs and young larvae (Mohyuddin & Greathead, 1970; Girling, 1978; Oloo, 1989). This is coupled with several attempts over the past 50 years to introduce exotic parasitoids for control of stemborers in Africa particularly for suppression of the invasive exotic stemborers like *C. partellus* on the mainland and *C. sacchariphagus indicus* (Kapur) on the Indian Ocean islands (Overholt, 1998).

In this region, it had been recognised that indigenous larval and pupal parasitoids were not sufficiently abundant to keep stemborer populations below economic injury levels (Oloo, 1989; Bonhof *et al*., 1997). In particular, parasitism by the most abundant indigenous larval endoparasitoid in sub-Saharan Africa, *Cotesia sesamiae* (Cameron) (Hymenoptera: Braconidae) typically never exceeds 5% at the Kenyan coast (Sallam *et al*., 1999).

The koinobiont larval endoparasitoid *Cotesia flavipes* Cameron (Hymenoptera: Braconidae) was released in Kenya in 1993 for control of the invasive exotic stemborer *C. partellus*; the economically most important pest of maize and sorghum in Eastern and Southern African lowlands (Overholt *et al*., 1994a, b; Overholt *et al*., 1997). *Cotesia flavipes* was selected as the preferred candidate because of its history of success and importance in the control of stemborers in its aboriginal home in Asia (Overholt *et al*., 1994a). This was to complement the activity of *C. sesamiae* which also attacks *C. partellus* but was initially associated with indigenous borer species such as the noctuids *S. calamistis* and *B. fusca* (Mohyuddin & Greathead, 1970; Overholt *et al*., 1994 a, b; Zhou *et al*., 2001; Songa *et al*., 2002).

Since its introduction at the Kenyan coast, *C. flavipes* has spread and become established in the entire country (Zhou *et al*., 2001; Songa *et al*., 2002; Omwega *et al*., 2006). At the coast, it took four years for the parasitoid to significantly affect stemborer densities. Since then, parasitism rates have been rising steadily and by 2000, *C. partellus* densities at the coast were reduced by 57% while maize yields increased by 10-15% (Zhou *et al*., 2001). As shown by Jiang *et al*. (2006) parasitism is still on the increase indicating that the pest-parasitoid system is not yet at equilibrium. Following its success in Kenya and western Tanzania (Omwega *et al*., 1995; 1997), the parasitoid was released in 11 other countries in Eastern and Southern Africa and has become established in 10 of these (Omwega *et al*., 2006).

1.3 Factors influencing the efficacy of parasitoids

A major factor affecting the efficacy of exotic parasitoids is the suitability of indigenous stemborer species and the host plants they feed on (Hailemichael *et al*., 2008). *Cotesia* species belong to a group of parasitoids known as koinobionts. These parasitoids allow their parasitized host larvae to continue feeding while the parasitoid immatures develop within the host.

Hailemichael *et al.* (2008) (for *C. sesamiae*) and Jiang *et al.* (2004) (for *C. flavipes*) showed that depending on the *Cotesia* species, parasitized stemborer larvae feed and continue growing at the same rate as unparasitized ones. In addition, their growth rate is greatly influenced by temperature and host age (Jiang *et al.*, 2004).
Such intimate parasitoid-host relationships expose young parasitoid life stages to the host's immune system (Godfray, 1994; Pennachio & Strand, 2006) in addition to allelochemicals in the host diet (Barbosa *et al.*, 1986, 1990; Cortesero *et al.*, 2000; Sznajder & Harvey, 2003; Ode, 2006). However, 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, in *C. flavipes*, survival was shown to be lower and immature developmental time longer when *C. partellus* was feeding on wild instead of cultivated plant species (Sétamou *et al.*, 2005).

During foraging, parasitoids use volatile chemical cues (infochemicals), to guide them to a specific host habitat and to eventually locate the host (Vinson, 1975). Successful parasitism of hosts is preceded by a sequence of events which include: host habitat location, host location, host acceptance and host suitability (Vinson, 1976). 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 preferred odours from stemborer-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).

Studies by Potting *et al.* (1993) and Ngi-Song *et al.* (1996) revealed that *C. flavipes* and *C. sesamiae* were remotely attracted to stemborer-infested plants regardless of the species (herbivore or host plant) used. Furthermore, the wasps could not discriminate between host plants infested by *C. partellus, C. orichalcociliellus, B. fusca* or *S. calamistis.* This implies that the parasitoids cannot remotely detect the suitability of stemborer species in the plants. Therefore, the volatiles do not carry any information on the damaging herbivore species (Ngi-Song & Overholt, 1997). It appears that discrimination of hosts occurs at "short-range" rather than "long-range", i.e., once the parasitoid has made contact with the herbivore larvae.

For biological control to be a reliable and effective method, 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).

1.4 *Cotesia sesamiae*

Cotesia sesamiae is one of the most important native larval parasitoids of stemborers in many countries of sub-Saharan Africa (Bonhof *et al*., 1997) attacking mid- to late larval instars of both exotic and indigenous borer species (Mohyuddin, 1971) (Fig. 1.4).
Across East Africa, *B. fusca* is reported to be one of the most destructive stemborers of maize and sorghum and is abundant in the highlands (Harris & Nwanze, 1992). In Kenya, it is found at elevations higher than 600 m above sea level (Nye, 1960). At such elevations, *C. sesamiae* is the main larval parasitoid attacking *B. fusca* (Overholt *et al*., 1994b). Despite being the most abundant larval parasitoid in Africa (Mohyuddin & Greathead, 1970; Polaszek & Walker, 1991), *C. sesamiae* is unable to effectively suppress *C. partellus* populations in Kenya (Overholt *et al*., 1994b). In Kenya, there exist at least two biotypes of *C. sesamiae*, coastal and inland, expressing differential abilities to develop in *B. fusca* (Ngi-Song *et al*., 1995). The inland biotype successfully develops in *B. fusca*, whereas the coastal one does not (Ngi-Song *et al*., 1995). This variation in parasitism of *B. fusca* by *C. sesamiae* is attributed to physiological suitability and an encapsulation mechanism by which oviposited eggs are melanised in *B. fusca* (Ngi-Song *et al*., 1995; Ngi-Song *et al*., 1998; Mochiah *et al*., 2002). Encapsulation of parasitoid eggs reduces the efficiency of a given parasitoid species especially in regions where the unsuitable host is the predominant pest species (Ngi-Song *et al*., 1995; Obonyo *et al*., 2008).

During oviposition, most parasitic hymenoptera co-inject with their eggs factors that are responsible for suppression of the host's immune response, including venom from their accessory glands (Asgari *et al*., 2003) as well as polydnaviruses that work in synergy to bring about host regulation and immune suppression (Richards & Parkinson, 2000). The presence of the virus is asymptomatic in the wasp but causes major physiological disturbances in host larvae in which several viral genes are expressed. The most commonly observed pathologies in infected caterpillars are suppressed immunity and developmental arrest prior to metamorphosis. These two conditions are essential for the survival and growth of the wasp larvae inside its hosts (Beckage & Gelman, 2004).

Calyx fluid experiments reveal that the substances co-injected by the inland strain of *C. sesamiae* during parasitism of *B. fusca* suppress the host immune system, while those from the coastal strain do not. Consequently, the two strains of *C. sesamiae* are termed, *B. fusca* (virulent and avirulent) respectively (Mochiah *et al*., 2002).

There is relatively less information on the biology of the indigenous parasitoid *C. sesamiae* as opposed to that of its exotic counterpart *C. flavipes*. This is because it came into the lime light during the initiation of the classical biological control program at ICIPE during 1993. Thus it was assumed that its biology and behavioural attributes resemble those of *C. flavipes.*

1.5 *Cotesia flavipes*

The biology of *C. flavipes* was initially studied and recorded by Gifford & Mann (1967), and later by Mohyuddin (1971). Briefly, the adult is a small wasp about 3-4 mm in length and lives for only a few days. Females lay about 15-65 eggs into the host larva and eggs hatch after about 3 days. The parasitoid larvae develop through three instars within the stemborer larva feeding on body fluids. The egg-larval period takes about 10-15 days at 25^{o}C, 50-80% relative humidity (RH), and a photoperiod of 12:12 (L:D) hr. The final larval instars of this parasitoid emerge from the host body by chewing through the stemborer larval integument and immediately spin a cocoon and pupate. Adult parasitoids emerge 6 days later at 25^{o}C, 50-80% RH, and a photoperiod of 12:12 (L:D) hr. Usually, adult parasitoids emerge in the morning hours of the day and mating begins soon afterwards (Smith *et al*., 1993).

Recent findings on the interaction of *C. flavipes* with native, non-target lepidopteran stemborer species in Africa showed that this exotic parasitoid has a high specificity for its aboriginal host *C. partellus* (Obonyo, 2005) and with minimal non-target harm (Obonyo *et al*., 2008). In addition, *C. flavipes* has a higher searching efficiency attacking more larvae than *C. sesamiae* when *C. partellus* is the host. This shows that it is a more efficient parasitoid against *C. partellus* than the indigenous *C. sesamiae* (Sallam *et al*., 1999). With exception of *B. fusca* in which *C. flavipes* eggs are encapsulated, the parasitoid attacks and successfully develops in several other stemborer species such as *C. partellus, C. orichalcociliellus* and *S. calamistis* (Ngi-Song *et al*., 1995).

1.6 Parasitoid host location and recognition

Despite their cryptic lifestyle, stemborers do not escape parasitism by their natural enemies. This is because parasitoids have evolved various strategies of attacking concealed hosts living inside plant stems. Smith *et al.* (1993) grouped the strategies employed by parasitoids to attack stemborer larvae into four categories: (i) "probe and sting" tactic, where parasitoids probe through the leaf sheath to find early instar larvae; while other species probe through the exit hole of the stem tunnel to find mature larvae and sting them. A related tactic is (ii) "wait and sting", where parasitoids insert their long ovipositors through one of the tunnel holes then wait till the host larva passes by and is close enough for oviposition to occur. Parasitoids with (iii) the "drill and sting" strategy have long and strong ovipositors to parasitise their hosts at a distance from outside the stalk. Parasitoid species such as *Cotesia* spp. which have small ovipositors adopt (iv) the "ingress and sting" tactic, since they are small enough they enter the stem tunnel and parasitise their hosts.

1.6.1 Host location

Host location is the process whereby parasitoids perceive and orient towards their hosts from a distance by responding to stimuli originating from the host or its products (Smith *et al*., 1993). During host location, parasitoids utilize both long and short-range chemical stimuli (infochemicals) arising from the host habitat or from the host itself (Godfray, 1994). Vinson (1976) categorised the process resulting in successful host parasitism by insect parasitoids into four steps: (i) host habitat location (ii) host location (iii) host acceptance, and (iv) host suitability. The first three steps constitute the host selection process. Habitat and host location constitute long-range approaching behaviour based upon reception of volatile compounds. In each of these, female parasitoids often use chemical stimuli to guide them in searching for suitable hosts. Perception of infochemicals is an important component in host location, selection, evaluation, actual handling and eventual parasitism (Dicke & Vet, 1999).

The sources of these infochemicals are also broadly categorised into three: (i) stimuli arising from the host microhabitat or food plant (ii) stimuli indirectly associated with the presence of the host, and (iii) stimuli arising from the host itself (Vinson, 1976).

Although these categories blend into each other, their ranking roughly reflects their increasing importance as indicators of host presence (Godfray, 1994). Chemical stimuli emanating from the hosts and their habitat are directly involved in communication and exert influence on other trophic levels. These tritrophic interactions involving plants, herbivores and their natural enemies represent an intricate array of chemical substances known as allelochemicals (Vinson, 1976). Long-range attractants arise from the host communication system and host food (Vinson, 1976). These volatile allelochemicals are the most reliable cues for the foraging parasitoid only if they are specific for the herbivore species or when the cues can be learned by a searching parasitoid (Dicke & Vet, 1999).

Damaged plant tissue plays a major role in narrowing the search area for natural enemies once located in the host community. Once near a potential host community, foraging parasitoids often fly over damaged plants landing briefly while antennating for useful cues, if the cues produced are not useful, the parasitoids immediately resume their search (Vinson, 1975). For example, the braconid parasitoid *Cardiochiles nigriceps* Viereck once situated in a tobacco field flies 2-3 cm above the plant, lands briefly and antennates damaged plant tissue. Should the damage be mechanical or not due to an insect, the parasitoid resumes her search. However, if the damage is due to a potential host, the behaviour of the parasitoid changes from flying to crawling on the plant (Vinson, 1975). The importance of plant cues in *C. flavipes* and *C. sesamiae* orientation toward various hosts has been observed from behavioural studies in the laboratory using olfactometric bioassays. For example, the female parasitoids prefer odours from stemborer-infested plants over those from their uninfested counterparts (Potting *et al*., 1993; Ngi-Song *et al*., 1996; Jembere *et al*., 2003; Obonyo, 2005; Obonyo *et al*., 2008).

Allelochemicals emitted by infested plants which are attractive to *C. flavipes* and *C. sesamiae* primarily consist of green leaf volatiles such as (E)-4,8-dimethyl-1,3,7-nonatriene and (Z)-hexenyl acetate and alcohols such as phenol (Ngi-Song *et al*., 2000; Obonyo, 2005; Obonyo *et al*., 2008). Plants accumulate compounds as “chemical reserves” in specialized glands and upon infestation their leaves produce green leaf volatiles (GLVs) by breaking down membrane lipids (blends of saturated and non-saturated C_6-alcohols, aldehydes and esters) (Paré & Tumlinson, 1999).

Several studies have shown quantitative and qualitative differences in volatiles between herbivore-infested plants and uninfested plants (Ramachandran & Norris, 1991; Turlings *et al*., 1991a, b; Tumlinson *et al*., 1992; Takabayashi *et al*., 1995; Turlings *et al*., 1998; Rose & Tumlinson, 2004) including maize, sorghum and Napier grass (Ngi-Song *et al*., 2000). These phyto-distress signals (allelochemical emission) resulting in active interaction between herbivore-damaged plants and a third trophic level, have been described for several agro-ecosystems and over 15 plant species involved in plant-spider mite-predatory mite and plant-caterpillar-parasitoid systems (Dicke & Van Loon, 2000).

Chemoreception is the primary sensory modality that natural enemies rely on to locate their hosts. This is because they encounter a wide variety of stimuli (both plants and herbivores) which may be potentially useful sources of information. Therefore, the appropriateness and usability of the information perceived ultimately depends on two factors: (i) its reliability in indicating host presence, accessibility, and suitability as well as, (ii) the degree to which the stimuli can be detected (Vet & Dicke, 1992). In the recruitment of *C. flavipes* and *C. sesamiae*, plant derived volatile compounds do not carry information on the damaging herbivore species (Ngi-Song & Overholt, 1997) and the parasitoids are often found attracted to plants containing unsuitable borer species (Ngi-Song & Overholt, 1997; Obonyo *et al*., 2008). Consequently, other volatiles must be exploited by the parasitoids for successful parasitism of hosts.

Cotesia flavipes and *C. sesamiae* are unable to discriminate between host plants infested by *C. partellus, C. orichalcociliellus, B. fusca* and *S. calamistis.* Furthermore, when offered frass from these species in an olfactometer, neither of the parasitoids could remotely discriminate between the hosts (Potting *et al*., 1993; Ngi-Song *et al*., 1996). In other studies, it was reported that host frass plays an important role in the recruitment of *C. flavipes* to an infested plant (Potting *et al*., 1995). However, this is not the case for the inland strain of *C. sesamiae*. Recent findings showed that upon close examination, the females are able to discriminate frass of the host from those of non-host stemborer species (Gitau unpublished data). Ramachandran & Norris (1991) observed that plant odours are made up of several chemicals, some of which may be unique to a single species while others are shared among many. It is possible that the emitted plant odours may not be important for host discrimination as they carry no information on the suitability of the stemborer species. As such, damaged plants odours merely inform natural enemies on herbivore presence but not suitability of the damaging species.

1.6.2 Host recognition

Once a parasitoid has located a potential host community, the female seeks cues to the recognition of its hosts. This usually involves "short-range" chemoreception of non-volatile products arising from the herbivore (Vinson, 1985). When approaching its host, a female is exposed to chemical cues that are host derived and at times may be host specific (Tumlinson *et al.*, 1992), most often, these chemicals are found in the host products like: (a) body odour, (b) frass or honeydew for phloem feeders, (c) webbing, (d) salivary constituents, (e) body scales, (f) egg chorions and, (g) host pheromones (Vinson, 1976; Vet & Dicke, 1992). In addition, oral extracts from larvae feeding on plants have been shown to have a potential of attracting parasitic wasps as well as inducing volatile emission in plants even in the absence of herbivores. However, the release of the volatiles is indirect because it is induced by volicitin [N-(17-hydroxylinolenoyl)-L-glutamine] a compound present in the regurgitant (Turlings *et al.*, 1990; Alborn *et al.*, 1997).

Short-range compounds are stimuli derived directly from the host and are thought to be most reliable in informing the parasitoid of host presence (Godfray, 1994). Studies in the laboratory have shown that *C. flavipes* probes and stings the unsuitable host *B. fusca* only in the presence of short-range contact cues (Ngi-Song *et al.*, 1995). This suggests that a closer host examination both externally and internally, is fundamental for parasitoids to discriminate between suitable and unsuitable hosts.

According to Vet & Dicke (1992), the major constraint to the usefulness of information released by herbivores is the low detectability-reliability problem. This is particularly more severe over a distance and is mainly due to two reasons. Firstly, herbivores are a small component of a complex environment and if they produce odours, these are usually in minute quantities. Secondly, continuous selection for inconspicuousness acts on herbivores as a way to escape parasitism and predation. Therefore, minimization of odour emission is one way to accomplish this goal. The more successful the herbivore is in avoiding information conveyance, the more natural enemies have to turn to information from plants (Vet & Dicke, 1992).

1.6.2.1 Host external examination

Chemical cues perceived *via* sense organs (antennae, tarsi and ovipositor) are important for host selection and acceptance (Godfray, 1994). Examination and recognition of non-volatile cues on the body surface of larvae is a crucial step mediating stemborer attack by parasitoids. This is achieved by the receptors on the parasitoid's sense organs. Among braconid parasitoids, the antennae are the most important structures involved in host location (Godfray, 1994). Canale & Raspi (2000) conducted a scanning electron microscopic examination of the last antenomere of *Opius concolor* (Szepligeti) (Hymenoptera: Braconidae) showing the presence of different sensilla types which may be involved in host location. Additionally, morphological examination of the tarsi revealed the presence of sensilla that could be involved in receiving vibrational signals.

In a study on the behaviour of *O. concolor*, females were tracked using a binocular microscope during host searching activity. It was observed that there exists a latency period of 40-45 seconds, during which females remained stationary without initiating searching. In this phase the antennae were maintained wide apart and raised above the surface. Afterwards the female walked rapidly, alternately drumming her antennae on the surface. The antennae were directed forwards with the apical portion curved outwards as it drummed on the surface (Canale & Raspi, 2000).

For the congeneric parasitoids *C. flavipes* and *C. sesamiae*, it is believed that the antennae (Ngi-Song & Overholt, 1997) and possibly the legs (Smith *et al*., 1993) are involved in host examination and recognition. The use of the antennae in *C. flavipes* and *C. sesamiae* was observed by presenting washed (in distilled water) and unwashed host larvae to the parasitoids. When encountering unwashed larva, a female parasitoid often approached it in a random manner but as it drew closer, the rate of antennating and walking increased and it soon stung the larva. However, when the host larva was washed, the female wasp often walked several times over it without showing any signs of increased searching behaviour (Ngi-Song & Overholt, 1997). It has been observed that female *C. flavipes* and *C. sesamiae* often oviposited more readily in unwashed larvae than in washed individuals (Ngi-Song & Overholt, 1997).

1.6.2.2 Host internal examination

Once a parasitoid has received sufficient stimuli related to the external cues of host larvae, the ovipositor is unsheathed and thrust into the larvae (Smith *et al*., 1993). For endoparasitoids, the cues to oviposition are detected while the ovipositor is inside the host (Vinson, 1985). Parasitoids have been observed to frequently insert their ovipositors into a host but without laying eggs. This is because their ovipositors are usually covered in sensilla that may be used in perceiving suitability of the host. It is very likely that the parasitoid may reject a host after perceiving that the host is unsuitable (Godfray, 1994). Internal examination of hosts has been reported in *O. concolor* parasitising *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) larvae. On arriving on the patch where the host is located, the parasitoid remains stationary and randomly inserts her ovipositor into the spot previously antennated. Having located the larva, it probes a potential host before deciding to oviposit. The wasp stings the larva with the ovipositor then either departs rapidly or goes ahead to lay eggs (Canale & Raspi, 2000).

Hosts may be rejected due to several conditions, internal marking pheromones or due to the fact that the host is already parasitized, or the host may be physiologically unsuitable and lacking the necessary cues that would indicate its suitability (Vinson, 1985). In other cases the host is rejected due to its chemical combination of amino acids and inorganic ions as compared to the haemolymph composition of what is perceived to be the true host (Godfray, 1994). Rejection of hosts after internal examination has not been reported in *C. flavipes* and *C. sesamiae* especially since reports have shown that they both oviposit in the unsuitable host such as *B. fusca* (Ngi-Song *et al*., 1995; Obonyo, 2005; Gitau, 2006). For example, in the noctuid, *S. nonagrioides* (Eastern biotype), *C. flavipes* probes and stings the larvae with the ovipositor but the parasitoid eggs were not observed after dissections, it is not clear whether the parasitoid rejected the stemborers (failed to lay eggs) after perceiving their unsuitability (Obonyo, 2005).

In other studies, the time taken prior to and during oviposition has been used to predict the success of oviposition. For example, in the parasitoid of *C. capitata, O. concolor,* stings resulting in successful oviposition generally lasted for 30-45 seconds, a time considerably longer than the possible preceding attempts in which oviposition is aborted. From these studies, it was concluded that *O. concolor* uses her ovipositor for host discrimination (Canale & Raspi, 2000).

Similarly, oviposition in *C. flavipes* and *C. sesamiae* occurs rapidly and is termed successful when the ovipositor remains thrust into the larva for about 3-5 seconds (Smith *et al*., 1993). However, it has also been observed that there is no difference in time taken by the females to oviposit in suitable or unsuitable hosts (Ngi-Song *et al*., 1995). This suggests that the duration taken during oviposition alone is not an accurate parameter in ascertaining the success of the event. It is not known if the ovipositors of these parasitoids have sensilla that function solely for the purpose of internal examination and whether they are useful for host discrimination.

1.7 Sensilla used in host examination by parasitoids

Parasitoid sensilla on the antennae, tarsi and ovipositor can be broadly categorised into three main groups on the basis of their morphological and ultrastructural characteristics (Zacharuk, 1985):

(i) Mechanoreceptors: non-porous and innervated by one neuron each.

(ii) Gustatory: uniporous on their tips and frequently associated with a mechanoreceptor neuron and are innervated by more than one neuron (Fig. 1.5b).

(iii) Olfactory: generally multi-porous and are innervated by several neurons (Fig. 1.5a).

1.7.1 Sensilla on the antennae

Female parasitoids use their antennae as the primary sensory organ for host external examination (Van Baaren, 1994). Among the Encyrtidae, searching females exploit external stimuli using both olfactory and gustatory sensilla, whereas the Myrmaridae only use gustatory sensilla. For braconid parasitoids, although olfactory organs are useful for long-range host location (Obonyo, 2005; Obonyo *et al*., 2008), gustatory sensilla appear useful for examination prior to oviposition (Canale & Raspi, 2000). The external morphology of the antennomeres among braconid parasitoids appear simpler and generally more uniform than those of chalcids (Van Baaren, 1994; Canale & Raspi, 2000). In the former, the distal antennomeres which are mainly involved in substrate drumming possess sensilla trichodea and placodea.

These two sensilla types are believed to play gustatory and olfactory roles, respectively (Barbarossa Tomassini *et al*., 1998).

1.7.2 Sensilla on the tarsi

In some parasitoid species, the pretarsi are directly involved in host location. In the family Eulophidae, *Sympiesis sericeicornis* Nees has been found to have both mechano- and chemo-receptors on the parasitoid claws which are believed to be important in host detection and reception of vibrational signals from the host (Meyhofer *et al*., 1997). Morphologically similar, tarsi of *O. concolor* have been suggested to play a similar role in receiving vibrational signals (Canale & Raspi, 2000).

1.7.3 Sensilla on the ovipositor

The ovipositors of parasitoids are considered to be among the main organs involved in host discrimination (Van Baaren, 1994). The ovipositor, in many parasitoid families including Braconidae, is primarily composed of (i) a sting (an organ which is inserted into the host and is usually enveloped in a pair of valves) and, (ii) the gonostyli which surround the sting (Hermann & Douglas, 1976). The sting is normally covered by campaniform sensilla which may function as chemoreceptors to detect oviposition-stimulo or deterrent factors associated with suitable and unsuitable hosts respectively (Greany *et al*., 1977; Le Ralec, 1991; Van Baaren, 1994; Canale & Raspi, 2000). These sensilla may also function as mechanoreceptors sensitive to tactile stimulations (Greany *et al*., 1977). The gonostyli are characterised by abundant trichoidea sensilla which are assumed to be stimulated during pre-stinging or pre-oviposition probing (Hermann & Douglas, 1976).

1.8 Kairomones stimulating oviposition in parasitoids

Kairomones stimulating oviposition in parasitic wasps comprise of proteins, glycopolypeptides or sericin-like polypeptides, free amino acids, sugars, sesquiterpens, alcohols, phenols and ketones (Table 1.1). They can be located in the haemolymph of host larvae (e.g., Lepidopteran larval haemolymph has been found to induce host acceptance in certain parasitic wasps [Tilden & Ferkovich, 1988]), in the mandibular glands, exuvia, frass or directly in the plant (Table 1.1).

To the best of our knowledge, kairomones responsible for inducing host acceptance in *C. sesamiae* and *C. flavipes* have not yet been identified.

1.9 Goal and objectives

The main goal of this study was to understand the basis of host recognition by the exotic and indigenous parasitoid *C. flavipes* and *C. sesamiae,* respectively.

Therefore, the study was conducted along four main objective lines and is reported as separate chapters of the entire thesis:

(i) To assess the host-handling behaviour of the parasitoids; a prerequisite was a detailed observation of the external oviposition behaviour on both host and non-host stemborer larvae;

(ii) To identify the sensory structures involved in host location, recognition and acceptance by these parasitic wasps;

(iii) To isolate contact kairomone(s) involved in parasitoid host location, recognition and acceptance;

(iv) To identify the kairomone(s) mediating host location and acceptance by these parasitic wasps.

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A

B

C

Figure 1.1. Damage to leaves (A), stems (B) and cob (C) caused by Lepidopteran stemborers (sources: ICIPE stemborer project (A and B) and Gitau, C.A.W. (C).

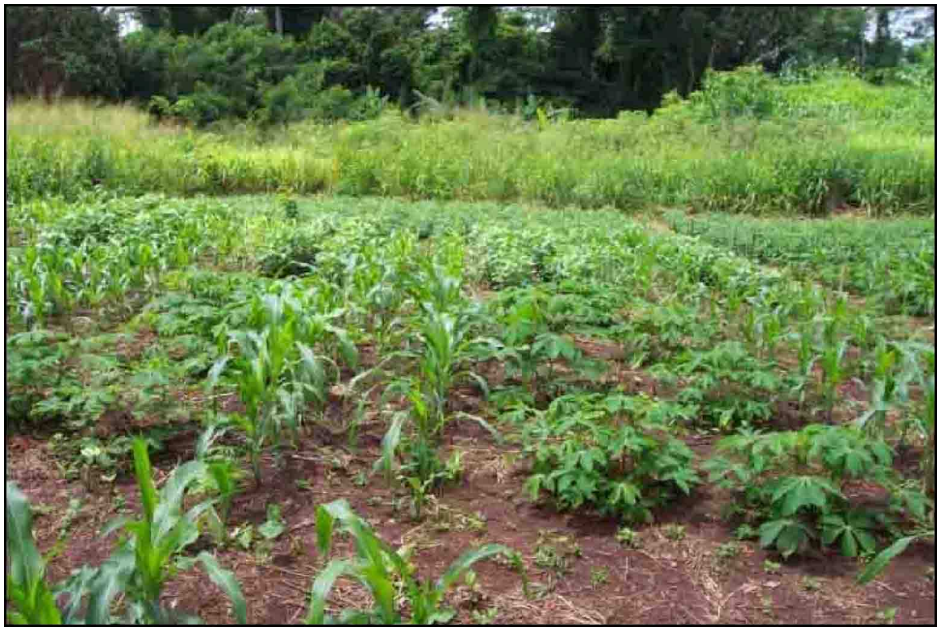

Figure 1.2. Maize plot surrounded by wild grasses (source: ICIPE stemborer project).

Figure 1.3. Chemical control in a maize plot (source: G. Ong'amo).

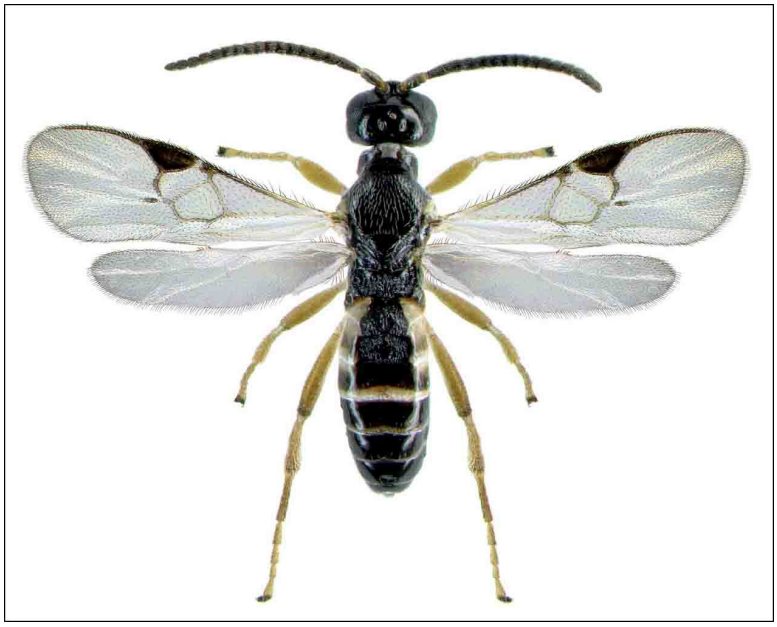

Figure 1.4. *Cotesia sesamiae* (source: ICIPE stemborer project).

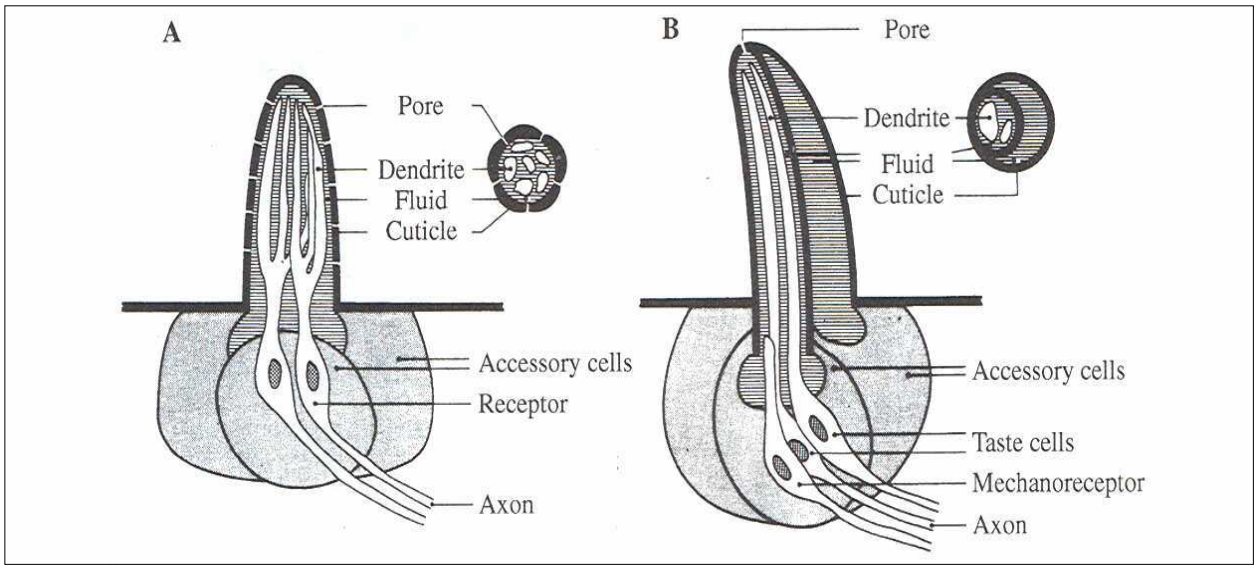

Figure 1.5. Schematic drawing of longitudinal and transverse section of (A) an olfactory sensillum and (B) a gustatory sensillum (source: Schoonhoven *et al*., 1998).

Table 1.1 Kairomones stimulating oviposition among parasitoids and their source.

Reference	**Parasitoid (Genus and Family)**	**Host (Genus and Family)**	**Source**	**Kairomone**
Bénédet *et al.* (1999a & b)	*Diadromus* (Ichneumonidae)	*Acrolepiopsis* (Yponomeutoidea)	silk cocoon	glycoproteins, glycopolypeptides
Burks & Nettles (1978)	*Eucelatoria* (Tachinidae)	*Heliothis* (Noctuidae)	cuticle of the larvae	-
Elzen *et al.* (1984)	*Campoletis* (Ichneumonidae)	*Heliothis* (Noctuidae)	plant (cotton)	sesquiterpens
Hassel (1968)	*Cyzeni* (Tachinidae)	*Operophtera* (Geometridae)	plant (Fagaceae)	sucrose, fructose
Gauthier *et al.* (2004)	*Diadromus* (Ichneumonidae)	*Acrolepiopsis* (Yponomeutoidea)	silk cocoon	polypeptides
Jones *et al.* (1971)	*Microplitis* (Braconidae)	*Heliothis* (Noctuidae)	frass, saliva, haemolymph	methylhentriacontane
Kuwahara *et al.* (1983)	*Venturia* (Ichneumonidae)	*Plodia* (Pyralidae)	Frass	2-palmitoyl- and 2-oleoyl-cyclohexane- l, 3-dione
Ma *et al*. (1992)	*Eriborus* (Ichneumonidae)	*Ostrinia* (Pyralidae)	frass, oral secretion	11 free amino acids including serine and glutamic acid
Mudd & Corbet (1982) Mudd *et al.* (1984)	*Venturia* (Ichneumonidae)	*Ephestia* (Pyralidae))	Mandibular glands	2-acylcyclohexane-1-3-diones
Nemoto *et al*. (1987)	*Venturia* (Ichneumonidae)	*Ephestia* (Pyralidae), *Plodia* (Pyralidae)	Frass	2-palmitoyl- 2-stearoylcyclohexane-1,3-dione
Nettles & Burks (1975)	*Archytas* (Tachinidae)	*Heliothis* (Noctuidae)	frass, haemolymph, entire larvae, pupae, emerged adults	protein (30 kD)
Roux *et al*. (2007)	*Cotesia* (Braconidae)	*Plutella* (Plutelidae)	larval cuticle	lipids
Strand *et al.* (1989)	*Bracon* (Braconidae)	*Ephestia* (Pyralidae)	Mandibular glands	2-acylcyclohexane-1-3-diones
Takabayashi & Takahashi (1989)	*Apanteles* (Braconidae)	*Pseudaletia* (Noctuidae)	Frass	2, 5-dialkyltetrahydrofuran
Thompson *et al.* (1983)	*Lixophaga* (Tachinidae)	*Diatraea* (Pyralidae)	frass, plant (sugarcane)	phenols, alcohols, sugars
Vinson *et al.* (1975)	*Cardiochiles* (Braconidae)	*Heliothis* (Noctuidae)	Mandibular glands	methyl hen-, di-, tritriacontane
Weseloh (1977)	*Cotesia* (Braconidae)	*Limantria* (Bombycidae)	silk producing glands	sericin or fibrinogen like protein

Chapter 2: Host Recognition Behaviour in *Cotesia sesamiae* and *Cotesia flavipes,* Parasitoids of Gramineous Stemborers in Africa

2.1 Abstract

Host recognition behaviour of two braconid larval parasitoids *Cotesia sesamiae* and *Cotesia flavipes* was studied using suitable stemborer hosts [i.e., *Busseola fusca* for *C. sesamiae*, and *Chilo partellus* for *C. flavipes*] and one non-host [*Eldana saccharina*]. A single 3rd-4th instar larva was introduced into a glass Petri dish together with a naïve, putatively mated female wasp and the behavioural events displayed by the wasp recorded until it stung the larva or for a maximum of 5 minutes where stinging did not occur. There was a clear hierarchy of behavioural steps which was similar for both parasitoid species. In the presence of suitable host larvae, after a latency period of 16-17 sec the wasps walked rapidly drumming their antennae on the larva. After host location, which lasted 60-70 sec, and antennal examination of about 30 sec, ovipositor insertion occurred. Stinging that resulted in successful oviposition usually lasted 5-6 sec. In the presence of non-host larvae, the latency period was between 25-70 sec, and the female parasitoids spent significantly more time walking and drumming their antennae on the larvae without showing oviposition behaviour. The two parasitoid species use their antennae for host recognition, and both the antennae and tarsi for final host acceptance for oviposition. Tactile and contact-chemoreception stimuli from the hosts seemed to play a major role in oviposition decision of *C. sesamiae* and *C. flavipes*.

2.2 Introduction

As mentioned in chapter one, in sub-Saharan Africa, lepidopteran stemborers of the Crambidae, Pyralidae and Noctuidae families are the economically most important pests of maize and sorghum (Harris, 1990; Polaszek, 1998; Kfir *et al*., 2002). The most cited species are the crambid *Chilo partellus* (Swinhoe), the noctuids *Busseola fusca* (Fuller) and *Sesamia calamistis* Hampson, and the pyralid *Eldana saccharina* (Walker) (Polaszek, 1998). With exception of *C. partellus*, which was accidentally introduced from Asia into Southern Africa sometimes before the 1930s (Tams, 1932), the others are indigenous to the African continent.

During the early 90s, the International Centre of Insect Physiology and Ecology (ICIPE) renewed emphasis on biological control activities. The most abundant larval parasitoid recovered from stemborers in East and Southern Africa is the gregarious braconid *C. sesamiae*. Parasitism is usually below 5% though in some localities it can attain 75% (Kfir, 1995; Sallam *et al*., 1999; Jiang *et al*., 2006; Songa *et al*., 2007). *Cotesia flavipes* Cameron, a close relative of *C. sesamiae*, was introduced into Kenya from Asia in 1991 and released against *C. partellus* in the coastal area in 1993 (Overholt *et al*., 1994b), where it reduced *C. partellus* densities by over 50% (Zhou *et al*., 2001; Jiang *et al*., 2006).

In order to establish, the parasitoid must first locate and be able to discriminate suitable from unsuitable hosts in its new habitat. Behavioural events leading to successful parasitism include host habitat location, host location, host acceptance and suitability (Vinson, 1976; 1985; Godfray, 1994). During location of hosts, they typically exploit long and short range stimuli emanating from the host habitat (Vinson, 1975; Godfray, 1994), followed by stimuli directly associated with the host and its products (Vinson, 1985; Vet & Dicke 1992; Godfray, 1994). However, stimuli from the habitat do not convey sufficiently reliable information on the suitability of host species but are mere indicators of herbivore presence (Ngi-Song & Overholt, 1997). As a result, *C. sesamiae* and *C. flavipes* are often attracted to plants harbouring unsuitable stemborer species (Potting *et al*., 1993; Ngi-Song *et al*., 1996). Therefore, it is suggested that *C. sesamiae* and *C. flavipes* females, though not capable of recognizing a host species from a distance, are able to distinguish suitable from non-suitable hosts at close contact (Obonyo *et al*., 2008).

This is especially crucial for the establishment and efficacy of the exotic *C. flavipes*, which encounters many new suitable and unsuitable hosts in it new environment in Africa (Le Rü *et al*., 2006).

In order to understand contact host discrimination behaviour of the congeneric species, this study attempted to identify the behavioural steps displayed by the females prior to host acceptance for oviposition. Host recognition and acceptance behaviour has been well studied for various parasitoids (e.g., Vinson, 1985; Godfray, 1994; Vinson, 1998; Canale & Raspi, 2000), but there is a lack of detailed information on the step-by-step process leading to oviposition by *C. sesamiae* and *C. flavipes*.

2.3 Materials and methods

2.3.1 Insects

The adults of *C. sesamiae* and *C. flavipes* were obtained from laboratory-reared colonies established at ICIPE, Nairobi, Kenya. The *C. sesamiae* colony was initiated with materials obtained from *B. fusca* collected from maize fields in Kitale, Western Kenya, in 2006, while *C. flavipes* was obtained from *C. partellus* in coastal Kenya in 2005. Twice a year, field collected parasitoids were added to rejuvenate the colonies. *Cotesia sesamiae* and *C. flavipes* were reared on larvae of their true hosts, *B. fusca* and *C. partellus*, respectively, according to the method described by Overholt *et al*. (1994a). Parasitoid cocoons were kept in Perspex cages (30 cm x 30 cm x 30 cm) until emergence. Adults were fed on a 20% honey-water solution imbibed in a cotton wool pad and kept under artificial light for 24 hrs to mate. In all experiments, only 1-day-old, naïve, putatively mated females were used. The experiments were carried out at 25 ± 2^{o}C, 50-80% RH, and a 12:12 hr (L:D) photoperiod.

Three stemborer species were used in the study: *B. fusca* and *C. partellus* as host of *C. sesamiae* and *C. flavipes*, respectively, and *E. saccharina* as a non-host of both parasitoid species. Preliminary tests showed that neither parasitoid attacked *E. saccharina* [Obonyo M., personal observation]. *Eldana saccharina* and *B. fusca* were collected from maize fields in the Western Province, while *C. partellus* originated from maize grown in the coastal region.

The larvae were reared on artificial diet according to the methods described by Ochieng *et al*. (1985) (for *C. partellus*) and Onyango & Ochieng'-Odero (1994) (for *B. fusca* and *E. saccharina*). Thrice a year feral stemborer larvae from their respective locations were added to rejuvenate the colonies.

2.3.2 Experimental procedure

Third and fourth instar larvae were introduced into jars (10 cm x 20 cm) containing pieces of maize stem and left for 24 hrs to feed and produce frass. Thereafter, a single larva was tansferred into a glass Petri dish of 7 cm diameter and 1 cm height together with a wasp. Recording of behavioural events displayed by the female wasp begun immediately after its release into the arena and continued until it stung the larva or for a maximum of 5 minutes if the wasp did not sting the larva.Some wasps were killed by the host before stinging the larvae. In this case, both the wasp and larvae were excluded from the analyses. For every replicate consisting of a single parasitoid and stemborer larva, the arena was used only once then cleaned in distilled water and air-dried. Similarly, a single wasp and larva was used only once. A total of 50 observations were made for each parasitoid/stemborer larva combination. All larvae that had been stung by the parasitoids were reared on artificial diet and monitored daily until cocoon formation, pupation or death.

A digital video camera (Panasonic, Japan) with an optical zoom system (Computer TV zoom Lens, 8-80 mm) connected to a VHS video recorder (time lapse video cassette recorder, Panasonic, AG-6730) was used to record the parasitoids' behavioural steps. In nature, adult *C. sesamiae* and *C. flavipes* are generally active throughout the photophase especially around 12:00hrs, under conditions of increased light intensity and temperatures (Mohyuddin, 1971). Thus, in the laboratory, their activity was induced by placing them under bright artificial light and temperature maintained at about 25°C (Overholt, 1993; Smith *et al*., 1993). The experiments were conducted between 10:00 and 14:00hrs. The arena was illuminated by a white 60 W Philips light bulb while maintaining the temperature at 25 ± 2°C.

2.3.3 Behavioural steps

The behaviour of a female wasp was described in terms of her body posture and movement when encountering a larva. The behavioural steps are mutually exclusive events described as a combination of orientation and movement of different body parts especially those bearing sensory structures (i.e., antennae, tarsi and ovipositor). These steps are the smallest scale of a composite, which could be reliably and repeatedly observed. The six behavioural steps displayed by the parasitoids were as follows (Fig. 2.1): (1) standing still with the antennae upright and apart - as soon as it had been dropped in the arena it would usually remain motionless parsitoid (ST); (2) grooming of rear legs and/or antennae - this is believed to involve the cleaning of the antennae to expose the sensory organs (G); (3) walking and antennal drumming of the arena - this is linked to the searching of the larvae in the arena (W); (4) walking and drumming of the larval body with the antennae - the parasitoid has locacted the larva and is walking on the larva examining the cuticle before deciding to sting (WB); (5) stinging attempt - usually occurs when the wasp tries to probe the larval body but the process is too short compared to normal oviposition (less than 2secs) (SA), i.e., unsuccessful or disrupted ovipositor insertion; (6) oviposition (O), i.e., successful ovipositor insertion, followed by voluntary parasitoid dispersal (the process is about 6 secs long). The total duration of each behavioural step was also recorded.

In the event that the wasp attempted to sting the larva (SA) or where it went ahead and succefully inserted her ovipositor (O), these larvae were reared individually in vials containing artifical diet while being monitored daily for cocoon formation, mortality or pupation.

2.4 Data analysis

The analysis of the behaviour of female parasitoids encountering stemborer larvae was performed by Proc CATMOD of the SAS system (SAS Institute, 2003). The analysis was conducted on the total number of transitions between all possible pairs of behavioural steps and the data were pooled for all females using transitional frequencies of all transitions between pairs of behavioural patterns.

Transitional frequencies were used to form a contingency table (p x q x r), in which the first variable p represented the possible “preceding” behaviour and the second variable q represents the possible “following” behaviour, while r represented the host larva.

"Logical zeroes" occur in the table, where one behavioural pattern cannot follow another, or between identical preceding and following behaviour.
Log-linear models were constructed based upon specific combinations of interactions to provide expected values of transitional frequencies for the contingency table. The rationale and method of analysis had previously been described by Parr *et al*. (1996) and Hora & Roessingh (1999). The goodness-of-fit of the log-linear model was assessed by likelihood ratio statistic (G tests).

Where the expected values of a model were not significantly different from the observed table, the assumptions of that model were accepted as necessary and sufficient to explain the observed data. In addition, an index was calculated to quantify the dissimilarity between the expected values under the assumptions of each model and the observed values. The probability of each transition given the preceding behaviour was also calculated. To identify the transitions that were a significant part of a sequence of behavioural steps, standardized residuals of the observed transitions were calculated; when compared to a model that did not include the assumption of dependence of following and preceding behavioural steps. Significant positive transitions – i.e., those for which positive standardized residuals were obtained, which were greater than the calculated threshold – were used to construct kinetograms of the host recognition and acceptance behaviour. Chi square-tests were used to compare the percentage occurrence of behavioural steps with respect to stemborer species. The Binomial proportions test of the SAS system was used to separate proportions of host larvae stung, forming cocoons, dying and pupating (SAS Institute, 2003)(Table 2.1). For the data of the total duration of behavioural steps, Tukey's studentized range test was used to separate the means using the GLM procedure of the SAS system (SAS Institute, 2003).

2.5 Results

When female *C. sesamiae* and *C. flavipes* encountered a larva, their behaviour varied depending on whether it was a host or non-host. However, no difference in behavioural steps was observed between the different parasitoid species. When approaching their respective hosts, the wasps walked in the arena drumming their antennae till they located the larva. They jumped on the larva, briefly antennated the larval body and then inserted the ovipositor.

On host larvae, increased WB culminated in O, while for non hosts a high WB value did not translate to O (Figs. 2.2 and 2.3). During WB, the antennae were directed forwards and the apical portion curved down and backwards as they drummed the surface. Only the distal ends of the antennae were observed to be in direct contact with either the arena or larval surface.
When *C. sesamiae* females were presented with *B. fusca*, they frequently displayed four pairs of behavioural transitions (probability of transitions > 0.01), i.e., (i) ST - W, (ii) W - WB, (iii) WB - O and, (iv) WB – W (Fig. 2.2). Among the wasps tested (N=50), the occurrence of O was 66%, while SA was 16 %(Fig. 2.2). Of all the stung larvae (N=41) 63% produced cocoons while 20% and 17% died and pupated respectively.

By contrast, when they encountered *C. partellus* and *E. saccharina*, no O was observed (2 x 3 contingency table $\chi^2 = 84.6$, d.f. = 2, $P < 0.0001$) neither was there a transition between WB and O (Figs. 2.2b and 2.2c). Moreover, the occurrence of SA was significantly higher when *C. sesamiae* encountered *B. fusca* than either *C. partellus* or *E. saccharina* ($\chi^2 = 10.5$, d.f. = 2, P = 0.005). However, the only larvae that had been stung (2%) in both species died afterwards. .With *C. partellus*, high probabilities of transitions (P > 0.01) were recorded only between two pairs of behavioural transitions, i.e., (i) ST - W and, (ii) W - WB (Fig. 2.2b). With *E. saccharina*, the kinetogram was more complex with highest probabilities of transitions (P > 0.01) that were not linked with oviposition behaviour. These were recorded between six pairs of behavioural transitions, i.e., (i) ST - W, (ii) G - W, (iii) W - WB, (iv) WB - W, (v) W - G, and, (vi) W - ST (Fig. 2.2c). The percentage of wasps that stood still (ST) was 42% when *B. fusca* was the host, but over 70% with *C. partellus* or *E. saccharina* as the host ($\chi^2 = 11.0$, d.f. = 2, P = 0.003). Similarly, G was less frequent when *C. sesamiae* encountered *B. fusca* than with *C. partellus* or *E. saccharina* ($\chi^2 = 6.595$, d.f. = 2, P = 0.0370). Consequently, on *B. fusca* larvae, *C. sesamiae* females spent significantly less time on ST and G ($P < 0.05$), preferring to spend significantly more time on O (Table 2.1).

When female *C. flavipes* encountered *C. partellus*, three pairs of behavioural transitions with high probabilities of transition (P > 0.01) were observed, i.e., (i) ST - W, (ii) W - WB and, (iii) WB and O (Fig. 2.3a). As for *B. fusca*, high probabilities of transitions (P > 0.01) were recorded between only two pairs of behavioural transitions, i.e., (i) W - WB and, (ii) WB - O (Fig. 2.3b). However, the kinetogram obtained with *B. fusca* was more complex than that with *C. partellus* having a higher number of transitions.

Comparison of *C. flavipes* encountering *C. partellus* and *B. fusca* reveals that, in *C. partellus* the occurrence of O was 58% and SA was 4% among all tested wasps (N=50). In addition, of all stung *C. partellus* larvae (N= 31) 74% produced cocoons while 10% and 16% died and pupated respectively. In *B. fusca*, O occurred in 52%, SA 10% [Z=0.00; P=1.00] of the wasps tested (N=50) while none of the stung larvae (N=31) produced cocoons [Z=6.03; P=0.00], 16% died [Z= 7.14; P=0.00] and 84% pupated [Z=6.14; P=0.00]. When female *C. flavipes* encountered the non-host *E. saccharina,* the wasps did not exhibit the O behaviour but instead SA 6%. However, of the three stung larvae, one died while the other two pupated. High transition probabilities (P > 0.01) were recorded between five behavioural steps, i.e., (i) ST - G, (ii) ST - W, (iii) G - W,(iv) W - G and (v) W - ST (Fig. 2.3c). In the presence of *E. saccharina* larvae, the percentage of females showing WB behaviour decreased while that of G and ST increased (for WB: χ^2 = 16.9, d.f. = 2, P < 0.0001; for G: χ^2 = 46.8, d.f. = 2, P < 0.0001; for ST: χ^2 = 11.0, d.f. = 2, P = 0.0041). With *C. partellus* larvae, the female *C. flavipes* spent less time on O than on ST and G than it did with *B. fusca.*

The wasps also spent significantly more time (P < 0.05) on ST, G and W in the presence of *E. saccharina* than they did with *C. partellus* (Table 2.1).

Generally, for both parasitoid species there was no relationship between time spent on WB and O. More time was spent on WB with non-host larvae but without the O behaviour (Table 2.1).

2.6 Discussion

There appears to be a relationship between the detection of the presence of a larva and the standing still behaviour displayed by *C. sesamiae* and *C. flavipes.* During this stationery phase of their behaviour, the wasps could have been able to detect the body odour emanating from the larvae or residues from feeding activity (e.g., frass). Ngi-Song & Overholt (1997) demonstrated that *C. sesamiae* and *C. flavipes* females were able to detect volatiles emitted from larval frass but were unable to discriminate between volatiles from frass of both host or non-host species. In the current study, the female wasps stood still for a shorter time when in the presence of suitable hosts than they did with non-host larvae. This probably indicates that they could discriminate between the body odour from host and non-host larvae.

Antennal drumming behaviour played an essential role in location, recognition and acceptance of hosts. Significantly more time was spent in antennal grooming when in the presence of a non-host. Grooming in insects, besides cleaning the outer body surface or organs, may also serve in functions such as courtship behaviour, social signalling, displacement activity or even de-arousal (Spruijt *et al*., 1992).The use of antennae by female parasitoids in host examination prior to ovipositor insertion is well documented in literature (Vinson, 1985; Godfray, 1994; Vinson, 1998; Canale & Raspi, 2000). In both *Cotesia* species, the distal antennomeres appeared to be intimately involved in host location and recognition process since they were observed to be in direct contact with the body surface of the larvae. This observation corroborates findings that the distal antennomeres of braconid parasitoids possess gustatory sensilla that allow for detection of chemical stimuli arising from the host (Navasero & Elzen, 1991; Barbarossa-Tomassini *et al*., 1998; Canale & Raspi, 2000; Ochieng *et al*., 2000).

Compared to other braconids, oviposition in *C. sesamiae* and *C. flavipes* occurred very rapidly (5-6 sec) and soon after antennal examination of the larval cuticle. In both congeneric species the ovipositor does not seem useful in host examination before oviposition. This is unlike other braconid parasitoids that use their ovipositors during host examination before oviposition, for example, *Opius concolor* Szepligeti, where oviposition lasts 30-40 sec (Canale & Raspi, 2000) and *Cotesia glomeratus* (Linnaeus) where it takes 16-20 sec (Tagawa *et al*., 1987). The ovipositors of these parasitoids are usually inserted repeatedly before oviposition. This is contrary to what was observed in *C. sesamiae* and *C. flavipes* whose females immediately inserted their ovipositors and laid eggs.

The behavior of the attacked larvae may influence the decision of a parasitoid during parasitisation. Cuticle penetration usually varies greatly among species and can be longer when a sessile host is attacked but often occurs very rapidly when mobile and defensive hosts are attacked (Vinson, 1985). Lepidopteran larvae feeding on plants whether externally or internally defend themselves against parasitoids either indirectly by hiding during times of vulnerability (Gross, 1993) or directly by biting, spitting on or flicking off the parasitoid (Potting *et al*., 1993; Takasu & Overholt, 1997). Thus, due to this aggressive behaviour of their hosts, *C. sesamiae* and *C. flavipes* females are under pressure to oviposit once they contact the host.

It appears that for both *C. sesamiae* and *C. flavipes*, host acceptance and the decision to insert their ovipositors occur during external antennal examination. Parasitoid ovipositors are thought to be involved in internal examination of the suitability of a host (Godfray, 1994). Thus, host acceptance or rejection may also depend on the internal markers perceived by the ovipositor of the female wasp (Godfray, 1994; Le Ralec *et al*., 1996; Van Lenteren *et al*., 2007). Internal signal processing may explain why some females rejected the non-host species *E. saccharina* after a short stint of ovipositor insertion. Only 2% and 6% of *C. sesamiae* and *C. flavipes*, respectively, attempted to insert their ovipositors albeit without cocoon formation. However, in the current study it could not be established whether or not the wasps laid eggs or not.

In addition to chemical stimuli, various other larval physical characteristics such as size, shape, texture, or movement can be involved in the host recognition process (Vinson, 1991; Godfray, 1994). During host encounter, the female parasitoids often jumped on the body of the larvae and walked all over it while inspecting cues on the larval cuticle. This suggests reception of vibrational signals through the tarsi because parasitoid tarsi bear contact mechanoreceptors that are useful in reception of vibrational signals upon contact with the larvae (Godfray, 1994). In addition, when the larvae were motionless during examination, the wasp took longer before ovipositing than when the larvae were moving or reacted to being contacted by the wasp. Similarly, for *O. concolor*, when in the presence of immobilised host larvae, the females took longer to locate the host, and frequently failed to do so (Canale & Raspi, 2000).

During external examination, the labial and maxillary palpi of *C. sesamiae* and *C. flavipes* were held perpendicular to the body surface of the larvae, and they did not appear to contact the larvae in the examination process. This behaviour may be associated with wasp feeding activity but not to host recognition. This also occurred when the females contacted the cotton wool pad imbibed in a sugar-based solution added as food source, even in the absence of larvae. By contrast *O. concolor* was observed to use the palpi in host searching (Canale & Raspi, 2000).

In conclusion, *C. sesamiae* and *C. flavipes* females are able to detect the presence of a larva in close proximity most probably by the odour emanating from the frass and/or its products resulting from the larval feeding activity. This detection allows the female wasp to locate their potential hosts when in close proximity.

Afterwards, antennal examination plays a crucial role for host recognition and acceptance. The tarsi appear important for the process of host acceptance before stinging by receiving vibrational signals when the wasps are walking on the larvae. We suppose that the stemborer larval body contact cues (whether physical and/or chemical) play a role in host acceptance for oviposition among the female parasitoids because the wasps must make contact with the cuticle before acceptance. As a result, the identification of these cues will enable isolation of kairomone(s) involved in host recognition and acceptance by *C. sesamiae* and *C. flavipes* – a goal which this study also targets to achieve.

2.7 References

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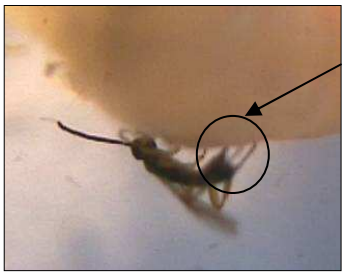
(1) Standing still (ST)

(2) Grooming the antennae and/or the legs (G)

(3) Walking in the arena (W)

(4) Walking on the larval body (WB)



(5) Oviposition (O)

Figure 2.1. Typical behavioural steps preceding oviposition displayed by female wasps (e.g., *Cotesia sesamiae* vs. *Busseola fusca*), showing (1) standing still with the antennae upright and apart (ST), (2) grooming the legs and/or the antennae (G), (3) walking in the arena drumming the surface with the antennae and with the tips curved (W), (4) walking on the larval body while drumming its surface with the antennae (WB) and (5) oviposition (O), i.e., successful ovipositor insertion followed by egg-laying.

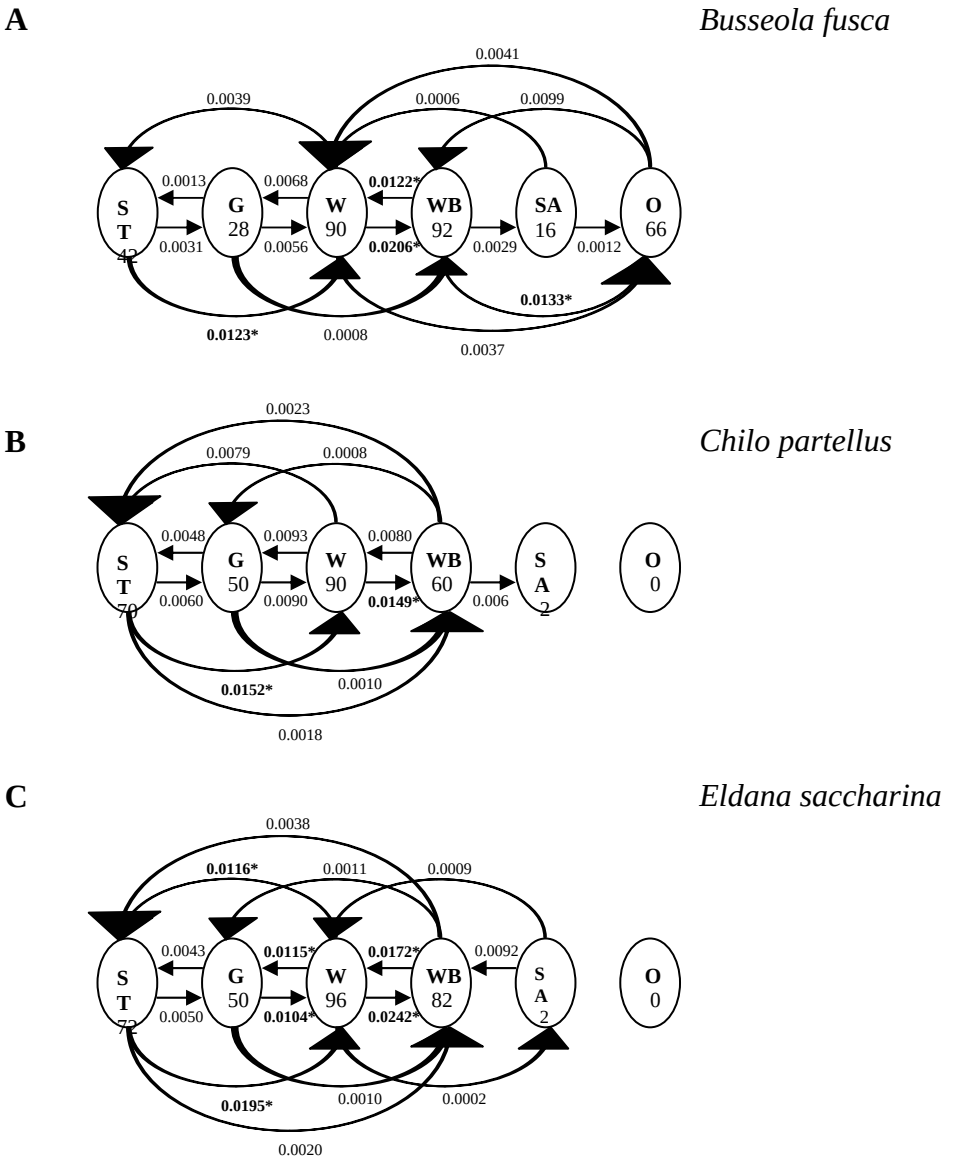


Figure 2.2. Behavioural steps exhibited by naive gravid female *Cotesia sesamiae* encountering *Busseola fusca* larvae (A), *Chilo partellus* larvae (B) and *Eldana saccharina* larvae (C). Values with arrows represent the probability of each significant transition given the preceding behaviour. For each experiment, fifty female wasps were observed. * = probability of transitions > 0.0100. The percentage of occurrence of each behavioural step is provided below its category definition.

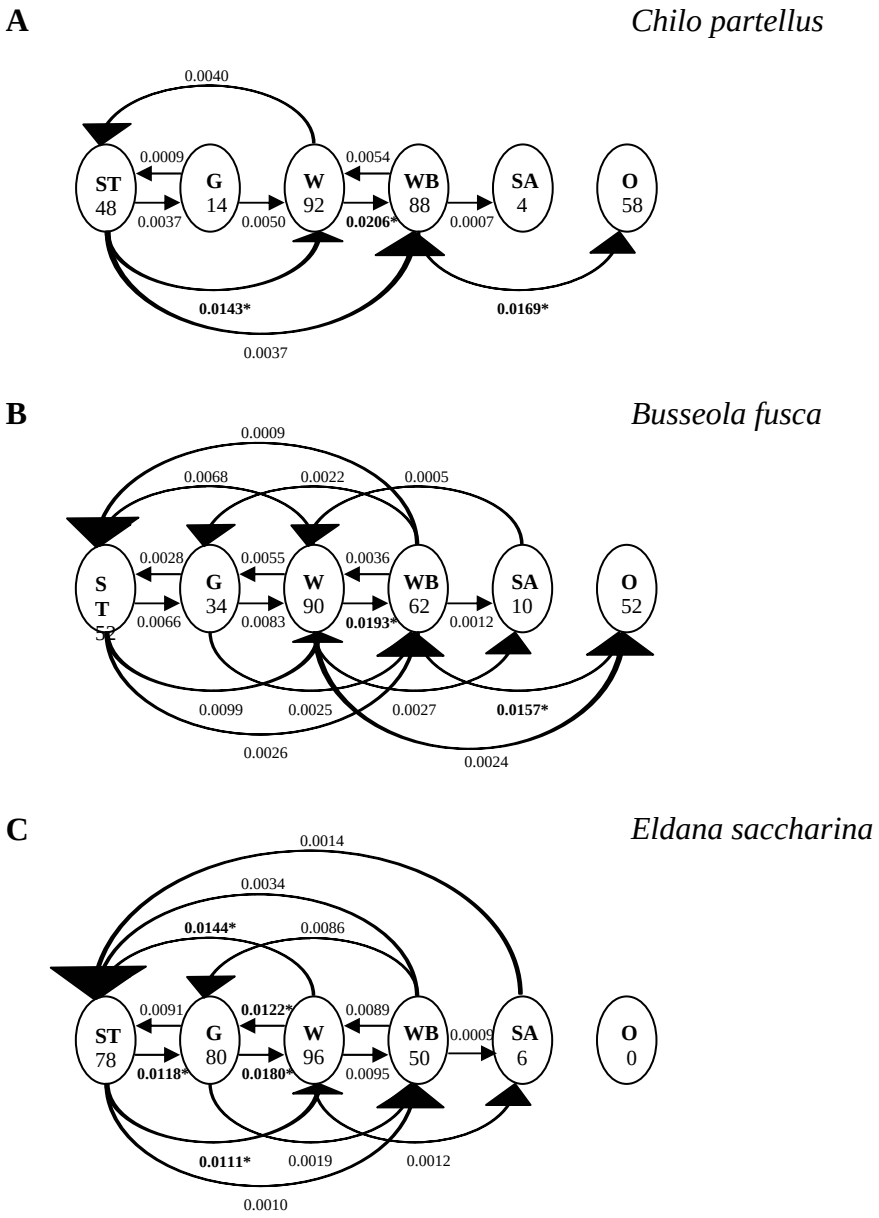


Figure 2.3. Behavioural steps exhibited by naive gravid female *Cotesia flavipes* encountering *Chilo partellus* larvae (A), *Busseola fusca* larvae (B) and *Eldana saccharina* larvae (C). Values with arrows represent the probability of each significant transition given the preceding behaviour. For each experiment, fifty female wasps were observed. * = probability of transitions > 0.0100. The percentage of occurrence of each behavioural step is provided below its category definition.

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Table 2.1 Total duration (seconds, mean ± SE, n=50) of each behavioural step exhibited by the females of *Cotesia sesamiae* and *Cotesia flavipes* on host or non-host larvae

Parasitoid species	Host tested	Behavioural steps					
		ST	G	W	WB	SA	O
C. sesamiae	*Busseola fusca*	16.5 ± 6.5a	10.7 ± 3.6a	73.0 ± 8.6a	32.1 ± 6.3a	0.5 ± 0.2a	6.5 ± 0.9
	Chilo partellus	64.5 ± 10.3b	38.4 ± 8.4b	95.5 ± 11.3a	17.8 ± 4.1a	0.2 ± 0.2a	0
	Eldana saccharina	24.4 ± 4.0a	14.6 ± 3.0a	156.3 ± 11.0b	71.7 ± 8.5b	2.4 ± 2.4a	0
	F,df,P	(12.04,2, 0.0001)	(7.26,2, 0.001)	(17.27,2, 0.0001)	(17.96,2, 0.0001)	(0.73,2, 0.5)	(48.77,2, .0001)
C. flavipes	*Chilo partellus*	17.2 ± 4.5a	2.6 ± 1.4a	63.6 ± 10.0a	30.0 ± 7.6b	0.1 ± 0.1a	4.9 ± 0.7a
	Busseola fusca	32.4 ± 6.8a	12.4 ± 3.4a	58.9 ± 8.5a	11.2 ± 2.4a	1.0 ± 0.6a	5.4 ± 0.8a
	Eldana saccharina	69.7 ± 8.2b	60.6 ± 8.4b	90.0 ± 8.4b	37.0 ± 6.7b	3.5 ± 2.0a	0
	F,df,P	(16.34,2, 0.0001)	(34.43,2, 0.0001)	(3.56,2, 0.0310)	(4.88,2, 0.0089)	(1.96,2,0.1441)	(21.43,2,0.0001)

For each parasitoid species, means within a column followed by different letters are significantly different at P=0.05 (Student-Newman-Keuls Test). ST: standing still; G: grooming the legs and/or the antennae; W: walking in the arena; WB: walking on the larval body; SA: stinging attempt; O: oviposition.

Chapter 3: Sensory Equipment on Antennae, Tarsi and Ovipositor of the Larval Braconid Wasps *Cotesia sesamiae* and *Cotesia flavipes*

3.1 Abstract

The external morphology and distribution pattern of sensilla present on antennae, tarsi and ovipositor of the braconid larval endoparasitoids *Cotesia sesamiae* and *Cotesia flavipes* was studied. Observations were conducted using scanning electron microscopy followed by selective staining with silver nitrate. The females of *C. sesamiae* and *C. flavipes* share the same type and distribution of sensilla enabling them to detect volatiles and contact chemical stimuli from their potential hosts. In both parasitoids, three sensillar types were identified on the last antennomeres: (i) non-porous sensilla trichoidea most probably involved in mechanoreception, (ii) uniporous sensilla chaetica which can play a gustatory function and, (iii) multiporous sensilla placodea which are likely to have an olfactory function. The tarsi possess a few sensilla chaetica which can play gustatory function and the manubrium is likely to be used during external examination (vibration detection) when the parasitoid is walking on potential host larvae before deciding to oviposit. The distal end of the ovipositor bears numerous multiporous dome-shaped sensilla. However, no sensilla coeloconica or styloconica, known to have a gustatory function, were observed.

3.2 Introduction

Lepidopteran stemborers are a major constraint to maize production in sub-Saharan Africa (Kfir *et al*., 2002). In eastern Africa, the braconid larval endoparasitoid, *Cotesia flavipes* Cameron (Hymenoptera: Braconidae) was introduced in a classical biocontrol program for the control of the invasive stemborer *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae). This was to complement the action of the closely related indigenous larval endoparasitoid *Cotesia sesamiae* (Cameron) (Hymenoptera: Braconidae) against this exotic pest (Overholt *et al*., 1994a, b; Overholt *et al*., 1997).

The efficiency of a natural enemy largely depends on its ability to locate, accept, and successfully parasitize hosts (Vinson, 1976; 1985; 1998; Godfray, 1994).

In chapter 2, each step of the behavioural sequence of host location and recognition behaviour of *C. sesamiae* and *C. flavipes* was described. It was shown that during host recognition and acceptance, these two congeneric parasitoids display an invariable hierarchy of behavioural steps. In addition, both parasitoids clearly depend on their antennae, particularly the distal antennomeres, for host recognition and both the antennae and the tarsi for definitive host acceptance for oviposition. In contrast, the use of the ovipositor in this process was not observed. Considering the similarity in the hierarchy of behavioural steps displayed by the two wasp species, it was necessary to conduct studies on their sensory equipment, particularly sensillar number, kind and distribution.

This study thus focuses on the external morphology of the sensory organs involved in each step of the behavioural sequence of host recognition and acceptance by *C. flavipes* and *C. sesamiae*. We studied the sensillar morphology of the last antennomere, ovipositor, fifth tarsomere and pretarsus of the prothoracic legs.

3.3 Materials and methods

3.3.1 Insects

Adults of *Cotesia sesamiae* and *C. flavipes* were obtained from laboratory-reared colonies established at ICIPE, Nairobi, Kenya. A *Cotesia sesamiae* colony was initiated with materials obtained from *B. fusca* collected from maize fields in Kitale, Western Kenya, in 2006, while *C. flavipes* was obtained from *C. partellus* from coastal Kenya in 2005. Twice a year, field collected parasitoids were added to rejuvenate the colonies. *Cotesia sesamiae* and *C. flavipes* were reared on larvae of their true hosts *B. fusca* and *C. partellus* respectively, according to the method described by Overholt *et al*. (1994a). The parasitoid cocoons were kept singly in glass vials (7.5 x 2.5 cm) until emergence and the males were immediately separated from the females before mating.

3.3.2 Organ length measurements

Five females of each parasitoid species were dissected and the lengths of their antenna, ovipositor, fifth tarsomere and pretarsus of the prothoracic legs were determined under a binocular Leica EZ4D system (magnification at 35x) including a standard software (Leica Application Suite, version 1.4.0).

3.3.3 Scanning electron microscopy

For each parasitoid species, ten live female adults were used for scanning electron microscopy. The female wasps were first placed in a 2.5% (vol/vol) glutaraldehyde, 0.1 M phosphate buffer (pH 7.4) solution and left overnight for fixation. The specimens were then dehydrated in a graded series of ethanol (70, 90 and 100%) and finally air-dried. The head, abdomen and forelegs of each wasp were separated and mounted on stubs with conductive double-side adhesive tape, sputter-coated with gold and examined with a JEOL JSM-T330A scanning electron microscope (JEOL, Tokyo, Japan) at 10 kV.

3.3.4 Silver nitrate staining

Silver nitrate staining was carried out to determine the presence of porous sensilla in the antenna, ovipositor and fifth tarsomere and pretarsus. Intact wasps were stained according to the method described by Nayak & Singh (1983) but with some modifications as follows: The wasps were first immersed in 70% ethanol containing 1 M silver nitrate for 1 hr then dehydrated in two concentrations of ethanol (90 and 100%). Afterwards, their heads, abdomens and forelegs were detached from the body and cleaned separately in xylene overnight. The specimens were mounted in Mountex (Histolab) for light microscope observations. A total of 10 females were examined for each parasitoid species.

3.4 Results

3.4.1 Antennae

The antennae of *C. sesamiae* and *C. flavipes* females were 1195.9 ± 25.5 and 1116.9 ± 35.2 µm (means ± SE) long respectively. Each parsitoid species had 16 antennomeres on the flagellum. In both species, scanning electron micrographs of the distal antennomeres revealed the existence of three different types of sensilla: trichoidea, chaetica and placodea (Figs. 3.1a and b). Among these, only sensilla chaetica and placodea appeared argyrophilic (i.e., stained in silver nitrate) (Fig. 3.2), showing their porous characteristic. In both parasitoid species numerous long, curved non-porous sensilla trichoidea (length ~ 24 µm) covered the antennomeres. At least two types of sensilla chaetica were observed on the distal antennomere, long and curved (about 5 and 9 in number, for *C. sesamiae* and *C. flavipes* respectively). Their length was about 20 µm long laterally and dorsally positioned at the apex of the antennomere (Figs. 3.1a and b). These were 4 (for *C. sesamiae*) or 3 (for *C. flavipes*) short, curved thick ones about 12 µm long on the ventral face of the distal antennomere and also visible on the ventral face of the adjacent antennomeres [Figs. 3.1e and f]) in both species. All these sensilla appeared uniporous on their tips (Figs. 3.1c and d). In both parasitoid species, the sensilla placodea occurred along the longitudinal axis of the antennomere.

3.4.2 Ovipositor

The lengths of the ovipositors were 369.6 ± 9.2 and 371.3 ± 16.9 µm (means ± SE) for *C. sesamiae* and *C. flavipes* respectively. On the paired valves of the ovipositors no sensilla were observed unlike on the unpaired [dorsal valve] which had numerous dome-shaped sensilla of different sizes concentrated on the tip (Figs. 3.3a and b). The dome-shaped sensilla were stained by silver nitrate solution (Figs. 3.4a and b). For both parasitoid species, the ovipositor is enclosed by valvulae whose tips are covered by long sensilla trichoidea (length ~ 30-45 µm) (Figs. 3.3c and d). These sensilla appeared non-porous since they did not stain in the silver nitrate solution.

3.4.3 Pretarsus and fifth tarsomere of the prothoracic legs

In *C. sesamiae* and *C. flavipes*, the total length of the fifth tarsomere and pretarsus was 158.6 ± 12.6 and 133.1 ± 7.1 µm (means ± SE) respectively. For both parasitoids, a single sensillum chaeticum (length ~ 20 µm) was observed on the medium dorsal side of each claw (Figs. 3.5a and b) and another one was located laterally on the fifth tarsomere even on the adjacent tarsomeres. All these sensilla were found to be argyrophilic (Figs. 3.5c and d).

3.5 Discussion

The distal antennomere, ovipositor, the fifth tarsomere and the pretarsus of the prothoracic legs of both *C. sesamiae* and *C. flavipes* females have the same type and distribution of sensilla. In chapter 2, the distal antennomeres of the two parasitoids were hypothesised to be the most important structures involved in host recognition before oviposition. The findings of the current study show that the distal antennomeres of the congeneric parasitoids possess uniporous sensilla chaetica. According to sensillar classification by Zakaruk (1980), these sensilla are mostly involved in gustation or taste reception. The fact that the involvement of the distal antennomeres in host recognition has been well demonstrated by ablation experiments among other parasitic Hymenoptera (Weseloh, 1972; Borden *et al*., 1973; Barlin *et al*., 1981; Bin, 1981); it may be hypothesised that for *C. sesamiae* and *C. flavipes* the distal antennomeres could serve in the perception of chemical cues on the cuticle of larvae during host examination.

Females of *C. sesamiae* and *C. flavipes* antennate (substrate drumming) the larval body before stinging. During this process the apical part of their antennae are curved to allow for maximum contact with the substrate (whether frass or host) [see chapter 2]. We believe that this behaviour is meant to expose the sensilla chaetica of the distal antennomeres to the chemical stimuli on the substrate. These findings corroborate those of Isidoro *et al*. (1996) in other parasitic Hymenoptera. Isidoro *et al*. (1996) further referred to the antennomeres bearing substrate-contacting sensilla as the “touch and taste areas”. This is because they are associated with the gustatory sensilla, which must “touch” active compounds in order to “taste” the proper chemical stimuli on the substrate during host recognition. In addition, such uniporous sensilla chaetica which bear socket-like insertions into the antennal cuticle are also mechanoreceptors (Zacharuk, 1980).

This dual gustatory and mechanoreceptory function of the sensilla chaetica has also been reported for *Trissolcus basalis* (Wollaston) (Hymenoptera: Platygastridae) (Isidoro *et al*., 1996) as well as *Microplitis croceipes* (Cresson) (Hymenoptera: Braconidae) (Ochieng' *et al*., 2000).

In both *C. sesamiae* and *C. flavipes*, the sensilla chaetica are surrounded by numerous sensilla trichoidea which did not stain in silver solution and are therefore non-porous.In contrast, Van Lenteren *et al*. (2007) reported numerous multiporous sensilla trichoidea on the antennae of *Leptopilina heterotoma* (Thompson) (Hymenoptera: Eucoilidae). According to the sensillar classification by Zakaruk (1980) these are assumed to be olfactoreceptors. In *C. sesamiae* and *C. flavipes*, these sensilla appear non-olfactory as has also been reported for other parasitic Hymenoptera (Norton & Vinson, 1974; Isidoro *et al*., 1996; Ochieng' *et al*., 2000). These sensilla trichoidea in *C. sesamiae* and *C. flavipes* are likely to be involved in mechanoreception.

Although electron microscopy did not reveal the nature of the sensilla placodea, it is possible to deduce on the basis of their argyrophilic characteristics that they are porous. Among other parasitoids, the sensilla placodea have been described as multiporous and function as olfactoreceptors (Barlin & Vinson, 1981a,b; Steinbrecht, 1984; Ochieng' *et al*., 2000). This could further explain their abundance on the antennae in the Braconidae family as well as other parasitoid families (Barlin & Vinson, 1981b). Thus, it is suggested that for *C. sesamiae* and *C. flavipes* the olfactory receptors of sensilla placodea may play an important role in remote host location whereas the gustatory receptors (sensilla chaetica) on the distal antennomere detect the non-volatiles cues on the host cuticle upon contact.

When walking on the host body the female wasps moved their tarsi, which could take up cues from the larvae [see chapter 2]. This is corroborated by the fact that both parasitoids had uniporous sensilla chaetica on the fifth tarsomere and pretarsus. Similarly, these sensilla chaetica are believed to have a gustatory or taste function (Zakaruk, 1980). The tarsi of both parasitoids are also very likely to be linked with the reception of cues *via* mechanoreceptors in reception of vibrational signals upon contact with the larvae [see chapter 2]. This is because, the female wasps very often moved their tarsi while in contact with the host body.

There exists a positive relationship between movement of host larvae and parasitoid acceptance i.e. mobile larvae were attacked faster than sessile ones especially when the parasitoid had already mounted a larva [see chapter 2]. Therefore, the tarsi contribute to mechanical sensation.

The mechanoreceptory sensilla on the arolium may be useful in perception of vibrational signals as has been reported for *Symplesis sericeicornis* Nees (Hymenoptera: Eulopidae) (Meyhofer *et al*., 1997).Earlier studies explained the basis of acceptance or rejection of hosts among female parasitoids as host internal markers perceived by the sensilla on the ovipositor (Godfray, 1994; Le Ralec *et al*., 1996). In general, among parasitic Hymenoptera chemoreceptors are concentrated around the ovipositor tips, are uniporous and are believed to be gustatory organs. These chemoreceptors have been referred to as sensilla coeloconica or styloconica (Quicke *et al*., 1999). Recently, Van Lenteren *et al*. (2007) demonstrated the first record of action potentials from a sensillum coeloconicum at the tip of the ovipositor of *Leptopilina heterotoma* (Thompson) (Hymenoptera: Eucoilidae).

This in itself confirmed the taste function of the ovipositor among parasitic Hymenoptera. However, in the ovipositor of *C. sesamiae* and *C. flavipes* no sensilla coeloconica or styloconica were observed except for the numerous porous dome-shaped sensilla at the tips of the dorsal valves. The ovipositors of various parasitoid species have numerous dome shaped sensilla concentrated around their tips (Quicke *et al*., 1999). The pores on these sensilla are hypothesised to allow for host haemolymph uptake by capillary action (Larocca *et al*., 2007). It is hypothesised here that these sensilla could be osmoreceptors.

In conclusion, the females of *C. sesamiae* and *C. flavipes* possess the same type and distribution of sensilla which enable them to detect volatiles and contact chemical stimuli from their potential hosts. Their olfactory receptors are restricted on the antennae while taste receptors are present both on the antennae and tarsi. However, the findings of these studies do not explain the ability to discriminate between cues. It is possible that the interpretation of information occurs internally, because sensillae are usually inervated by neurons.

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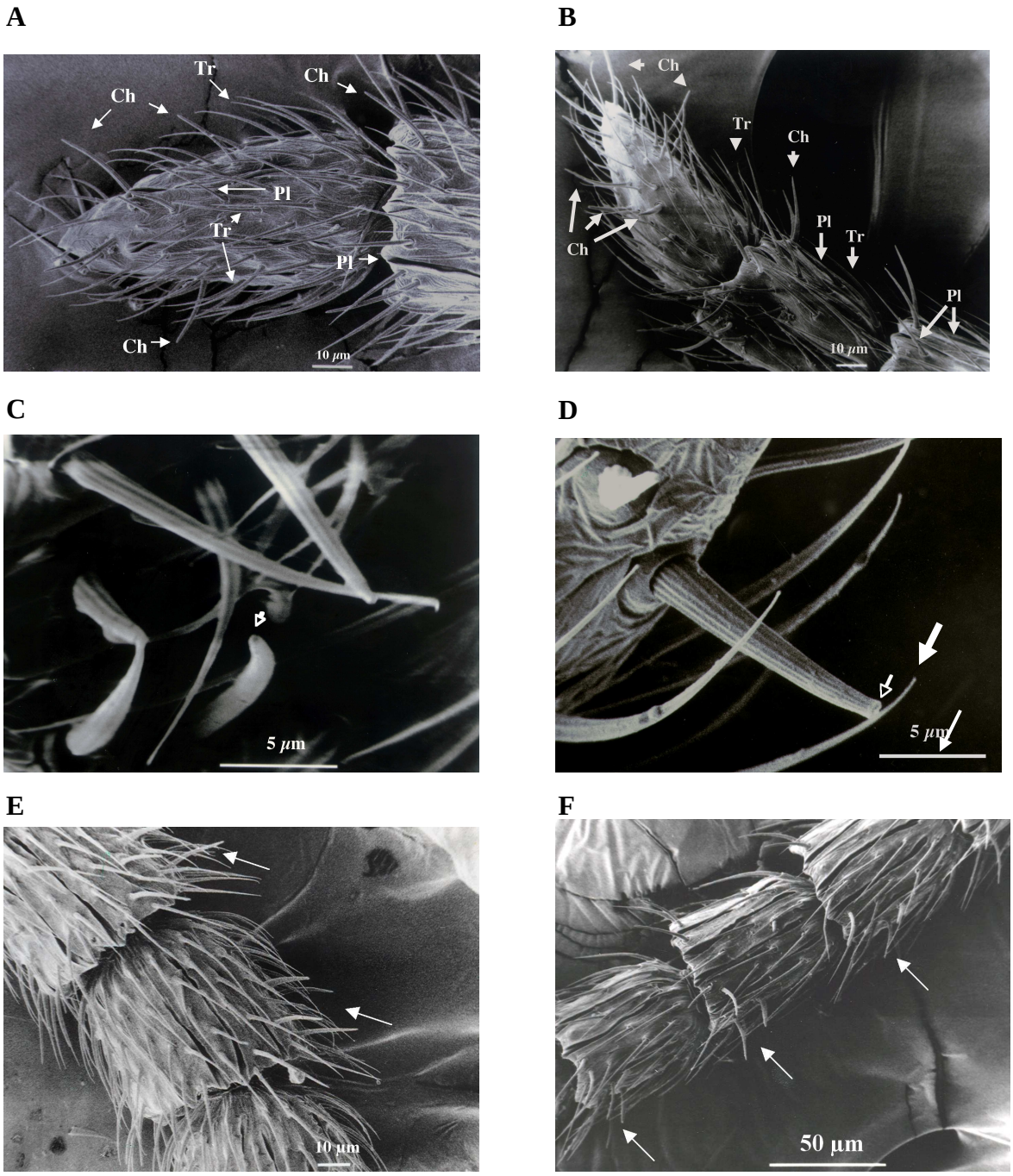

Figure 3.1. Distal antennomeres of adult females of *Cotesia sesamiae* (A) and *Cotesia flavipes* (B) observed by scanning electron microscopy, showing the uniporous sensilla chaetica (Ch), the sensilla trichoidea (Tr) and the sensilla placodea (Pl) along the longitudinal axis. Close-up of a long (C, the apex of the sensillum) and a short (D) sensilla chaetica located on the last antennomere of *C. sesamiae* showing a single pore on their tip (white arrowhead). Portion of the antennae (ventral view of intermediate antennomeres) showing the 4 or 3 small and curved sensilla chaetica for *C. sesamiae* (E) and *C. flavipes* (F), respectively (see arrows).

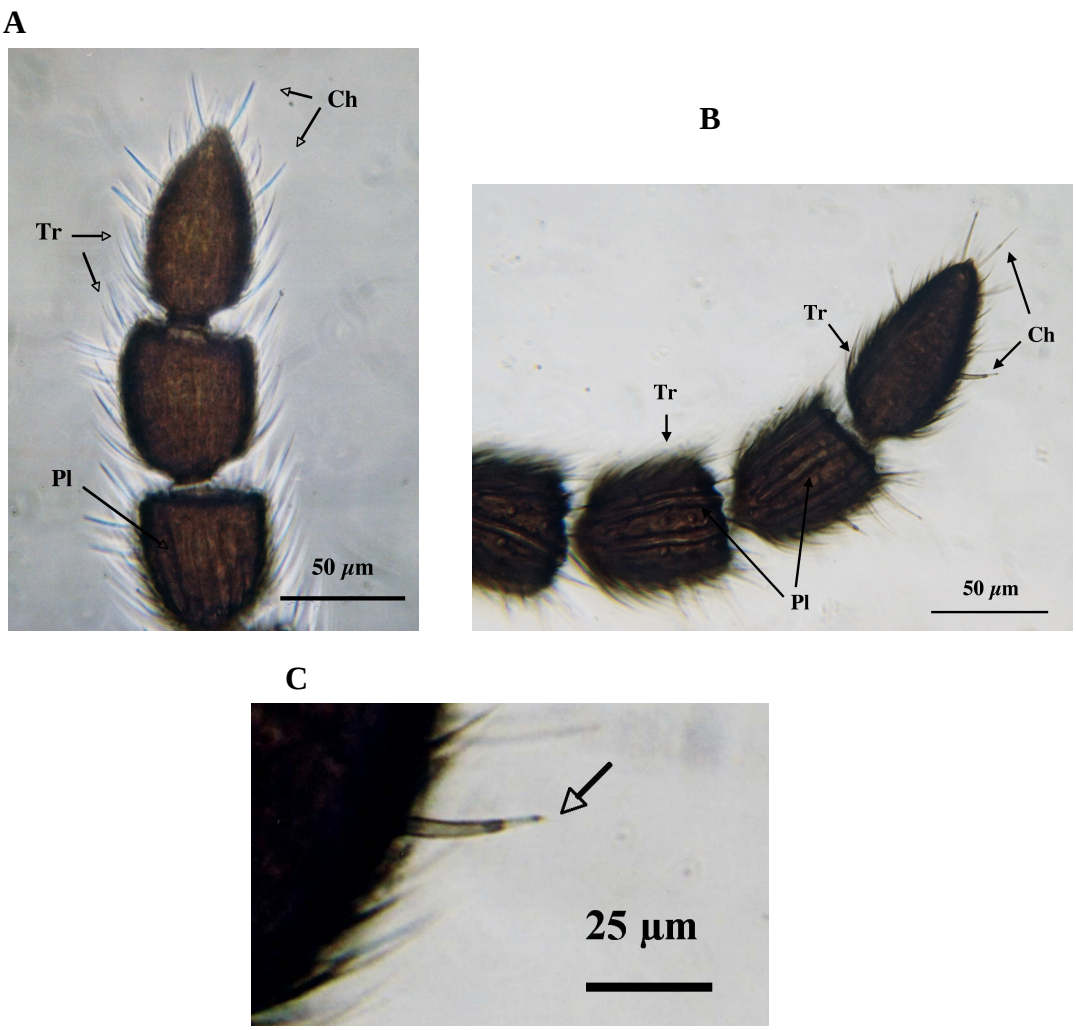

Figure 3.2. Distal antennomeres of adult females of *Cotesia sesamiae* (A) and *Cotesia flavipes* (B) observed by optic microscopy after silver staining procedure, showing the uniporous sensilla chaetica (Ch) and the sensilla placodea (Pl) along the longitudinal axis silver stained. Close-up of a long and curved sensillum chaeticum (C) located on the last antennomere of *C. flavipes* showing the silver nitrate which penetrated from the single pore on its tip (see arrow).

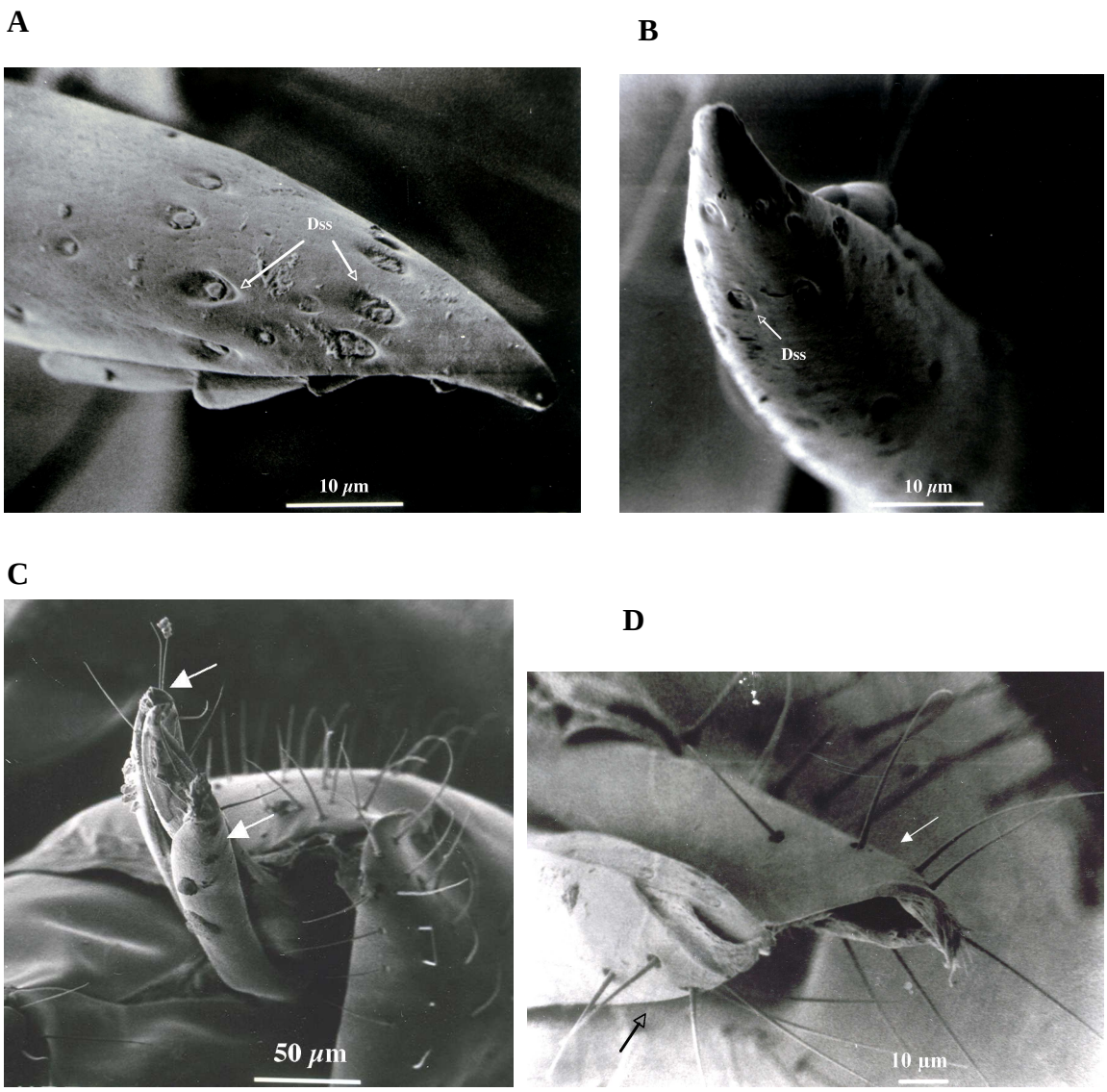

Figure 3.3. Distal portion of the ovipositor of *Cotesia sesamiae* (A) and *Cotesia flavipes* (B) observed in a dorsal view by scanning electron microscopy, showing the dome-shaped sensilla (Dss) near the apical part of the ovipositor. View of the ovipositor enclosed by valvulae (see arrows) covered by long non-porous sensilla trichoidea of *C. sesamiae* (C) and *C. flavipes* (D).

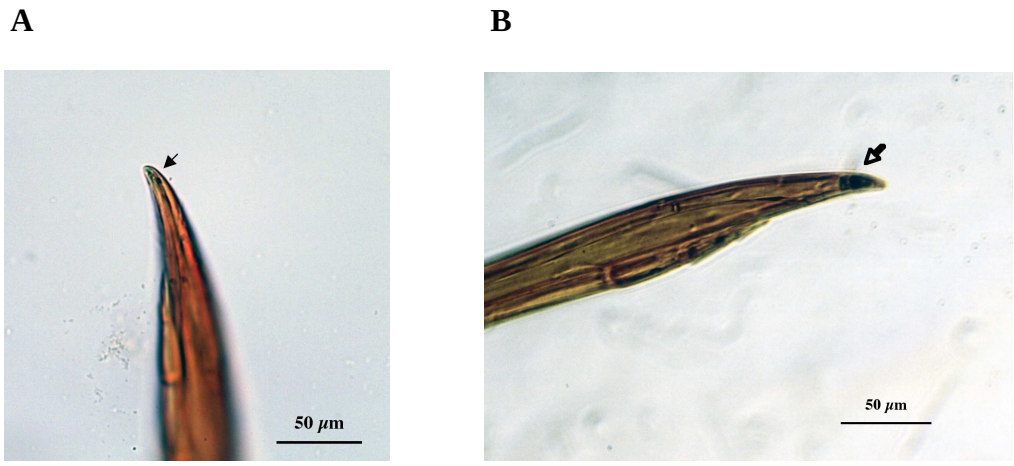

Figure 3.4. Unpaired (dorsal valve) of *Cotesia sesamiae* (A) and *Cotesia flavipes* (B) after silver staining. For each parasitoid species, the tip appeared silver stained (see arrow) due to numerous dome-shaped sensilla concentrated on it.

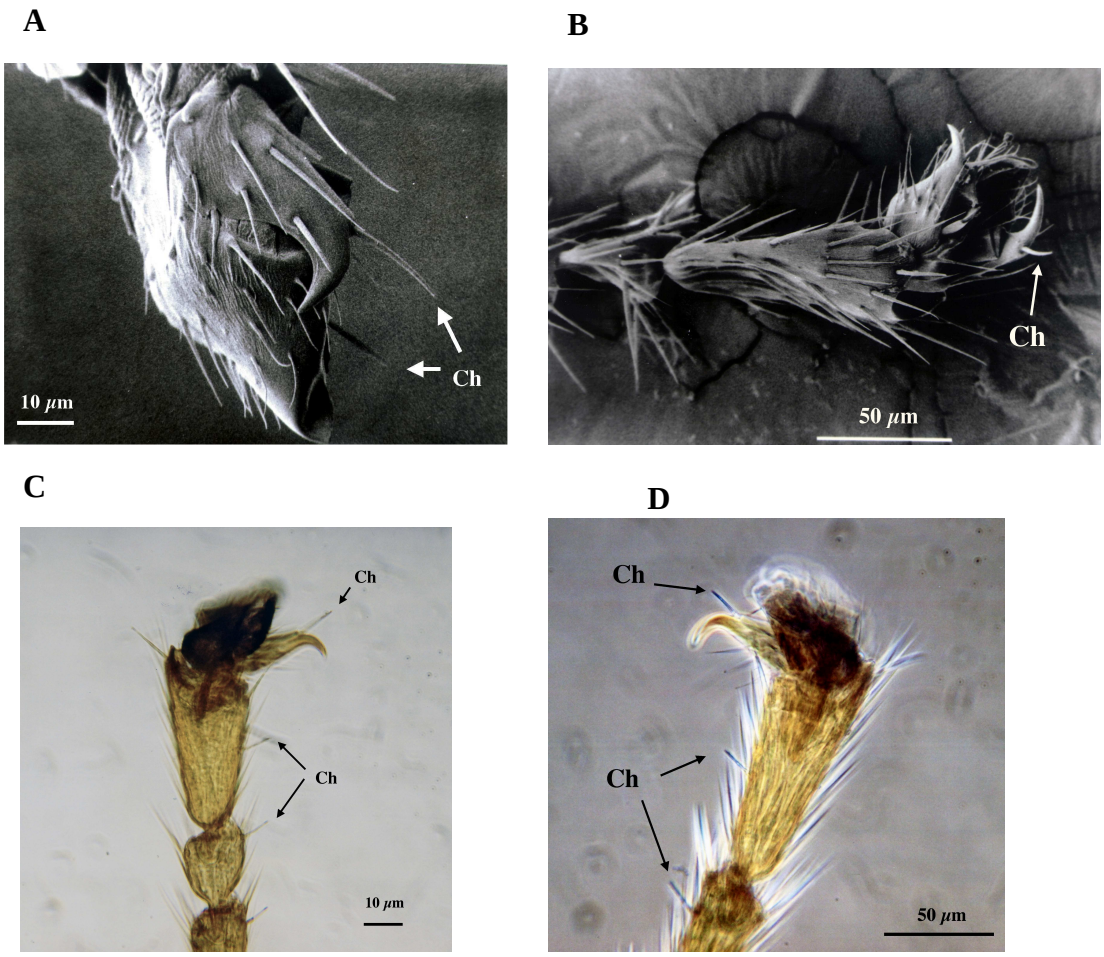

Figure 3.5. Distal portion of the pretarsus of *Cotesia sesamiae* (A, in lateral view) and the pretarsus and last tarsomere of *Cotesia flavipes* (B, in dorsal view) observed by scanning electron microscopy, showing the uniporous sensilla chaetica (Ch). Last tarsomere and pretarsus of *C. sesamiae* (C) and *C. flavipes* (D) showing the silver stained sensilla chaetica (Ch) observed by optic microscopy.

Chapter 4: Importance of Contact Chemical Cues in Host Recognition and Acceptance by the Braconid Larval Endoparasitoids *Cotesia sesamiae* and *Cotesia flavipes*

4.1 Abstract

The ability of the congeneric parasitoids *Cotesia sesamiae* and *Cotesia flavipes* to discriminate between stemborer larval cues upon contact was studied using suitable hosts (i.e. *Busseola fusca* for *C. sesamiae*, and *Chilo partellus* for *C. flavipes*) and a non-host (*Eldana saccharina*). When the suitable host larvae were washed in distilled water, the behaviour of the parasitoid was similar to that displayed when in contact with the non-host larvae, characterised by the absence of ovipositor insertion. When the stemborer larvae (washed suitable host or non-host larvae) were painted with water extracts of their respective suitable host-larvae, the parasitoids showed a significant increase in ovipositor insertions. However, the water extracts of suitable host-larvae deposited on cotton wool balls did not induce ovipositor insertion for both *C. sesamiae* and *C. flavipes*. Instead, these extracts enabeled them to discriminate between suitable host and non-hosts, intensely antennating those of their suitable hosts more than those of the non-hosts. Similarly, the role of larval frass and regurgitant in host discrimination and acceptance was assessed.The frass was important to both parasitoid species in host recognition during short-range examination, intensely antennating frass of the suitable hosts more than non-host frass. The regurgitant of both *B. fusca* and *C. partellus* appeared not useful in discrimination between the two species for both parasitoid species. However, both parasitoids could discriminate the regurgitant of the non-host, *E. saccharina* over the other stemborer species showing no antennation on cotton wool balls containing its regurgitant. Moreover, the regurgitant of *B. fusca* and *C. partellus* induced ovipositor insertion in *C. flavipes* while only antennation was observed among those of *C. sesamiae*.

4.2 Introduction

In sub-Saharan Africa, lepidopteran stemborers of the Crambidae, Pyralidae and Noctuidae families are economically the most important pests of maize and sorghum (Harris, 1990; Polaszek, 1998; Kfir *et al*., 2002). Due to their widespread distribution and destructive nature, stemborers have been the subject of extensive research in Africa (Calatayud *et al*., 2006). The most cited species are the crambid *Chilo partellus* (Swinhoe), the noctuids *Busseola fusca* (Fuller) as well as *Sesamia calamistis* Hampson, and the pyralid *Eldana saccharina* (Walker) (Polaszek, 1998). With exception of *C. partellus*, which was accidentally introduced from Asia into Southern Africa before the 1930s (Tams, 1932), the others are indigenous to the African continent. In East and Southern Africa, *B. fusca* and *C. partellus* are the most important pests of cereal crops (Seshu Reddy, 1983; Zhou *et al*., 2001a).

During the early 1990s, the International Centre of Insect Physiology and Ecology (ICIPE) renewed emphasis on biological control activities with the introduction of *Cotesia flavipes* Cameron (Hymenoptera: Braconidae) into Kenya from Asia in 1991. The exotic parasitoid was released against *C. partellus* in the coastal area in 1993 (Overholt *et al*., 1994b), where it reduced *C. partellus* densities by over 50% (Zhou *et al*., 2001b; Jiang *et al*., 2006). This was to complement the action of the closely related and most abundant indigenous larval parasitoid in East and Southern Africa, *Cotesia sesamiae* (Cameron) (Hymenoptera: Braconidae). Parasitism by the indigenous parasitoid is usually below 5% though in some localities it can attain 75% (Kfir, 1995; Sallam *et al*., 1999; Jiang *et al*., 2006; Songa *et al*., 2007).

The ability of parasitic wasps to successfully utilize cues arising from within their habitat in location and discrimination between suitable and unsuitable hosts is vital in determining their efficiency (Vinson, 1985; Godfray, 1994). This usually involves host habitat location, host location, host acceptance and suitability (Vinson, 1976; 1985; Godfray, 1994). During location of hosts, they typically exploit long and short range stimuli emanating from the host habitat (Vinson, 1975; Godfray, 1994), followed by stimuli directly associated with the host and its products (Vinson, 1985; Vet & Dicke 1992; Godfray, 1994).

However, stimuli from the habitat do not convey sufficiently reliable information on the suitability of host species but are mere indicators of herbivore presence (Ngi-Song & Overholt, 1997). As a result, *C. sesamiae* and *C. flavipes* are often attracted to plants harbouring unsuitable stemborer species (Potting *et al*., 1993; Ngi-Song *et al*., 1996; Obonyo *et al*., 2008). We hypothesise that the females of *C. sesamiae* and *C. flavipes* though incapable of discriminating between host species from a distance do so at close contact.

In the laboratory, host acceptance and oviposition by the congeneric wasps is induced by contact with the host frass and/or host products (Ngi-Song & Overholt, 1997). It appears that this response is dependent on certain specific chemicals because it can either be disrupted when the host frass is withdrawn or swopped with that of a non-host (Gitau Catherine. ICIPE, personal communication), or lost entirely when the host larva is washed in distilled water (Ngi-Song & Overholt, 1997). This indicates that chemicals from the larval body are involved in host acceptance and oviposition by both *C. sesamiae* and *C. flavipes*.

In chapter 3, uniporous sensilla chaetica which play gustatory/taste function were shown to be located on the distal antennomeres and the tarsi of both *C. sesamiae* and *C. flavipes*. In addition, it was observed that both organs are used by the parasitoids during external examination while the parasitoid walks on the potential host larvae before deciding to oviposit. The purpose of this study was to show the influence of contact chemical cues from stemborer larvae allowing the parasitoids to recognise and accept the larvae.

4.3 Materials and methods

4.3.1 Insects

The females of *Cotesia sesamiae* and *C. flavipes* were obtained from laboratory-reared colonies established at ICIPE, Nairobi, Kenya. A *Cotesia sesamiae* colony was initiated with materials obtained from *B. fusca* collected from maize fields in Kitale, Western Kenya, in 2006, while *C. flavipes* was obtained from *C. partellus* in coastal Kenya in 2005. Twice a year, field collected parasitoids were added to rejuvenate the colonies. *Cotesia sesamiae* and *C. flavipes* were reared on larvae of their true hosts, *B. fusca* and *C. partellus*, respectively, according to the method described by Overholt *et al*. (1994a). Parasitoid cocoons were kept in Perspex cages (30 cm x 30 cm x 30 cm) until emergence.

Adults were fed on a 20% honey-water solution imbibed in a cotton wool pad and kept under artificial light for 24 hrs to mate. In all experiments, only 1-day-old, naïve, putatively mated females were used. The experiments were carried out at 25 ± 2^{o}C, 50-80% RH, and a 12:12 hr (L:D) photoperiod.

Three stemborer species were used in the study: *B. fusca* and *C. partellus* as host to *C. sesamiae* and *C. flavipes,* respectively, and *E. saccharina* as a non-host to both parasitoid species. Preliminary tests conducted prior to this study showed that neither parasitoid attacked *E. saccharina* , thereby allowing the use of this stemborer as a non host in the current experiments (Obonyo M. ICIPE, personal observation). *Eldana saccharina* and *B. fusca* were collected from maize fields in Western Province, while *C. partellus* originated from maize grown in the coastal region of Kenya. The larvae were reared on artificial diet according to the methods described by Ochieng *et al*. (1985) (for *C. partellus*) and Onyango & Ochieng'-Odero (1994) (for *B. fusca* and *E. saccharina*). Thrice a year feral stemborer larvae from their respective locations were added to rejuvenate the colonies. From the colonies, third and fourth stemborer larval instars of *B. fusca*, *E. saccharina* and *C. partellus* were introduced into jars (10 cm x 20 cm) each containing pieces of maize stem and left for 24 hrs to feed and produce frass. Thereafter, the larvae and the frass were used in the experiments.

4.3.2 Experimental procedure

4.3.2.1 Washing and painting of larvae

In order to verify the influence of chemical cues on the cuticle of the larvae, a total of 200 larvae per stemborer species were washed separately in 200 ml of distilled water. The 200 ml of body extract (stock solution) was filtered (Whatman # 1) to remove any debris and used in the experiments. In order to conserve their activity, the extracts were collected in a cold room (around 10°C) and the stock solutions were kept frozen before being used.

Prior to painting, third and fourth instar larvae from maize stems were individually introduced into glass vials (7.5 cm x 2.5 cm) half filled with distilled water then gently swirled and the water poured out. This procedure was repeated five times for thorough removal of cues. Afterwards, the larvae were dried on paper towel and allowed to crawl to enhance the drying.

The larvae were then directly introduced into a glass Petri dish arena (5.5 cm diameter x 1.5 cm height) for use in the bioassays (washed *B. fusca*, washed *C. partellus*, painted larvae) as presetend in Table 4.1. The painting of larvae was done as follows: an aliquot of the stock solution containing the body extract was used to bathe the larva. A single larva was dipped in the extracts five times for about two seconds each time. Application of the extract was further enhanced by using a camel hair brush to paint the larva. The brush was dipped into the extract and painted on the larva. The larva was then placed on paper towel to drain-off excess water and transferred into the arena. The behaviour of the wasp encountering the larva was monitored immediately after its release into the arena for a maximum of 60 sec (in this case, it had been observed that after 60 sec, the activity of the extracts on larvae greatly diminished). Twenty replicates consisting of a single wasp and larva were used in the experiments. The wasps and larva were used only once. The percentage of wasps probing the larvae with their ovipositor was calculated.

4.3.2.2 Influence of larval body extracts

An extract of whole larval body similar to the one explained above was obtained from 100 larvae freshly recovered from maize stems and then filtered (Whatman # 1). The extracts were collected in a cold room (around 10°C) and freeze dried. Thereafter, they were resuspended in distilled water (1.5 ml) and aliquoted into several portions (working solutions). A 20 µl portion of the extract was deposited on a cotton wool ball (3 mm across) and presented to a single wasp in the arena (5.5 cm diameter x 1.5 cm height). The behavior of the wasp was then monitored for a maximum of 120 sec (the activity of the extracts on the cotton wool balls was residual for about 120 sec). For each wasp, the duration of antennation (behavior involved in host recognition [see chapter 2]) was recorded. The percentage of stinging was calculated from twenty replicates of a single wasp and extract sample. Both the wasp and the cotton wool ball with extracts were replaced each time.

4.3.2.3 Influence of fresh frass

About 0.01 g of frass-produced after 48hrs of larval feeding- was collected from each stemborer species and put separately into Petri dishes (7 cm diameter x 1 cm height) then a wasp was introduced and covered for the bioassay. Each wasp was given a maximum of 120 sec in the arena to respond to the frass. Only the time the wasp was in contact with the frass was considered in the analysis. For each wasp, the duration of antennation on the frass was recorded after which both the wasp and the frass were replaced for each replicate The mean duration of antennation was calculated from twenty replicates each consiting of a wasp and frass.

4.3.2.4 Influence of frass extract

About 1.0 g of fresh frass, produced after 48hrs of larval feeding, was collected from each stemborer species and suspended in 10 ml of distilled water, vortexed and filtered (Whatman # 1). The extracts were collected in a cold room (around 10°C) and freeze dried. Thereafter each extract was resuspended in 1 ml of distilled water to form a stock solution. The stock solution was aliquoted into portions of working solution (100 µl) in 0.2 ml tubes while the rest was kept frozen until use. During the experiments, 20 µl of the frass extract of a particular stemborer species was deposited on a ball of cotton wool (3 mm) in the glass petri dish (5.5 cm diameter x 1.5 cm height) followed by a wasp and covered. Recording of time begun soon after the wasp was in the arena for a maximum of 120 sec afterwhich both the wasp and odour source were replaced. For each wasp, the duration of antennation on the cotton wool ball was recorded. The mean duration of response was calculated from twenty replicates each consisting of wasp and odour source. Both the wasp and the cotton wool ball with extracts were replaced each time.

4.3.2.5 Influence of regurgitant

For each stemborer species, third and fourth instar larvae were recovered from the maize stems after 48 hours of feeding (sufficient time to clear remains of artificial diet from their gut). A single larva held by a soft forceps was gently squeezed (behind the head) and a capillary tube used to collect oral extract produced. The process was repeated for several larvae and the total mass of the extract recorded each time.

The collection of regurgitant was done in a cold room (around 10°C). The extract was then diluted into 200 µl distilled water in a 1 ml tube. and then freeze dried. It was later resuspended into distilled water to obtain a solution of about 20 µl of regurgitant per 100 µl for each stemborer species. This 20 µl aliquot was deposited on a ball of cotton wool (3mm) each time and exposed to the parasitoid.Due to the rapid loss of regurgitant activity, the wasps were given 60 sec to respond to the regurgitant. For each wasp, the duration of antennation was recorded. From twenty replicate trials of each consisting of a wasp and extract, the percentage of wasps probing the cotton wool balls containing the regurgitant was calculated. Both the wasp and cotton wool ball were replaced for each replicate.

4.3.3 Data Analysis

All means were separated by Student-Newman-Keuls Test following one way analysis of variance (ANOVA). The data presented in the tables are the untransformed results except for the fresh frass and frass extract which were log (x+1) transformed then subjected to ANOVA.These statistical tests were done using the SAS system software (USA).

4.4 Results

4.4.1 Washing and painting of larvae

When *B. fusca* larvae were washed, no stinging or ovipositor insertion was observed among the tested *C. sesamiae* females (Table 4.1). However, when washed *B. fusca* was painted by *B. fusca* body extracts a 65% of the females stung the larvae.There was a marked reduction in the incidence of stinging by *C. sesamiae* (from 65 to 25%) when *B. fusca* was painted by the body extracts of the non-host *E. saccharina.* In addition, stings recorded for *E. saccharina* painted by *B. fusca* body extracts did not differ significantly from those of washed *B. fusca* painted by *B. fusca* body extracts($F= 9.33$; $df=3$; $P= 0.0001$).

Similarly, when *C. partellus* larvae were washed no stinging or ovipositor insertion was recorded among *C. flavipes* (Table 4.1). The percentage stinging decreased significantly from (75 to 30%) when washed *C. partellus* larvae were painted with *E. saccharina* body extracts.

No significant difference in percentage stinging was observed between *E. saccharina* larvae painted by *C. partellus* body extracts and washed *C. partellus* larvae painted by *C. partellus* body extracts (F= 14.29; df=3; P= 0.0001).

4.4.2 Influence of larval body extracts

For *C. sesamiae*, there was no significant difference in duration of antennating the body extracts of either *C. partellus* or *E. saccharina*. By contrast, the wasps spent significantly more time antennating the body extracts of their suitable host *B. fusca* (F=24.08; df=3; P=0.0001) (Table 4.2). Similarly, *C. flavipes* spent least time on *E. saccharina* extracts. By contrast, the females spent significantly more time antennating *B. fusca* and *C. partellus* body extracts (F=10.87; df= 3; P=0.0001). Comparison of the two parsitoid species reveals that there was no significant difference in the response to the body extracts of *B. fusca* (F=1.68;df=1;P=0.2030), *C. partellus* (F=2.36;df=1; P=0.1328) and *E. saccharina*(F=2.54;df=1;P=0.1195).

4.4.3 Influence of fresh frass, frass extracts and regurgitant

When *C. sesamiae* females were separately exposed to the fresh frass of *E. saccharina, C. partellus* or *B. fusca* they spent significantly more time antennating the fresh frass of *B. fusca* (the suitable host) as compared to the other two species (F=22.26;df=2; P=0.0001) (Table 4.3). On the other hand, *C. flavipes* females spent more time on the fresh frass of their suitable host *C. partellus* followed by *B. fusca*. However, in both parasitoid species, the duration of antennation on fresh frass was not significantly different between the *B. fusca* and *C. partellus*; whereas it was significantly low on the frass of the non-host *E. saccharina* (F=4.26;df=2; P=0.0188) (Table 4.3).

The duration of antennation on the frass extract of *B. fusca, E. saccharina* and *C. partellus* by *C. sesamiae* females was in reducing order with the lowest being C. partellus (F=3.54;df=2; P=0.0354). As for *C. flavipes* females, the duration of antennation was highest in *B. fusca* followed by *C. partellus* and least *E. saccharina* although the difference in response was not significantly different (F=1.12; df=2; P=0.3330).

The regurgitant of *E. saccharina* elicited the no response from both parasitoid species when compared to that of *B. fusca,* and *C. partellus* which were similar (F=8.05;df=3; P=0.0001) (Table 4.3).

Cotesia flavipes females antennated the cotton wool balls of *C. partellus* longer than those of *B. fusca* althought the difference was not significant (F=6.82,df= 3,P= 0.0004) (Table 4.3). In addition, the females of *C. sesamiae* did not probe the cotton wool balls imbibed in the regurgitant of *B. fusca, E. saccharina* and *C. partellus.* For *C. flavipes,* only 40% of the wasps probed cotton wool balls containing the regurgitant of *C. partellus* or *B. fusca* (F=8.44,df=3, P=0.0001).

4.5 Discussion

The present findings support the hypothesis that the females of *C. sesamiae* and *C. flavipes*, though incapable of discriminating between suitable and unsuitable host species from a distance, can do so at close contact. This is because the wasps recognised the body extracts of their preferred suitable hosts, which they probed with their ovipositors. Furthermore, both species oviposited in the non-host *E. saccharina* when it was painted with extracts of host larvae [see chapter 2]. On the other hand, a marked reduction in oviposition on their true hosts occurred when they were painted with extracts of the non-host larvae. This further underscores the importance of host recognition cues.

Potting *et al.* (1995) reasoned that due to the short lifespan of *C. flavipes,* the parasitoid is expected to encounter only a few hosts in its lifetime; thus, *C. flavipes* was expected to have low host specificity. This could possibly explain the inability of the exotic wasp to distinguish between the body extracts of *B. fusca* and *C. partellus*. However, this may not apply for the indigenous wasp *C. sesamiae* which was able to discriminate between the extracts of the two species. As opposed to *C. flavipes, C. sesamiae* has been reported to be a highly host selective species avoiding the exotic pest *C. partellus* (Ngi-Song & Overholt, 1997). This behaviour corroborates earlier observation that *C. sesamiae* does not sting *C. partellus* [chapter 2]. The above observations can also be explained by looking at the evolutionary history of the two parasitoids: (i) *C. sesamiae* co-evolved with *B. fusca* (from which it was recovered) and is likely to be adapted to recognising it over *C. partellus* which is exotic; (ii) the exotic wasp *C. flavipes* co-evolved with *C. partellus* (originated from the same geographic area [Asia] and from which it was recovered), which may explain its preference for the extracts over that of the indigenous borer *B. fusca* (Overholt *et al*., 1994a, b; Overholt *et al*., 1998). Consequently, the orientation of these parasitoids to their hosts may be linked to their evolutionary history.

In earlier olfactometeric studies, it was thought that these two wasp species were more habitat than host specific because they could not discriminate between same host plants infested by different stemborer species (Ngi-Song & Overholt, 1997). We suggest that at close contact, the reverse is true (van Leerdam *et al*., 1985), because the wasps spent more time searching cotton wool imbibed with body extracts of their respective suitable hosts than those of the non-host. In the current experiments, the wasps did not sting the cotton wool balls imbibed with extracts of their hosts. This is believed to be due to the absence of tactile stimulation (mimicking movement of larvae), which is necessary for final acceptance and oviposition [see chapter 3]; in fact, such extract used on a mobile larva induced oviposition (Table 4.1).
The water extracts of larval bodies can be expected to contain compounds mainly arising from the feeding activity of the larva (i.e. frass and regurgitant). The present study confirms that the compounds present in the frass and the regurgitant influenced the behaviour of *C. sesamiae* and *C. flavipes* by inducing antennation and/or oviposition.

Specialisation of parasitic wasps towards their hosts may explain why the fresh frass of *B. fusca* was most attractive to *C. sesamiae* and likewise that of *C. partellus* to *C. flavipes.* This can be advantageous to foraging parasitoids because once they have contacted the frass they may be able to distinguish between stem tunnels containing suitable and unsuitable larvae. Whereas in Kenya *B. fusca* prevail the high altitude areas, *C. partellus* is predominant in the low-lands and mid-altitudes (Seshu Reddy, 1983). Multi-species infestations by *B. fusca*, *C. partellus* and *E. saccharina* are common in the mid-altitudes although the latter species is scarce (Ong'amo *et al*., 2006). Ngi-Song *et al*. (2001) showed that *B. fusca* was not suitable to *C. flavipes* and successful parasitism was possible only in cases of multi-parasitism. In which case *C. sesamiae* had oviposited first and lowered the immune response of the host thereby preventing encapsulation of *C. flavipes* eggs (Ngi-Song *et al*., 1995). As a result, *B. fusca* is often found parasitized by *C. flavipes* in the field (Matama-Kauma *et al*., 2007). Thus, the attraction of *C. flavipes* to *B. fusca* and its association to this host is likely to have an effect on biological control programs in localities where both parasitoids exist in sympatry and *B. fusca* is a dominant host.

The ability to discriminate hosts is crucial for the establishment of the exotic *C. flavipes*, which encounters many new suitable and unsuitable hosts in its new environment in Africa (Le Rü *et al*., 2006 a,b).

Considering that as many as 50% of foraging wasps are killed in stem tunnels due to the aggressive behavior of hosts by biting or spitting (Takasu & Overholt, 1997), the foraging wasps may be under selection pressure to recognise their host with minimal risk of injury. When a parasitoid has a high mortality risk at each oviposition, life history theory predicts a high selectivity to avoid waste of progeny (Ward, 1992).

During stem tunnelling, the frass is usually pushed outside as larvae feed inside the stem. It was observed that host seeking behaviour in *C. flavipes* is mediated by water soluble chemical substance present in the frass, which when extracted, elicits a characteristic host searching response (van Leerdam *et al.,* 1985). The behaviour of *C. sesamaie* and *C. flavipes* antennating cotton wool balls containing water extracts of their respective hosts in the current study corroborate the above. Despite the females retaining preference for their host's extracts, their response was not significantly different among the stemborer species. This is likely to be caused by low activity of the water extract due to insolubility (in water) of some components of frass such as fatty acids and lipids (Kuwahara *et al.,* 1983; Takabayashi & Takahashi, 1989; Roux *et al.,* 2007). Furthermore, the parasitoids could have adjusted their foraging behaviour by reducing their response to changes in the quality of the extract. Potting *et al*. (1995) observed that these parasitoids respond to host abundance and quality in the habitat, and are often less attracted to frass that is dry or even forage less in a dry habitat. As such, frass is believed to be a directive mediator in the host finding process by *C. sesamiae* and *C. flavipes* (Ngi-Song & Overholt, 1997).

Spitting among lepidopteran stemborer larvae is often a defence mechanism in response to attack by parasitoids (Takasu & Overholt, 1997). However, the host regurgitant produced is by itself an important source of short-range attractants and arrestants for parasitoids (Cobert, 1971). The fact that the regurgitant is intimately related to larval species and is produced at the final stage of host encounter during oviposition attempts suggests that contact with the regurgitant should induce oviposition behaviour as observed in *C. flavipes*. This encounter with their hosts usually implies that *C. sesamiae* and *C. flavipes* in turn must evolve the ability to recognise the regurgitant of their respective hosts. This explains the stinging activity observed when exposed to the host of *B. fusca* and *C. partellus.* However, we do not have an explanation as to why the indigenous parasitoid, *C. sesamiae*, did not sting the cotton wool balls imbibed in the regurgitant of *B. fusca*.

These findings corroborate those of Potting *et al.* (1997) showing that extracts from the mandibular glands of *C. partellus* elicit behavioural response among *C. flavipes* females.

The similar duration of antennation of the regurgitant of both *C. partellus* and *B. fusca* could indicate that the two are close in chemical composition while that of *E. saccharina*, which did not elicit any response from the wasps, is different. However further experiments may be required in order to confirm this.

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Table 4.1. Percentage stinging (%, mean ± SE, n=20, by the female wasps in response to the treated stemborer larvae

Parasitoid species	Larvae treatment	% stinging
Cotesia sesamiae	Washed *B. fusca*	0a
	B. fusca painted with *B. fusca* extract	65 ± 11c
	B. fusca painted with *E. saccharina* extract	25 ± 10ab
	E. saccharina painted with *B. fusca* extract	50 ± 11bc
		(F= 9.33; df=3; P= 0.0001)
Cotesia flavipes	Washed *C. partellus*	0a
	C. partellus painted with *C. partellus* extract	75 ± 10c
	C. partellus painted with *E. saccharina* extract	30 ± 10.5b
	E. saccharina painted with *C. partellus* extract	65 ± 11c
		(F= 14.29; df=3; P= 0.0001)

Means within a column for each parasitoid species followed by different letters are significantly different at P=0.05 level (Student-Newman-Keuls Test following ANOVA).

Table 4.2. Duration of antennation (in seconds, mean ± SE, n=20) by female wasps in response to extract solution of host larvae deposited on ball of cotton wool

	Parasitoid species		
Extract tested	*Cotesia sesamiae*	*Cotesia flavipes*	(F,df, P) [2]
H_2O	0a	0a	(0,1,0),
B. fusca body	31.6 ± 6.3bA	20.9 ± 5.3cA	(1.68,1,0.2030)
C. partellus body	0.2 ± 0.1aA	39.5 ± 9.7cA	(2.36,1, 0.1328)
E. saccharina body	1.0 ± 0.6aA	2.8 ± 1.0bA	(2.54,1,0.1195)
(F,df, P) [1]	(24.08, 3, 0.0001)	(10.87, 3, 0.0001)	

[1]Means within a column for each parasitoid species followed by different letters (abc) are significantly different at P=0.05 level (Student-Newman-Keuls Test following ANOVA).
[2] Means between columns for each parasitoid species followed by different letters (ABC) are significantly different at 5% level (Student-Newman-Keuls Test following ANOVA).

Table 4.3. Duration of antennation (in seconds, mean ± SE, n=20) by female parasitoids in response to sample tested

Sample tested	Host species	*C. sesamiae*	*C. flavipes*	(F, df, P) [2]
Fresh frass	*E. saccharina*	18.0 ± 5.6aB	61.3 ±17.8aA	5.37,1,0.0260
	C. partellus	21.7 ± 6.4aB	142.9 ±25.6bA	21.14,1,0.0001
	B. fusca	117.9 ±19.0bA	112.6 ±15.7bA	0.05,1,0.8324
	(F, df, P)[1]	6.75, 2 , 0.0023	4.26, 2, 0.0188	
Frass extract	*E. saccharina*	1.7 ± 0.4abB	10.1 ± 3.6aA	8.71,1,0.0054
	C. partellus	1.1 ± 0.3aB	21.7 ± 5.9aA	34.50,1,0.0001
	B. fusca	2.7 ± 0.6bB	26.1 ± 11.6aA	6.36,1,0.0160
	(F, df, P)[1]	3.54,2, 0.0354	1.12,2,0.3330	
Regurgitant	H_2O	0a	0a	
	E. saccharina	0a	0a	
	C. partellus	1.4 ± 0.6bB	25.3 ± 7.1bA	11.16,1,0.0019
	B. fusca	1.4 ± 0.2bB	16.7 ± 5.3bA	8.27,1,0.0066
	(F, df, P)[1]	8.05,3, 0.0001	6.82,3,0.0004	

[1]For each parasitoid species means within a column followed by different letters (abc) are significantly different at P=0.05level (Student-Newman-Keuls Test following ANOVA).
[2]For each parsitoid species means between columns follwed by different letters (ABC) are significantly different at P=0.05level (Student-Newman-Keuls Test following ANOVA).

Chapter 5: Preliminary Identification of Kairomone(s) Involved in oviposition by the Braconid Larval Endoparasitoid *Cotesia flavipes*

5.1 Abstract

In chapter 4, it was demonstrated that the regurgitant of the suitable hosts induced antennation by both *C. sesamiae* and *C. flavipes* and insertion of the ovipositor for *C. flavipes* only. Thus, the component of C. partellus regurgitant appears to be involved in host recognition and acceptance by *C. flavipes*. This study was conducted to identify the compounds responsible for ovipositor insertion by *C. flavipes*. When the regurgitant was subjected to dialysis (retaining compounds with molecular weight > 8 kD) stinging activity was retained. However, when the regurgitant was boiled ovipositor probing did not occur. Gel Electrophoresis revealed the presence of numerous protein bands in the regurgitant of a suitable stemborer host some of which differed from those of the non-host *E. saccharina*. These results indicate the involvement of protein(s) in host recognition and acceptance by *C. flavipes*. More studies are already underway to confirm this and to identify the protein(s).

5.2 Introduction

In the evolutionary arms race between herbivores and their natural enemies, herbivores have evolved several traits to reduce their vulnerability to enemies. One of these traits can be a specific behaviour that reduces their recognition and detection by natural enemies. It has been argued that the stemboring habit of many herbivore species avoids proximity of feeding damage and reduces accessibility for natural enemies which may have been one of the reasons for its evolution (Strong *et al*., 1984).

However absolute safety from natural enemies is virtually impossible. Although attack by enemies imposes selection pressure on victims to reduce vulnerability, counter selection works on enemies to improve their efficiency in finding hiding hosts (Jeffries & Lawton, 1984). It is as a result of such continued selection pressures that we believe the parasitoids have been able to utilise the regurgitant as a cue (intimately related to the host) in discriminating their potential hosts from non hosts [chapter 4].

As already mentioned, the ability of parasitic wasps to successfully utilize more host specific cues in their habitat in location and discrimination between suitable and unsuitable hosts contributes to their efficiency (Vinson, 1985; Godfray, 1994). In this regard, we found that the larval regurgitant is an important cue to the searching natural enemy. This is partly because the natural enemy usually comes into contact with the regurgitant after examining the frass at the entrance of the tunnel [see chapter 4]. It was further confirmed that the regurgitant induced a host seeking behaviour (antennation) by both *C. sesamiae* and *C. flavipes* and ovipositor probing in *C. flavipes* [see chapter 4].

In the current study, a preliminary attempt was made to identify the components of the regurgitant that induce oviposition by *C. flavipes* are made.

5.3 Materials and methods

5.3.1 Insects

The rearing conditions and origin for the parasitoids (*C. sesamiae* and *C. flavipes*) as well as those of the stemborers (*B. fusca*, *C. partellus* and *E. saccharina*) were similar to those described in chapter 4. Similarly, for the parasitoids 1-day-old naïve, putatively mated females were used. The third and fourth larval instars of *B. fusca*, *E. saccharina* and *C. partellus* were introduced into jars (10 cm x 20 cm) each containing pieces of maize stem and left for a 48 hr feeding period before being used in the experiments [see chapter 4].

5.3.2 Experimental procedure

For each host stemborer species, a single larva held by a soft forceps was gently squeezed (behind the head) and a capillary tube used to collect the oral extract produced. The process was repeated for several larvae and the total mass of the extract each time recorded. The collection of regurgitant was done in a cold room (around 10°C) in order to conserve activity. This was filtered (Whatman # 1) and dialyzed against water in an MWCO 6-8 kD cellulose membrane for 3 days at (2-4°C) then freeze-dried.

The sample was later resuspended into distilled water to obtain a solution of about 20 µl of regurgitant per 100 µl for each stemborer species. In the first setup, the solutions were directly used for bioassays, while in the second setup the freshly collected regurgitant in their tubes were directly transferred into a water bath to boil at 100°C for 10 minutes.

A 20 µl aliquot of each type of extract was deposited on a ball of cotton wool (3 mm) and exposed to the parasitoid. Each wasp was observed for a maximum of 60 sec [similar to chapter 4]. The percentage of wasps probing the cotton wool ball was calculated from twenty replicates of a single wasp each. Both the wasp and cotton wool ball with regurgitant were used once and replaced after each replicate.

5.3.3 Electrophoresis

In all gels, the Hoefer Scientific Instrument (HIS PS 1500, San Francisco, USA) was used. About 3 mg/ml of total proteins of frass extracts and 1 mg/ml of total proteins of regurgitant extracts of *B. fusca*, *C. partellus* and *E. saccharina* obtained from the samples of chapter 4 were run on 4-20% polyacrylamide gradient gel under native conditions at low temperature (2-4°C). Thereafter, the gel was stained by the silver nitrate method described by Oakley *et al.* (1980).

5.3.4 Partial purification

About 10 mg/ml of regurgitant of *C. partellus* suspended into Tris-HCl 20 mM, pH 8 buffer was loaded on a diethylaminoethyl Sephacel (Sigma, 057K0700) column (105 mm x 6 mm) and the column eluted with the same buffer. The absorbance at 280 nm was measured in the eluted fractions. The fractions containing protein peaks were combined. Thereafter, the column was eluted with NaCl 0.5 M in Tris-HCl 20 mM, pH 8 buffer. Similarly the absorbance at 280 nm was measured in the eluted fractions and the fractions containing peaks of proteins were combined. Both combined fractions (- or + NaCl) were dialyzed for 3 days at low temperature (2-4°C) with MWCO 6-8 kD cellulose membrane against water then freeze-dried.

A 20 µl aliquot of each type of extract was deposited on a ball of cotton wool (3 mm) and exposed to the parasitoid. The response of each wasp was observed during 60 sec. The percentage of wasps probing the cotton wool ball was calculated from twenty replicates of a wasp and cotton wool ball each. Both the wasp and cotton wool ball with extracts were replaced after each replicate.

5.3.4 Data Analysis

The means of the percentage stinging activity on the cotton woll balls containing boiled and dialysed regurgitants were separated by Student-Newman-Keuls Test. The statictical tests were conducted using SAS software USA.

5.4 Results

The dialysed regurgitant of the suitable host *C. partellus* induced stinging activity on the cotton wool balls in about (55±11.4) % of female *C. flavipes*. However, the female parasitoids showed no response to the cotton wool balls containing the boiled regurgitant (F=23.22,df=1, P=0.0001).

Electrophoresis of the frass extracts and regurgitant of the three stemborer species: *B. fusca*, *E. saccharina* and *C. partellus* revealed the presence of numerous proteins (Fig. 5.1). However, more proteins were revealed in the regurgitant extracts than in the frass extracts regardless of the stemborer species used. For each stemborer species some of the proteins present in the frass were also found in the regurgitant. However, the protein profiles were for the three stemborer species.After elution on a diethylaminoethyl Sephacel column with or without NaCl, of dialysed regurgitant of *C. partellus*, no stinging activity was observed for *C. flavipes*, even when the two types of extract were mixed together. It was therefore not possible to continue the purification process of the regurgitant of *C. partellus*.

5.5 Discussion

Caterpillars exhibit a broad array of behaviours, morphologies and physiologies; and it can be difficult to discern which of these, if any, actually function as defenses against their many vertebrate, invertebrate, and microbial enemies (Evans & Schmidt 1991; Gross, 1993; Godfray, 1994; Dyer, 1995). There are at least two main strategies of defense followed by lepidopteran stemborer larvae: (i) primary defenses that include behavioral and physiological responses that are activated once larvae have been encountered or attacked by an enemy. Here the larvae aggressively defended themselves against attacking wasps by biting, spitting or flicking-off the wasp (Takasu & Overholt, 1997) and, (ii) secondary defenses that include immune system responses by hemocytes against endoparasitoid eggs or larvae once parasitized (Gross, 1993; Godfray, 1994).

While the production of the host regurgitant serves as a defence strategy, it also enables the parasitoid to recognise its suitable host because of a component in the regurgitant that attracts and arrests the parasitoids (Cobert, 1971). The regurgitant has elicited much attention from the scientific community because of the diverse reactions that it is inducing among parasitoids and the plants.

The fact that fresh regurgitant rapidly lost activity at room temperature (M. Obonyo, personal observation) could indicate the presence of an enzyme in the extract. For example a β-glucosidase that rapidly degrades the regurgitant of *Pieris brassicae* L. (Lepidoptera: Pieridae) larvae was reported by Mattiacci *et al.* (1995). It is possible that boiling of the crude extracts of the regurgitant denatures the active compounds leading to the loss of activity but this also could indicate the presence of a protein like substance. In addition, the observation that the regurgitant extract remained active after dialysis using MWCO 6-8 kD cellulose membrane implies that its active components inducing oviposition by *C. flavipes* are have molecular weight greater than 8 kD and were retained in the membrane. For example, a 30 kD protein from the frass, haemolymph and the entire larvae of *Heliothis virescens* (Fabricius) (Lepidoptera: Noctuidae) has been identified as a kairomone inducing oviposition by *Archytas marmoratus* (Townsend) (Diptera: Tachinidae) (Nettles & Burks, 1975).

According to the electrophoresis results, the proteins present in the regurgitant appear characteristic to the stemborer species. In actual fact, the protein profile was different among the three stemborer species (Fig. 5.1). Nevertheless, when the regurgitant extract was loaded on a diethylaminoethyl Sephacel column, the extract lost activity. There are two possible reasons for this (i), the process probably denatures the active protein(s) and/or (ii), the extract obtained after elution is too dilute to show any activity (the activity of a kairomone is frequently concentration dependent). Another purification method avoiding this dilution phenomenon or risk of protein denaturation is necessary to allow us to purify and identify the potential protein(s) inducing oviposition in *C. flavipes*. This aspect of the research is already underway.

For *C. sesamiae*, although the regurgitant extract of its suitable host (even the frass extract) induced antennation (indicator of host recognition) [see chapters 2 and 4], it is not inducing oviposition. Therefore, the compounds present in the regurgitant of its suitable host are not by themselves inducing oviposition. It is possible that such induction occurred in combination with the movement of the larvae [cf. tactile stimulation mentioned in chapter 4]. Another hypothesis is that other kind(s) of compound(s) different from the ones used by *C. flavipes* are involved in host acceptance for oviposition by *C. sesamiae*. Other compounds including sugars, free amino acids, sesquiterpenes and lipids have been evidenced as kairomones stimulating oviposition among parasitoids [see chapter 1].

5.6 References

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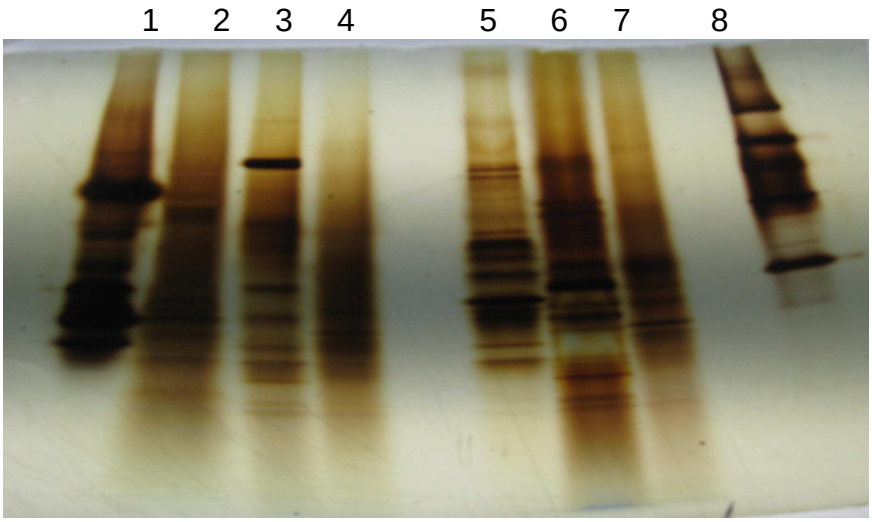

Figure 5.1 Electrophoresis using a 4-20% gradient polyacrylamide native gel of proteins present in the frass extract of *B. fusca* (lane 2), *E. saccharina* (lane 3) and *C. partellus* (lane 4) and in the regurgitant of *B. fusca* (lane 5), *E. saccharina* (lane 6) and *C. partellus* (lane 7) (lanes 1 and 8: low and high molecular weight markers, respectively). The gel was stained with silver nitrate.

Chapter 6: Conclusion

In the evolutionary arms race between herbivores and their natural enemies, herbivores have evolved several traits to reduce their vulnerability to enemies. This is achieved by the reduction of their detection and recognition by natural enemies. Strong *et al.* (1984) reasoned that the stemboring habit of many herbivore species enables them to avoid proximity of feeding and reduces accessibility for natural enemies. However absolute safety from natural enemies is virtually impossible. Although attack by enemies imposes selection on victims to reduce vulnerability, counter selection works on enemies to improve their efficiency in finding hiding hosts (Jeffries & Lawton, 1984). As a consequence of this evolutionary process, parasitoids have also developed behavioural and morphological characteristics allowing them to attack hosts with a concealed lifestyle (Smith *et al*., 1993).

Information is available giving candid detail on the use of plant volatiles in long-range foraging behaviour by the congeneric parasitoids *C. sesamiae* and *C. flavipes* during the searching for hosts (Potting *et al.,* 1995; Obonyo *et al.,* 2008) as well the usefulness of volatiles to discriminate different host and non-host pants (Jembere *et al.,* 2003), including those that had been infested by different suitable and not suitable stemborer host species (Ngi-Song *et al*., 1996; Ngi-Song & Overholt, 1997). However, stimuli from the habitat or host products do not convey sufficiently reliable information on the suitability of the damaging species for the parasitic wasps; but are mere indicators of herbivore presence (Ngi-Song & Overholt, 1997). As a result, *C. sesamiae* and *C. flavipes* are often attracted to plants harbouring unsuitable stemborer species, which act as a reproductive sink (Potting *et al*., 1993; Ngi-Song *et al*., 1996; Obonyo *et al*., 2008). Thus the females of *C. sesamiae* and *C. flavipes* are often unable to recognise their hosts from a distance but rather by close contact. For that reason, the purpose of this study was to understand the basis of host discrimination upon contact by the two parasitoid species. It was incumbent to compare suitable hosts (i.e. *Busseola fusca* for *C. sesamiae*, and *Chilo partellus* for *C. flavipes*) and a non-host (*Eldana saccharina*).

The findings in this thesis showed that *C. sesamiae* and *C. flavipes* females are able to detect the presence of a larva in cloze proximity, probably by the volatiles emitted by the frass and/or products related to feeding of the larva. This detection in close proximity permits female wasps to locate their potential hosts.

Afterwards, antennal examination played a crucial role for host recognition and acceptance. Whereas the tars probably through reception of vibrational signals seemed to be important or even essential for final host acceptance before stinging. The stemborer larval contact cues (both physical and chemical) seemed to play a major role in host acceptance for oviposition by the female wasps. The identification of these cues will help us to isolate the kairomone(s) involved in host recognition and acceptance by *C. sesamiae* and *C. flavipes.*

In addition, the females of *C. sesamiae* and *C. flavipes* share the same type and distribution of sensilla enabling them to detect volatiles and contact chemical stimuli from potential hosts. The olfactory receptors are restricted to the antennae while taste receptors are present on the antennae and tarsi.

When suitable host larvae were washed in distilled water, the behaviour of the parasitoid was similar to that displayed when in contact with the non-host larvae, characterised by the absence of ovipositor probing. When the stemborer larvae (washed suitable host or non-host larvae) were painted with water extracts of their respective suitable host-larvae, the parasitoids showed a significant increase in ovipositor probing. This indicates the importance of water-soluble contact chemical cues from the stemborer larvae in host recognition and acceptance by the parasitic wasps. However, water extracts of suitable host-larvae deposited in cotton wool balls did not induce ovipositor insertion for both *C. sesamiae* and *C. flavipes*. These extracts allowed them to discriminate between suitable host and non-host, antennating more intensely on their respective suitable hosts than on their non-host water extracts.

It was further shown that the parasitic wasps utilise frass for host recognition during short-range examination because they intensely antennated the frass of their respective suitable hosts more than that of the non-host. The attraction of wasp species is very likely to be consistent with oviposition record; i.e., the wasps would not prefer the frass of species they would not oviposit in. This is further supported by the fact that the frass is usually a product of the feeding activity of the larvae. Consequently, the frass may be intimately related with the larva's regurgitant explaining the inability of both parasitoid species to discriminate between *B. fusca* and *C. partellus.* It was also observed that the regurgitant of *C. partellus* by itself induced oviposition by *C. flavipes*.

This was not the case with *C. sesamiae* when using regurgitant of *B. fusca*. However, the regurgitant of the suitable hosts induced antennation (a behavior involved in host recognition) by both *Cotesia* species.

As for the composition of the regurgitant, it is very likely that there is/are protein(s) from the host stemborers that is/are responsible for ovipositor probing by *C. flavipes*. However, further experiments are required to confirm this and to identify the protein(s). Similarly, the results of this study do not allow for explanation as to why the indigenous parasitoid, *C. sesamiae*, did not sting the cotton wool balls imbibed in the regurgitant of *B. fusca*. Additional experiments are needed to isolate and identify the host kairomone(s) involved in host recognition and acceptance by *C. sesamiae*.

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