PERFORMANCE OF *COTESIA FLAVIPES* CAMERON (HYMENOPTERA: BRACONIDAE) ON STEM BORERS OF CEREALS AND WILD CROPS.

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A thesis submitted in partial fulfilment of the academic requirements for the award of the degree of Master of Science in Biochemistry of Jomo Kenyatta University of Agriculture and Technology (JKUAT).

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DECLARATION

I, Meshack O Obonyo, declare that this thesis is my original work and has not been presented for a degree in any other form to another university.

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DEDICATION

This thesis is dedicated to my parents Mr and Mrs George Obonyo and the entire family for the never-ending love and continual motivation.

ABSTRACT

The braconid larval parasitoid *Cotesia flavipes* Cameron (Hymenoptera**:** Braconidae) was introduced into Kenya from Pakistan and India for the control of the exotic crambid stem borer *Chilo partellus* Swinhoe (Lepidoptera: Crambidae). In Africa, maize fields are usually islands surrounded by land occupied by wild gramineous plants. Many of the wild plants harbour borer species not found on crops. It is not known if the exotic parasitoid *C. flavipes* follows these borers into the wild habitat and whether they are suitable for its development. Thus, the purpose of this study was: i. to assess the suitability of some borer species found in crops and wild host plants for the development of *C. flavipes*, ii.to study the host-foraging behaviour of the parasitoid and iii. to isolate and identify plant volatiles that could mediate host finding by *C. flavipes*. Seven stem borer species were used: *C. partellus* and *Busseola fusca* Fuller (control species), *Sesamia calamistis* Hampson, two populations of *S. nonagrioides* Tams & Bowden (from Eastern and Western Kenya), *B. phaia* Bowden and *Sciomesa piscator* Tams. *C. partellus*, *S. calamistis* and *B. fusca* are mostly found on cultivated crops whereas *S. nonagrioides* (especially eastern population), *B. phaia* and *S. piscator* are mostly found on wild host plants.

The stem borer species exposed to *C. flavipes* were equally acceptable for oviposition. However, the suitability varied with species. Parasitoid emergence occurred only on *C. partellus*, *S. calamistis* and the *S. nonagrioides* West population, while species feeding on wild hosts plants were not suitable. *C. flavipes* females were significantly more attracted to volatiles from stem borer-infested plants than to volatiles from uninfested plants regardless of stem borer and the plant species used. This was probably due to the richer chemical profile of stem borer-infested plants and especially in green leaf volatiles and terpenoids compared to uninfested plants. It can be concluded that the unsuitable borer species used in the present experiment form a reproductive sink.

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ABBREVIATIONS

BC	Biological control
CIMMYT	International Maize and Wheat Improvement Center
FID	Flame ionisation detector
GC	Gas chromatography
GC-MS	Gas chromatography coupled to mass spectrometry
GMOs	Genetically modified organisms
HPR	Host plant resistance
PDMS/DVB	Polydimethylsiloxane/divinylbenzene
PVCs	Plant volatile compounds
RH	Relative humidity
SPME	Solid phase micro-extraction technique

CHAPTER ONE

INTRODUCTION

1.1 General introduction.

As the case may be, the most important event in the history of man was the invention of agriculture, but with drastic consequences on the natural environment. Increased agricultural production and the entailing manipulation of land have been environmentally costly. Loss of natural habitat and the associated species of plants and animals has resulted in diminished and modified biodiversity. It is therefore no longer acceptable to modify landscape solely for the purpose of increasing agricultural production. Consequently, we face the challenge of working not against, but with the environment in order to incorporate an ecological aspect during the planning of maintained or increased production.

Over the last decade, the role of biodiversity and population dynamics of insects in the functioning of natural and managed ecosystems has become a central topic in agriculture.

Understanding how evolutionary, ecological and socio-economic processes drive biodiversity changes and how knowledge of these changes must be incorporated into production planning, is a main goal for the future. Despite considerable progress made by conservation biologists, there is still no general theoretical framework that can predict the effects of loss or increase in biodiversity on both natural and cultivated systems. Existing studies have reported neutral, negative and positive effects. This reflects the unpredictable nature of multi-trophic interactions and as a result, each system must be evaluated individually.

Lepidopteran stem borers are a major impediment to cereal crop production in sub-Saharan Africa. They account for a significant reduction in yield by the damage they cause.

Despite several control measures employed, reduction of the impact of stem borers still remains a subject of research. Therefore, it was recognized that the conventional means of control, among them host plant resistance (HPR) alone would not solve the problem of pests and as such, forms of biological control (BC) were developed as a way of complementation.

Biological control has reported considerable success in several areas because of the specificity of natural enemies on target organisms. As a result, increase in yield has been recorded and the method is being implemented in areas where impact assessment had taken place previously.

Among the natural enemies that have been used to control cereal stem borers is the larval parasitoid *Cotesia flavipes* Cameron (Hymenoptera: Braconidae). This wasp was introduced in a classical biocontrol programme for the control of the exotic crambid stem borer *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae). This was to complement the action of the indigenous *Cotesia sesamiae* (Cameron) (Hymenoptera: Braconidae), which attacks both the exotic and indigenous borer species such as the noctuids *Sesamia calamistis* Hampson and *Busseola fusca* Fuller (Lepidoptera: Noctuidae).

This study aimed at investigating the PVCs which attract, hence augment, the performance of *C. flavipes* in wild and cultivated systems in Kenya. At the same time look at the suitability of borers found on plants in these systems for the development of the parasitoid.

1.2 Literature review

Lepidopteran stem borers are generally considered to be the most destructive group of insect pests attacking cereal crops in Africa. Yield losses due to these insects in Kenya alone, fluctuates around 14% (De Groote, 2001). Several species of these insects exist, often occurring on the same field at the same time [see website: (http://stem borer.icipe.org/). Due to their widespread distribution and destructive nature, stem borers have been the subject of extensive research in Africa. Primarily, they have been classified into three families: Crambidae, Pyralidae and Noctuidae (Bleszynki, 1969; Harris, 1990).

Earlier work had reported a complex of 12 species from cereal crops in East Africa with *Chilo partellus, Chilo orichalcociliellus* (Strand), *Busseola fusca, Sesamiae calamistis* Hampson and *Eldana saccharina* (Walker) being among the most significant and widely distributed (Nye, 1960; Youdeowei, 1989). However, more recent work at ICIPE shows that the species diversity, especially on wild hosts, is much higher than had earlier been thought (http://stem borer.icipe.org/).

1.2.1 Management of stem borers

In Africa, maize is usually grown in small plots surrounded by a mosaic of wild gramineous plants. This scenario has also contributed in part to the complex nature of the habitat.

Diverse methods have been employed in an attempt to control stem borers and research is in progress to improve the existing ones while developing other new strategies.

Habitat management strategies, which aim to control the pest via increased plant diversity, have also been put into use. The entailed techniques aim at altering the natural habitat as a means of controlling the stem borers.

However, information such as the effect of adjacent wild plants on the population dynamics of both the predator and the pest in the main crop is yet to be unravelled.

Moreover, wild host plants have been thought to be refugia for susceptible borer strains and therefore could be candidates for use in resistance management (Fitt *et al*., 2004). This information appears necessary particularly at the helm of the likely introduction of *Bt* maize in Kenya in the year 2005. It has been proposed that wild grasses can be a reservoir for stem borers and are responsible for pest outbreaks on crops (Bowden, 1976; Sampson and Kumar, 1986). Contradicting this hypothesis, Schulthess *et al*. (1997) explained the role of wild grasses in three ways: (a) grasses harbour natural enemies that prevent stem borers from reaching damaging levels on maize (b) grasses act as trap plants, or (c) both. Losey *et al*. (2001) explained the possibility of non-host plants for European corn borer serving as "nursery plants" which support partial but not complete development of the young forms. In view of this, habitat management cannot be used in isolation since such vital information is not yet explicit.

Host Plant Resistance is a method endeavouring to develop plant varieties with intrinsic resistance to pests. The method has been suggested to be ideal for the control of pests, posing no environmental hazard and being generally compatible with other control methods (Bosque-Perez and Schulthess, 1998).

A holistic breeding strategy which aimed at developing varieties with acceptable agronomic characteristics and yield, as well as resistance to the major diseases, yielded moderate resistance to borers only (Bosque-Pérez *et al*., 1997; Schulthess and Ajala, 1999). An important issue with host plant resistance (HPR) 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). Research is needed to develop cultivars resistant to polyphagous pest but more often than not, strong antibiosis is achieved at the cost of yield. Crop varieties resistant to pests are still not widely available (Kfir *et al*., 2002).

Cultural practices involving the manipulation of the environment (both crop and land) so as to render it unsuitable to the pest.

These practices alter the condition of the host plant, or the behaviour of the pest to prevent or suppress an infestation. They disrupt the normal relationship between the pest and the host plant and make the pest less likely to survive, grow or reproduce. This method is the most relevant and economical means of control for resource–poor farmers in Africa consisting of crop rotation, sowing date manipulation among others (Kfir *et al*., 2002). However, cultural control is bedevilled by the inability of farmers to implement the entailed practices over and above their being labour intensive (Van den Berg *et al*., 1998).

Pesticides are chemicals used to destroy pests, control their activity, or prevent them from causing damage. They are generally the fastest means of control. However, their use requires frequent application due to continuous infestation and the relatively short time of larval exposure. Control using systemic insecticides provides only protection against early attacks but not against borers feeding in the cob (Sétamou *et al*., 1995; Ndemah and Schulthess, 2002). Furthermore, broad-spectrum insecticides such as carbofuran, with high trans-dermal toxicity, are environmentally damaging and a serious health hazard in the hands of untrained farmers.

Commercially produced alternative pesticides such as neem tree (*Azadirachta indica* A. Juss) products or the bacteria *Bacillus thuringiensis*, have a potential but are not readily available or are too costly (Brownbridge, 1991; ICIPE, 1995).

There is also conflicting evidence on pesticide–yield relationship. It has been reported that, despite heavy attack by *C. partellus* in Uganda and India respectively, the infested crops compared favourably with the insecticide-protected plots in terms of grain yield hence no significant difference was observed (Ingram, 1958; Trehan and Butani, 1949).

In contrast, Jotwani and Young (1972) reported that application of a number of insecticides to whorls of certain hybrids and improved varieties in India gave effective control and significant yield increase in the experimental plots. Other workers have also shown that pesticides are not effective against larvae feeding in the ear (Ndemah and Schulthess, 2002). Pest management techniques that are inexpensive and limit the use of insecticides while improving the competence of cultural approaches deserve attention.

There has been renewed interest in the use of biological control agents to reduce stem borer population densities including ants, spiders and earwigs believed to cause high mortality of stem borer eggs and young larvae (Mohyuddin and Greathead, 1970; Girling, 1978; Oloo, 1989). Indigenous larval and pupal parasitoids have been shown to be unable to keep stem borer populations below economic injury levels (Oloo, 1989). *Cotesia sesamiae* being the most common larval parasitoid in sub Saharan Africa attacks various stem borers in gramineous crops including *B. fusca* and *S. calamistis* (Mohyuddin and Greathead, 1970).

Busseola fusca is reported to be among the most damaging stem borers of maize and sorghum throughout East Africa, it is abundant in the highlands (Harris and Nwanze, 1992) where it is the main host of *C. sesamiae* (Overholt *et al*., 1994a). The coastal population of *C. sesamiae* does not develop in *B. fusca*, unlike the inland grade. Apparently, there exists two biotypes of the parasitoid (Ngi-Song *et al*., 1995).

A classical biological control program was implemented in Kenya in 1993, to complement the action of the native parasitoids. The braconid larval parasitoid *C. flavipes* was introduced for the control of the exotic crambid stem borer *C. partellus* (Swinhoe), to complement the action of the indigenous *C. sesamiae* which attacks both the exotic and indigenous borer species such as the noctuids *S. calamistis* and *B. fusca.* Since its introduction at the coast, *Cotesia flavipes* has become permanently established in the country, increasing maize yields by 10-15 %, on average (Overholt *et al*., 1994a, b; Zhou *et al*., 2001; Songa *et al*., 2002).

Following its success in Kenya and western Tanzania (Omwega *et al*., 1995; 1997), the parasitoid has been released in nine other countries in East and Southern Africa.

Parasitization rates have been rising steadily and *C. flavipes* has become the most prevalent larval parasitoid species in the area (Overholt *et al*., 1997).

1.2.2 Tritrophic interactions

The success of classical biological control program is attributed to the performance of the natural enemy in the environment. This largely depends on its ability to locate, accept and successfully develop within the host system.

Tritrophic interactions involving plants, herbivores and their natural enemies are primarily intricate arrays of chemical substances which aid the parasitoid during foraging.

These chemicals arise either from: (a) the herbivore itself, (b) the host plant or (c) interaction between the host plant and the herbivore (Gohole and Ngi-Song, 2001). Successful parasitism of the host is preceded by a sequence of events including: (a) host habitat location, (b) host location, (c) host acceptance and (d) host suitability (Vinson, 1985; Smith *et al*., 1993).

Plant volatile compounds involved in communication can have direct and/or indirect beneficial or detrimental effects on herbivores and their natural enemies. They convey information to the parasitoid on the likely presence of the host insect (Dicke and Sabelis, 1988; Dicke and Vet, 1999). More useful for parasitoid host location are those produced by plants in response to herbivore attack (Turlings *et al*., 1995).

Females of *C. flavipes* and *C. sesamiae* prefer odours from stem borer infested plants over those from uninfested plants, suggesting that damaged plants provide important cues to the searching parasitoid (Ngi-Song *et al*., 1996).

Chemical substances produced by the stem borers are used by their parasitoids to locate and identify a potential host directly.

They arise from among many sources including; (a) body odour, (b) frass, (c) webbing, (d) salivary constituents, (e) honeydew, (f) body scales, (g) egg chorions and (h) host pheromones (Vinson, 1976; Lewis and Martin, 1990; Vet and Dicke, 1992; Fiaboé *et al*., 2003). Some of the compounds involved in recruiting larval parasitoid *C. flavipes* have been identified and include: (a) heptanal, (b) (*Z*)-3-hexenylacetate, (c) (*E*)-ß-ocimene, (d) linalool and (d) (*E*)-4.8-dimehtyl-1,3,7-nonatriene, (e) anisole and (f) (*E*)-ß-farnesene (Ngi-Song and Overholt 1997; Ngi-Song *et al*., 2000).

Studies by Potting *et al.* (1993) and Ngi-Song *et al.* (1996) revealed that *C. flavipes* and C. *sesamiae* were unable to discriminate between host plants infested by *C. partellus*, *C. orichalcociliellus, B. fusca* and *S. calamistis.* Furthermore, when offered frass from these species, neither of the parasitoids could discriminate between the hosts. This indicates that, the volatiles do not carry any information on the herbivore species (Ngi-Song and Overholt, 1997). This could be disadvantageous for *C. flavipes* since it accepts but does not successfully develop in *B. fusca* in which encapsulation of the parasitoid eggs has been observed (Ngi-Song *et al*., 1995). Plant volatile compounds are also known to have a negative effect on the behaviour of insects. For example, some emissions have been found to deter conspecific female moths from ovipositing on previously damaged plants (Kessler and Baldwin, 2001; De Moraes *et al*., 2001).

The breeding strategy of combining multiple forms of resistance into a single variety is termed pyramiding. Pyramids of traditional and transgenic varieties may limit stem borer population shift toward increased tolerance. Identification of PVCs may enable breeders select novel forms of resistance to be combined with transgenic resistance into a single pyramid.

1.3 Justification

In Africa, maize fields are usually "islands" surrounded by land occupied by wild gramineous plants. Some of these plants have been found to produce volatile compounds which attract ovipositing female moths but they may not be suitable for offspring development. For example, Haile and Hofsvang (2002) found Sudan grass (*Sorghum vulgare* Pers. var. *sudanense*) and colombus grass (*Sorghum alum* Parodi) to have very high larval mortality despite a high oviposition, thus, they form a reproductive sink (Schulthess *et al*., 1997). On the other hand, wild hosts are stable refugia perennating natural enemy populations and therefore increase biological control activity in the system (Ndemah *et al*., 2003).

Many of the wild plants harbour borer species not found on crops. It is not known if the exotic parasitoid *C. flavipes* follows these borers into the wild habitat, and whether they are suitable for its development. Such knowledge appears necessary, particularly in view of the highly probable of introduction of *Bt*-transgenic maize in Kenya by CIMMYT in the year 2005. A prerequisite for the assessment of the environmental impact of GMOs is knowledge of the potential movement of parasitoids into the wild habitat before those cultivars are planted on a large scale.

Wild host plants have been suggested to be a refugia for susceptible borer strains and therefore could be used in resistance management (Fitt *et al*., 2004). However, in view of the scarcity of both *B. fusca* and *C. partellus* on wild hosts, with the exception of wild sorghum, this is questionable (see website for details: http://stem borer.icipe.org/).

1.4 Research goal

To understand the role of wild and cultivated grasses in the population dynamics of the larval parasitoid C. *flavipes* (Hymenoptera: Braconidae).

1.5 Objectives

1. To assess the suitability of some borer species found in crops and wild grasses for the development of *C. flavipes*;

2. To determine the host foraging ability of the *C. flavipes*

3 To identify volatile compounds involved in recruitment of the parasitoid.

1.6 Hypothesis

Stem borer species on wild host plants are not suitable for the development of the endoparasitoid *Cotesia flavipe*s and therefore form a reproductive sink.

CHAPTER TWO

Host suitability of stem borers from cereal and wild crops to *Cotesia flavipes*

2.1 Introduction

The braconid larval parasitoid *C. flavipes* was introduced into Kenya from Pakistan and India for the control of the exotic crambid stem borer *C. partellus*. Since its introduction in 1993 at the coast, *C. flavipes* has become permanently established in the country, increasing maize yields by 10-15 %, on average (Overholt *et al*., 1994 a, b; Zhou *et al*., 2001; Songa *et al*., 2002).

Hosts encountered by parasitoids often vary in their quality as food for the developing offspring; this necessitates examination of the potential host. The wasp is thus faced by a sequence of decisions to make regarding oviposition. Should the host be used for oviposition (accepted), or should it be ignored (Godfray 1994)? Host acceptance therefore is a key step preceding parasitism and the eventual suitabilityof the stemborer to its natural enemies.

Stem borer suitability is determined by the ability of the parasitoid to complete its development (egg-adult) within the host. Hosts attacked, but in which the wasp does not complete development, are thus termed as unsuitable. Host unsuitability arises due to immunological response of the herbivore against the natural enemy. Ngi-Song *et al*. (1995) observed this phenomenon in *B. fusca* parasitized by *C. flavipes*, in which the eggs of the wasp are encapsulated. *Cotesia flavipes* is a koinobiont and will therefore not kill hosts until her larvae emerge to pupate outside the hosts; consequently, food consumed by the herbivore indirectly affects the endoparasitoid. Toxic plant metabolites are often sequestered by herbivores for defence against its natural enemies (Cortesero *et al*., 2000).

Kester and Barbosa (1991) reported that caterpillars of *Manduca sexta* (Linnaeus) (Lepidoptera: Sphingidae) feeding on tobacco plants retain nicotine in their haemolymph, which is detrimental to the performance and survival of the endoparasitic wasp *Cotesia congregata* (Say) (Hymenoptera: Braconidae). It is difficult to find *C. flavipes* from stem borer species living on wild grasses (Le Rü B., personal communication). Such a phenomenon could be due to the unsuitability of these borers due to their immune responses or by their ingesting toxic plant metabolites that make them unfit for endoparasitoid habitation. Setamou *et al*. (2004) found the immature developmental time of *C. flavipes* to be higher on wild than on cultivated plants and that the larvae living on wild plants died faster than those on cultivated plants.

This study was conducted to understand whether the stem borer species from cultivated and wild plant habitats would be acceptable and suitable for the introduced natural enemy *C. flavipes*.

2.2 Materials and methods:

2.2.1 *Cotesia flavipes*

Specimens of *C. partellus* and *C. flavipes* were obtained from a laboratory colony established at the International Centre of Insect Physiology and Ecology (ICIPE). The colony originated from field-collected individuals in the Coastal regions of Kenya in 1998. Feral individuals were added to the colonies twice a year to maintain genetic diversity.

Parasitoid cocoons were provided by ICIPE's mass-rearing unit, collected in glass vials and kept in a clean Perspex cage for emergence.

The adults were provided with a 20% honey/water solution as diet and allowed 24 hr for mating. Afterwards they were maintained at 25^{o}C, 50-80% Relative humidity (RH), and a photoperiod of 12:12 (L:D) hr (Overholt *et al*., 1994b). One-day-old mated, naive female parasitoids were used in all experiments.

2.2.2 Host stem borers

The stem borer species used in this study and their origin is given below (Table 1). According to mitochondrial DNA analysis (P. Moyal, unpublished data), *S. nonagrioides* populations can be separated into two mitochondrial grades: one from western and another one from eastern Kenya. The west population is found on *Sorghum bicolour* whereas the east one originated from *Typha domingensis*. They will be herein referred to as 'East' and 'West'. The ones that were not obtained from ICIPE's mass-rearing unit were collected from their respective host plants in the field. All stem borer larvae were reared on the same artificial diet developed by Ochieng' *et al*., (1985) following the procedure described by Onyango and Ochieng'-Odero (1994).

Table 1. List of host stem borer species used and their origin.

Stem borer species	**Origin**
Sesamia nonagrioides (west population) Tams & Bowden	*Sorghum bicolor* [L.] Moench
Sesamia nonagrioides (east population) Tams & Bowden	*Typha domingensis* Pers
Sesamia calamistis Hampson	ICIPE mass rearing unit
Busseola phaia Bowden	*Pennisetum purpureum* Schumach
Busseola fusca Fuller	ICIPE mass rearing unit
Sciomesa piscator Tams	*Pennisetum purpureum* Schumach
Chilo partellus Swinhoe	ICIPE mass rearing unit

2.2.3 Host acceptability and suitability

The borer species from the wild and the ICIPE mass rearing unit were kept on artificial diet and reared in the laboratory for 3-4 days at 25^{o}C, 50-80% RH, and a photoperiod of 12:12 (L:D) hr to acclimatise. Only the large larvae suitable for parasitism were used for experimental purposes. On average, the number of larvae exposed to *C. flavipes* was forty except for *B. fusca* where only twenty were used. *Busseola fusca* and *C. partellus* were the negative and the positive control respectively; unparasitized larvae were used as a control to check for naturally occurring mortality. Prior to their exposure, stem borer larvae were removed from the artificial diet and fed on stem cuttings of their respective host plants for 24 hrs (Table 1); *S. calamistis*, *B. fusca* and *C. partellus* from the artificial diet were offered *Sorghum bicolor* [L.] Moench. For exposure to the parasitoids, hand stinging procedure was adopted (Overholt 1993).

A single borer held by a soft forceps was exposed to a single parasitoid in a cage; host probing was not timed because the hand stinging technique was used. As a result, observation of ovipositor insertion was assumed to correspond with acceptance. After stinging, the parasitoid was removed from the cage and the borers were put singly into vials containing artificial diet.

Larval: mortality, cocoon formation, pupation and adult emergence as well as parasitoid emergence, total progeny per host larvae, the sex ratio (proportion of female progeny) and parasitoid mortality were computed. For every stem borer species parasitized, 12.5 % of the larvae were dissected 48hrs after being parasitized to check for egg encapsulation. Reasons for mortality of parasitoid larvae were determined by dissecting the host 20 days after parasitization, if no parasitoid emerged. In addition, host larvae, from which parasitoids emerged, were dissected 2 days after parasitoid egression to look for dead parasitoid larvae. Finally, the number of parasitoid larvae that exited the host but failed to form cocoons were counted. All tests were conducted in the laboratory at 25^{o}C, 50-80% RH, and a photoperiod of 12:12 (L:D) hr.

2.2.4 Data analysis

Statistical tests were done using the Stat View software (Abacus Concept, USA). Homogeneity of variance and data normality were examined by the F-test and Kolmogorov-Smirnov method, respectively, before running the one-way analysis of variance (ANOVA). The number of parasitoid larvae dying and the proportion of female progeny were transformed to log (X+1) and arcsin (X/100) respectively, before being subjected to ANOVA. Because the number of replicates was not similar, means were separated by Turkey-Kramer test when the ANOVA was significant ($P < 0.05$). Chi-square test [contingency tables (2 x 7)] was used to compare the proportions.

2.4 Results

2.4.1 Host acceptability and suitability

Chilo partellus, S. calamistis and *S. nonagrioides* (West) were found to be acceptable and suitable for *C. flavipes*. On the contrary to the cultivated borers, no parasitoid progeny was obtained from the wild borer species: *S. nonagrioides* East, *B. fusca, B. phaia* and *S. piscator*, despite their being acceptable. Egg encapsulation was observed in the proportions of 7.5% in *S. piscator,* 12.5% in *B. phaia* and 100% in *B. fusca*. However, no parasitoid eggs were observed in *S. nonagrioides* (East). Of the borers living on *S. bicolor C. partellus* produced the highest number of progeny followed by *S. calamistis* whereas the least number of progeny was obtained from *S. nonagrioides* (West) (Table 2).

Table 2. Stem borer suitability for the development of *C. flavipes*. Means followed by different letters are significantly different at 5% level (Turkey-Kramer test following ANOVA).Number of emerging parasitoid progeny per female: F = 17.450; df = 6; P < 0.0001. Developmental time: F = 14.154; df = 2; P < 0.0001. Number of parasitoid larvae dying: F = 5.507; df = 2; P = 0.0069. Proportion of female progeny: F = 3.304; df = 2; P = 0.0449.

Host Borer	*N*	No. of emerging parasitoid prog. per female (means ± SE)	*N*	Developmental time in days (means ± SE)	No. of dying parasitoid larvae (means ± SE)	% female progeny (means ± SE)
C. partellus	37	21.5 ± 3.3c	26	19.0 ± 0.2b	1.0 ± 0.3a	72.5 ± 3.1b
S. calamistis	50	12.0 ± 2.4b	20	17.8 ± 0.1a	3.6 ± 0.7b	51.8 ± 7.3a
S. nonagrioides (West)	43	5.9 ± 2.4a	7	20.0 ± 0.5b	0.8 ± 0.6a	67.0 ± 11.7ab
S. nonagrioides (East)	40	0.0a	-	-	-	-
B. fusca	20	0.0a	-	-	-	-
B. phaia	40	0.0a	-	-	-	-
S. piscator	40	0.0a	-	-	-	-

However, the data presented for *S. nonagrioides* West stemmed from only seven females out of the total forty-three exposed. The other biological parameters were similar between *C. partellus* and *S. nonagrioides* West Kenya. The *S. calamistis* host was significantly distinguished from the others by exhibiting a shorter egg-adult development of *C. flavipes*, a higher mean number of parasitoid larvae that died in the host and a lower proportion of female progeny emerged.

2.4.2 Host fate after parasitism by *Cotesia flavipes*

Among the species: *S. nonagrioides* (East), *B. fusca*, *B. phaia* and *S. piscator* survival after being probed by *C. flavipes* was between 80-100% (Table 3).

Table 3. Host fate after parasitism by *Cotesia flavipes*

Host Borer	N	% of the host successfully parasitized	% of the host killed	% of the host alive
C. partellus	37	70.3	29.7	0.0
S. calamistis	50	40.0	44.0	16.0
S. nonagrioides (West)	43	16.3	48.8	34.9
S. nonagrioides (East)	40	0.0	0.0	100.0
B. fusca	20	0.0	0.0	100.0
B. phaia	40	0.0	20.0	80.0
S. piscator	40	0.0	5.0	95.0

All proportions were significantly influenced by the host borer factor [percentage of successfully parasitized host: $\chi^2 = 107.795$; df = 6; $P < 0.0001$; percentage of host killed: $\chi^2 = 169.056$; df = 6; $P < 0.0001$; percentage of host alive: $\chi^2 = 53.833$; df = 6; $P < 0.0001$; contingency tables (2 x 7)]. The highest percentage of larvae successfully parasitized was obtained with *C. partellus*, which also had the lowest survival. Then, the percentage of larvae successfully parasitized decreased with *S. calamistis* and *S. nonagriodes* (west population).

2.5 Discussion

The seven stem borer species exposed to *C. flavipes* were equally acceptable for oviposition, having been stung by the parasitoids except for *S. nonagrioides* East in which we did not find any eggs, we are therefore not certain whether the stinging corresponded with egg-laying. However, the suitability varied with species. Not surprisingly, *C. partellus* is the most suitable host of the *C. flavipes.* The effect of parasitism decreased on *S. calamistis* and was lowest on the *S. nonagrioides* West found on cultivated sorghum. As had already been shown by Ngi-Song *et al. (*1995), *B. fusca* was totally unsuitable for *C. flavipes* development, and so were the species from wild habitat such as *S. nonagrioides* East, *B. phaia* and *S. piscator*.

Ngi-Song *et al*. (1995) upon dissection of *B. fusca* larvae observed that larval forms of *C. flavipes* were encapsulated. In our study, dissection of stem borer larvae 48hrs after parasitism showed egg encapsulation as follows: 7.5% *S. piscator,* 12.5% *B. phaia* and 100% *B. fusca* of the exposed and dissected insects.

Although Jiang *et al*. (2004) observed up to 18% of *C. partellus* encapsulating *C. flavipes,* in this work no such observation was made and all the *C. partellus* developed fully. This corroborates the findings of Mbapila and Overholt (2001) that encapsulation is uncommon for *C. flavipes* parasitizing *C. partellus*.

In *S. nonagrioides* East, upon dissection parasitoid no eggs were found indicating that it was not an acceptable host. The mechanism by which this herbivore escapes parasitism is not known. This phenomenon could be explained in terms of the possibility of host rejection after ovipositor insertion. Parasitoid ovipositor is normally covered in sensillae and it seems likely that the insect is rejecting the host after perceiving that it is unsuitable for its development. It is likely that the wasp examines the chemical composition of the haemolymph using its ovipositor prior to oviposition (Godfray, 1994).

The findings on suitability of borers from wild and cultivated habitat agree with earlier studies which generally showed that *C. flavipes* would accept a variety of hosts in the laboratory but only complete development in a few (Beg and Inayatullah, 1980; Gifford and Mann, 1967). Furthermore, the borers from the wild habitat by virtue of their unsuitability appear to form a reproductive sink because despite their being acceptable to *C. flavipes,* the parasitoid was not able to develop.

In conclusion, the performance of *C. flavipes* on the wild habitat stem borers is poor as they are better defended against the parasitoid and have a mechanism by which they evade parasitism. Unlike the cultivated stem borers, which are susceptible to *C. flavipes* even though the performance of the parasitiod is variable.

CHAPTER THREE

Cotesia flavipes host location.

3.1 Introduction

Herbivore attack triggers metabolic changes in a plant leading to increased or *de novo* production of secondary compounds and the ultimate emission of plant volatile compounds (PVCs). The emission of these compounds has diverse effects that are beneficial and/or detrimental across tritrophic systems involving: plants, herbivores and their natural enemies. Consequently, their terminology, assigned on the basis of their effect on the receiver, is as follows: Kairomones are those allelochemicals that are pertinent to the biology of an organism (usually the borer) which, when in contact with an individual of another species (the parasitoid), evokes in the receiver a behavioural or physiological response that is adaptively favourable to the parasitoid but not to the borer. Synomones are systemic allelochemicals, which are pertinent to the biology of an organism (usually the plant) which, when in contact with an individual of another species (the parasitoid), evokes in the receiver a behavioural or physiological response that is adaptively favourable to the parasitoid (Dicke and Sabelis, 1988).

Host selection process appears to be divided into environmental and host factors that involve both chemical and physical cues. Plants have been implicated in parasitoid orientation, Vinson (1975) working with *Cardiochiles nigriceps* Viereck (Hymenoptera: Braconidae) found that damaged plant tissue plays a role in narrowing the search area for the parasitoid once situated near a potential host community. Once in a tobacco field *C. nigriceps* flies 2-3 cm above the plant, lands briefly and antennates damaged plant tissue. If the damage is mechanical or due to non-insect, the parasitoid resumes searching. However, if the damage is due to a potential host, the behaviour of the parasitoid changes from flying to crawling on the plant.

Workers conducting choice tests have made noteworthy observations on the ability of natural enemies to discriminate between plants damaged by herbivores and those that were not (Potting *et al*., 1993; Dicke and Vet, 1999). Moreover, their preference appears to be consistent with host/host plant records from the field (Rutledge and Weidenmann, 1999; Jembere *et al*., 2003). Ngi-Song *et al*. (1996; 1997) showed that *C. sesamiae* and *C. flavipes* were significantly more attracted to maize plants infested with stem borers than to their noninfested ones and that the parasitoids could not distinguish between borer species inhabiting the plant. It is well known that plant volatiles released from herbivore-damaged plants are more attractive to searching parasitoids than their undamaged counterparts (Turlings *et al*., 1991a, b; Turlings *et al*., 1998; Dicke and Vet, 1999).

As shown in the preceding chapter, the stem borer species generally encountered in the wild habitat are not suitable for the development of the parasitoid *C. flavipes* as opposed to the ones found in the cultivated habitat, with the exception of *B. fusca*.

Using the same stem borer species, this study aimed to provide evidence on the ability of *C. flavipes* to discriminate between unifested host and plants infested with their respective stem borer species. For this reason, olfactometric bioassays were conducted to assess the attraction of parasitoids to the different plant cues. A simple method was used for the analysis of the emitted PVCs arising from the infested as well as the uninfested plant species.

3.2 Materials and methods

3.2.1 Insects

Cotesia flavipes were obtained from ICIPE mass rearing unit and maintained in the laboratory under the same conditions as described in chapter two. The same stem borer species and populations used in this experiment were also provided by ICIPE mass-rearing unit or collected from the field on their respective host plants.

3.2.2 Plants

The wild plants used in these experiments were collected directly from their natural habitat; while *Sorghum bicolor* (Cv. seredo) was grown in pots for about six weeks before being used.

Individual plants were infested artificially by attaching a glass vial containing five medium-sized larvae (around the third instar) onto the stem using parafilm. The set up was left for 24 hrs, sufficient time to injure the plant. This same set-up was made for both the Y-tube bioassay and for volatile micro-extraction. Prior to the experiments, both the uninfested and infested plants were cut at the stem base and the point of incision wrapped in wet cotton wool and covered by aluminium foil, then the vials removed from the infested plants.

3.2.3 Bioassays

The Y-Tube olfactometer (Figure 1) developed by Ngi-Song *et al.* (1996) was used to test responsiveness of *C. flavipes* females to plant volatile cues. Samples were introduced into Perspex cages measuring (30 x 30 x 120 cm) one on each arm of the olfactometer. To make the system air tight, the open end of the chamber was placed over the test material standing in water held in a plastic basin.

The two chambers were connected to the arms of the Y-tube with Tygon tubing from the top of the experimental chambers. A vacuum pump (Cole-Parmer Air-Cadet, Chicago, IL) drew and pushed air into the system through activated charcoal. Clean air was drawn into the chambers containing the test material through the arms of the olfactometer. Air flow was set at 2.5 litre/min/arm and measured by flow meters connected between the chambers and the activated charcoal. The set up was left to run for thirty minutes before carrying out any tests. One-day-old naïve *C. flavipes* females were introduced singly into the olfactometer and allowed a maximum of 5 min to move into either of the two arms. The position of the tube was turned at 180º after every five parasitoids to avoid any directional bias; at the same time the odour sources were changed every ten parasitoids to minimise asymmetries-if any. Parasitoids passing the finish line (4 cm past the inter section) and remained arrested for more than 20 seconds in the olfactometer were recorded as having made a choice; those that made no choice were excluded from the analyses.

Prior to the experiment, the parasitoid was given the choice between two empty Perspex cages to check if the location of the arms affected choice. For every plant species and the associated stem borer, the choice was between infested and uninfested plant. In a second set of experiments, infested and uninfested plants were cut just above the point where the former was infested to check if the plant produced any characteristic systemic emissions upon attack by the borer.

The experiments were carried out in the laboratory at 24 ± 1.5^{o}C, 65-80%RH and a L:D 12:12 hr photoperiod and light intensity of 350-450 lux between 08:00 to 13:00 hrs. The proportions of parasitoids choosing (infested or uninfested plants) as well as the proportion that made no choice were computed.

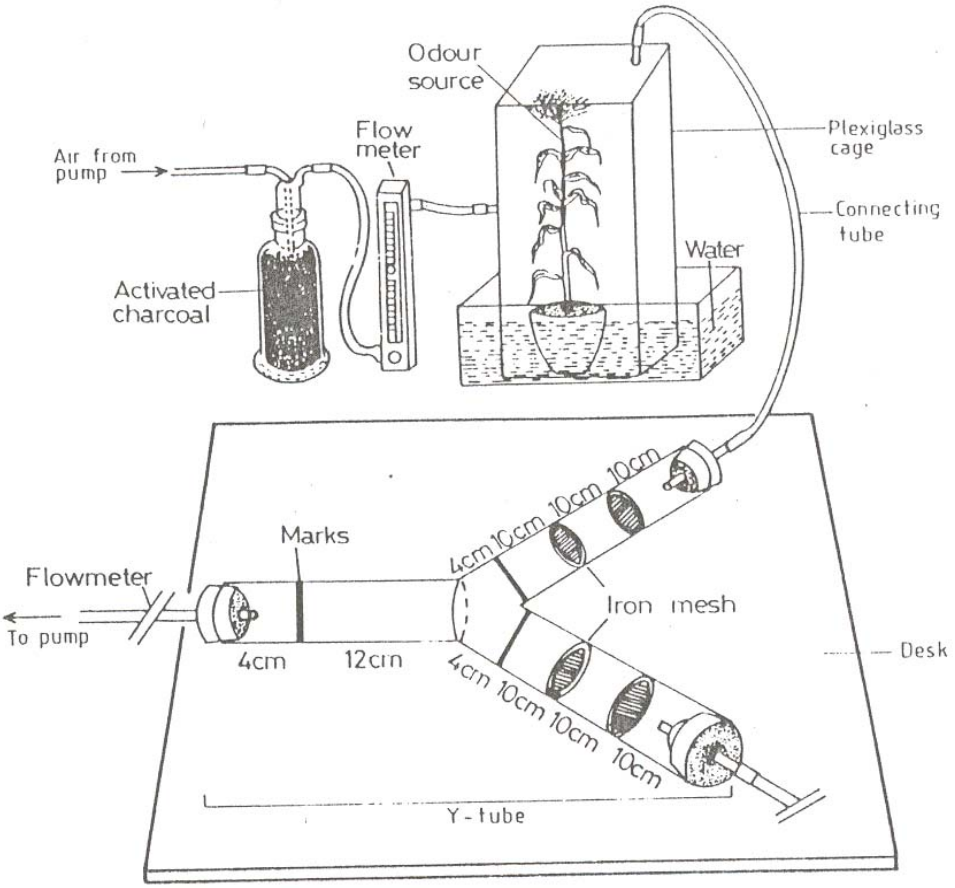


FIG. 1. A schematic diagram of the Y-tube olfactometer.

3.2.4 Collection of plant volatile compounds

Headspace plant volatile compounds (HS-PVCs) were collected by solid phase micro-extraction technique (SPME) (Figure 2). This is an extraction technique developed by Arthur and Pawliszyn (1990) and was recently addressed as an authentic sampling technique (Shang *et al*., 2002; Flamini *et al.,* 2003).

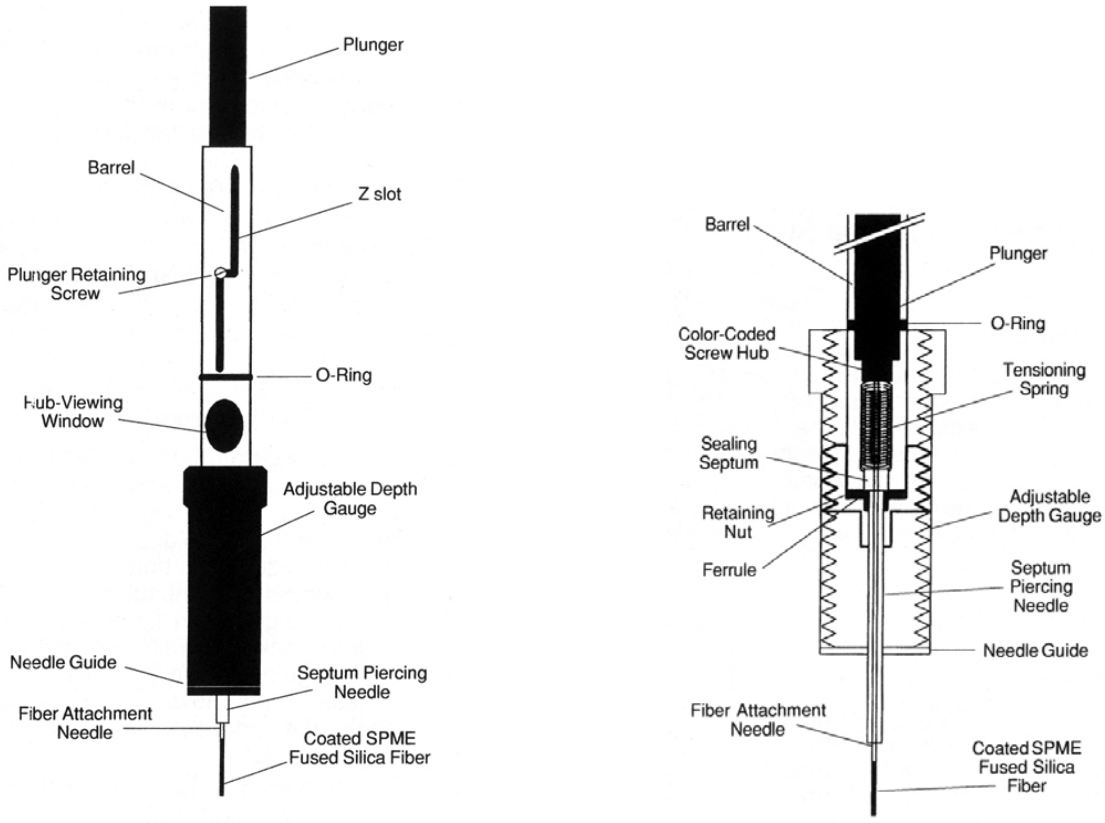
The SPME manual holder device (Supelco, Bellefonte, PA, USA) equipped with a fused-silica fibre, coated with a 65 µm layer of polydimethylsiloxane/divinylbenzene (PDMS/DVB), was used to adsorb the volatile components of the plant headspace.

For every stem borer/host-plant combination as shown in (Table 1) as well as uninfested host plant species, three plants from the field (wild plant) or in pots for *S. bicolor* were excised at the stem base at around 08:00 to 13.00 hrs, and the excision point wrapped immediately with moist cotton wool covered in aluminium foil. Samples were put in a 2 litre measuring cylinder, whose top was tightly secured by aluminium foil made to resemble a cylinder cap using parafilm. Sandwiched between the foil cap was a self sealing gas tight septum through which the SPME septum-piercing needle was driven into the plant headspace.

The SPME fibre desorbed at 250 ^{o}C for 20 mins in a GC injection liner was used for the micro-extraction of PVCs. Adsorption of plant emissions was done for two hours in the laboratory after which, the fibre was extruded from the plant headspace and immediately introduced into the GC injector port and allowed to desorb for five minutes.

Figure 2. SPME fiber holder and fiber assembly (A) with its section view (B)

A B



3.2.5 Analysis and identification of plant volatile compounds

The headspace volatile components were separated using a HP 5890 series II gas chromatograph equipped with HP capillary column (ultra-1-crosslinked methyl silicone, 50 m x 0.2 mm I.D. x 0.33 μL film thickness) using nitrogen-carrier gas at a flow rate of 0.35 ml/min with flame ionisation detector (FID) heated at 270°C. The fibre was immediately inserted into the GC injector port operating in a split less mode and the analytes thermally desorbed for 5 minutes this duration was made to correspond to the split less time. The GC temperature program used for analyses was as follows: initial temperature of 60 ^{o}C increasing to 280 ^{o}C at a rate of 5 ^{o}C/min, and maintained at 280 ^{o}C for 20 minutes.

The injection port septum purge flow was programmed to turn off and on, at the beginning and the end of the desorption process, respectively.

The identification of chromatographically separated volatile compounds was carried out by comparing GC retention times to those of authentic samples run at under the same conditions, comparison of mass spectral data to those of mass spectral libraries (WILEY and NIST registry of mass spectral data, Washington DC, USA), comparison of order of elution and by comparison of their mass spectra with those in literature (Kjaer *et al*., 1963; Spencer and Daxenbichler, 1980). The ratios of compounds were represented by GC peak areas, normalized so that the peaks of interest equalled 100%.

The test samples were compared against a control (empty apparatus) to assign chemicals specific to each treatment. The percentage relative proportion (peak area/total peak area of present compounds) was calculated; for the repetitively detected compounds and for the major compounds emitted in all plant species.

The analytes were identified using coupled gas chromatography-mass spectrometry GC-MS. Hewlett Packard 5790 gas chromatograph coupled to a VG MassLab 12-250 mass spectrometer operating in an electron impact mode (70eV). The analytes were separated using similar column and conditions as used in GC analysis.

3.2.6 Data Analysis

Statistical tests were performed by Stat view software (Abacus Concept, USA). Chi-square tests were used to compare proportions of insects responding to the plant cues in the Y-tube. The insects that did not respond were excluded from the analyses.

3.3 Results

3.3.1 Y-tube olfactometric bioassays

In two-choice olfactometric assays, volatiles from stem borer-infested plants significantly attracted more *C. flavipes* females ($P < 0.01$) than those from uninfested plants (Figure: 3).

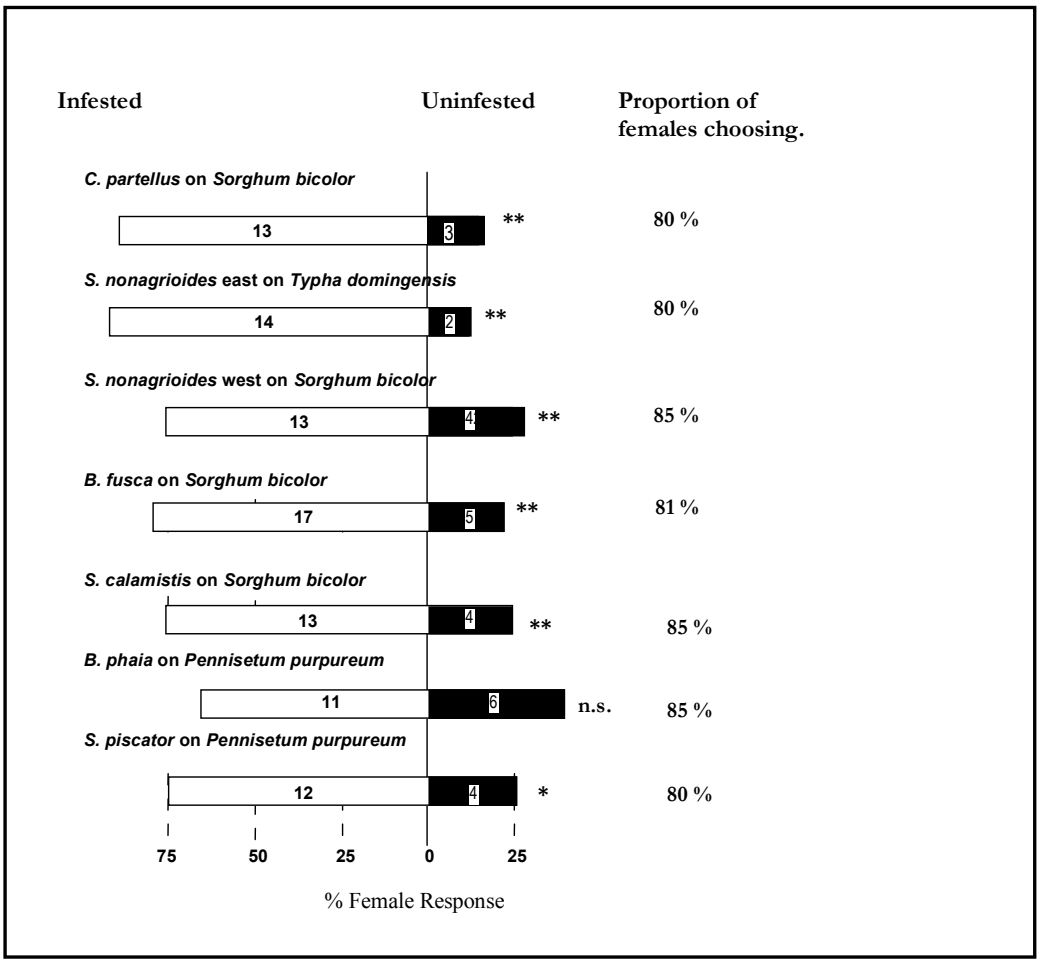


Figure 3. Response of *C. flavipes* in a Y-tube olfactometer to the odours of infested and uninfested plants. In each bar the actual number of wasps that made a particular choice is given, while the X axis indicates the percentages of choosing wasps as well as the proportion of females that made a choice given ($P<0.01$; $N=20$ for each combination, except $N=27$ for *B. fusca* on *S. bicolor*).

For *B. phaia* and *S. piscator* on *P. purpureum*, although the same trend was observed, no such statistical significance was obtained probably due to too low a number of replicates in these cases caused by limited supply of insects.

3.3.2 Analysis of volatiles emitted by uninfested and infested plants

For *S. bicolor*, the chemical profile of stem borer-infested plants obtained by GC and GC-MS analyses was richer in green leaf volatiles (C_5-C_6 alcohols and esters), terpenoids and phenols than that of uninfested plants (Figure 4).

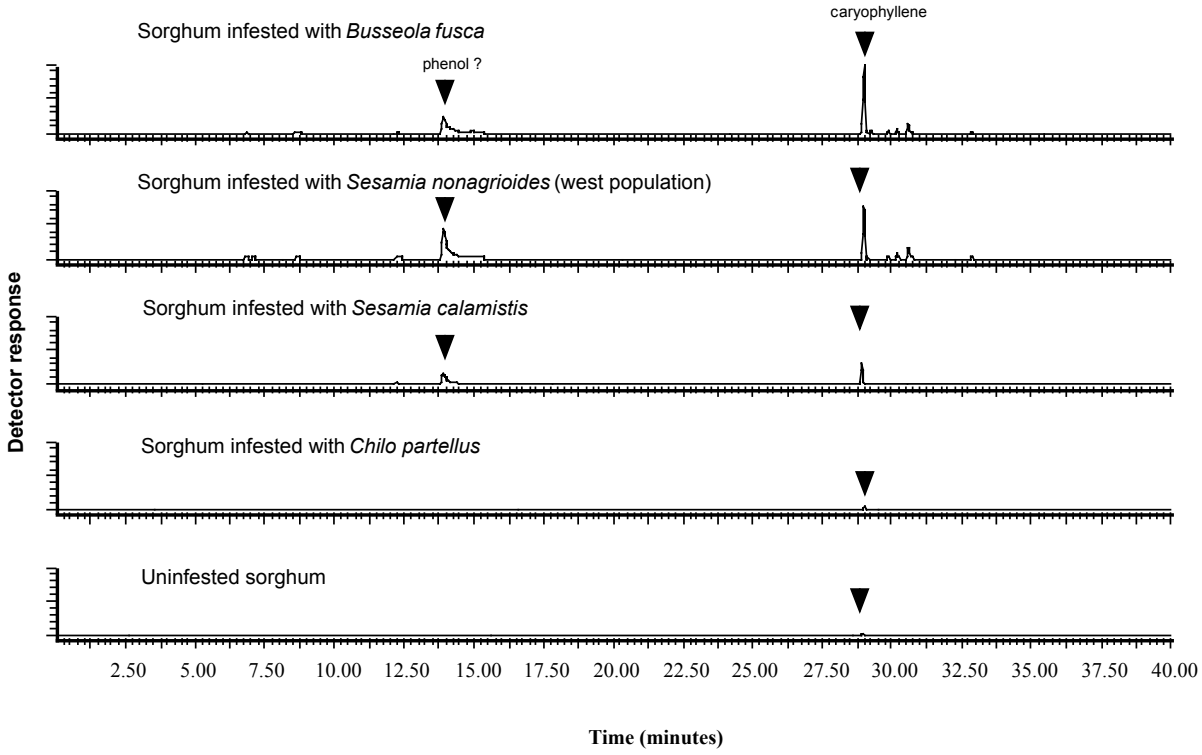


Figure 4. Chromatogram of sorghum volatiles-uninfested and infested with four respective stem borer species. Sample spectra of identified compounds compared to library spectra; and caryophyllene confirmed by injection with authentic sample.

The sample spectra of identified compounds were clearly visible upon tenfold amplification of the detector response (Figure 5).

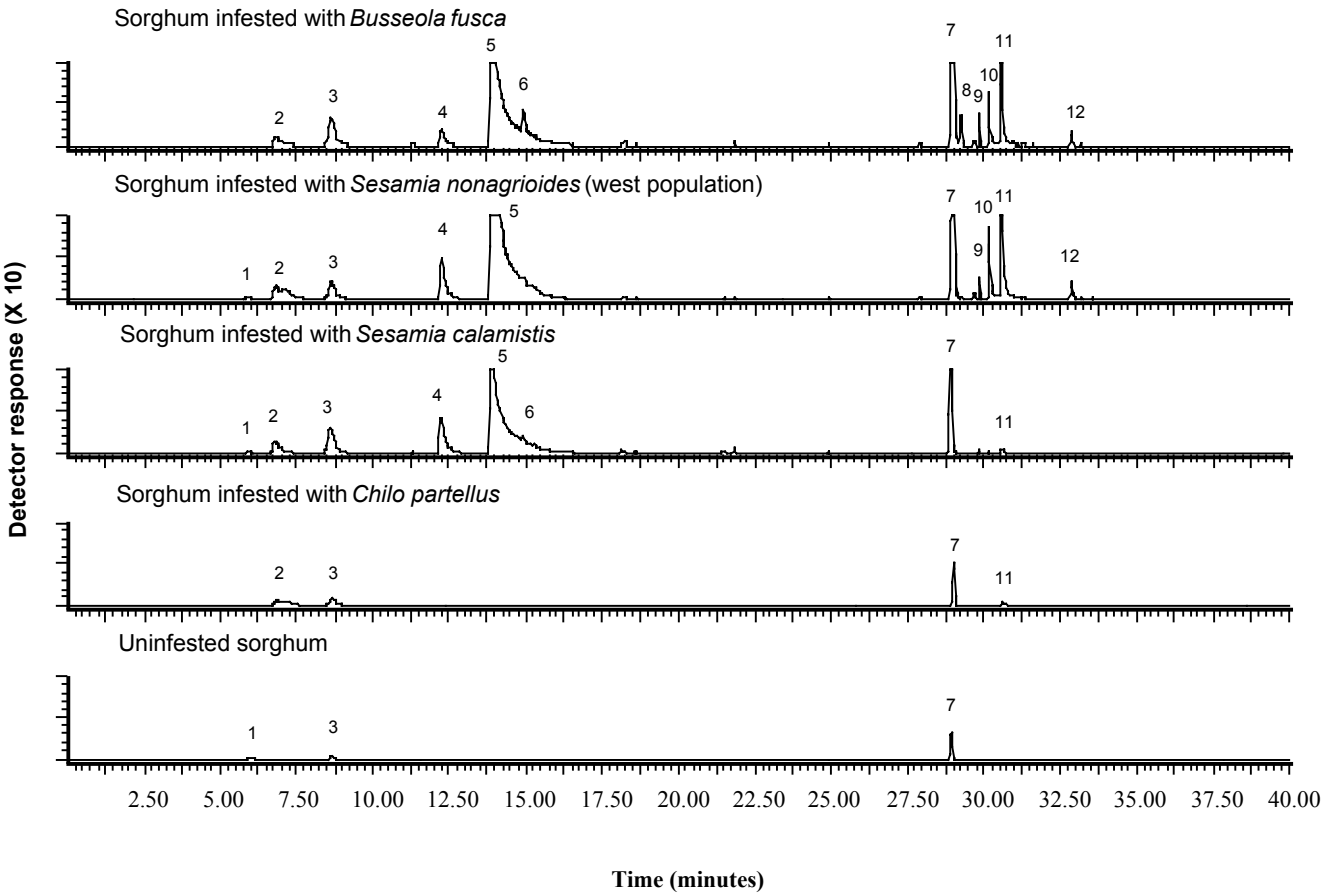


Figure 5. Representative gas chromatogram of Figure 4 enlarged (detector response x 10**)** Compounds: 1 and 2, unknown; 3, 2,3-epoxyhexanol; 4, anisole; 5, phenol; 6, (*Z*)-3-hexenyl acetate; 7, caryophyllene; 8, ß-farnesene; 9, α-humulene; 10, α-farnesene; 11, farnesene; 12, α-patchoulene. Compounds identified were compared to library spectra and confirmed by injection with authentic samples were only 6, 7 and 8.

Ten of the identified compounds, which were proposed by comparing with spectral data from the MS libraries were (a) 2,3-epoxyhexanol, (b) anisole, (c) phenol, (d) (*Z*)-3-hexenyl acetate, (e) caryophyllene, (f) ß-farnesene, (g) α-humulene, (i) α-farnesene, (j) farnesene and (k) α-patchoulene, whereas those confirmed by injection with synthetic compounds were: (a) caryophyllene, (b) ß-farnesene, and (c) (*Z*)-3-hexenyl acetate. The chemical profile of *S. bicolor* infested by *C. partellus* was not as rich as those infested by other stem borer species. This observation may be due to the small size of L3 does not induce a good amount of volatiles after only 24 hrs of infestation. In general, almost similar profile of compounds is obtained regardless of the stem borer used for infestation.

In napier grass, the chemical profile of stem borer-infested plants obtained was also richer in green leaf volatiles (C_5-C_6 alcohols and esters) and terpenoids than those of uninfested plants; however, unlike in sorghum there was no phenol production. (Figure 6).

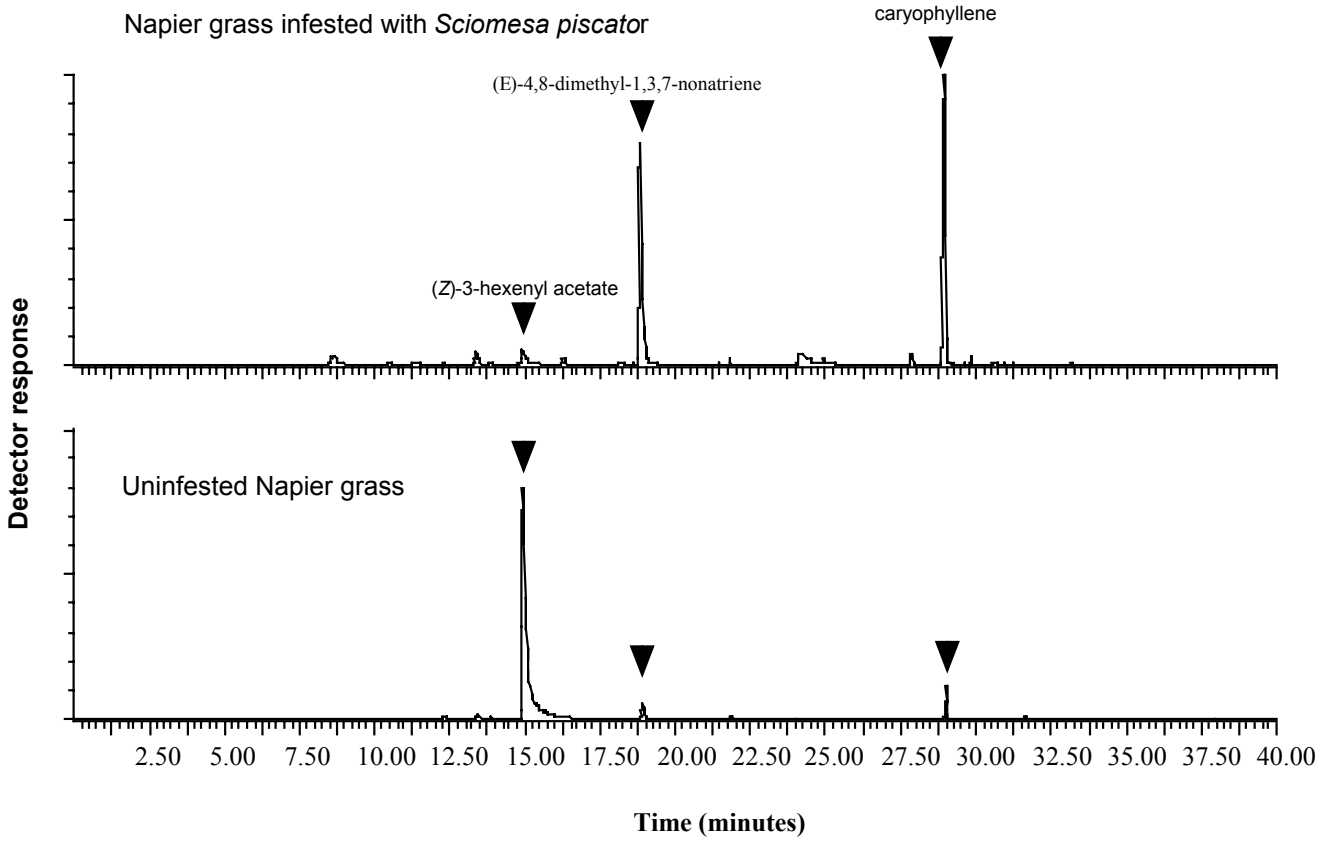


Figure 6. Chromatogram of volatiles from uninfested and infested (*S. piscator*.) napier grass.

The chemical profiles of the other compounds were visible upon tenfold amplification of the detector signal (Figure 7).

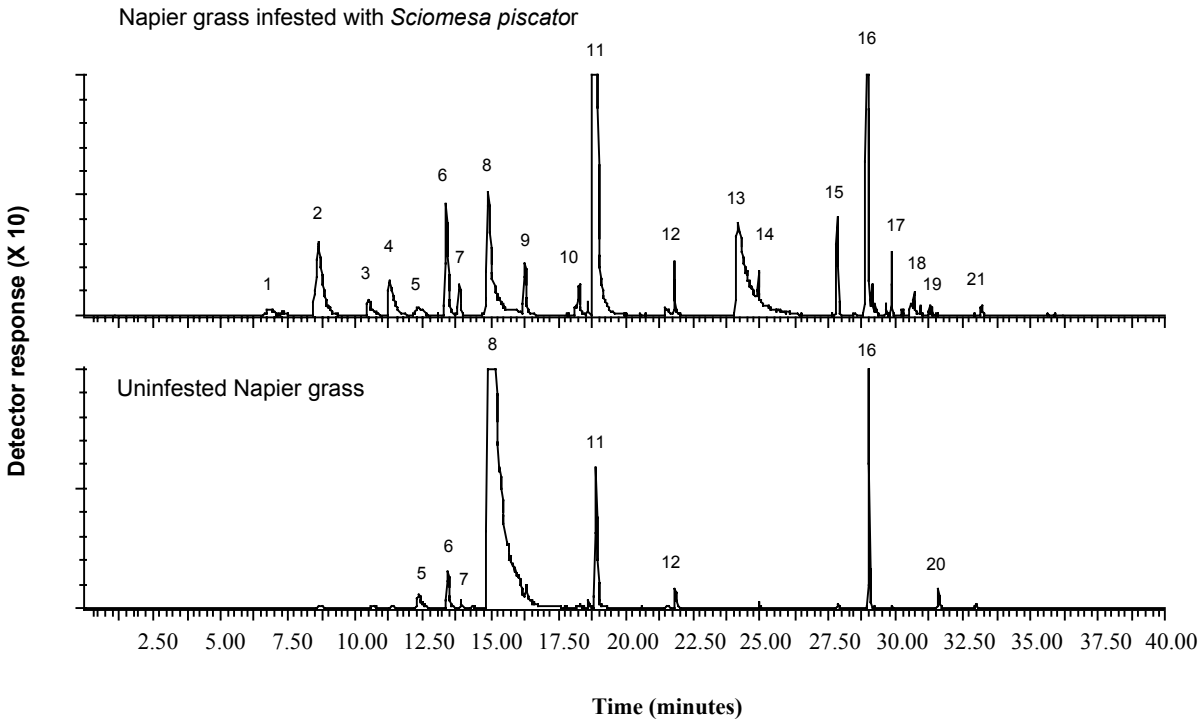


Figure 7: Representative gas chromatogram of figure 6 enlarged (detector response x 10).1, unknown; 2, 2,3-epoxyhexanol; 3, 4-cyclopentene-1,3-diol; 4, 2-ethyl-1-butanol; 5, anisole; 6, α-pinene; 7, camphene; 8, (*Z*)-3-hexenyl acetate; 9, limonene; 10, 4-methyl-1,5-heptadiene; 11, (*E*)-4,8-dimethyl-1,3,7-nonatriene; 12, n-dodecane; 13, indole; 14, 6-ethyl-2-methyldecane; 15, ß-elemene; 16, caryophyllene; 17, α-humulene; 18, 4-methyl-2,6-di-tert-butyl phenol; 19, germacrene; 20, unknown; 21, docosane. Sample spectra of identified compounds were compared to library spectra and, for compounds 6, 8, 11 and 16, confirmed by injection with authentic samples.

Nineteen compounds were identified and proposed by comparison with spectral data in the library; as follows: (a) 2,3-epoxyhexanol, (b) 4-cyclopentene-1,3-diol, (c) 2-ethyl-1-butanol, (d) anisole, (e) α-pinene, (f) camphene, (g) (*Z*)-3-hexenyl acetate, (h) limonene, (i) 4-methyl-1,5-heptadiene, (j) (*E*)-4,8-dimethyl-1,3,7-nonatriene, (k) n-dodecane, (l) indole, (m) 6-ethyl-2-methyldecane, (n) ß-elemene, (o) caryophyllene, (p) α-humulene, (q) 4-methyl-2,6-di-tert-butyl phenol, (r) germacrene, and (s) docosane. Those confirmed by injection with synthetic compounds were only, (a) α-pinene, (b) (*Z*)-3-hexenyl acetate, (c) (*E*)-4,8-dimethyl-1,3,7-nonatriene and (d) caryophyllene.

Infested sorghum and napier grass emitted; similar compounds; these were also green leaf volatiles (alcohols and ester) and terpenoids and included 2,3-epoxyhexanol, anisole, (*Z*)-3-hexenyl acetate, and caryophyllene and α-humulene.

However, unavailability of insects in the field made it impossible to analyse the combinations: *T. domingensis* infested by *S. nonagrioides* (east population) as well as *P. purpureum* infested by *B. phaia*.

3.4 Discussion

The present study demonstrates that females of *C. flavipes* were significantly more attracted to borer-infested than uninfested plants regardless of the borer or plant species used. This corroborates results by Potting *et al*. (1993), Ngi-Song *et al*. (1996) working with *C. flavipes* and *C. sesamiae* on sorghum, maize and Napier grass and Jembere *et al*. (2003) who used *C. flavipes* on the same host plants and other non host plants. In this case the herbivore induced plant volatile compounds (PVCs) attract natural enemies. This could imply that the volatile compounds are pertinent to the biology of the plant and their production is typically induced by herbivory. Their effect is beneficial to the third trophic level (the parasitoid) as they aid it in host finding. It is sufficient to assume that these compounds are pertinent to the plant as other workers working on other plant-borer species combination have found same compounds. Kessler and Baldwin (2001) working with *Nicotiana attenuata* plants infested by three different herbivore species. Flamini *et al*. (2003) obtained these same compounds from uninfested flowers and flower parts of *Citrus deliciosa* Ten (Rutaceae).

The present study has identified the compounds that are emitted during plant infestation. As a result, further research ought to aim at finding the exact source of these compounds; whether the borer itself or the plant. Further experiments should also show whether these compounds are systemic and thus arise from all the plant parts. Notably, the time of infestation before the plant parts are used in the olfactometer should be sufficient to induce a systemic plant volatile emission. Basing on the classification by Dicke and Sabelis (1988); whether or not these compounds are Kairomones is unclear because they act on the third trophic level. Thus, studies are necessary to ascertain their effect on the herbivore (second trophic level)

The ability of host-seeking insects to recognise and respond to such chemical cues and differentiate them from background odours indicates that insect-damaged plants emit volatile compounds that are clearly distinguishable from those released from undamaged plants. The profound difference in the emission profile from the uninfested and the infested plants evidenced that herbivory induced the emission of PVCs.

It is well documented in literature (Turlings *et al*., 1991 a, b; Tumlinson *et al*., 1992) that plant odours play an important role in communication in tritrophic systems; the regions of damage normally release small amounts of volatile compounds that increase markedly upon herbivore infestation. These phyto-distress signals which result in an active interaction between herbivore-damaged plants and a third trophic level, have been described for several agro-ecosystems including more than 15 plant species involved in plant-spider mite-predatory mite, plant-caterpillar-parasitoid systems (Dicke and Van Loon, 2000**).**

Ngi-Song *et al*. (1997) reported that *C. flavipes* was not able to differentiate between maize and Napier infested by different borer species. This could be explained by the fact that both plant species produced very closely related blends of compounds but the differences were in the amount of volatiles produced. In napier grass, we found that caryophyllene, (*E*)-4,8-dimethyl-1,3,7-nonatriene and (*Z*)-hexenyl acetate were present in both infested and non infested plants but they differed in quantities. Ngi-Song *et al*. (2000) found the latter two compounds to be electro-physiologically active on the antennae of females of *C. flavipes*. These workers also showed that a blend containing such green leaf volatiles and terpenoids emitted in Figures 5 to 7 at increased doses emitted from infested plants are attractive to females of *C. flavipes*.

Different feeding habits inflict different types of damage on plants leading to the production of different quantity and/or quality of volatile compounds (Turlings *et al*., 1998).

Herbivore infested plants emit volatiles that are derived mainly from three biochemical pathways: green leaf volatiles C_6-alcohols and aldehydes, emitted rapidly after damage and produced from α-linoleic acid and linoleic acid via their hydro-peroxides in the octadecanoid pathway; terpenoids derived from the two isoprenoid pathways (mevalonate and nonmevalonate) are emitted slowly, typically 24 hrs after attack (Kessler and Baldwin 2002). Finally, the third pathway involves the shikimate pathway whose products transiently increase immediately after herbivore attack (Kessler and Baldwin 2001). Our results indicate that the chemical profile of stem borer-infested plants was comparatively richer in green leaf volatiles and terpenoids than uninfested plants corroborating results by Turlings *et al*. (1991 a, b), Tumlinson *et al*. (1992), and Takabayashi *et al*. (1995).

In conclusion, *C. flavipes* cannot discriminate between plant species infested by different suitable or unsuitable stem borer species. When infested plants were offered to the insect in a cage, they landed on the plant and entered the tunnel (Meshack, pers. observations). It is possible that determination of the suitability of hosts occurs once the parasitoid encounters the host inside the tunnel, preventing it from ovipositing. However, Potting *et al.* (1993) and Takasu and Overholt (1997) reported that *C. flavipes* were often killed in the maize stem when foraging for *C. partellus*, probably by biting or spitting of the host. Thus, following any host into the tunnel may act as a reproductive sink, although in the laboratory the parasitoid attacks several hosts.

CHAPTER FOUR

CONCLUSION

4.1 Conclusion

Invasive species threaten natural habitats world wide, and active human management is required to prevent invasion, contain spread, or remediate ecosystems following habitat degradation. One effective technology for invasive species management in sensitive habitats is biological control: the use of carefully selected upper-trophic–level organisms that utilise the exotic pests as a resource thereby reducing it to less harmful densities. Most biological control programs address the following simple premise: an exotic organism becomes an invasive pest, in part, because it has left behind the guild of natural enemies that regulated its population growth in its home range (Hoddle, 2004). The most fundamental question in considering deliberate introduction of exotic species is whether the outcomes can be precisely enough from known causes to imagined effects to know with certainty that the benefits will outweigh the environmental costs.

The braconid larval parasitoid *C. flavipes* was introduced into Kenya from Pakistan and India for the control of the exotic crambid stem borer *C. partellus*, to complement the action of the native parasitoids. In Africa, maize fields are usually surrounded by land occupied by wild gramineous plants. Many of the wild plants harbour borer species not found on crops. It is not known if the exotic parasitoid *C. flavipes* can follow these borers into the wild habitat, and whether they are suitable for its development. In this context, the purpose of this study was to assess the host foraging ability of, and suitability of some borers (cultivated and wild) for the development of *C. flavipes*. For this reason, we also identified plant volatiles compounds that could mediate host finding.

Seven stem borer species were used: *Chilo partellus* and *Busseola fusca* (control species), *Sesamia calamistis*, *S. nonagrioides* western Kenya mostly found on cultivated crops; as well as: *Busseola phaia*, *S. nonagrioides* (east population) and *Sciomesa piscator* mostly found on wild grasses. All these stem borer species exposed to *C. flavipes* were equally acceptable for oviposition, having been stung by the parasitoids except for *S. nonagrioides* east in which we did not find any eggs.

The parasitoid appears to have evolved the ability to find the habitat using an array of chemical information from the plants. They are more attracted to the stem borer-infested plants than to their uninfested counterparts regardless of the stem borer and the plant species used. This is probably due to a richer chemical profile from infested plants -chiefly composed of green leaf volatiles and terpenoids over the healthy plants.

Despite her foraging ability, *C. flavipes* is still not well adapted to develop on the herbivores living in the wild habitat. It is evident that *C. flavipes* would accept a variety of hosts in the laboratory but complete development in a limited few. Wild borers by virtue of their unsuitability, form a reproductive sink. This is disadvantageous as the parasitoid will not establish on these areas.

The mechanism by which the parastoid recognises her host is not known and whether *C. flavipes* rejects the wild habitat stem borers (in particular *S. nonagrioides* east population) after internal examination are potential research areas. The role of the wild habitat in the perennation of stem borer natural enemies still lies unknown.

4.2 Recommendations

In this study, the borer species generally found in the cultivated habitat (*C. partellus*, *S. calamistis*, *S. nonagrioides* (west population) and *B. fusca*) were obtained from ICIPE's mass rearing unit and maintained on artificial diet and not on their natural host plants. We therefore recommend that the same experiments on suitability be carried out under the same conditions using borers grown on their natural host plants. It is also requisite to complement these findings with the analysis of *Typha domingensis* infested by *S. nonagrioides* (east population) as well as *Pennisetum purpureum* infested by *B. phaia* which were not available at the time of this study due to adverse weather conditions.

Further research should aim at finding the exact source of these compounds; whether the borer itself or the plant. Supplementary experiments should also show whether these compounds are systemic and thus arise from all the plant parts. Furthermore, research ought to be directed towards dose-dependent response of the parasitoid to each of the identified chemical signals. This would not only identify the most potent attractant in the mixture and also establish the effective concentration ranges of the identified compounds.

In view of these findings, it appears necessary to determine the mechanism by which *C. flavipes* recognises her hosts as well as the chemicals involved in its oviposition. In particular, it will be useful to develop a tool for analysing the egg oviposition by the parasitic wasp (host acceptance) into the larvae whether suitable or not.

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