

**ASSESSMENT OF KAIROMONES AS A MONITORING TOOL FOR
COTESIA FLAVIPES CAMERON (HYMENOPTERA: BRACONIDAE)**

BY

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**A thesis submitted in partial fulfillment for the award of the degree of
Master of Science in Biotechnology of Kenyatta University**

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DECLARATION

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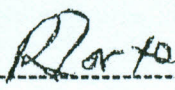
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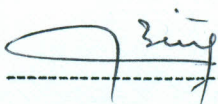
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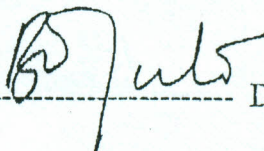
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DEDICATION

My parents, Mr. and Mrs. Harrison Ngumbi
 whose love, care and support have been fountains
 of inspiration to my chosen career.

ABSTRACT

Chilo partellus (Swinhoe) is a major pest of maize in East Africa. Its indigenous natural enemies are unable to regulate its densities to a level acceptable to farmers. *Cotesia flavipes* Cameron (Hymenoptera: Braconidae), a larval parasitoid of *C. partellus* now established in Kenya, was released in 1993 from Pakistan for the control of this pest. A major constraint in evaluating the establishment of *C. flavipes*, is the great amount of time spent for its field collection and identification. Use of traps may facilitate the assessment of the establishment and spread of this parasitoid. A number of chemical compounds released by *C. partellus* infested maize plants influence the searching behaviour of *C. flavipes*. This work investigated the potential of these kairomones and other green leaf volatiles for use as bait in traps for the purpose of monitoring the establishment of *C. flavipes* in released areas.

The attraction of these kairomones, which include (Z)-3-Hexenylacetate, (E)- β -Farnesene, 4,8-Dimethyl-1,3,7-nonatriene, Thomac oil (natural oil containing 85% (E)- β -Farnesene), heptanal, and a blend of (Z)-3-Hexenylacetate and Thomac oil (1:1), to *C. flavipes* female parasitoids was studied in the Y-tube olfactometer. Results showed that parasitoids were attracted to all the kairomones; however, this attraction varied with the dose of the kairomone. Odours from host plant complex (HPC), obtained from maize stalk in which *C. partellus* had been feeding for 24 hours and parasitoid diet (20% honey/water solution) were also very attractive to *C. flavipes*.

A preliminary field test evaluating the delta trap baited with HPC for its potential in trapping *C. flavipes* under field conditions showed that *C. flavipes* was caught in 2 out of the 12 fields used for the study, suggesting that with some improvement an effective trap could be developed for use as a monitoring tool for *C. flavipes*. To improve on the trap catches, different other traps including water traps, delta traps, plastic plates, plastic cups and modified delta traps, and different baits including parasitoid diet and HPC were evaluated. The vertical sticky trap was the most effective both in the laboratory and under semi-field conditions; however, no difference was observed between the two baits tested. The effect of the number of traps placed in a cage on the number of insects caught was assessed. A positive correlation between the density of traps and the number of *C. flavipes* trapped in a cage was noted.

The vertical sticky trap baited with different kairomones was also evaluated under semi-field conditions for 8 hours. Results indicated that all the kairomones tested were able to attract *C. flavipes* to the trap. A blend of (Z)-3-Hexenylacetate and Thomac oil (1:1) (150 μ g per disc) attracted more parasitoids.

In conclusion, traps baited with herbivore-induced kairomones can effectively be used in trapping *C. flavipes* under semi-field conditions; however their effectiveness under large field areas needs to be investigated before they are used as bait in traps for monitoring purposes. Evaluation of the trap and monitoring of *Cotesia flavipes* could also be done using HPC or parasitoid diet as baits since they are inexpensive and are attractive to the parasitoid.

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CHAPTER 1

1.0 General introduction

Maize (*Zea mays* L.) is a major food crop for millions of people in eastern Africa (Warui and Kuria, 1983; Minja, 1990). In Kenya alone, 1.4 million hectares of maize were estimated to be under cultivation between 1994 to 1998, with an average total grain production of 2.5 million tones per year; this resulted in an average grain yield of approximately 1.8 tones/ ha (Table 1) (FAO, 1999). The crop is primarily grown by subsistence farmers and provides food, animal fodder, and building materials. The production of maize is low due to several constraints including climatic factors, plant diseases, weeds and damage due to insect pests.

Lepidopteran stem borers are insect pests of maize, which are considered to be geographically wide spread. They are the most damaging insect pests of maize in East Africa (Ingram, 1958; Nye, 1960; Youdeowei, 1989). Nearly all farmers report stem borers in their fields (Chitere and Omolo, 1993; Grisley, 1997; Bonhof *et al.*, 1997). Yield losses due to these pests fluctuate around 14% (De Groot, 2001). Most stem borer species inflict similar injury to host plants. Newly hatched larvae feed initially by scrapping in the leaf whorl of young plants producing characteristic 'window panning' and 'pin holes'. Later the larvae tunnel into the stem and larval feeding might result in the destruction of the growing point typically referred to as 'dead hearts'. At later stages the tunneling and girdling activities of the larvae often result in stalk breakage. Plants thus have a poor growth

Table 1. Area under maize production, yield per hectare, total production, total human population, production per capita and production losses due to stem borer damage (assuming 15% yield loss) of Kenya, Tanzania and Uganda and of the whole Africa. Figures are averages of 1974 to 1978 and 1994 to 1998 (FAOSTAT Statistics database)

Country	Period	Area (ha x 10 ⁶)	Yield (tonnes/ha)	Total production (tonnes x 10 ⁶)	Total human population ^a (x 10 ⁶)	Production (Kgs) per capita	Production loss per capita due to stem borers
Africa	1974-1978	1.86	1.46	2.71	413.89	5.36	0.94
	1994-1998	2.57	1.56	4.04	719.50	5.62	1.35
Kenya	1974-1978	1.50	1.47	2.22	13.74	161.57	28.5
	1994-1998	1.44	1.75	2.52	27.15	92.82	13.92
Tanzania	1974-1978	1.22	1.09	1.34	15.90	84.28	12.55
	1994-1998	1.72	1.40	2.40	30.03	79.92	12.03
Uganda	1974-1978	0.45	1.25	0.57	11.18	50.98	7.55
	1994-1998	0.58	1.38	0.80	19.69	40.63	6.10

^aTotal population based on figures for 1975 or 1995.

and reduced yields and are more susceptible to wind damage and secondary infections (Seshu Reddy, 1998). Larvae also bore into the maize cobs and feed on the developing grains.

In East Africa a complex of 12 stem borer species has been reported from cereal crops, with *Chilo partellus* (Swinhoe), *Chilo orichalcociliellus* (Strand), *Busseola fusca* (Fuller), *Sesamia calamistis* (Hampson), *Sesamia cretica* (Lederer), and *Eldana saccharina* (Walker) being the most important and widely distributed species (Nye, 1960; Youdeowei, 1989; Seshu Reddy, 1998).

Chilo partellus is indigenous to Asia and its first record in Africa was in Malawi in 1932 (Tams, 1932). Since the early 1950's, it has become well established as a pest throughout East and Southern Africa (Mohyuddin and Greathead, 1970; Sithole, 1989; Harris, 1990; Overholt *et al.*, 1994a; Kfir, 1997a). Recent surveys indicate that in Malawi, Kenya, Uganda, and Zambia, *C. partellus* accounted for over 75% of the stem borer population at lower altitudes (Overholt *et al.*, 1994a). At higher altitudes, *B. fusca* is the most important stem borer species (Sithole, 1989) although reports from South Africa suggest that *C. partellus* is gradually replacing *B. fusca* at higher elevations (Kfir, 1997b). *Eldana saccharina* is widely spread South of the Sahara and is known as a stem borer of sugar cane although maize and other cereals are also attacked.

Sesamia calamistis is widespread but usually not abundant while *Sesamia cretica* occurs mainly in the savanna regions of Northeast Africa and Northern Kenya. *Chilo orichalcociliellus* occurs at altitudes lower than 300m. There is evidence that *C. orichalcociliellus* is being displaced by *C. partellus* in Kenya (Overholt *et al.*, 1994a; Ofomata *et al.*, 1999; 2000).

Different control measures have been used in an attempt to reduce losses due to these stem borers. These include cultural practices, use of host plant resistance, chemical and biological control. Chemical control is the commonly recommended method for stem borer control and research has shown its efficacy (Mathez, 1972; Warui and Kuria, 1983). However, pesticides must be applied frequently due to continuous infestation and the relatively short time larvae are exposed. Chemical control is time consuming and expensive. It is therefore not appropriate for the subsistence farmers of Africa. Commercially produced alternative pesticides for example the products from the neem tree (*Azadirachta indica* A. Juss) or the bacteria, *Bacillus thuringiensis* Bercher, have potential (Brownbridge, 1991; ICIPE, 1995) but these products are not yet readily available and may be costly too (Mihm, 1994).

Farmers, for various reasons, have used cultural practices such as intercropping with non-cereals and early planting. The efficiency of these cultivation practices against stem borers is often questionable. Some effective cultural control strategies have been identified, but not all are feasible for subsistence farming systems. Other effective yet labour-intensive practices include the removal of infested plants, planting of trap or repellent crops (Khan *et al.*, 1997) and deep tillage. Host plant resistance is promising but agronomically acceptable maize varieties with adequate levels of stem borer resistance are not yet available (Leuschner *et al.*, 1985; Nwanze and Youm, 1994). An extensive review of these and other cultural control methods is given by Van den berg *et al.* (1998).

There is renewed interest in the use of biological control agents to reduce stem borer population densities. Information about a wide range of egg and larval parasitoids of stem borers and the occurrence of predators, nematodes and microbial pathogens is available (Bonhof *et al.*, 1997). The most abundant and widespread parasitoids in the East Africa region are the egg parasitoids *Telenomus* spp. and *Trichogramma* spp., the larval parasitoids *Cotesia sesamiae* (Cameron) (Hymenoptera: Braconidae) and *Sturmiopsis parasitica* (Curran) (Diptera: Tachinidae) and the pupal parasitoids *Pediobius furvus* Gahan (Hymenoptera: Eulophidae) and *Dentichasmias busseolae* Heinrich (Hymenoptera: Chalcididae) (Bonhof *et al.*, 1997). Ants, spiders and earwigs are believed to cause high mortality on stem borer eggs and young larvae (Mohyuddin and Greathead, 1970; Girling, 1978; Oloo, 1989; Bonhof, 1998). Nematodes and microbial pathogens have been reported to infect all life stages, but their impact is low under natural conditions (Odindo *et al.*, 1989). Indigenous natural enemies are not able to keep stem borer populations below economic injury levels (Oloo, 1989; Overholt *et al.*, 1994a).

Due to the low impact of native natural enemies on the stem borer populations, a classical biological control program was initiated in 1968. Nine parasitoids were released in Kenya, Tanzania and Uganda but none of the species established (CIBC, 1968-1972) and stem borers continued to be an important pest (Sithole, 1989; Overholt *et al.*, 1994a; Kfir, 1997b). In 1990, a second biological control programme began in Kenya. The exotic larval parasitoid *Cotesia flavipes*

Cameron (Hymenoptera: Braconidae) was released in the coastal area of Kenya in 1993. The parasitoid has now established at the coast (Overholt *et al.*, 1994a), south western Kenya and western Tanzania (Omwega *et al.*, 1995, 1997). Parasitism by *C. flavipes* has been rising steadily and may eventually become an important factor in some areas (Overholt *et al.*, 1997). *Cotesia flavipes* has been released and will be released in several other Eastern and Southern African countries.

The level of success of classical biological control programme has been attributed partly to the searching efficiency of the natural enemy. The ability of a natural enemy to locate its host in a complex environment is critical to successful establishment in a new environment (Nordlund *et al.*, 1988). It is known that both host and host plants play an important role in host selection by parasitoids. Chemicals emanating from host plants are utilized by parasitoids in locating and recognizing their host (Nordlund, 1981). Volatile chemicals that attract parasitoids over long and short distances could be emitted by the host habitat or host itself or by the host by-products. Knowledge of the nature of these chemicals and their role in host location and recognition is important in the effective design of biological control programmes (Lewis and Martin, 1990).

A major constraint in evaluating the establishment of *Cotesia flavipes* after its introduction to Kenya in different areas is the great amount of time spent in its field collection and identification. Sampling of *C. flavipes* involves collection of larvae from host plants and the rearing of the collected larvae until the wasp emerges. Previous work has shown that the parasitoid responds strongly to various

maize-produced kairomones. *Cotesia flavipes* is able to search for and locate its host *C. partellus* in maize following odours released from its habitat (Ngi-Song, 1995). Some of the volatile compounds from maize plants infested with *C. partellus* that were found to be attractive to *C. flavipes* are: (E)-(β)-farnesene, (Z)-3-Hexenylacetate, (E, E)-4,8-dimethyl-1,3,7-nonatriene, anisole and heptanal (Ngi-Song, 1995; Ngi-Song *et al.*, 2000). The use of traps baited with these kairomones may facilitate the assessment of the establishment and the spread of the wasp. The assessment and optimization of these kairomones as candidate tools for monitoring *C. flavipes* will help provide information on the versatility of the attractants. The development of suitable traps for monitoring *C. flavipes* could therefore reduce the amount of time spent in collecting and rearing stem borers to evaluate establishment.

1.1 Justification

After the introduction of *Cotesia flavipes* into Kenya for the bio-control of *Chilo partellus*, a major constraint in evaluating the establishment of this parasitoid in different areas is the amount of time spent in its field collection and identification. Sampling of *C. flavipes* involves the collection of the larvae from host plants and the rearing of the collected larvae until the wasp emerges. *Cotesia flavipes* is currently being released in several other countries in Eastern and Southern Africa. A quick method to measure establishment is required. Previous studies have shown that the parasitoid responds strongly to various maize-produced

kairomones. The assessment and optimization of the kairomones attractive to *C. flavipes* will help to develop suitable traps for use in evaluating the establishment of the parasitoid in the areas of its release. Time spent in collecting and rearing the stem borers to evaluate establishment could therefore be reduced. In addition, it will provide information on the versatility of these attractants.

1.2 HYPOTHESIS

Chemical analysis and bioassays have shown that the stem borer damaged plants are a source of compounds that attract *C. flavipes*. It is thus hypothesized that these compounds that attract *C. flavipes* can be used as bait in traps that could be used for the purpose of monitoring establishment of this parasitoid.

1.3 Objectives

The main objective of the present study was to assess the efficacy of herbivore-induced kairomones from maize plants for use as bait in traps for the purpose of monitoring the establishment of *C. flavipes* in released areas.

Specific objectives:

1. To assess attraction of different maize volatile compounds to *C. flavipes* at different doses in the laboratory.
2. To explore methods to optimize the efficacy of five types of traps.
3. To evaluate potential of kairomone-baited traps in semi-field conditions.

CHAPTER TWO

LITERATURE REVIEW

2.1 Biology and distribution of stem borers

Stem borers, which attack cereals, are primarily Lepidoptera belonging to the families Crambidae, Pyralidae and Noctuidae (Bleszynki, 1969; Harris, 1990). Lepidopteran stem borers are considered to be the most damaging insect pests (Nye, 1960; Youdeowei, 1989).

In East Africa, a complex of 12 stem borer species has been reported from cereal crops with *C. partellus*, *C. orichalcociliellus*, *B. fusca*, *S. calamistis*, *S. cretica* and *E. saccharina* being the most important and widely distributed species (Nye, 1960; Youdeowei, 1989; Seshu Reddy, 1998).

The life cycle of the stem borer is completed in 25-50 days when conditions are favourable (Harris, 1990). *Chilo partellus* moths emerge in the late afternoon and early evening. Mating occurs soon after emergence and on two to three subsequent nights (Berger, 1989), egg batches of 10-80 overlapping eggs are laid on the undersides or upper sides of leaves, often near mid ribs (Pat and Ekbohm, 1994). The fecundity of *C. partellus* is approximately 434 eggs per female (Berger, 1989). Adults live for approximately two to seven days (Alghali, 1988) and normally do not disperse far from the emergence sites. Eggs hatch in the early morning (6.00 to 8.00 h), four to eight days after being oviposited (Berger, 1989).

Young larvae ascend plants to enter the leaf whorls, where they start to feed. Older larvae tunnel into the stem tissue and pupate after feeding for 2-3 weeks, unless they go to quiescence. Moths eclose from the pupae after 4-8 days. During the growing season, three or more successive generations may develop. Although the life cycle may be continuous when favourable conditions for host plant growth exist, it is usually interrupted by a cold or dry season. To overcome this period, the mature larvae enter diapause inside old stems or stubble, and pupate on return of favourable conditions (Scheltes, 1978).

2.2 Parasitoids of stem borers

Despite their cryptic lifestyle, stem borers do not escape parasitism. Parasitoids have evolved several amazing strategies to attack their concealed hosts inside the plant stem. Smith *et al.* (1993) divided the attack tactics of parasitoids of stem borer larvae into six categories. Parasitoids with the 'probe and sting' tactic, probe with their ovipositor into the leaf sheath to find early instar larvae. Other species probe through the exit hole of the tunnel to find mature larvae. A related tactic is the 'wait and sting' strategy, where the parasitoid inserts her long ovipositor through one of the tunnel holes and then waits till the host larvae passes by and is close enough for oviposition. Parasitoids with the 'drill and sting' strategy have long and strong ovipositors to parasitize the host at a distance, from outside the stalk. Several tachnid parasites have the 'planidial ingress' tactic, where the female larviposits a mobile maggot at the tunnel entrance that will actively search

for the host larva. Other tachnid parasitoids with the 'bait and wait' tactic lay their eggs at the tunnel entrance and require that the eggs be ingested by the host for parasitism to occur. Finally, some small parasitoid species with the 'ingress and sting' tactic are small enough to enter the tunnel and parasitize the host there. *Cotesia flavipes*, is a parasitoid with the 'ingress and sting' tactic. Polaszek (1997) has written a review of parasitoids attacking stem borers.

2.2.1 *Cotesia flavipes*

The biology of *Cotesia flavipes* has been studied by several workers (Moutia and Courtois, 1952; Li, 1965; Gifford and Mann, 1967; Kajita and Drake, 1969; Mohyuddin, 1971; Shami, 1990). Briefly the adult *C. flavipes* is a small wasp about 3-4 mm in length which lives for only a few days. Females lay about 15-65 eggs within the host body and eggs hatch after 3 days. The larvae develop through three instars within the host where they feed on body fluids. The egg-larval period takes 10-15 days depending on the temperature. The last instar of the parasitoid emerge from the host by chewing their way through the stem borer integument after which they immediately spin cocoons and pupate. Adult parasitoids emerge 6 days later depending on the temperature.

2.3 Host selection in insect parasitoids

Doutt (1964) and Vinson (1976) divided the process resulting in successful parasitism by insect parasitoids in to four steps: host habitat location, host location,

host acceptance and host suitability. The first three steps constitute the host selection process. In each of these steps, the female parasitoids often use chemical stimuli to guide her in the search of a suitable host. Tritrophic interactions that involve plants, herbivores and natural enemies are to a large extent intricate arrays of chemical substances referred to as allelochemicals. Plant substances that are involved in communication can have direct and / or indirect, and beneficial or detrimental effects on herbivores and their natural enemies. Likewise substances from herbivores and their natural enemies can influence other trophic levels. Elucidating these relationships of allelochemicals increases our understanding and provides potential for the manipulation of these communication systems (Whitman, 1988). Parasitoids use many strategies to locate their potential hosts and this depends on the type of semiochemicals provided by the host or its environment.

Semiochemicals are chemicals that mediate inter-specific (allelochemicals) and intra-specific (pheromones) interactions (Nordlund and Lewis, 1976). These semiochemicals are divided into two groups: pheromones, which mediate interactions between organisms of the same species, and allelochemicals, which mediate interspecific interactions (Nordlund and Lewis, 1976). These chemicals are categorized based on the nature of the interaction (producer/acquirer) and cost benefit analysis (Nordlund and Lewis, 1976; Dicke and Sabelis, 1988a). Dicke and Sabelis (1988a) proposed the term 'infochemicals' for information conveying chemicals. The infochemical terminology is given in Table 2.

Table 2. Infochemical terminology (Dicke and Sabelis, 1988a)

INFOCHEMICAL: A chemical that, in the natural context, conveys information in an interaction between two individuals, evoking in the receiver, a behavioural or physiological response that is adaptive to either the interactants or both.

PHEROMONE: An infochemical that mediates an interaction between organisms of the same species whereby the benefit is to the origin-related organism ((+ . -) pheromone), to the receiver ((- , +) pheromone), or both ((+ , +) pheromone).

ALLELOCHEMICAL: An infochemical that mediates an interaction between two individuals that belong to different species.

ALLOMONE: An allelochemical that is pertinent to the biology of an organism (organism 1) and that, when it contacts an individual of another species (organism 2) evokes in the receiver a behavioral or physiological response that is adaptively favourable to organism 1, but not organism 2.

KAIROMONE: An allelochemical that is pertinent to the biology of an organism (organism 1) and that, when it contacts an individual of another species (organism 2), evokes in the receiver a behavioural or physiological response that is adaptively favorable to organism 2 but not organism 1.

SYNOMONE: An allelochemical that is pertinent to the biology of an organism (organism 1) and that, when it contacts an individual of another species (organism 2), evokes in the receiver a behavioural or physiological response that is adaptively favorable to both organism 1 and 2.

Host selection by insect parasitoids is strongly influenced by 'infochemicals' or chemicals that convey information about the location, identity and suitability of potential host species (Dicke and Sabelis, 1988a; Vet and Dicke, 1992; Turlings *et al.*, 1993; Vet *et al.*, 1995). Kairomones are widely used by parasitic insects to locate and identify their hosts. The source of such kairomones are diverse and include body odour, frass, webbing, salivary constituents, honey dew, body scales, egg chorions and some host pheromones (Vinson, 1976; Weseloh, 1981; Whitman, 1988; Lewis and Martin, 1990; Vet and Dicke, 1992; Rutledge, 1996). Parasitoids learn to respond to the different blends of these chemicals that indicate the location of their hosts (Van Alphen *et al.*, 1986; Noldus, 1989; Papaj and Vet, 1990; Vet *et al.*, 1990ab; Turlings *et al.*, 1991a; Tumlinson *et al.*, 1993; Vet *et al.*, 1995; Dicke and Vet, 1999).

2.3.1 Host habitat location

Habitat preference exhibited by parasitoids is a major factor in determining the type of habitat searched and the host thus located or selected. Location of the host habitat by the searching parasitoids may be influenced by different stimuli from several sources, the first to be encountered being those in the environment in which the adult parasitoids emerge (Vinson, 1985; Van Alphen and Vet, 1986). Temperature, humidity, light intensity, wind and food sources as well as flying and crawling habits of the parasitoid (Vinson, 1991) are physical factors that play a

major role in causing parasitoids to aggregate in a particular micro-habitat, thus influencing host selection. Other factors that are involved in habitat location include biological factors, such as adult food sources, refuge sites and the presence or absence of competitors as well as predators. Chemical stimuli are considered to be the most important cues utilized by parasitoids during foraging.

Plant volatiles emanating from host food or food plants and food odours have been shown to be important cues in host habitat location for a number of hymenopteran parasitoids (Vinson *et al.*, 1975; Powell and Zhang Zhi-Li, 1983; Elzen *et al.*, 1983; 1984; 1986; Martin *et al.*, 1990; Takabayashi and Dicke, 1992; Ngi-Song *et al.*, 1996; Rutledge, 1996). Aldehydes, alcohols and sulfur containing compounds have been identified as the volatiles used in the host habitat location stage (Rutledge, 1996). For example, *Cardiochiles nigriceps* (Viereck) appears to cue first on plant factors but once in proper habitat, may cue on injured plant tissue (Vinson *et al.*, 1975). Volatiles from uninfested plants have been found to be long-range attractants for parasitoids (Vinson, 1985; Nordlund *et al.*, 1988; Whitman, 1988; Lewis *et al.*, 1990). *Ostrinia nubilalis* (Hubner) was attracted to several plants including its food (maize) (Ding *et al.*, 1989). Females of *Eucelatoria byani* Sabrosky, a parasitoid of the *Heliothis* spp. responded to volatiles emanating from 13 out of 19 fresh plant materials tested, all of which were food sources for *Heliothis* spp. (Martin *et al.*, 1990). Response of parasitoids to whole cotton plant odours and odours from different plant parts have been reported by Elzen *et al.*, (1983 1986 1987); Baehrecke *et al.* (1990); Mc Auslane *et al.* (1990, 1991) and

Vinson and Williams, (1991). Females of *Microplitis croceipes* Cresson have also been reported to respond to volatiles emanating from damaged host plants (Whitman and Eller, 1992). Plants therefore provide important cues to the searching parasitoid and thus guide parasitoids to identify the habitat for their hosts.

2.3.2 Host location

The parasitoids having reached a potential host habitat must find the host. Host location is defined as the perception and orientation by parasitoids to their hosts from a distance, by responding to stimuli originating from the host or from host products (Weseloh, 1981; Lewis and Martin, 1990; Godfray, 1994). The parasitoid utilizes both long-range volatile compounds and short range chemical stimuli coming from the host habitat or from the host itself. Long range attractants could be released from the host communication system, the host food, or by the organisms associated with the host. Short-range attractants may be released by the host or from the host by-products.

Plant volatiles released by herbivore-damaged plants are used as host location cues by foraging parasitoids (Steinberg *et al.*, 1993; Tumlinson *et al.*, 1993; Takabayashi *et al.*, 1994; Turlings *et al.*, 1995; Rutledge, 1996; De Moraes *et al.*, 1998). These volatiles are the most reliable cues for the foraging parasitoid if the released compounds are specific for the herbivore species or when the cues can be learned by a parasitoid (Dicke and Vet, 1999). These chemical signals are mixtures of volatile terpenoids and green leaf volatiles ((C5-C6) alcohols and

esters) (Whitman and Eller, 1990; Tumlinson *et al.*, 1992; Takabayashi *et al.*, 1995; Ngi-Song, 1995; Rutledge, 1996; Ngi-Song *et al.*, 2000). Chemicals from the herbivores are thus the most reliable indicators of the presence of the host (Turlings *et al.*, 1995; Pare and Tumlinson, 1997; Turlings and Benrey, 1998). These chemicals that result in active interaction between herbivore-damaged plants and a third trophic level have been described for several plant species including Lima beans that produce volatiles that attract the predatory mite *Phytoseiulus persimilis* when damaged by the spidermite *Tetranychus urticae* (Dicke *et al.*, 1993; Geervliet, 1994) or maize plants that produce volatiles that attract the hymenopterous larval parasitoids *Cotesia marginiventris* (Cresson) and *Microplitis croceipes* when under attack by *Spodoptera exigua* (Hubner) caterpillars (Turlings *et al.*, 1991a, Turlings and Tumlinson, 1991). Tobacco, cotton and maize plants have also been shown to produce attractive chemicals that attract the specialist parasitoid wasp *Cardiochiles nigriceps* to its host *Heliothis virescens* F. (De Moraes *et al.*, 1998). These chemicals have also been described in several tritrophic systems involving maize-herbivore-parasitoid interactions (Turlings *et al.*, 1991ab; Takabayashi *et al.*, 1995; 1996; Rutledge, 1996; Takabayashi *et al.*, 1998; Turlings *et al.*, 1998; Ngi-Song *et al.*, 2000).

2.4 Attractive allelochemicals

2.4.1 Long range plant produced attractants (synomones)

Vinson (1991), Tumlinson *et al.* (1992), Dicke (1994), Takabayashi *et al.* (1994), Ngi-Song (1995), Rutledge (1996), Turlings *et al.* (1998) and Ngi-Song *et al.* (2000) have reported on semiochemical complexes involved in tritrophic interactions. Plant compounds attractive to some parasitoids have been identified. The parasitoid, *Campoletis sonorensis* (Cameron) was reported to be attracted to sesquiterpenes isolated from cotton essential oils (Elzen *et al.*, 1984). The sesquiterpenes included α -humulene, γ -bisabolene, β -caryophyllene oxide, spathulenol, and β -bisabolol and gossanorol. These compounds were isolated from ethyl ether washes of freshly cut flowers and buds of cotton, which is a food plant of *Heliothis virescens*. It was demonstrated that parasitoids respond to chemicals from damaged plants.

Some plants, when damaged have been reported to actively produce volatile chemicals in response to a substance produced by the attacking herbivore (Turlings *et al.*, 1990b, 1991b). The presence of beta-glucosidase activity in saliva from *Pieris brassicae* larvae, which feed on cabbage and induce the release of volatiles was reported recently (Mattiacci *et al.*, 1995).

Turlings *et al.* (1991b) isolated and identified allelochemicals that attract *C. marginiventris* to the microhabitat of its host. In their study eleven compounds were identified (1) Z-3-hexenal, (2) (E)-2-hexanal, (3) (Z)-3-hexenol, (4) (Z)-3-hexen-1-yl acetate, (5) Linalool, (6) (3E)-4,8-dimethyl-1,3,7-nonatriene, (7)

Indole, (8) α -trans-bergamotene, (9) (E)- β -farnesene, (10) (E)-nerolidol, and (11) (3E, 7E)-4,8,12-trimethyl-1,3,7,11-tridecatetraene. These allelochemicals were released by damaged leaves. Results similar to Turlings findings were obtained by Steinberg *et al.* (1993) who found out that caterpillar infested cabbages initiate the release of volatile allelochemicals that play important role in long range host location by *Cotesia glomerata* (L). Repeatedly (3E)-4,8-dimethyl-1,3,7-nonatriene and (3E, 7E)-4,8,12-trimethyl-1,3,7,11-tridecatetraene have been identified in the headspace of herbivore infested plants including Lima bean, cucumber, maize and cotton (Turlings *et al.*, 1991b; Dicke, 1994; Loughrin *et al.*, 1994). The composition of the volatiles varied with larval feeding times. Immediately after the larvae began feeding, green leaf aldehyde and alcohol volatiles were released and continued to be released as long as the larvae were actively feeding (Tumlinson *et al.*, 1992). After several hours of feeding, the compounds of higher molecular weight, primarily terpenoids were released (compounds 5-11 above) (Tumlinson *et al.*, 1992). Undamaged plants released very little of these compounds. When artificially damaged plants were treated with regurgitant from larvae, release of similar amounts of compounds 5-11 was observed. Whitman and Eller (1990) also reported attraction of green leaf volatiles (GLVs). They reported that undamaged plants emitted low levels of GLVs, while naturally and artificially damaged plants emitted relatively higher levels of certain GLVs. In Beet armyworm-damaged cotton plants during the early stages of damage, high levels of lipoxygenase-derived compounds ((Z)-3-hexanal, (Z)-3-hexenylacetate) and several terpene

hydrocarbons (E- β -ocimene, E- β -farnesene) were emitted (Loughrin *et al.*, 1994). Females of the braconid parasitoid, *Microplitis croceipes* were attracted to individual GLVs in a wind tunnel. The GLVs tested were: hexenal, (E)-2-hexenal, (Z)-3-hexen-1-ol, (Z)-3-hexenylacetate, (E)-2-hexenylacetate, (Z)-3-hexenylpropionate, and (Z)-3-hexenylbutyrate.

In a study consisting of *Cotesia rubecula* Marshall-*Pieris rapae* L. and cabbage, done by Agelopoulos and Keller (1994a) it was reported that the volatiles emitted by undamaged plants were α -pinene, β -pinene, myrcene, 1,8-cineole, n-hexylacetate, cis-3-hexen-1-yl acetate and dimethyltrisulfide. Mechanical damage induced the release of two more compounds, trans-2-hexenal and 1-methoxy-3-methylene-2-pentanone. Feeding by the larvae *P. rapae* induced the release of all compounds released by the mechanical damage and additionally, 4-methyl-3-pentanal and allylthiocyanate (Agelopoulos and Keller, 1994c).

The attractive volatiles in the faeces of herbivores may be derived from plants or other substrates on which the herbivores feed. It is also possible that microorganisms in the insect gut modify plant constituents or faeces to produce long range kairomones (Tumlinson *et al.*, 1992). Eller (1990) reported that the parasitoid *M. croceipes* was attracted to a compound identified as *trans*-phytol isolated from the hexane extract of the frass from *Heliothis zea* Boddie larvae fed on cowpea. It was suggested that long range kairomones produced by herbivores are released from their faeces. Agelopoulos *et al.* (1995) reported that the

chemical profile emitted from faeces of *P. rapae* and *P. brassicae* after feeding on Brussels sprouts plants was species specific in both quality and quantity.

Substances in the oral secretions of lepidopterous larvae and other insects could be a source of the volatile signals that attract parasitoids (Dicke and Dijkman, 1992; Turlings *et al.*, 1993). The elicitor from the oral secretions of Beet armyworm, *Spodoptera exigua* (Hubner) has been isolated, identified and synthesized (Alborn *et al.*, 1997). This compound N (17-hydroxylinolenoyl) (L)-glutamine, when applied to maize seedlings induces biosynthesis and release of a volatile blend identical to that induced by Beet armyworm feeding.

Several other systems have shown quantitative and qualitative differences in the volatile composition of stem borer infested and uninfested maize plants using caterpillars of *S. exigua*, *C. partellus*, *Ostrinia nubilalis* (Hubner) and *Mythimna separata* Walker (Turling *et al.*, 1991a, 1991b, 1998; Tumlinson *et al.*, 1992; Takabayashi *et al.*, 1995). The volatile composition of the plant has been shown to change after several hours of larval damage from a signal containing low molecular weight green leaf volatiles to a signal containing higher molecular weight compounds characteristic of terpenoids. Mechanically damaged plants also release volatiles which, differ in both quantity and quality from insect-damaged plants. Low molecular weight green leaf volatiles are released soon after mechanical damage (Agelopoulos *et al.*, 1999).

2.4.2 Host produced attractants (kairomones)

2.4.2.1 Long range attractants

Hosts previously parasitised are marked by the attacking female with a pheromone. These marking pheromones may occur at various levels in the host selection process but are most often on the host itself. Some parasitoids thus exploit the pheromonal system of their host during foraging. *Spathius benefactor* (Matthews), *Cheiopachus colon* (L.), *Entedon leucogramma* (Ratzeburg), *Dendrosoter protuberans* (Nees) are parasitoids that have been reported to be attracted to the synthetic multistriatin, 4-methyl-3-heptanol, and cubebene, components of the aggregation pheromone of their host, the elm bark beetle, *Scolytus multistriatus* (Marsham) (Kennedy, 1984). Egg parasitoids have been reported to use host sex pheromone as an indicator for the probable presence of host eggs (Noldus, 1988). *Aphytis melinus* (DeBach) has been reported to use learned volatile cues from host plant as long-range attractants to potential habitats of the California red scale (Morgan and Hare, 1998).

2.4.2.2 Short-range attractants

Stimuli derived directly from the host are the most reliable source of information because they can inform the parasitoid of the presence, identity, availability and suitability of the host (Vet *et al.*, 1991). As the parasitoid approaches its host, it is exposed to chemical cues that are host specific. These chemicals are most often found in the host by-products (Tumlinson *et al.*, 1992).

2,5-dialkyltetra-hydrofurans that have been isolated from buccal secretions, faeces and the body surface of *Pseudaletia separata* (Walker) are arrestants for the larval parasitoid *Apanteles kariyai* (Watanabe) (Takabayashi and Takahashi, 1986). In another study, these compounds were found in the faeces of *Acantholeucania loreyi* (Duponchel), although this host is not suitable for the parasitoid development (Takabayashi and Takahashi, 1990). Volatiles emitted by faeces of larvae may also be involved in the orientation to host-infested plants since many species of parasitoids are known to be attracted to faeces of their host larvae (Eller *et al.*, 1988ab; Steinberg *et al.*, 1993; Agelopoulos and Keller, 1994a; Geervliet *et al.*, 1994; Agelopoulos *et al.*, 1995). A component from the mandibular gland secretion of *H. virescens* that elicited short-range attraction of its parasitoid *C. nigriceps* was isolated and identified by Vinson *et al.* (1975).

2.5 Chemical ecology of *Cotesia flavipes*

The stimuli involved in the searching behaviour of *Cotesia flavipes* has been studied by several authors (Mohyuddin, 1971; Mohyuddin *et al.*, 1981; Van Leerdam *et al.*, 1985; Potting *et al.*, 1993, 1995; Ngi-Song, 1995; Ngi-Song *et al.*, 1996; Ngi-Song and Overholt, 1997; Ngi-Song *et al.*, 2000). *Cotesia flavipes* was found to be more attracted to volatiles emanating from maize infested with *Chilo partellus* than uninfested maize stems or any other component of the plant-host complex (Potting *et al.*, 1995). However, frass and leaves of plants damaged by stem borers were also very attractive (Potting *et al.*, 1995).

Larval frass has been reported to act as a directive mediator in the host finding process of *C. flavipes* (Van Leerdam *et al.*, 1985; Potting *et al.*, 1995; Ngi-Song *et al.*, 1996; Ngi-Song and Overholt, 1997). *Cotesia flavipes* was able to detect the entrance of a tunnel made by feeding of stem borer larvae by using fresh frass presence at the entrance of the tunnel (Potting, 1996). Bioassays done using frass indicated that *C. flavipes* was attracted to volatile kairomones from frass regardless of the stem borer species that produced the volatiles or the plant species upon which the host had fed (Ngi-Song and Overholt, 1997).

Cotesia flavipes was imported from Pakistan where it was collected from *Chilo partellus* in maize fields. Ngi-Song (1995) reported that *C. flavipes* was strongly attracted to selected gramineous plants (maize, sorghum and a wild host, napier grass) which are host plants of stem borers. In all single choice tests, the gramineous plant was selected regardless of the plant species. *Cotesia flavipes* showed a preference for maize over sorghum in the dual choice experiments. The preference may be a reflection of a genetic adaptation to searching in maize (Ngi-Song, 1995). In a related study done by Rutledge and Wiedenmann (1999), it was reported that *C. flavipes* was attracted to odours from sorghum and maize which are habitats of its host.

Cotesia flavipes preferred odours from infested plants over uninfested plants, indicating that the parasitoids were able to identify plants attacked by their host. Work conducted on the isolation and identification of allelochemicals involved in the host selection process of *C. flavipes* by Ngi-Song (1995), indicated

that although *C. flavipes* was attracted to odours from uninfested maize plants, host frass or artificially damaged plants (Potting *et al.*, 1995), the parasitoid was preferentially attracted to volatiles from maize plants on which stem borers had been feeding (HPC). This was supported by olfactometric data on tests with volatile extracts from infested and uninfested maize plants which were also differentially attractive to *C. flavipes*, albeit in a dose-independent manner. The attractive volatiles identified from infested maize plants were anisole, (E)- β -farnesene, (Z)-3-hexenylacetate, myrcene, 2-heptanone, 4,8-dimethyl-1,3,7-nonatriene, (Z)-2-hexenal, (Z)-3-hexen-1-ol, cyclosativen, cedrene and α -copaene (Ngi-Song, 1995). Further work on behavioural bioassays showed that *C. flavipes* was attracted to synthetic anisole, (E)- β -farnesene and (Z)-3-hexenylacetate (Ngi-Song, 1995). In a study carried out by Ngi-Song *et al.* (2000) the attractive volatiles identified from maize infested with *C. partellus* larvae were (Z)-3-hexenylacetate, Linalool, (E)-4,8-dimethyl-1,3,7-nonatriene, heptanal and (E)- β -ocimene. Y-tube olfactometer assays and gas chromatography-electroantennographic detector (GC-EAD) were used to confirm the attractiveness of the compounds to *C. flavipes* (Ngi-Song *et al.*, 2000).

The chemical data obtained has shown that the stem borer damaged plants are a source of compounds attractive to *C. flavipes*. In particular, terpenoids and green leaf volatiles (GLVs) were among chemicals obtained from the host plant complex. Green leaf volatiles are saturated and unsaturated six carbon alcohol's, aldehydes and derived esters formed by oxidative degradation of plant lipids

through the ' lipoxygenase pathway' (Hatanaka, 1993). They have been reported as volatile components of numerous plants from several families (Visser *et al.*, 1979; Rutledge, 1996) and have been shown to play a role in host detection by phytophagous insects (Visser, 1986; Rutledge, 1996). The green leaf volatiles identified from *C. partellus* infested plants in a study carried out by Ngi-Song (1995) are (E)-2-hexenal, (Z)-3-hexen-1-ol and (Z)-3-hexenylacetate. These compounds were also identified in a related study involving maize seedlings, the Beet armyworm and the parasitoid *C. marginiventris* (Turlings *et al.*, 1991b). (Z)-3-hexenylacetate was identified in a study involving maize seedlings infested by *C. partellus* by Ngi-Song *et al.* (2000).

In addition to green leaf volatiles, terpenoids have been implicated in the searching behaviour of parasitoids. The terpenoids identified from *C. partellus* infested maize plants were Myrcene, 4,8-dimethyl-1, 3,7-nonatriene, cyclosativen, (E)- β -farnesene, cedrene and α -copaene (Ngi-Song, 1995).

(E)- β -farnesene was the most abundant terpenoid in volatile extracts obtained from maize plants infested with *C. partellus* and is a host-induced synomone. A similar observation was made by Turlings *et al.* (1991b) in the maize-Beet armyworm-*C. marginiventris* system. Buttery and Ling (1984) also identified this compound in the volatiles of maize leaves. (E)- β -farnesene was identified in Beet armyworm-damaged cotton plants (Loughrin *et al.*, 1994). Recently it was identified in cotton plants under herbivore attack (Rose *et al.*, 1998). (E)- β -farnesene is known to attract the chalcid wasps (Kamm and Buttery, 1983), and

serves as an alarm pheromone for aphids (Bowers *et al.*, 1972; Edwards *et al.*, 1973; Wohlers, 1981).

4,8-dimethyl-1, 3,7-nonatriene is a terpenoid which has been listed among herbivore-induced synomones that attract natural enemies (Dicke, 1994). According to Dicke (1994), 4,8-dimethyl-1, 3,7-nonatriene is also synthesized by many species of plants without any mediation by herbivory from the terpene alcohol's, nerolidol and geranylinalool. This terpenoid has been reported from oil of *Elettaria cardamomum* (cardamom oil) (Maurer *et al.*, 1986) and from night-scented flowers of different plant species that are pollinated by moths (Kaiser, 1987). It was first reported in maize by Turlings *et al.*, (1991b). This compound is also released by lima bean leaves (Dicke *et al.*, 1990a) and cucumber leaves (Dicke *et al.*, 1990b) that have been subjected to spidermite infestation. 4,8-dimethyl-1, 3,7-nonatriene has also been found to be released by cotton plants under herbivore attack (Rose *et al.*, 1998) and was recently reported in *C. partellus* infested maize seedlings (Ngi-Song *et al.*, 2000).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Bioassays

3.1.1 Parasitoids

A colony of *Cotesia flavipes* was initiated from the material collected from *Chilo partellus* at Rawalpindi, Pakistan by the International Institute of Biological Control (IIBC). *Cotesia flavipes* were reared on *C. partellus* larvae according to the method described by Overholt *et al.* (1994b). After parasitization, *C. partellus* were maintained on an artificial diet at 25°C, 65-70% relative humidity and 12: 12 (L: D) photoperiod. Parasitoid cocoons were collected in glass vials and kept in a clean Perspex cage until emergence. On emergence, adult parasitoids were provided with a 20% honey/water solution as diet. One-day-old-naïve females of *C. flavipes* were used in all the experiments.

3.1.2 Y-tube olfactometer

The Y-tube-olfactometer (Figure 1) used in this study has been described previously by Ngi-Song (1995). The odour sources were placed in glass chambers (Figure 2). The two glass chambers were connected to the arms of the Y-tube with tygon tubing from the chambers. A vacuum pump (Cole-Parmer Air-cadet) drew and pushed air through the system.

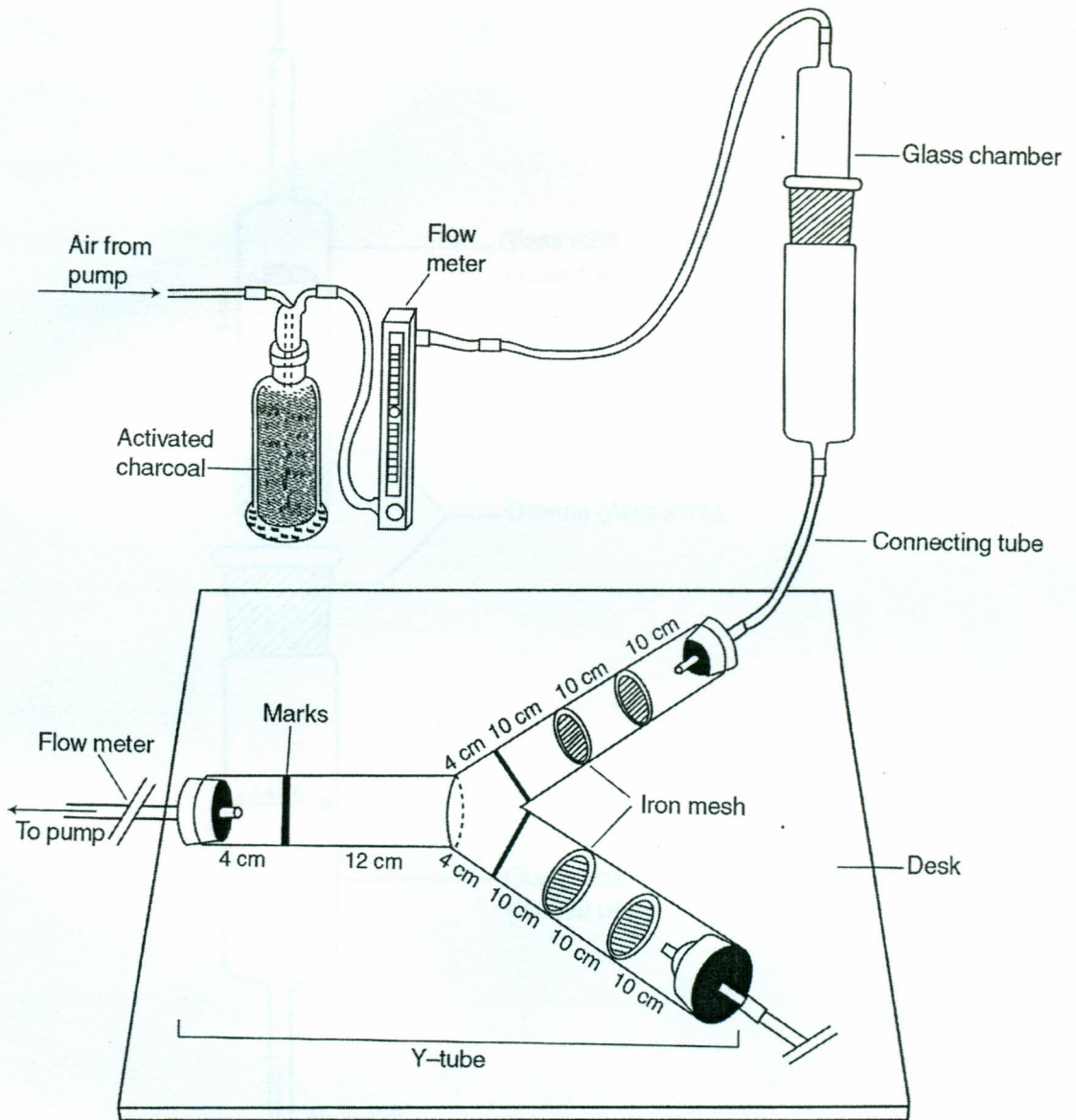


Figure 1. A schematic representation of the Y-tube olfactometer (Ngi-Song, 1995)

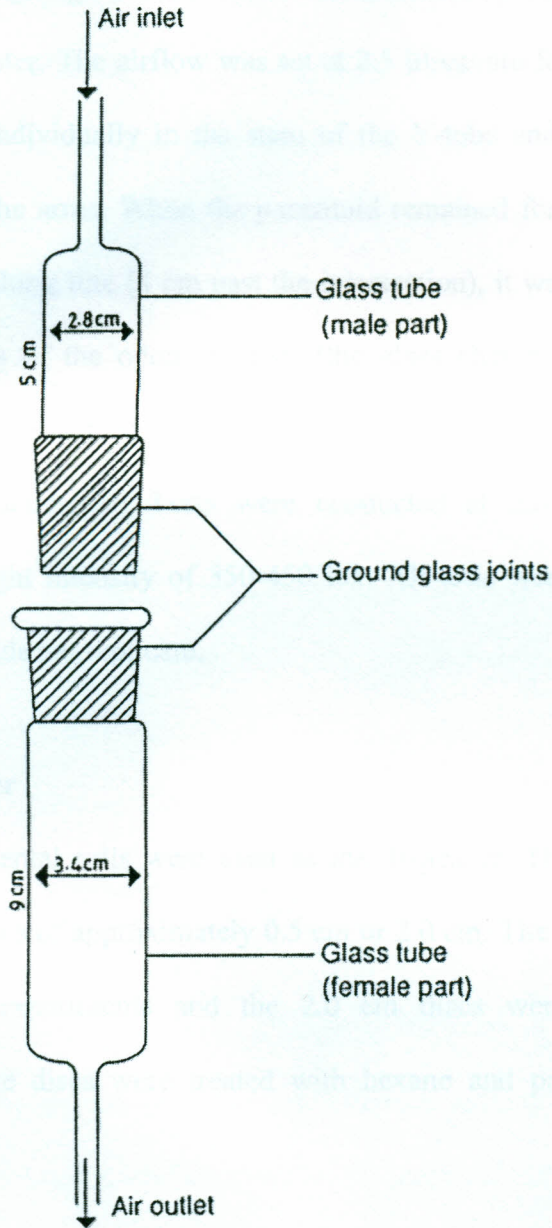


Figure 2. Glass chamber for placing test material (plant volatiles)

Air was pushed through the activated charcoal filter into the Y-shaped glass tubing of the olfactometer. The airflow was set at 2.5 litres/min for each arm. Parasitoids were released individually in the stem of the Y-tube and allowed 5 minutes to choose one of the arms. When the parasitoid remained for more than 15 seconds beyond the finishing line (4 cm past the intersection), it was recorded as a choice. The connections of the odour sources (the glass chambers) to the arms of the olfactometer were reversed after testing 5 parasitoids to rule out any asymmetrical bias in the olfactometer. Tests were conducted at 23-26°C, 65-75% relative humidity and light intensity of 350-450 lux. All tests were replicated four times with 15 parasitoids per replicate.

3.1.3 Dispenser

Cotton dental rolls were used as the dispenser. These cotton dental rolls were cut into discs of approximately 0.5 cm or 2.0 cm. The 0.5 cm discs were used for laboratory experiments and the 2.0 cm discs were used for semi-field experiments. The discs were treated with hexane and paraffin oil to minimise volatilization.

3.1.4 Behavioural response of *C. flavipes* to the synthetic volatiles

The kairomones tested in the Y-tube olfactometer were (E)- β -Farnesene, (Z)-3-hexenylacetate, (E)-4,8-dimethyl-1,3,7-nonatriene, heptanal, Thomac oil (a natural oil containing 85% (E)- β -Farnesene and a mixture of anti-oxidants obtained

from a South African plant commonly known as Thomac and a blend of (Z)-3-hexenylacetate + Thomac oil (1:1 v/v). (E)- β -Farnesene, (E)-4,8-dimethyl-1,3,7-nonatriene and Thomac oil were obtained from Dr. Lester Wadhams (IACR-Rothamsted, UK), while (Z)-3-hexenylacetate and heptanal were purchased from Lancaster Synthesis Ltd, and Aldrich, UK, respectively. These chemical compounds with the exception of Thomac oil were mixed with paraffin oil and hexane in the ratio of 1:1 (v/v). The kairomones were tested individually to assess their attraction to *Cotesia flavipes* in the Y-tube olfactometer. Kairomones were applied to dispensers which were placed in glass chambers (Figure 2). All the compounds were tested singly in a Y-tube olfactometer as described in Section 3.1.2. Doses tested were expressed in micrograms per disc and these were 5, 10, 15, 20 and 25 micrograms per disc. The control disc contained equivalent amounts of solvent (hexane) and paraffin oil only.

3.1.5 Behavioural response of *C. flavipes* to the frass of *Chilo partellus*

Bioassays were conducted to determine the attraction of *Cotesia flavipes* to frass produced by *Chilo partellus* when fed on maize. Tests were conducted in a Y-tube olfactometer (as described in Section 3.1.2). *Chilo partellus* was fed on fresh maize plant material and the frass produced after 48 hours was used for the experiments. Fresh frass obtained from *C. partellus* was tested against clean air (control). This was replicated four times with 15 parasitoids per replicate.

3.1.6 Behavioural response of *C. flavipes* to its diet

Bioassays were conducted to determine the attraction of *Cotesia flavipes* to their diet (20% honey/water solution). Tests were conducted in a Y-tube olfactometer. The parasitoid diet applied to cotton wool was tested against clean air. This was replicated 4 times with 15 parasitoids being tested per replicate. For data analysis see Section 3.6.

3.2 Preliminary assessment of baited delta trap in the field

Preliminary field trapping experiments were conducted at the South Coast (Kwale District) of Kenya, approximately 480 kms from Nairobi between June and July 2000 during the long rainy season. Experiments were done in maize fields where *Cotesia flavipes* is established (Overholt *et al.*, 1997). Twelve fields ranging between 0.5 to 1.5 acres were used to carry out the study. Delta traps (Plate 5) were placed in all fields. Delta traps were locally made from white cardboard (8 inches x 4.5 inches). The white cardboard was folded at an angle of 90° to form a tent. The bait used was the host plant complex, obtained from maize stalk (approximately 1.5 inches long) on which *C. partellus* larvae had been feeding on for 24 hours. The host plant complex was glued at 4 cm beneath the center of the delta trap. The traps were then hung on the maize plants at a distance of 1m above the ground. The number of traps placed in a field was dependent on the field size. In the small plots (0.5-0.7 acres), 4 baited and 4 unbaited traps were utilized and were placed randomly, while in large plots (1-1.5 acres), 9 baited and 9 unbaited traps were

placed similarly. Traps were placed at a distance of 7 meters from each other. Baited and unbaited traps were examined after 24 hours and the captured insects identified at the ICIPE Biosystematics Unit.

3.3 Development and selection of a trap for semi-field experiments

These experiments were done to develop and select the best trap to use for trapping *C. flavipes* in semi-field experiments after preliminary trapping of the parasitoid in the field showed low levels of catches with delta traps baited with host plant complex (see Results Section 4.4). Tests were conducted in a Perspex cage (45 x 45 x 45 cm). The delta trap (Plate 1) was modified to a vertical trap (Plate 2) by changing the angle of orientation from 90 °C to 180 °C. Water traps (Plate 3), plastic plates (Plate 4), and plastic cups (Plate 5) were used as traps while the parasitoid diet (20% honey/water solution) was used as the bait. Vertical and plastic cup traps were hung at the centre of the Perspex cage while the water and plastic plate traps were placed at the center of the Perspex cage. All the tests were set at 9:00 am. Fifty *C. flavipes* were released and the number of *C. flavipes* caught by 5:00 pm the same day was recorded. The tests were replicated six times.

3.4 Semi-field experiments

3.4.1 Growing of maize plants

Maize seeds (*Zea mays* L., (hybrid 5-13)) from Kenya Seed Company Ltd Nairobi, were grown in cages of dimensions 2.5m x 2.5m x 2m at the International

Centre of Insect Physiology and Ecology (ICIPE). After germination, plants were kept under fine mesh cages to protect them from insect attack and to prevent the released parasitoids from escaping.

3.4.2 Semi-field trapping of *C. flavipes* with the baits

The modified delta trap (Plate 2) was found to be the most suitable for use in trapping *C. flavipes* and was therefore used in subsequent experiments. To protect it from getting soaked by the rain, the outer cardboard was covered with plastic sheet. Tangle trap (Tangle foot company, Michigan, USA) was applied to the inner side of the trap so that captured parasitoids could be counted. A series of experiments were conducted.

Experiment 1 tested the modified delta trap with two types of baits; the host plant complex and the parasitoid diet (20% honey/water solution). The host plant complex (HPC) was prepared by introducing one *Chilo partellus* larvae into a maize stalk (1.5 inches), 48 hours prior to the setting of the trap in an incubator maintained at 28 °C. After this period, the maize stalks with frass were wrapped with a thin wire mesh and placed at 4cm beneath the center of the trap. Traps were hung 1m above the ground by tying them on a 1.5m hard wood stick. One hundred *C. flavipes* were released in the different cages at 9:00 am and the number captured was recorded at 5:00 pm the same day. Six cages were used to carry out the experiment and eight replicates were conducted.

Experiment 2 tested the optimum number of traps to be used in a cage. Vertical traps baited with parasitoid diet were used. Experiments were conducted in cages as indicated in Section 3.4.1. Varying numbers of traps (1, 2, 4, 6 and 8) were placed in the different cages. One hundred *C. flavipes* were released in the different cages at 9:00 am and the number captured was recorded at 5:00 pm the same day. Eight replicates were done.

3.5 Evaluating the potential of kairomone baited trap under semi-field conditions

To evaluate the potential of kairomone baited traps, preliminary experiments were conducted to determine the release rates of diet and the kairomones. The release rates of diet and kairomones loaded onto cotton dental roll dispensers were determined by measuring the weight changes of the loaded dispenser after every 1 hour. Four candidate kairomones including (Z)-3-Hexenylacetate (150 µg per disc), (E)- β-Farnesene (100 µg per disc), Thomac oil (150 µg per disc) and a blend of (Z)-3-Hexenylacetate and Thomac oil (1:1) (150 µg per disc) were tested based on the olfactometer results. An anti-oxidant, Butylated Hydroxytoluene (BHT) (0.2 mg) was added to each solution. Release rates were measured from 9:00 am to 5:00 pm. A total of 10 replicates were done for each kairomone and diet.

The candidate kairomone and the anti-oxidant were first prepared in hexane and then transferred into paraffin oil to minimize volatilization. The solution was

loaded onto 2.0 cm-long discs of cotton dental roll dispensers. The dispensers were glued at the center of the trap. Six vertical sticky traps were hung 1m above the ground by tying them on a 1.5m hard wood stick. All the experiments were set at 9:00 am. One hundred parasitoids were released in the cages containing 4-5 weeks old maize plants (see Section 3.4.1). The number of *C. flavipes* trapped were counted at 5:00 pm. One control trap with no bait was also set up in each cage. For each test compound eight replicates were carried out.

3.6 Data analyses

Data from laboratory bioassay (Y-tube olfactometer) were analysed using Chi-square (SAS 1999-2000). Parasitoids that did not respond ('No response' group) were excluded from the analyses.

Data from semi-field experiments were transformed to arcsine prior to analysis. Analysis of variance (ANOVA) PROC GLM, (SAS 1999-2000) was performed to compare the number of *C. flavipes* trapped using the different kairomones. Means were separated by Student-Newman-Keul's (SNK) multiple range test when the ANOVA was significant ($p < 0.05$).



Plate 1. Delta trap for trapping *C. flavipes* placed on a maize field

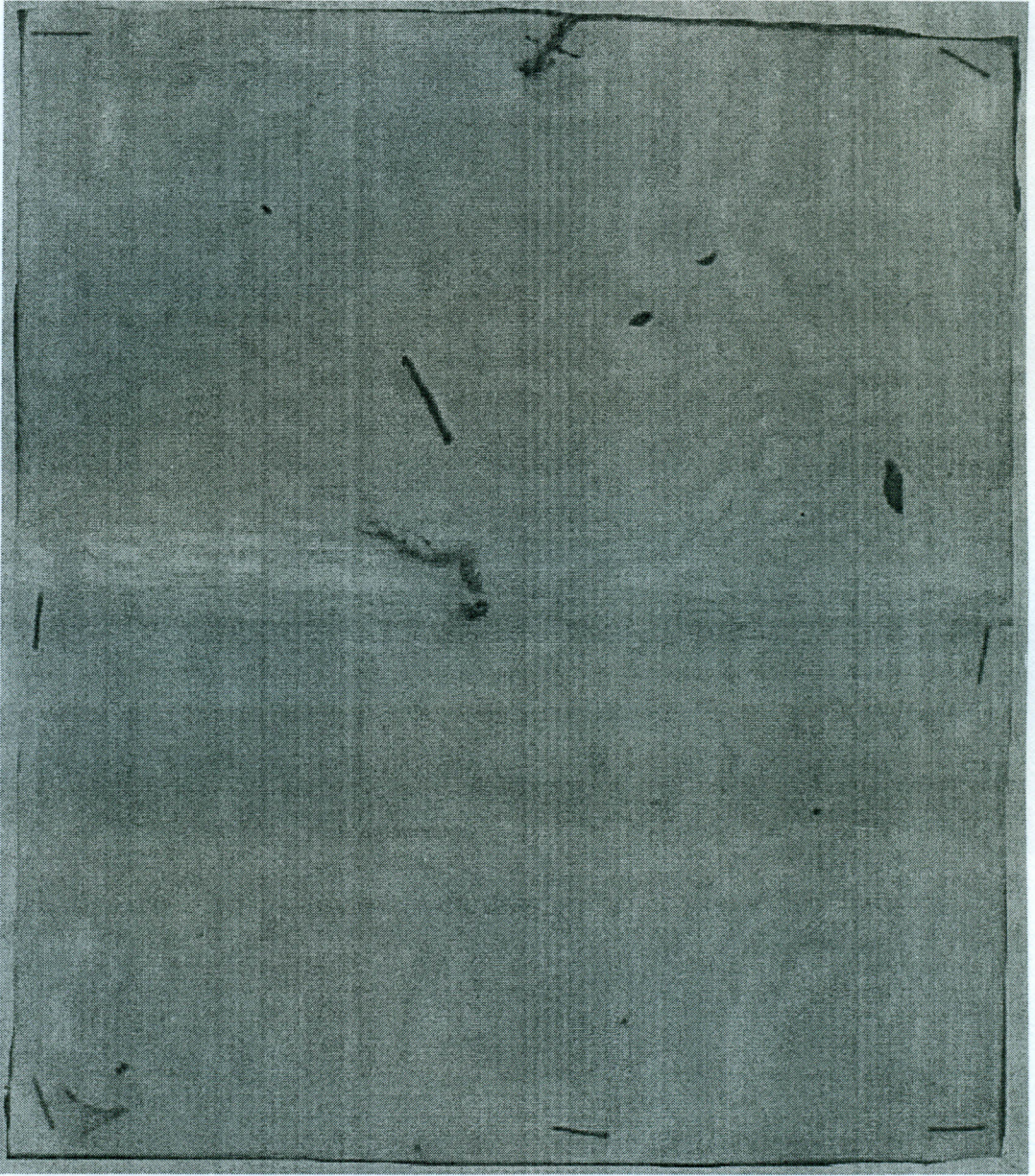


Plate 2. Modified delta trap for trapping *C. flavipes*

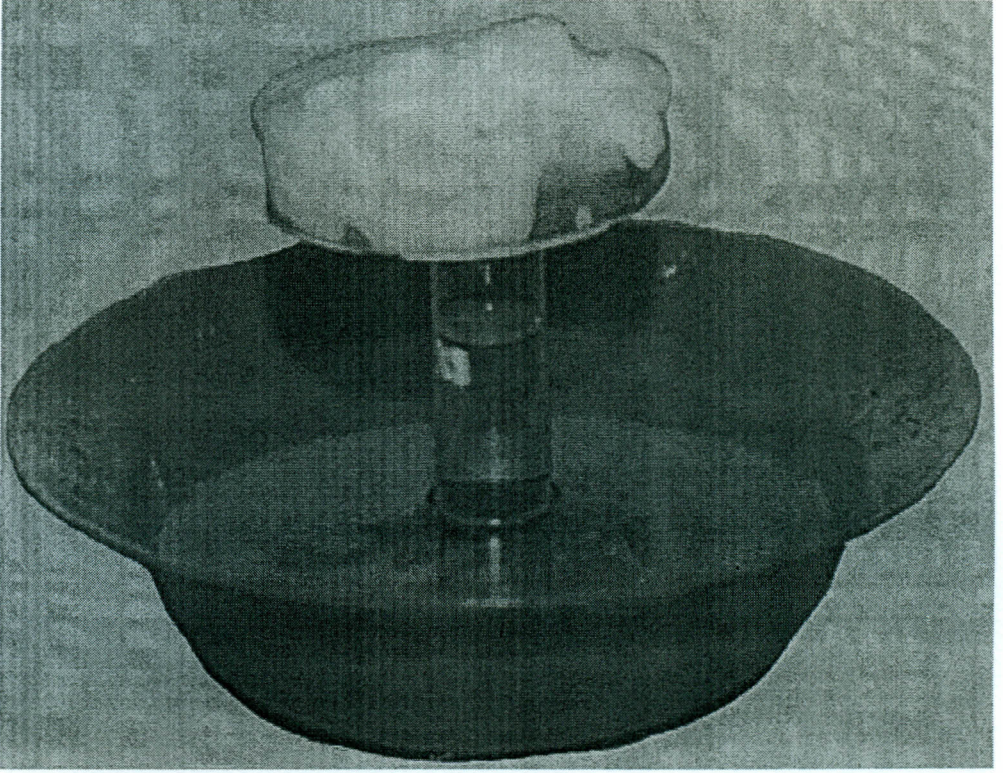


Plate 3. Water trap for trapping *C. flavipes*

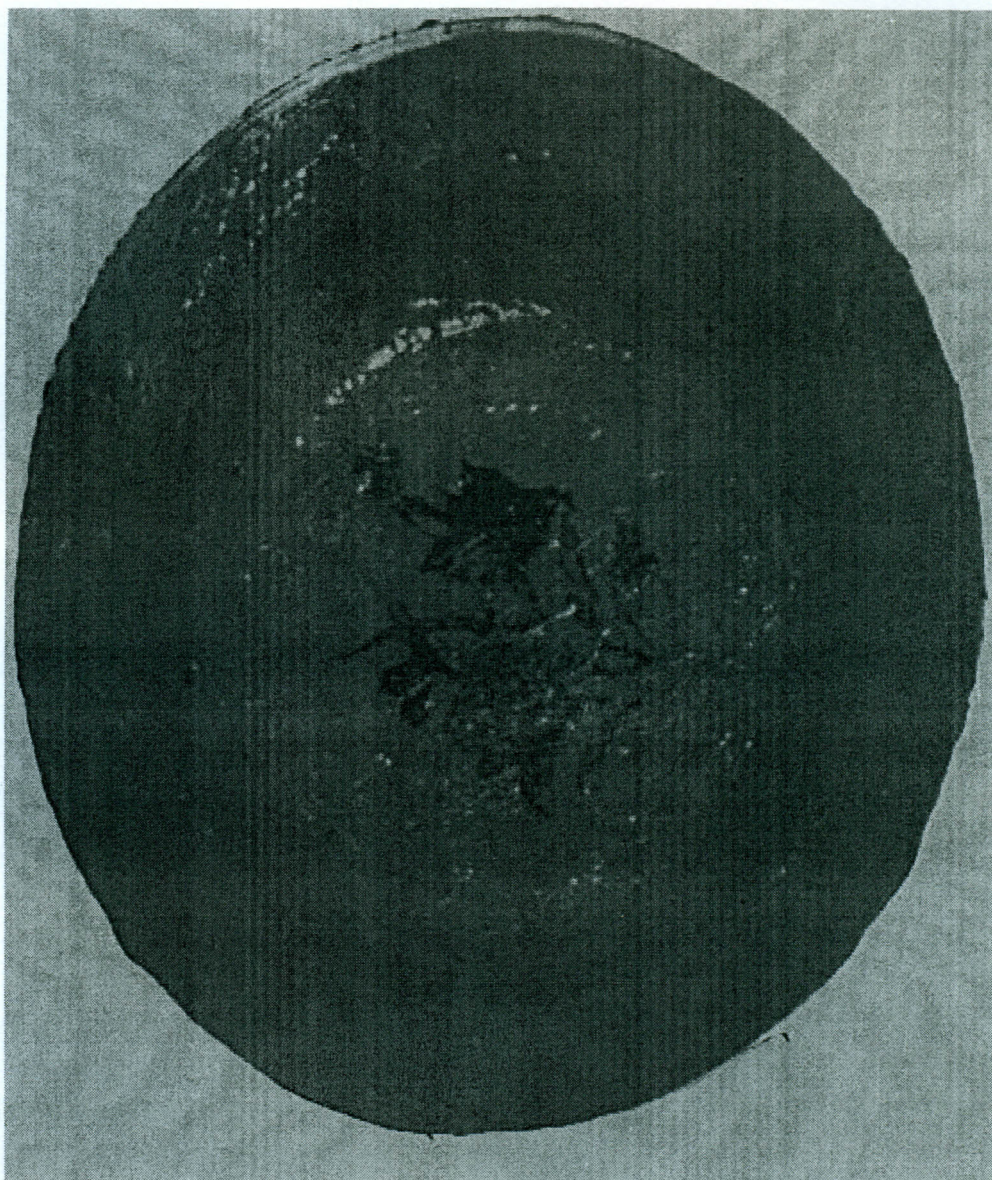


Plate 4. Plastic plate trap for trapping *C. flavipes*

CHAPTER VIII

RESULTS

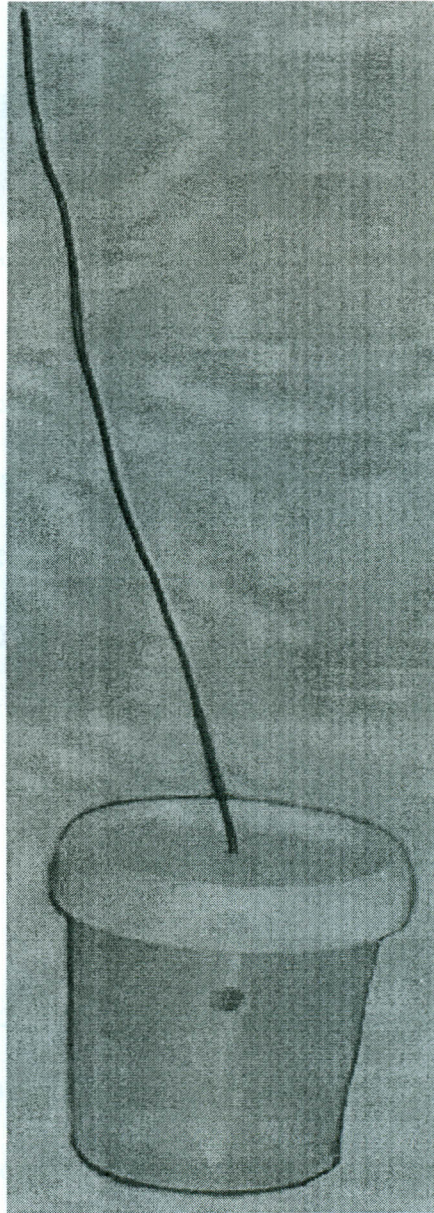


Plate 5. Plastic cup for trapping *C. flavipes*

CHAPTER FOUR

RESULTS

4.1 Behavioural response of *C. flavipes* to synthetic volatiles

Results of the experiments conducted on the behavioural response of *C. flavipes* to synthetic and natural volatiles are presented in Figures 3,4,5,6,7 and 8 respectively. All the five compounds tested in this experiment were attractive to *Cotesia flavipes*. However, the attractiveness of the compounds varied with the dose. Heptanal was most attractive at 10 µg per disc ($\chi^2 = 9.68$; $P = 0.002$) (Figure 3), while the monoterpene, 4,8-dimethyl-1,3,7-nonatriene was attractive at doses of 20 and 25 µg per disc ($\chi^2 = 7.41$; $P = 0.006$, $\chi^2 = 5.16$; $P = 0.023$) (Figure 4).

(E)- β -farnesene significantly attracted the parasitoid at 10 µg and 15 µg ($\chi^2 = 12.79$; $P = 0.001$, $\chi^2 = 18$; $P = 0.001$,) with 70% and 67% activity, respectively (Figure 5). At 10 and 15 µg per disc the attractiveness of (Z)-3-hexenylacetate and the natural oil, Thomac oil which contains 85% (E)- β -farnesene was similar (Figure 6 & 7). A blend of (Z)-3-Hexenylacetate and Thomac oil in a ratio of 1:1 (v/v) was attractive at 15 and 20 µg (Figure 8) ($\chi^2 = 17.65$; $P < 0.0001$, $\chi^2 = 11.36$; $P = 0.0007$). In some compounds (Heptanal, (Z)-3-hexenylacetate and the blend of (Z)-3-Hexenylacetate and Thomac oil in a ratio of 1:1 (v/v)) the lower doses were repellent to the parasitoid (Figure 4,6 and 8) while Thomac oil was repellent at the highest dose of 25 µg (Figure 7).

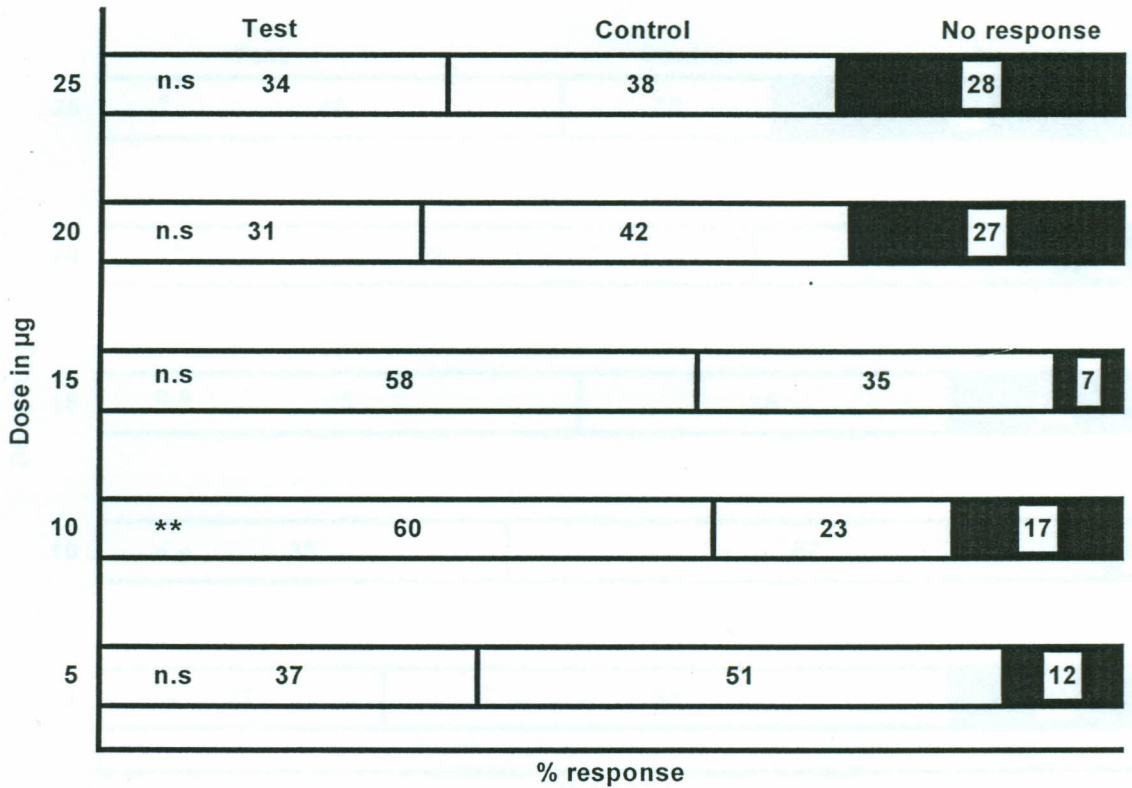
4.2 Behavioural response of *C. flavipes* to the frass of *Chilo partellus*

Results of the behavioural response of *C. flavipes* to the frass of *Chilo partellus* are presented in Figures 9. In this experiment, the frass of *C. partellus* fed on maize was found to be highly attractive with a response of 88% to *C. flavipes* ($\chi^2 = 39.72$; $P < 0001$) (Figure 9).

4.3 Behavioural response of *C. flavipes* to its diet

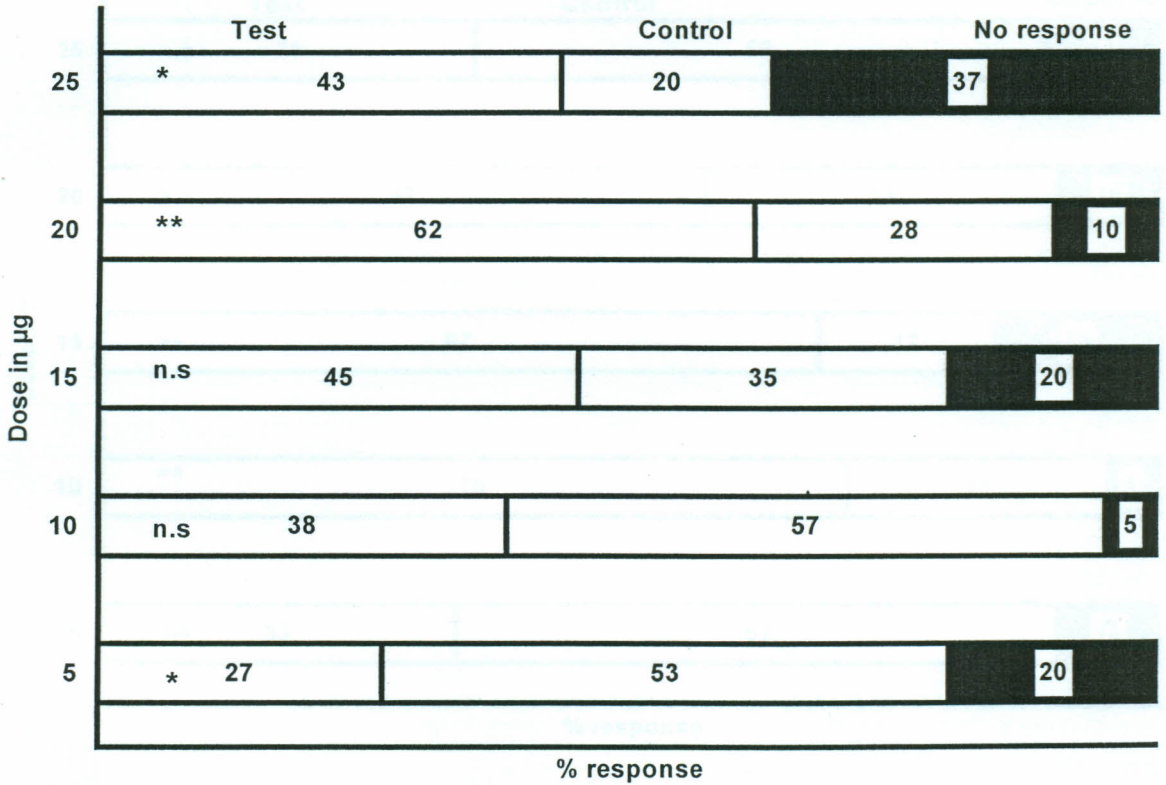
Results on the behavioural response of *C. flavipes* to its diet are presented in Figures 10. The diet of 20% honey/ water solution was moderately attractive and evolved a response of 65% to the parasitoid ($\chi^2 = 11.79$; $P < 0.005$) (Figure 10).

Figure 3. Percentage reponse of *C. flavipes* to the varying doses of heptanal in the Y-tube olfactometer



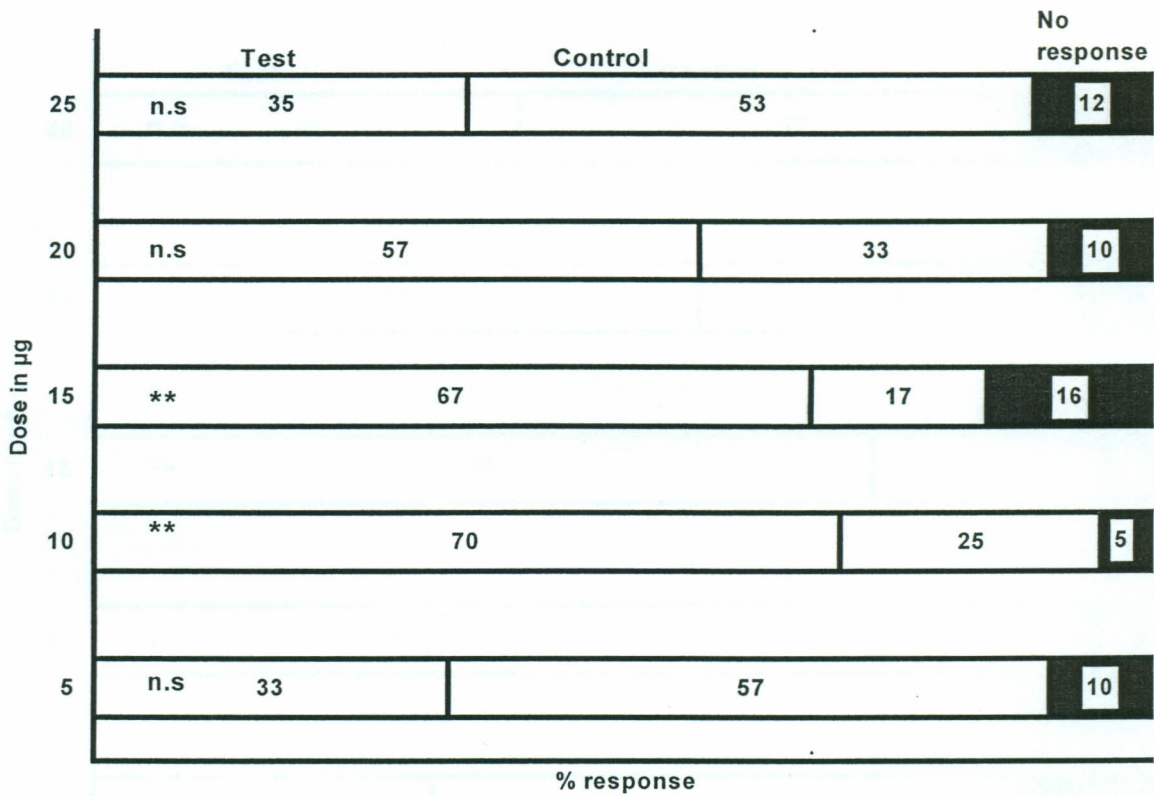
Numbers inside the bars indicate the percentage of parasitoids that made a choice for one of the two odour sources or did not make a choice at all ($n=60$). The three percentages add up to 100%. Asterisks indicate significant differences within the choice test: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$, n.s = not significant

Figure 4. Percentage response of *C. flavipes* to the varying doses of 4,8-dimethyl-1,3,7-nonatriene in the Y-tube olfactometer



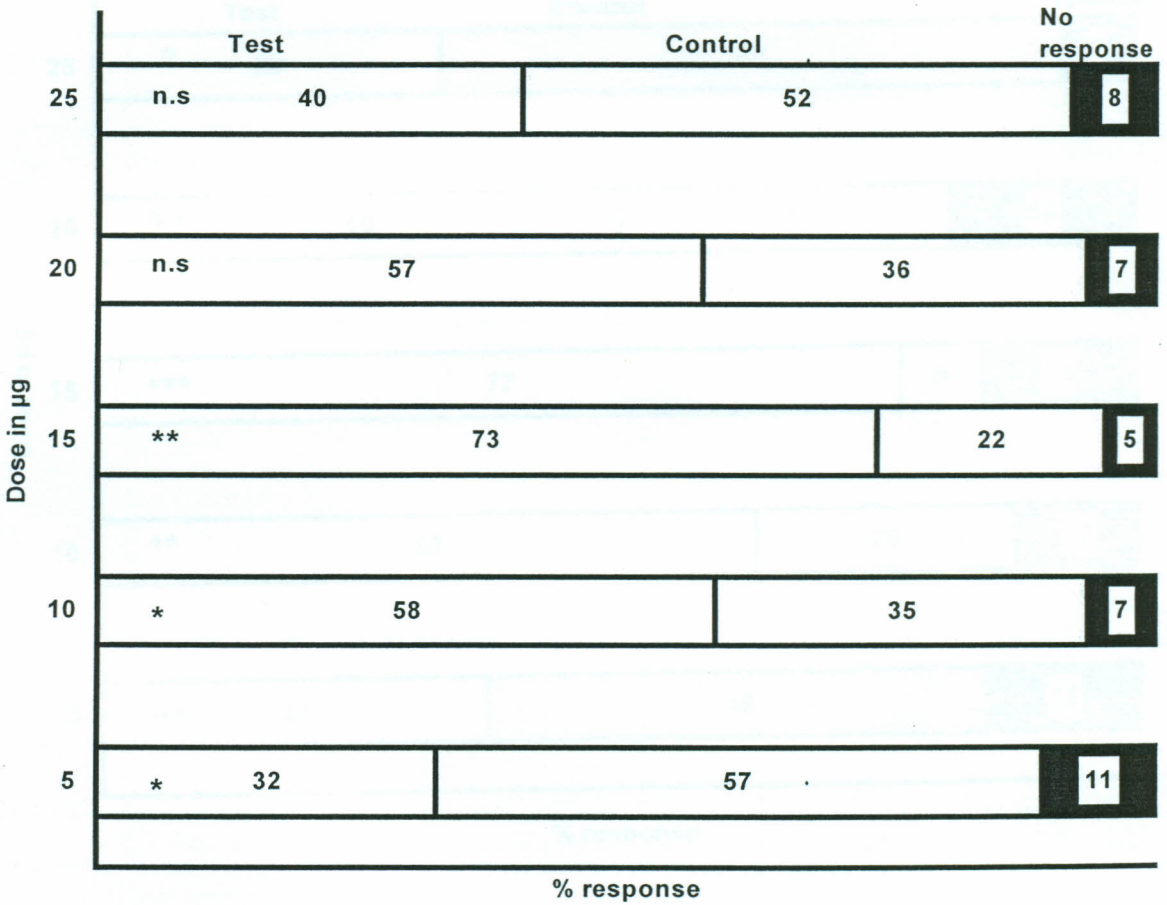
Numbers inside the bars indicate the percentage of parasitoids that made a choice for one of the two odour choices or did not make a choice at all (n=60). The three percentages add up to 100%. Asterisks indicate significant differences within the choice test: * $P < 0.05$, ** $P < 0.01$; *** $P < 0.001$, n.s = not significant

Figure 5. Percentage response of *C. flavipes* to the varying doses of (E)- β -farnesene in the Y-tube olfactometer



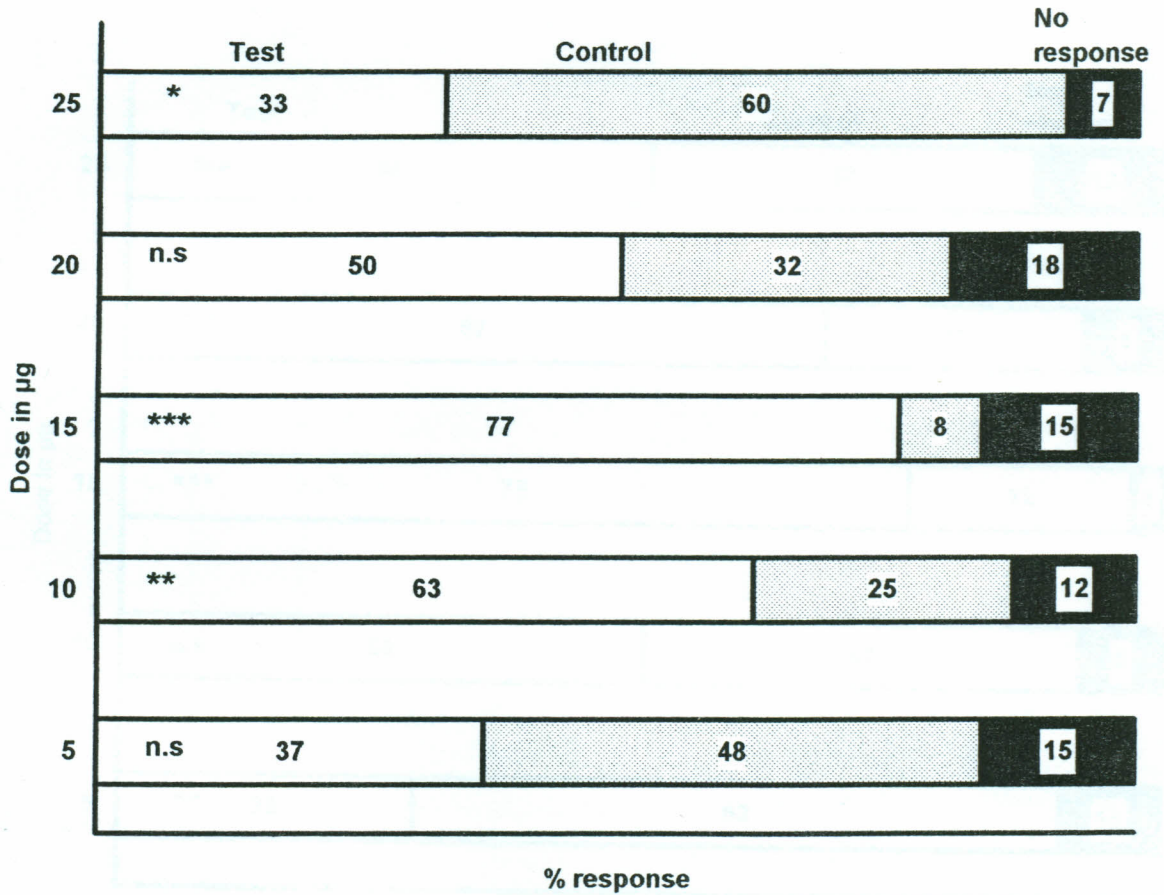
Numbers inside the bars indicate the percentage of parasitoids that made a choice for one of the two odour choices or did not make a choice at all ($n=60$). The three percentages add up to 100%. Asterisks indicate significant differences within the choice test: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$, n.s = not significant

Figure 6. Percentage response of *C. flavipes* to the varying doses of (Z)-3-hexenylacetate in the Y-tube olfactometer



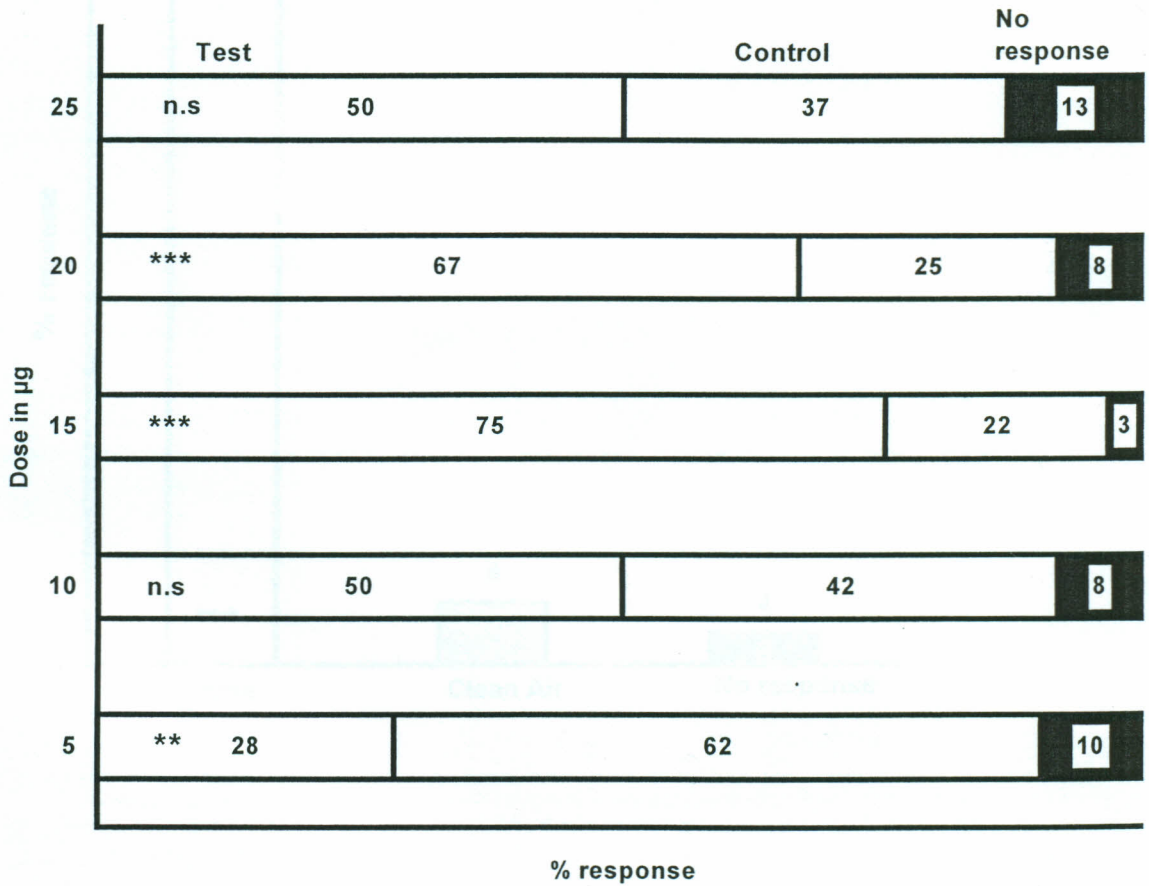
Numbers inside the bars indicate the percentage of parasitoids that made a choice for one of the two odour choices or did not make a choice at all ($n=60$). The three percentages add up to 100%. Asterisks indicate significant differences within the choice test: * $P < 0.05$, ** $P < 0.01$; *** $P < 0.001$, n.s = not significant

Figure 7. Percentage response of *C. flavipes* to the varying doses of Thomac oil in the Y-tube olfactometer



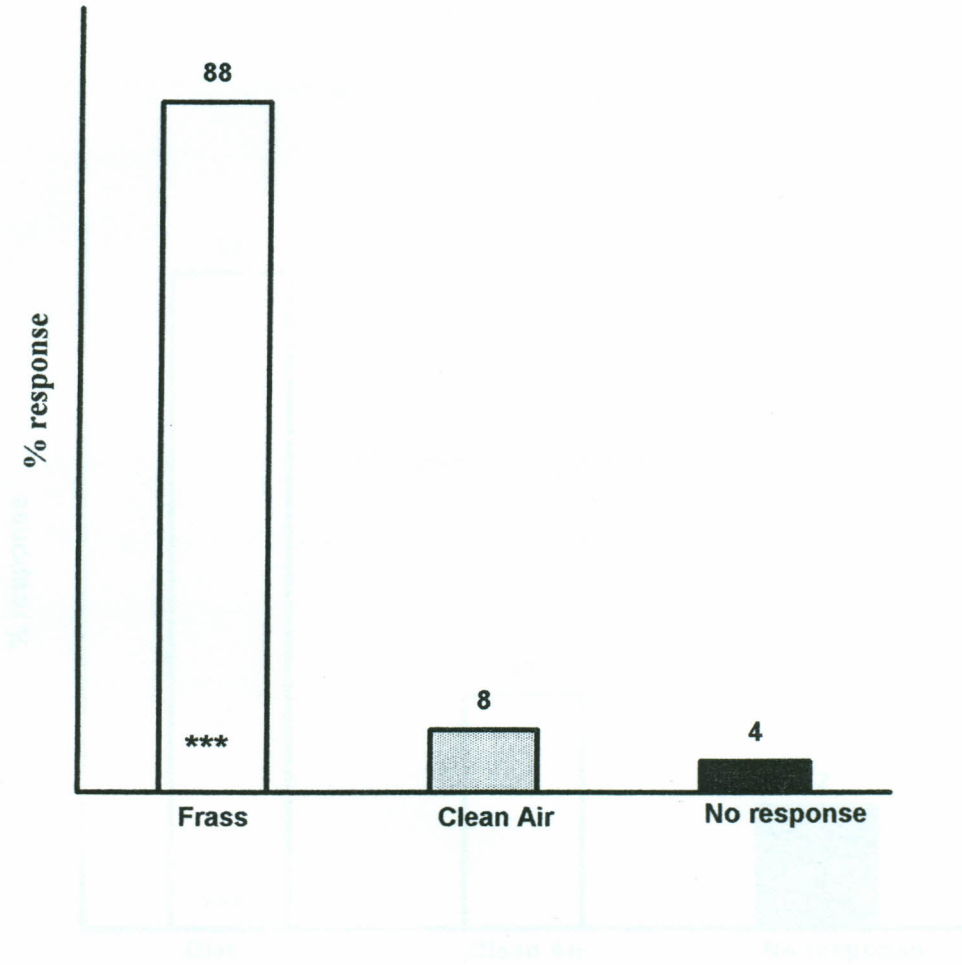
Numbers inside the bars indicate the percentage of parasitoids that made a choice for one of the two odour choices or did not make a choice at all (n=60). The three percentages add up to 100%. Asterisks indicate significant differences within the choice test: * $P < 0.05$, ** $P < 0.01$; *** $P < 0.001$, n.s = not significant

Figure 8. Percentage response of *C. flavipes* to the varying doses of hexenylacetate + Thomac oil 1:1 (v/v) in the Y-tube olfactometer



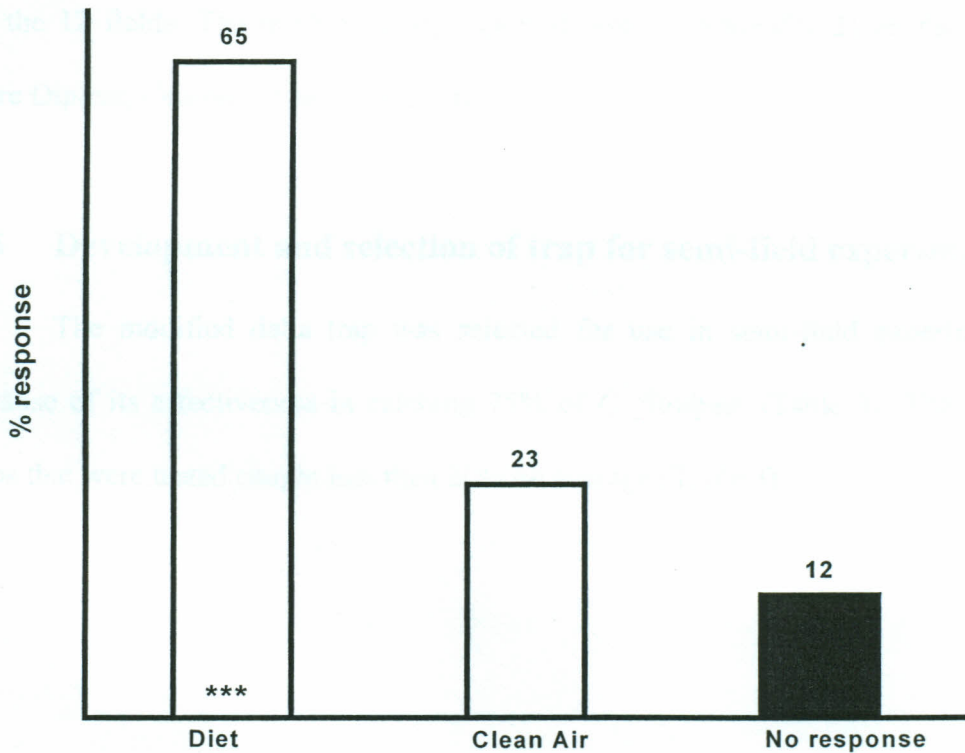
Numbers inside the bars indicate the percentage of parasitoids that made a choice for one of the two odour choices or did not make a choice at all ($n=60$). The three percentages add up to 100%. Asterisks indicate significant differences within the choice test: * $P < 0.05$, ** $P < 0.01$; *** $P < 0.001$, n.s = not significant

Figure 9. Percentage response of *C. flavipes* to *C. partellus* frass in the Y-tube olfactometer



Numbers outside the bars indicate the percentage of parasitoids that made a choice for one of the two odour choices or did not make a choice at all (n=60). The three percentages add up to 100%. Asterisks indicate significant differences within the choice test: * P < 0.05, ** P < 0.01; *** P < 0.001, n.s = not significant

Figure 10. Percentage response of *C. flavipes* to the rearing diet in the Y-tube olfactometer



Numbers outside the bars indicate the percentage of parasitoids that made a choice for one of the two odour choices or did not make a choice at all (n=60). The three percentages add up to 100%. Asterisks indicate significant differences within the choice test: * $P < 0.05$, ** $P < 0.01$; *** $P < 0.001$, n.s = not significant

4.4 Preliminary assessment of baited delta trap in the field

Results from the preliminary field trapping experiments showed that *C. flavipes* were very weakly attracted to the baited delta trap. Only 2 out of the 12 fields (Appendix 2) appeared to show the presence of *C. flavipes* in the traps although manual dissections of maize stalks showed the presence of *C. flavipes* in all the 12 fields. The predominantly captured insects (Appendix 2) on the traps were Diptera, Coleoptera and Hemiptera.

4.5 Development and selection of trap for semi-field experiments

The modified delta trap was selected for use in semi-field experiments because of its effectiveness in catching 75% of *C. flavipes*. (Table 3). The other traps that were tested caught less than 20% on average (Table 3).

Table 3. Perspex cage experiments using different traps baited with parasitoid diet

Trap design	Bait	Percentage of <i>C. flavipes</i> trapped
Water traps	Diet	12
Plastic plates	Diet	15
Plastic cups	Diet	8
Delta trap	Diet	28
Modified delta trap	Diet	75

4.6 Semi-field trapping of *C. flavipes* with baits

When modified delta trap was tested in the large cages using diet and host plant complex (HPC) as the bait it proved effective with 45% *C. flavipes* being caught. There was no significant difference between the two baits used ($\chi^2 = 0.1905$; $P = 0.6625$)

In experiment 2, increasing the number of traps per cage increased trap catches. The number of *C. flavipes* trapped was significantly higher ($F = 561.96$; $df = 4, 235$; $P = 0.0001$) using 6 and 8 traps in a cage compared to when 1, 2 and 4 traps were put in a cage. In addition 4 traps caught more *C. flavipes* than 2 traps and 2 traps caught more *C. flavipes* than a single trap (Table 4).

Table 4. The number of *Cotesia flavipes* trapped when varying number of traps were placed in a cage

Number of traps placed in a cage	N	Percentage of <i>C. flavipes</i> trapped (mean \pm SE)
8	48	57 \pm 1.1 a
6	48	54 \pm 0.6a
4	48	35 \pm 1.0b
2	48	19 \pm 0.9c
1	48	10 \pm 0.5d

Numbers followed by the same letter in a column are not significantly different (SNK); $F = 597.93$; $df = 4, 235$; $P = 0.0001$

4.7 Evaluating the potential of kairomone baited traps under semi-field conditions

Results from the preliminary experiments conducted to determine the release rates of the diet and the kairomones were 18.83 $\mu\text{g}/\text{hour}$ for (Z)-3-Hexenylacetate (Figure 11), 18.83 $\mu\text{g}/\text{hour}$ for the blend of (Z)-3-Hexenylacetate and Thomac oil (1:1) (Figure 12), 18.73 $\mu\text{g}/\text{hour}$ for Thomac oil (Figure 13), 12.27 $\mu\text{g}/\text{hour}$ for (E)- β -Farnesene (Figure 14) and 1.4 g/hour for *C. flavipes* diet (Figure 15). The preliminary release rate experiment done for both the kairomones and diet

indicated that within the eight hours of the semi-field experiment, there were traces of kairomones and diet remaining on the traps which could attract the parasitoids.

When trapping was done using kairomones, the number of *Cotesia flavipes* trapped was significantly higher ($F = 11.28$; $df = 3,188$; $P = 0.0001$) using a blend of (Z)-3-Hexenylacetate and Thomac oil (1:1) (150 μg per disc) compared to (Z)-3-Hexenylacetate (150 μg per disc), (E)- β -Farnesene (100 μg per disc) and Thomac oil (150 μg per disc) tested alone (Table 5). There was no difference in the number of *C. flavipes* trapped when (Z)-3-Hexenylacetate (150 μg per disc), (E)- β -Farnesene (100 μg per disc) and Thomac oil (150 μg per disc) were used as the bait (Table 5). Control traps failed to trap any *C. flavipes* during the experimental period.

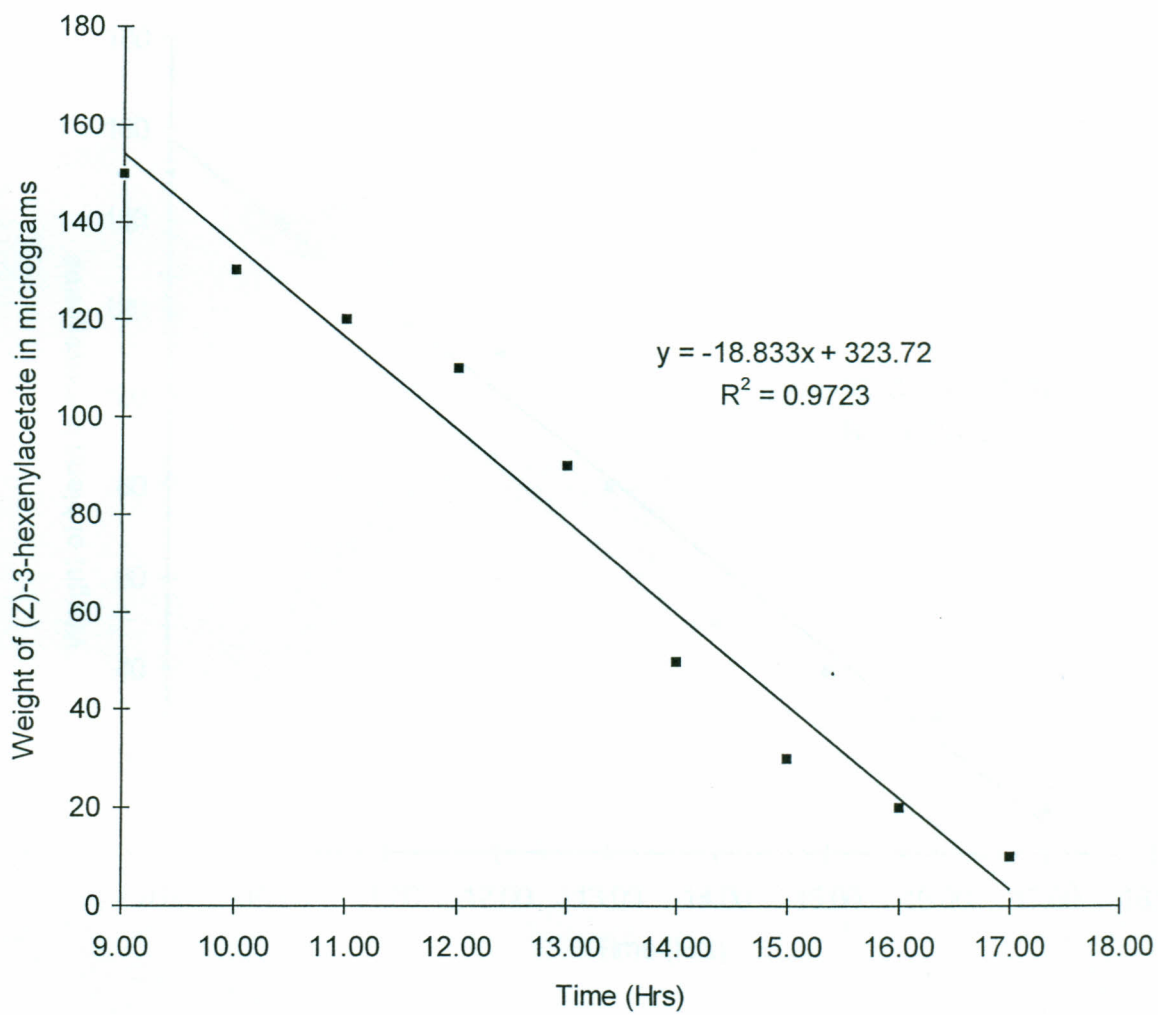
Figure 11. Release rate of 150 μg of (Z)-3-Hexenylacetate

Figure 12. Release rate of 150 μg of a blend of (Z)-3-Hexenylacetate and Thymac oil 1:1 (v/v)

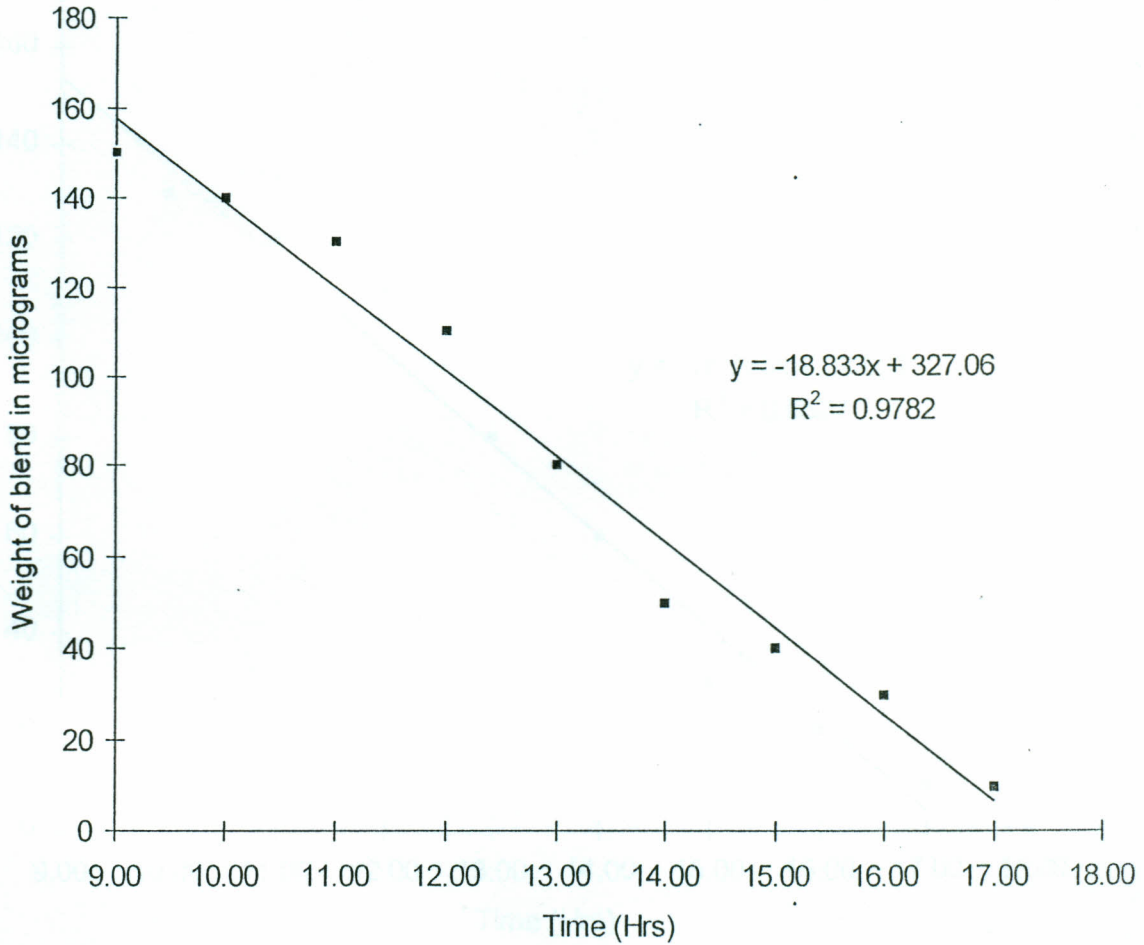


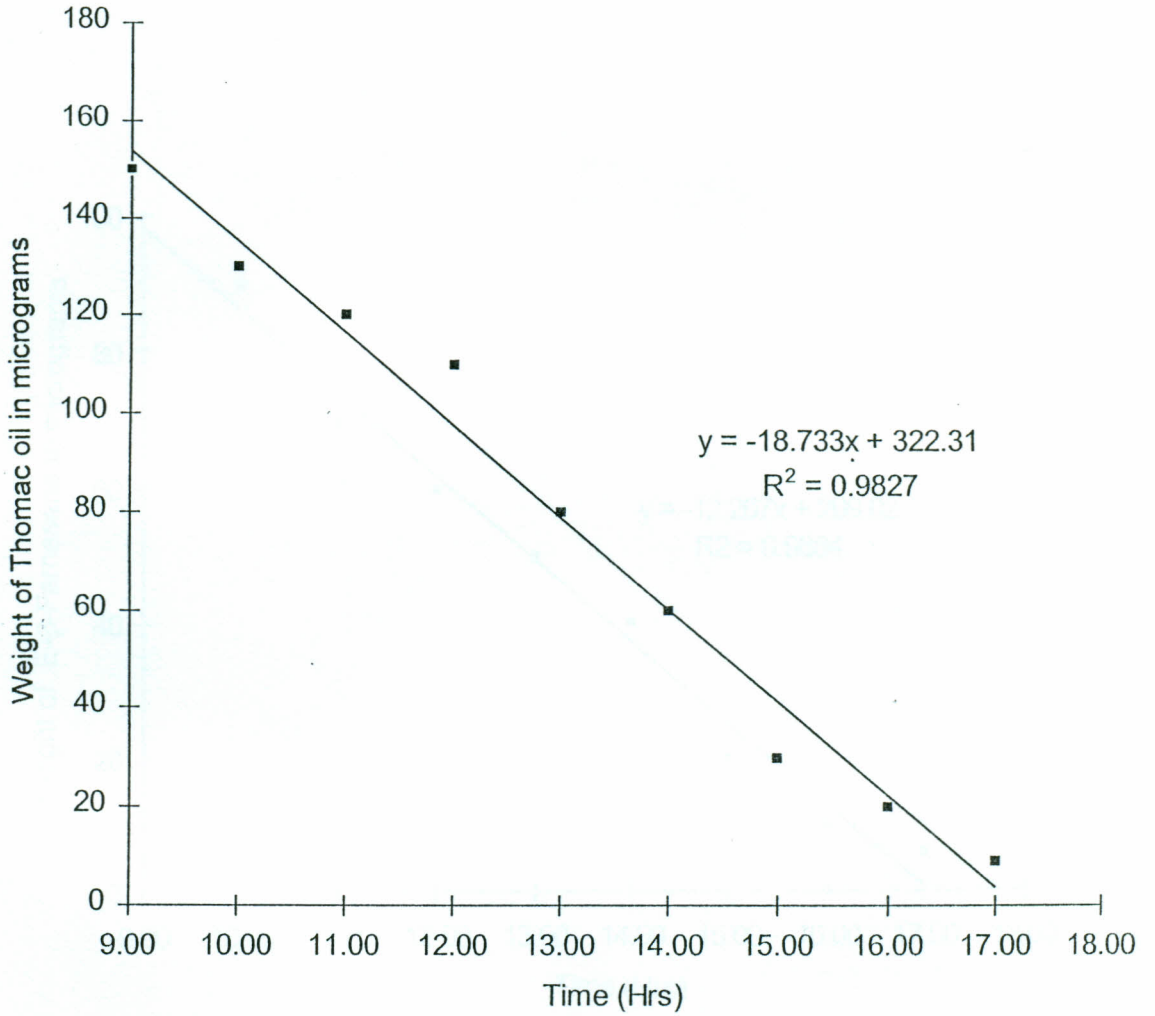
Figure 13. Release rate of 150 μg of Thomac oil

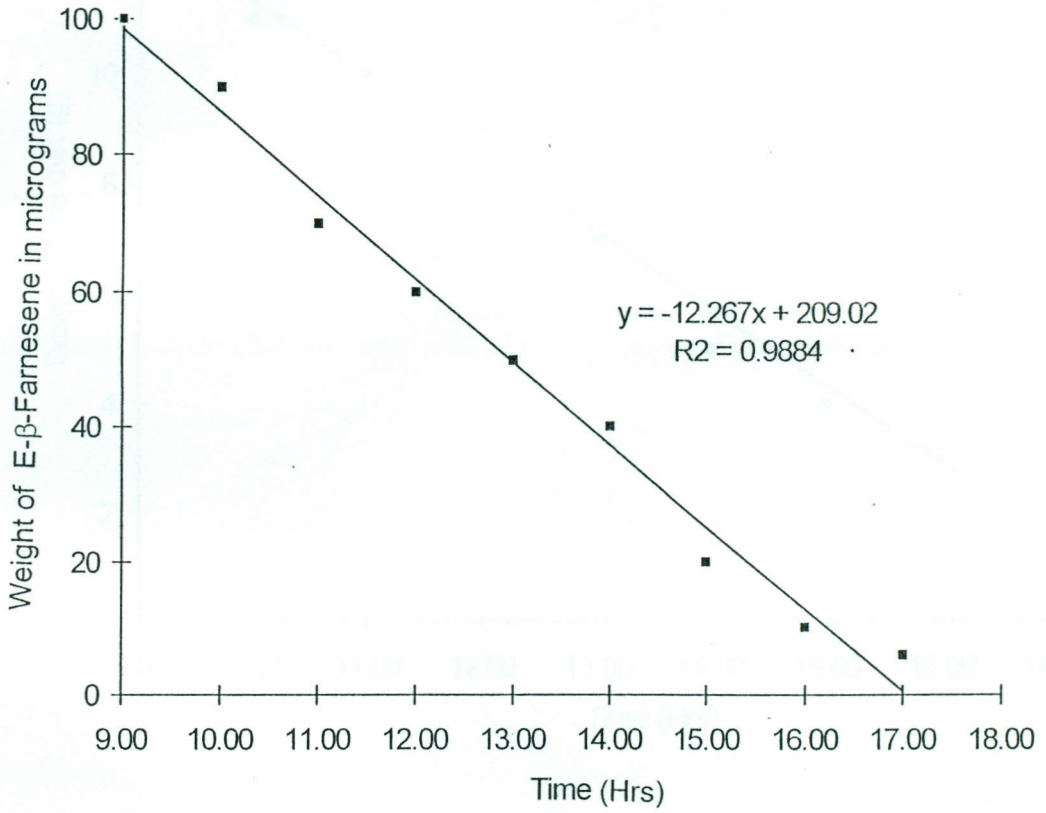
Figure 14. Release rate of 100 μg of (E)- β -farnesene

Figure 15. Release rate of 13 grams of diet

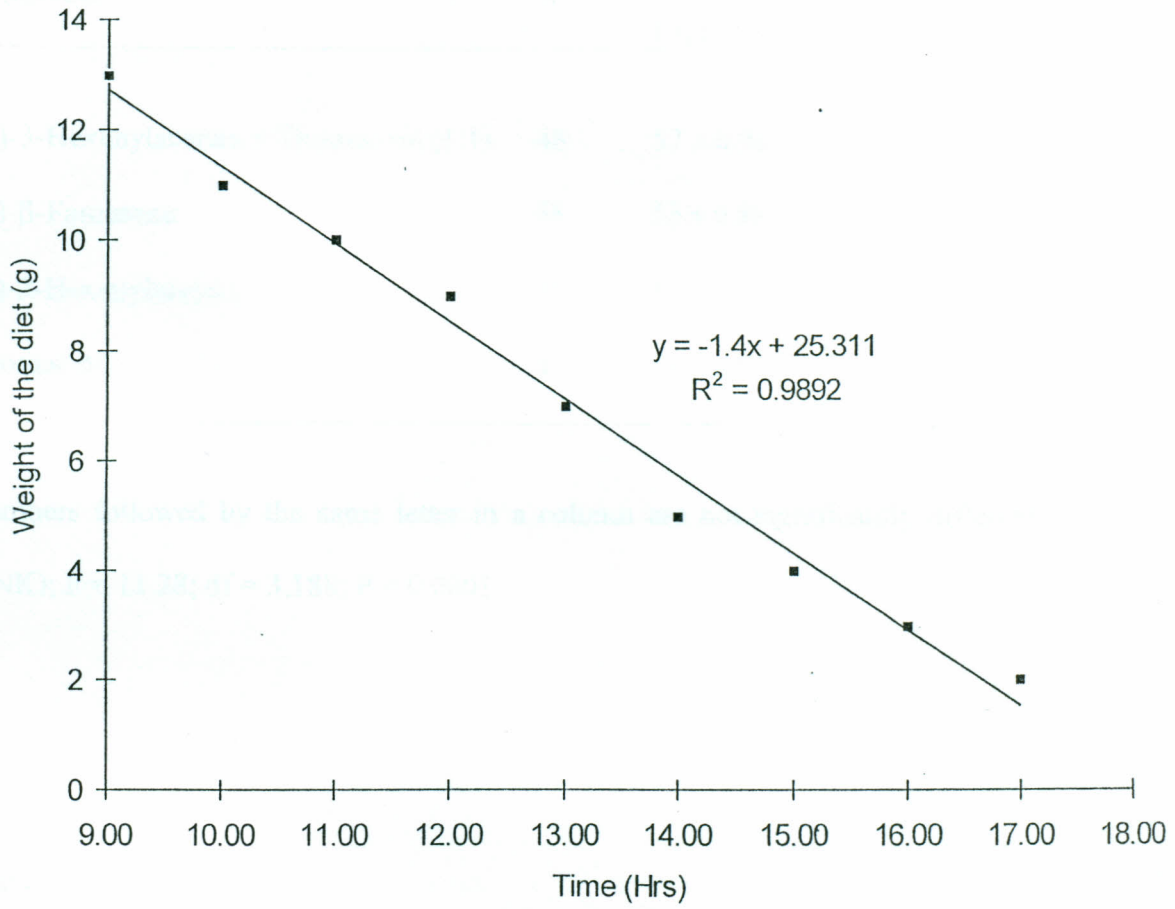


Table 5. The number of *Cotesia flavipes* trapped using different kairomones in semi-field experiment

Compound	N	Percentage of <i>C. flavipes</i> trapped (mean \pm SE)
(Z)-3-Hexenylacetate + Thomac oil (1:1)	48	57 \pm 0.9a
(E)- β -Farnesene	48	53 \pm 0.8b
(Z)-3-Hexenylacetate	48	51 \pm 0.7b
Thomac oil	48	51 \pm 0.8b

Numbers followed by the same letter in a column are not significantly different (SNK); F = 11.28; df = 3,188; P = 0.0001

CHAPTER FIVE

5.0 Discussion

Tests conducted in the laboratory in this study confirm previous reports by Ngi-Song (1995) and Ngi-Song *et al.* (2000) that heptanal, (E)- β -farnesene, 4,8-dimethyl-1,3,7-nonatriene and (Z)-3-hexenylacetate identified in the volatiles from host plant complex (HPC) were attractive to *Cotesia flavipes*. The attractiveness varied with dose of the compounds. Frass, a component of HPC, was also attractive to *C. flavipes* as observed in previous studies by Potting *et al.* (1995) and Ngi-Song and Overholt (1997).

Studies involving several tritrophic systems have demonstrated that feeding by the herbivore induces the release of new volatile compounds (herbivore-induced synomones) that attract predators and /or parasitoids of the herbivore (Sabelis *et al.*, 1984; Dicke and Sabelis, 1988b; Dicke *et al.*, 1990b; Turlings *et al.*, 1990ab, 1991b; Agelopoulos and Keller, 1994ab; Mattiacci *et al.*, 1994). These volatiles have also been found to be attractants for certain phytophagous insect species (Visser, 1986; Hopkins and Young, 1990) and some hymenopteran parasitoids (Whitman and Eller, 1990; Turlings *et al.*, 1991ab; Steinberg *et al.*, 1993; Agelopoulos and Keller, 1994a; Geervliet *et al.*, 1994; Takabayashi *et al.*, 1995; Rose *et al.*, 1998; Pare and Tumlinson, 1999).

Preliminary tests conducted in the field using a trap made from white cardboard (Delta trap) baited with pieces of maize stem infested with *C. partellus* (HPC) showed that *C. flavipes* was weakly attracted to the baited trap. In previous studies by Potting *et al.* (1995), Ngi-Song *et al.* (1996) and Ngi-Song *et al.* (2000), it was shown that the stem borer infested stem was a major source of volatiles that were highly attractive to *C. flavipes*. The present delta traps baited with HPC trapped only few parasitoids. This was contrary to the results of the sampling studies carried out alongside the field trapping experiments which indicated a high number of parasitoids from the manually dissected stem borer infested plants from field plots (Overholt unpublished). The low trap catches observed from the field tests could therefore be due to the trap design which indicated that more work was needed to improve the efficiency of the trap.

The design of the trap has been shown to influence trap attraction and efficiency (Lewis and Macaulay, 1976). To improve the efficiency of the trap in the present study, attempts were made to develop and select a suitable trap in the laboratory for detailed assessment in the semi-field. Four different designs of traps were used. Among the trap designs compared, the modified delta trap performed consistently better than all the other traps tested (Table 3). The water traps tested were not effective in catching *C. flavipes* because the parasitoid simply walked out of water. Plastic cups were not effective, as it was difficult for the parasitoid to find the bait. The modified delta trap which was flat and suspended vertically straight (Plate 2), caught more parasitoids. The increased contact area may have improved

the efficiency of the trap to catch flying insects. Trap efficiency is affected by trap shape and entrance and bait characteristics (Ali Niaze, 1983; Lewis and Macaulay, 1976). A previous study (Unnithan and Saxena, 1990) conducted on the trapping of *C. partellus* using pheromones with modified delta traps, plastic cup traps or water traps showed that the delta trap caught more *C. partellus* than any other trap. Provision of more entrances to the moth improved trap efficiency. Similarly converting the standard delta trap into a flat vertical trap in the present study increased parasitoid catch.

In the semi-field environment, the flat vertical trap baited with parasitoid diet or HPC was effective in trapping the parasitoid although there was no significant difference between the performance of the baits. It was also observed that the number of *C. flavipes* caught varied proportionally. In this study although trap catches were highest with 8 traps per cage, more work is needed to identify the optimum number of traps required to effectively trap the parasitoid in the semi-field.

Several variables have been reported to influence the performance of traps in trap development. These include trap design, trap colour, trap placement, trap density, air, temperature, wind, kairomone concentration and dispenser (Riedl, 1980). In this study, the factors taken into account in trap development were; trap design, trap density, and concentration of the kairomone. Sticky traps have been used in other trapping systems. Sticky traps baited with kairomones have effectively been used in trapping the Northern corn rootworm beetles *Diabrotica*

barberi Smith and Lawrence and Western corn rootworm, *Diabrotica virgifera virgifera* LeConte (Hammack, 1996). Sticky traps have also been used in pheromonal trapping studies for *C. flavipes* (Kimani and Overholt, 1995). Other insects that have been monitored using sticky traps include winged aphids, whiteflies and thrips.

In addition to the chemical composition, the amount of substance released is of great importance. The preliminary study on release rates of the kairomones that were to be used as bait from the dispenser indicated that more than 70% of the kairomone in a trap evaporated before midday. The effect of temperature on release rate has been reported recently (Johansson *et al.*, 2001) in a study which compared release rates, duration in the field and catch efficiency of polyethylene and cotton dental roll dispensers for the sex pheromones of saw flies. The release rate from the cotton dental roll dispenser was found to be dependent on temperature. This study has shown that the time period of 8 hours was the effective window for the kairomone to be dispensed in the paraffin oil carrier. However, more work on the release rates is needed to be carried out alongside with field experiments to determine the actual release rates of diet and the kairomones. In chemical ecological studies, the constancy and predictability of release rate is important. An ideal dispenser is expected to have a constant release rate to dispense the compound in relation to the target insect (Byers, 1988). In a previous study (Anderbrant *et al.*, 1992) the release rate of 3,7-dimethyl-2-pentadecanol was tested in a glass capillary and cotton dental roll. The cotton dental roll was found to be more effective in

dispensing the compound. Cotton dental rolls have been used with satisfactory results in a number of studies on the pine sawfly *Neodiprion sertifer* (Anderbrant *et al.*, 1992; Jonsson and Anderbrant, 1993; Wedding *et al.*, 1995; Simandl and Anderbrant, 1995) and the Northern and Southern corn rootworm beetles (Hammack, 1996, 1997, 2001). Other dispensers reported to be commonly used depending upon the insect include; rubber septa, polyethylene vials and micropipettes.

Results of the semi-field trapping experiments of *Cotesia flavipes* using synthetic kairomones identified from *C. partellus* infested plants (Ngi-Song, 1995) and green leaf volatiles indicated that significantly more *C. flavipes* were attracted to traps baited with a blend of (Z)-3-Hexenylacetate and Thomac oil (1:1) (150 µg per disc) compared to (Z)-3-Hexenylacetate, (150 µg per disc), (E)-β-Farnesene (100 µg per disc) and Thomac oil tested alone at 150 µg per disc. These confirmed previous results which showed that volatiles from herbivore-damaged plants were cues used by parasitoids to locate the habitats of their hosts and the hosts themselves (Vet and Dicke, 1992; Ngi-Song, 1995; Ngi-Song *et al.*, 2000). Similarly, synthetic maize volatiles have been effectively used as baits for trapping corn rootworm beetles (Hammack, 1996). In a study carried out on trapping of Diabroticite corn rootworm beetles using sticky traps baited with single and blended maize volatiles (Hammack, 1996; 1997; 2001) it was shown that blended maize volatiles trapped more beetles than the volatiles tested singly. Likewise, in our study, the blend of (Z)-3-Hexenylacetate and Thomac oil (1:1) attracted more

C. flavipes than the other volatiles, tested singly indicating that lure efficacy can be increased by blending attractants (Metcalf and Lampman, 1997; Petroski and Hammack, 1998). More work is needed to identify more suitable maize volatile blends for the trapping of *C. flavipes*.

In conclusion, the results from this study have confirmed earlier results that synthetic kairomones are attractive to *C. flavipes* in the Y-tube olfactometer. Semi-field experiments have shown the potential of these synthetic kairomones as baits for use with flat vertical sticky traps for rapid monitoring of the presence of *C. flavipes* in release; however more work needs to be done in the field to validate the semi-field experiments. In addition to the volatiles from the HPC system, it was interesting to note that *C. flavipes* was attracted to its own food (honey/ water solution). The attractiveness was comparable to that of kairomones tested. Thus both kairomones and the rearing food could serve as potential baits in the trapping of the parasitoid. Food has effectively been used as a bait in *Maladera matida* beetles (Falach and Shani, 2001). Evaluation of the traps and monitoring of *Cotesia flavipes* could be done using as bait HPC or parasitoid diet that might be easier to obtain by resources-limited farmers than the synthetic compounds of the kairomone tested.

5.1 SUMMARY

1. *Cotesia flavipes* was attracted to heptanal, (E)- β -Farnesene, (E)-4,8-dimethyl-1,3,7-nonatriene, (Z)-3-Hexenylacetate, volatiles isolated from *Chilo partellus* infested maize plants in the Y-tube olfactometer in a dose-dependent manner.
2. Odours from frass were highly attractive to *Cotesia flavipes*.
3. Odours from parasitoid diet (20% honey/water solution) were attractive to *Cotesia flavipes*.
4. The delta trap baited with maize infested with *C. partellus* was able to trap *C. flavipes* under field conditions.
5. Kairomone-baited traps have the potential to trap *Cotesia flavipes* under semi-field conditions.
6. The vertical sticky trap was more effective in trapping *Cotesia flavipes* compared to the delta trap.

5.2 RECOMMENDATIONS

1. The effect of trap colour on the number of *C. flavipes* catches needs to be investigated.
2. Before the trap developed in this work could be used under field conditions several aspects needs to be studied including; kairomone persistence and release rates in the field, efficiency of the trap under different weather conditions, effect of crop density and diversity on the number of wasps caught, effect of trap heights, inter-trap distance and position on catches.
3. The identification of attractive compounds present in the volatiles from frass is needed to fully understand their origin and importance in developing an effective bait.
4. The versatility of the kairomones tested needs to be studied fully.
5. The 20% honey/ water solution should be considered as a cheaper and equally good bait to be used in traps for the monitoring of *Cotesia flavipes*.
6. The selectivity of the trap needs to be studied so that the trap developed only traps *Cotesia flavipes* or *C. flavipes* and a far related species.

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APPENDICES

Appendix 1 Response of *Cotesia flavipes* females in a Y-tube olfactometer to compounds identified from *Chilo partellus* infested maize plants

Compound	N	Dose (μg) per disc	Wasps attracted to the test (%)	Wasps attracted to the control (%)	Unresponsive wasps (%)	P-values
Heptanal	60	5	36.67	51.67	11.67	0.216 n.s
		10	60	23.33	16.67	0.002 **
		15	58.33	35	6.67	0.061 n.s
		20	31.67	41.67	26.67	0.366 n.s
		25	33.33	38.33	28.33	0.647 n.s
(E)-4,8-dimethyl- 1,3,7-nonatriene	60	5	26.67	53.33	20	0.021 *
		10	38.33	56.67	5	0.145 n.s
		15	45	35	20	0.386 n.s
		20	61.67	28.33	10	0.006 **
		25	43.33	20	36.67	0.023 *
(E)- β -farnesene	60	5	33.33	56.67	10	0.057 n.s
		10	70	25	5	0.001 **
		15	66.67	16.67	16.67	0.001 **
		20	56.67	33.33	10	0.057 n.s
		25	35	53.33	11.67	0.141 n.s
(Z)-3-Hexenyl acetate	60	5	31.67	56.67	11.67	0.039 *
		10	58.33	35	6.67	0.061 n.s
		15	73.33	21.67	5	0.001 **
		20	56.67	36.67	6.67	0.109 n.s
		25	40	51.67	8.33	0.345 n.s

Compound	N	Dose (μ g) per disc	Wasps attracted to the test (%)	Wasps attracted to the control (%)	Unresponsive wasps (%)	p-values
Thomac oil	60	5	37	48	15	0.327 n.s
		10	63	25	12	0.002 **
		15	77	8	15	0.001 **
		20	50	32	18	0.116 n.s
		25	33	60	7	0.033 *
(Z)-3-Hexenylacetate + Thomac oil (1:1)	60	5	28.33	61.67	10	0.0065 **
		10	50	41.67	8.33	0.5002 n.s
		15	75	21.67	3.33	<.0001 ***
		20	66.67	25	8.33	0.0007 ***
		25	50	36.67	13.33	0.2673 n.s
Diet	60		65	14	12	0.0006 ***
Frass	60		83.33	8.33	3.33	<.0001 ***

N= Number of replicates; *Significantly different at $p < 0.05$; **Significantly different at $p < 0.01$;

***Significantly different at $p < 0.001$; n.s- not significant (Chi-square test)

Appendix 2 Table showing insects caught in delta traps baited with host plant complex (HPC) under field conditions

LOCATION OF THE FIELD	TYPE OF INSECT COLLECTED
Taru-Mwambani (03 ^o 45.10S, 039 ^o 02.14E)	<p>Hymenoptera: Braconidae-<i>Cotesia flavipes</i></p> <p>Hymenoptera-Ichneumonidae</p> <p>Hymenoptera-Encyrtidae</p> <p>Coleoptera-Coccinilidae</p> <p>Diptera-Phoridae</p> <p>Hemiptera</p> <p>Diptera-Culicidae</p> <p>Diptera-Tephritidae</p> <p>Diptera-Tachinidae</p>
Mwereni-Maledi (04 ^o 26.85S, 039 ^o 07.78E)	<p>Hymenoptera: Braconidae-<i>Cotesia flavipes</i></p> <p>Diptera-Culicidae</p> <p>Hemiptera</p> <p>Coleoptera-Coccinilidae</p>

Appendix 3 Table showing the weight change of (Z)-3-Hexenyl acetate (150µg)

with time.

Rep Time	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	Average
9:00 AM	150	150	150	150	150	150	150	150	150	150	150
10:00 AM	140	130	130	140	130	140	130	140	130	130	130
11:00 AM	120	120	130	120	130	120	120	130	120	120	120
12:00 PM	100	100	110	110	100	110	110	100	100	110	110
1:00 PM	90	80	90	80	80	80	90	80	90	90	90
2:00 PM	50	60	50	50	60	50	50	50	60	60	50
3:00 PM	40	30	30	40	40	30	30	40	30	30	30
4:00 PM	30	30	20	30	20	30	20	20	20	20	20
5:00 PM	10	9	10	10	10	9	10	10	9	9	10

Appendix 4 Table showing the weight change of a blend of (Z)-3-Hexenyl acetate and Thomac oil (150 μ g) with time.

Rep Time	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	Average
9:00 AM	150	150	150	150	150	150	150	150	150	150	150
10:00 AM	140	140	130	140	140	140	140	140	140	140	140
11:00 AM	130	120	130	130	130	130	130	130	130	130	130
12:00 PM	100	100	110	110	100	110	110	100	100	110	110
1:00 PM	80	80	80	80	80	80	90	80	80	80	80
2:00 PM	50	60	50	50	60	50	50	50	60	60	50
3:00 PM	40	40	40	40	40	40	40	40	40	40	40
4:00 PM	30	30	30	30	30	30	30	20	30	30	30
5:00 PM	10	10	10	10	10	9	10	10	10	10	10

Appendix 5 Table showing the weight change of Thomac oil (150 μ g) with time.

Rep Time	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	Average
9:00 AM	150	150	150	150	150	150	150	150	150	150	150
10:00 AM	140	130	130	140	130	140	130	140	130	130	130
11:00 AM	120	120	130	120	130	120	120	130	120	120	120
12:00 PM	100	100	110	110	100	110	110	100	100	110	110
1:00 PM	90	80	90	80	80	80	80	80	80	80	80
2:00 PM	60	60	60	60	60	50	60	60	60	60	60
3:00 PM	40	30	30	40	40	30	30	40	30	30	30
4:00 PM	30	30	20	30	20	30	20	20	20	20	20
5:00 PM	10	9	8	10	10	9	10	10	9	9	9

Appendix 6 Table showing the weight change of (E)- β -Farnesene (100 μ g) with time.

Rep Time	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	Average
9:00 AM	100	100	100	100	100	100	100	100	100	100	100
10:00 AM	90	80	90	90	90	90	80	90	90	90	90
11:00 AM	70	70	70	80	70	70	80	70	70	70	70
12:00 PM	50	60	60	60	50	60	60	50	60	60	60
1:00 PM	50	50	50	40	40	50	50	40	50	50	50
2:00 PM	40	40	40	30	40	40	40	40	30	30	40
3:00 PM	20	20	20	20	10	10	20	20	20	20	20
4:00 PM	10	10	9	10	10	10	10	10	10	9	10
5:00 PM	7	6	7	7	6	5	6	5	4	6	6

Appendix 7 Table showing the weight change of Diet (13g) with time.

Rep \ Time	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	Average
9:00 AM	13	13	13	13	13	13	13	13	13	13	13
10:00 AM	12	11	12	10	12	10	12	12	11	12	11
11:00 AM	10	10	11	11	10	10	10	11	10	11	10
12:00 PM	9	8	9	9	9	8	9	9	8	9	9
1:00 PM	7	7	7	7	6	7	6	7	7	7	7
2:00 PM	5	6	5	5	6	5	5	6	5	5	5
3:00 PM	4	4	4	4	3	4	4	4	4	4	4
4:00 PM	3	3	3	3	3	4	4	3	3	3	3
5:00 PM	2	2	1	2	1	1	1	2	2	2	2

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