PARASITIZATION OF SELECTED AFRICAN STEMBORERS BY COTESIA FLAVIPES CAMERON AND COTESIA SESAMIAE (CAMERON) (HYMENOPTERA:BRACONIDAE) WITH EMPHASIS ON HOST SELECTION AND HOST SUITABILITY.

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Crop Science (Entomology)

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DECLARATION

I hereby declare that the work herein now submitted as a thesis for the Doctor of Philosophy Degree in Crop Science (Entomology) is the result of my own investigations and has not been submitted for a similar degree in any other University.

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To my father, **Ndjee Ndebi Theodore Blaise**

whose love, care, and support

were fountains of inspiration to my chosen career,

but never lived to see me through.

ABSTRACT

Chilo partellus (Swinhoe) is a major pest of maize and sorghum in East Africa and indigenous natural enemies have been unable to maintain the pest population at a level acceptable to farmers. In Kenya, a classical biological control programme was initiated in an attempt to increase natural suppression of *Chilo partellus* and other stemborers. To do this, a natural enemy of *Chilo partellus*, *Cotesia flavipes* Cameron (Hymenoptera: Braconidae), has been introduced into Kenya from Pakistan since 1991. This work investigated the host and host habitat location, the acceptability and suitability of *Chilo partellus* and indigenous stemborer hosts for the development of *Cotesia flavipes* and a local natural enemy, *Cotesia sesamiae* (Cameron) (Hymenoptera: Braconidae), as well as the semiochemicals involved in host finding.

The host selection process of the larval parasitoids *Cotesia flavipes* and *Cotesia sesamiae* was studied in the laboratory. Female parasitoids were attracted to odours from uninfested maize, sorghum and napier grass in a Y-tube olfactometer. In a dual choice test, the three plant species infested with *Chilo partellus*, *Chilo orichalcociliellus* Strand, *Busseola fusca* (Fuller) or *Sesamia calamistis* Hampson larvae were more attractive than uninfested plants. *Cotesia flavipes* and *Cotesia sesamiae* did not show preference for any of the stemborer species under study in dual choice tests. Odours from frass, produced by the four stemborer species fed on maize, sorghum, and napier grass were attractive to

both parasitoid species. *Cotesia flavipes* was more attracted to frass produced by *Chilo* species than frass produced by *B. fusca* in dual choice tests. No preference was observed in other combinations. *Cotesia sesamiae* was equally attracted to all types of frass.

Attractive volatiles from infested plants were collected using activated charcoal traps and were subjected to gas chromatography -electroantennography (GC-EAD) and gas chromatography-mass spectrometry (GC-MS). Twelve GC-EAD active peaks were observed. Three of the peaks were identified as anisole, (E)-β-farnesene, and (Z)-3-hexenyl acetate. Other chemicals identified from maize infested with *Chilo partellus* included myrcene, 2-heptanone, 4,8-dimethyl-1,3,7-nonatriene, (Z)-2-hexenal, (Z)-3-hexen-1-ol, cyclosativen, cedrene and α-copaene.

GC comparison of infested and uninfested maize seedlings showed a quantitative and qualitative difference in the volatile composition. Anisole, (E)-β-farnesene and 4,8-dimethyl-1,3,7-nonatriene were absent in the volatile collection of uninfested maize. The chemical composition of the headspace of artificially damaged maize seedlings treated with larval regurgitant was similar to that of infested maize seedlings. Behavioural bioassays showed that *Cotesia flavipes* was attracted to volatile extracts of infested and uninfested maize and that they were attracted to synthetic anisole, (E)-β-farnesene, (Z)-3-hexenyl acetate.

Chilo partellus, *Chilo orichalcociliellus*, *B. fusca* and *S. calamistis*, were exposed to female of *Cotesia flavipes* and *Cotesia sesamiae* to assess their acceptability for oviposition and suitability for the development of the parasitoids.

There were no differences in the acceptability of the four hosts exposed to *Cotesia flavipes*. In contrast, *Cotesia sesamiae* preferred *S. calamistis* larvae (92%), followed by the two *Chilo* species. *Busseola fusca* larvae were least attacked (48.8%) by *Cotesia sesamiae*. The suitability of the four hosts also varied with the parasitoid species. In *B. fusca* both parasitoid species did not develop and egg encapsulation was observed. *Chilo partellus*, *Chilo orichalcocilielius* and *S. calamistis* were suitable hosts for the development of *Cotesia flavipes*. However, a higher mortality of immature parasitoids was observed in *S. calamistis* as compared to *Chilo partellus*. No differences were found in the mean number of progeny per female, or the sex ratio. The most suitable host for *Cotesia sesamiae* was *S. calamistis*, followed by the two *Chilo* species, which were equally suitable. When third, fourth, fifthand sixth instars of *Chilo partellus* were exposed to *Cotesia flavipes* females, third instars were less suitable than fourth, fifthor sixth instars as measured by immature parasitoid mortality. Total haemocyte counts increased in the blood counts in parasitized larvae two to six days after parasitization, suggesting an active immune interaction between the suitable host, *Chilo partellus*, and the invading parasitoids.

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CHAPTER ONE

1.1 General introduction

In Africa south of the Sahara, sorghum and maize are among the main food crops (Hill,1983; Dogget, 1988; CIMMYT,1992). In Kenya alone, nearly 1,500,000 hectares of maize are under cultivation (FAO, 1991). Maize and sorghum are also used for making flour, beer, as building materials, as a fuel source and as food for livestock. The production of maize and sorghum is low due to several constraints including, climatic factors, plant diseases, weeds and damage due to insect pests.

In the tropics, over 150 species of insect pests are known to damage these two crops (Ingram, 1958; Mohyuddin and Greathead, 1970; Young and Teetes, 1977; Seshu Reddy and Davies, 1979; Hill, 1983; Seshu Reddy, 1991). Lepidopteran stemborers are generally considered to be the most important. Plant injury by stemborers is caused by larvae feeding in the plant's whorl and later through stem tunneling. Infested plants have poor growth, reduced yield and are more susceptible to lodging and secondary infections. At the early stage of plant growth a heavy infestation can cause 'dead hearts' and sometimes lead to total crop failure. Losses due to stemborers as high as 88-100% in sorghum, and 23-100% in maize have been reported (Whealey, 1961; Seshu Reddy, 1983, 1988; Ampofo, 1986; Seshu Reddy and Walker, 1990). Several species of noctuid and pyralid stemborers occur in mainland Africa and, with exception of *Chilo partellus*

(Swinhoe) (Lepidoptera: Pyralidae), all are thought to be indigenous. *Chilo partellus*, which is native to Asia, was first reported in Africa in the early 1930's in Malawi (Tams, 1932), and was later recorded in Uganda (Ingram, 1958) and Tanzania (Duerdon, 1953). By 1977, the distribution included Ethiopia, Kenya, Malawi, Mozambique, Somalia, South Africa, Sudan, Tanzania and Uganda (CAB, 1987). *Chilo partellus* is still spreading; Botswana, Swaziland, Togo and Cameroon have been added to the list of invaded areas more recently (IAPSC, 1985; Harris, 1990). The authenticity of the West African reports is questionable because recent surveys in the region failed to recover *Chilo partellus* (Bosque-Perez and Mareck, 1990; Schulthess *et al.*, 1991; Shanower *et al.*, 1991; Gounou *et al.*, 1993).

Chilo orichalcociliellus Strand is an important native stemborer in coastal East Africa (Warui and Kuria, 1983) and it is also found in several African countries including Zaire, Malawi, Kenya, South Africa, and Madagascar at altitudes below 600 m (Bleszynski, 1970; Delobel, 1975). On the Kenyan coast, *Chilo orichalcociliellus* was the predominant pyralid stemborer before about 1970, but appears to becoming progressively less important, possibly due to displacement by *Chilo partellus* (Overholt *et al.*, 1994b).

In addition to *Chilo orichalcociliellus*, several other indigenous stemborers are considered to be economically important, including the noctuids *Sesamia calamistis* Hampson and *Busseola fusca* Fuller, which occur throughout sub-Saharan Africa. In East Africa, *B. fusca* is mainly found at higher altitudes (>600

m) (Nye, 1960; Sithole, 1989). It is the predominant stemborer species in the western and central parts of Kenya (Figure 1.1).

Stemborer larvae feed in communities of wild and cultivated grasses which have stem sufficiently large to accommodate stemborer feeding behaviour (Harris, 1990). In addition to grasses, sedges (Cyperaceae) and cat-tails (Typhaceae) are important wild host plants of some stemborers (Jepson, 1954; Seshu Reddy, 1989; Conlong, 1990). Native grass, sedges and cat-tails are presumably the aboriginal host plants for indigenous stemborers in Africa, and many species are attacked. However, densities in wild hosts do not reach the levels observed in cultivated crops (Mathez, 1972). The introduction and widespread cultivation of maize in Africa (Purseglove, 1972) is likely to have had an impact on the abundance of graminaceous stemborers by providing a highly nutritious and readily available food source with little inherent resistance to stemborers (Overholt, 1994).

Various control measures have been used in attempts to reduce the losses due to these stemborers, including chemical control, cultural practices, the use of host plant resistance, and biological control. Noctuid stemborers have been controlled with insecticidal dusts applied against young larvae entering the plants (Swaine, 1957; Walker, 1961), but this method is not often economical for subsistence farmers. Although insecticides may be effective, they are expensive and their application must be precisely timed to control borers before they enter the stalk (Mathez, 1972; Ingram, 1983).

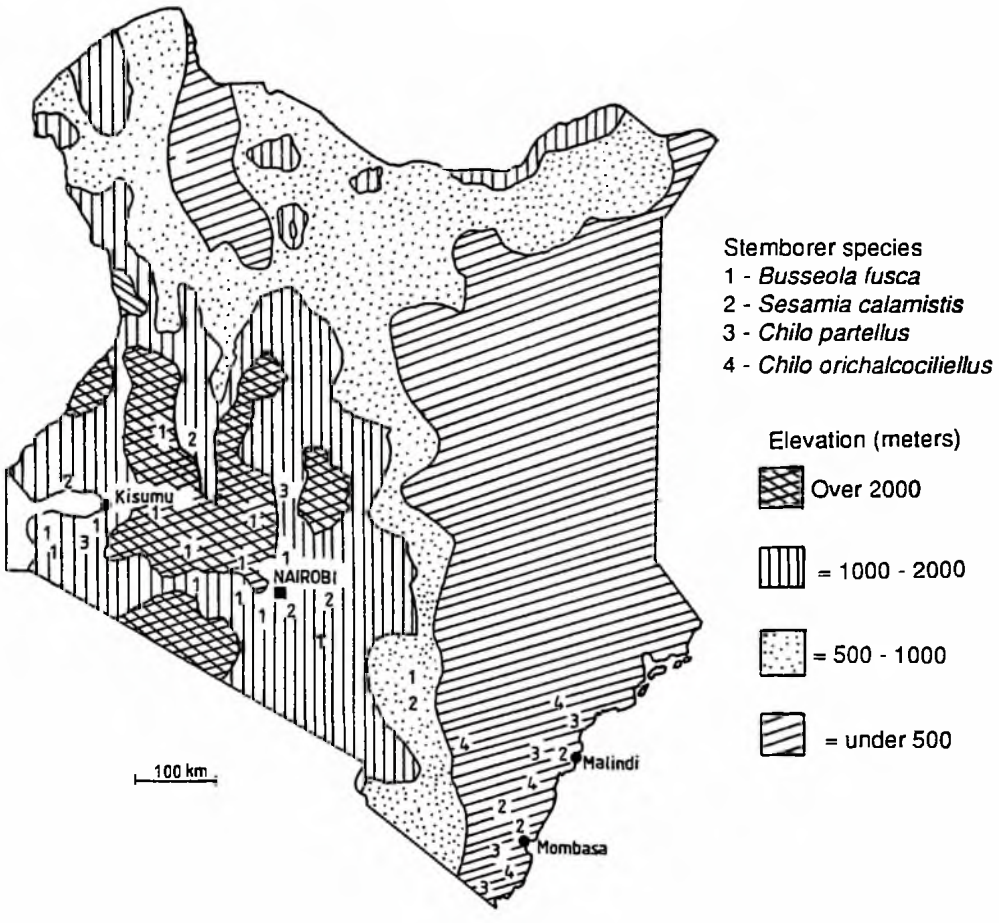

Figure 1.1 Distribution of 4 major stemborer species in Kenya (Nye, 1960)

Cultural practices have been attempted with limited success. Attempts include the destruction of crop residues after harvest by farmers (Minja, 1990), destruction of wild host plants in the proximity of fields (Ingram, 1958), and intercropping (Minja, 1990; Pats, 1994).

Varietal resistance to attack by borers may also reduce the intensity of infestation by stemborers (Saxena *et al.*, 1992). However, agronomically acceptable varieties with adequate levels of stemborer resistance are not yet available (Leuschner *et al.*, 1985; Nwanze and Youm, 1994).

For the purpose of biological control, the role of local parasitoids and predators in suppressing stemborer populations in East Africa has been examined, and some species of natural enemies have been identified, including several species of larval and pupal parasitoids (Mohyuddin, 1970; Ingram, 1983; Mohyuddin, 1990; Overholt, 1994). *Cotesia sesamiae* is the most common larval parasitoid and is widely distributed in Africa (Mohyuddin, 1990; Polaszek and Walker, 1991) where it attacks *Chilo partellus*, *Eldana saccharina* (Walker), *Maliarpha separatella* Ragonot, *S. calamistis*, and *B. fusca* (Mohyuddin, 1971; Shami and Mohyuddin, 1986; Polaszek and Walker, 1991). The biology of this insect has been studied by Ullyett (1935) and Mohyuddin (1971). *Cotesia sesamiae* does not appear to effectively regulate *Chilo partellus* populations in Kenya (Oloo, 1989; Oloo and Ogeda, 1990; Overholt *et al.*, 1994a). In Africa, there are few reports of augmentation biological control using natural enemies. Most studies have concentrated on the bionomics and biology of parasitoids and

predators (Bahana, 1985, 1987; Ochiel, 1989). Lu (1988) studying the dispersal of *Trichogramma* sp. nr. *mwanzai*, in Western Kenya, reported that this parasitoid had a good potential for inundative release. However, subsequent field and caged experiments showed that the parasitoid had a poor searching ability resulting in very low parasitism (Chacko and Ogedah, 1990; Dwumfour and Chacko, 1990).

Augmentation biological control using pathogens has also been attempted. Recent work at the ICIPE has demonstrated the potential use of the microsporidian protozoan, *Nosema* sp., which led to an 80% increase in yield on experimental plots when plants were artificially infested with *Chilo partellus* larvae (Odindo, 1991). In glass house experiments, yields of sorghum protected with a spray of a local isolate of *Bacillus thuringiensis* Beliner were five to seven times higher than those from unprotected sorghum (Brownbridge, 1991). In laboratory experiments, eggs of *Chilo partellus* were reported to be susceptible to some ICIPE isolates of *Beauveria bassiana* Vuillemin, *Metarhizium anisopliae* Sorokin and *Paecilomyces fumosoroseus* Brown and Smith (Maniania, 1991).

Classical biological control which utilizes exotic natural enemies to regulate pest populations (DeBach and Rosen, 1991) has also been attempted against stemborers in Africa (Moutia and Courtois, 1952; Betbeder-Matibet and Malinge, 1968; Appert *et al.*, 1969; Greathead, 1971; Bordat 1983; Kfir, 1994; Overholt, 1994). The most commonly released parasitoid has been *Cotesia flavipes* which is indigenous to the Indo-Australian region where it attacks a number of pyralid and noctuid stemborers (Mohyuddin, 1971; 1978; 1989; 1990). *Cotesia flavipes* has

been introduced into many countries including Pakistan, India, Brazil, Madagascar, Barbados, Peru, Gaudeloupe, Bahamas, Mauritius, USA, Ghana, Ivory Coast and Senegal, with variable levels of success (Greathead, 1971; Mohyuddin, 1971: Alam *et al.*, 1972; Simmonds, 1974; Attique *et al.*, 1980; Macedo *et al.*, 1984; Breniere *et al.*, 1985; Youm *et al.*, 1988).

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; Subba Rao *et al.*, 1969; Mohyuddin, 1971; Shami, 1990). Briefly, the adult *Cotesia 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 three days. The larvae develop through 3 instars within the host where they feed on body fluids. The egg-larval period is 10-15 days. The last instar of the parasitoid emerge from the host by chewing their way through the stemborer integument, after which they immediately spin cocoons and pupate. Adult parasitoids emerge about six days later.

Biological control of introduced stemborers using exotic parasitoids, has been highly successful in some countries (Alam *et al.*, 1972; Attique *et al.*, 1980; Macedo *et al.*, 1984; Youm *et al.*, 1988). The success of classical biological control agents has been attributed in part to the high searching efficiency of the natural enemy for its host. The ability of a natural enemy to locate a suitable host in a complex environment is critical to its successful establishment in a new environment (Nordlund *et al.*, 1988). It is known that both the host and host plants

play an important role in host selection by parasitoids and chemicals emanating from the host plant 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, the host itself or by host by-products, such as faeces, silk and honeydew. 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) and may also assist in understanding the factors underlying the eventual success or failure of a biological control programme.

A programme to introduce *Cotesia flavipes* into Kenya for the control of *Chilo partellus* was initiated in 1991. The efficiency of *Cotesia flavipes* in controlling stemborers may depend on its ability to establish in wild grasses and periodically invading cultivated grasses during the growing season. Additionally, it also depends on the degree to which the parasitoid is able to find its hosts and successfully parasitize them. Some aspects of the host selection behaviour of *Cotesia flavipes* have been examined (Mohyuddin *et al.*, 1981; Inayatullah, 1983; Van Leerdam *et al.*, 1985; Shami and Mohyuddin, 1992; Potting *et al.*, 1993). Most of these studies investigated the effect of stemborer diet on host and host plant preference of the parasitoids with respect to attraction to different volatile emanations from frass (Mohyuddin *et al.*, 1981; Inayatullah, 1983; Shami and Mohyuddin, 1992). Recent studies by Potting *et al.* (1995) have indicated that *Cotesia flavipes* was attracted to leaves and infested maize stems, although odours

from infested stems attracted the majority of the parasitoids. However, no attempts were made to elucidate the sources and/or characterize the nature and the role of the attractive allelochemicals involved in the host selection behaviour of *Cotesia flavipes*. Long range and short range chemical cues used by *Cotesia flavipes* during the host location process have not been identified. After successful location of the host, it is not known whether the indigenous African stemborers would be acceptable or suitable for the development of the exotic parasitoid. On the other hand, it is not clear why *Cotesia sesamiae* is not able to keep *Chilo partellus* populations at low levels. Bennett (1993) suggested that a native parasitoid, *Apanteles diatraeae* Muesebeck (Hymenoptera: Braconidae), may have been extirpated from maize ecosystem in Trinidad when the ecologically similar species, *Cotesia flavipes* was introduced for the biological control of *Diatraea saccharalis (F.)*, in sugarcane, but that *A. diatraeae* is probably still found in other habitats. *Cotesia flavipes* and *Cotesia sesamiae* are also ecologically similar species that are thought to be closely related (Polaszek and Walker, 1991). However, the degree of competition between the two species, and the likely result of the competition are unknown. Therefore, studies on host habitat finding, host finding, host acceptance and suitability will provide information on the amount of niche overlap between the two species. These investigations will shed light on the factors that influence the foraging behaviour of these two parasitoids.

1.2 Objectives:

The main objectives of the present study are to investigate the host selection process of *Cotesia flavipes*, and to conduct a comparative study of the suitability of exotic and indigenous stemborers for the development of *Cotesia flavipes* and *Cotesia sesamiae*.

Specific objectives :

1. Study of the host selection process of *Cotesia flavipes* and *Cotesia sesamiae*.
2. Isolation and identification of infochemicals involved in the host selection process of *Cotesia flavipes*.
3. Investigation of the acceptability of four African stemborers for parasitization by *Cotesia flavipes* and *Cotesia sesamiae*.
4. Examination the suitability of the above four stemborers for the development of *Cotesia flavipes* and *Cotesia sesamiae*.

CHAPTER TWO

2. Literature review

2.1 Host selection process in insect parasitoids

The selection of the most effective species or strain of entomophagous insects for use in classical biological control is critical to the success of the programme (Nordlund *et al.*, 1988). Although extensive biological data are often used in selecting the best candidates (Waage, 1990), micro-habitat preference is not generally considered (Nordlund *et al.*, 1988). Tritrophic interactions involving plants, herbivores, and natural enemies are, to a large extent, intricate arrays of chemical substances referred to as allelochemicals. Plant substances 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 complex relationships of allelochemicals not only increase our understanding, but also provides potential for the manipulation of these communication systems (Whitman, 1988).

Since the early reviews of Salt (1935), Flanders (1953), and Doutt (1964), the understanding of the host selection process of insect parasitoids has greatly expanded (Vinson, 1991; Vet and Dicke, 1992; Godfray, 1994). Information about the complex factors that result in a host being located and successfully attacked is, however, inadequate. The stimuli utilized by a female parasitoid are many and

varied, and the behaviour they elicit in various species of parasitoids are equally varied. The nature of the stimuli depends on the biological characteristics and the life strategies of the host, as well as on the morphological, physiological and behavioural characteristics of the parasitoid under study (Vinson, 1991).

Semiochemicals are chemicals that mediate inter- and intraspecific interactions (Nordlund, 1981). 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 have been 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 a physiological response that is adaptive to either of 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).
ALLELOCHEMICALS: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 behavioural or physiological response that is adaptively favourable to organism 1 but not to 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 favourable to organism 2 but not to organism 1.

SYNOMOME: 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 favourable to both organism 1 and 2.

The chemical cues utilized by parasitoids and the behavioural sequences they elicit have been discussed by various authors (Whittaker, 1970; Vinson, 1976, 1985; Arthur, 1981; Jones, 1981; Weseloh, 1981; Van Alphen and Vet, 1986; Kainoh, 1990). Doutt (1964) divided the process of locating and parasitizing hosts by parasitoids into three steps: (1) location of the host habitat, (2) location of the host, and (3) acceptance of the host. Infochemicals may be utilized in all these steps.

2.1.1 Host habitat location

Habitat location by 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). Vinson (1991) suggested that physical factors such as temperature, light, wind and humidity play a major role in causing parasitoids to aggregate in a particular microhabitat, thus influencing host selection. Other factors 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, each of which plays a role in the distribution of parasitoids in a particular habitat. However, chemical stimuli are considered the most important cues utilized by parasitoids during foraging.

The complex of volatile stimuli released may depend on the physiological state of the plant (Hedin, 1976), including age or health (Denno and Mc Clure, 1983), and also the cultivar (Elzen *et al.*, 1985). Chemicals emanating from these habitats and associated organisms may result in an accumulation of parasitoids. Chemical extracts of weeds were shown to increase parasitism of *Heliothis* sp. in cotton (Altieri *et al.*, 1981). However, the chemical nature and behavioural specificity of these cues have not been characterized (Vinson, 1991).

Flower scents attract and/or arrest movement of potential natural enemies of herbivores (Whitman, 1988). Many natural enemies are carnivorous only as larvae, while as adults they require nutrition from flowers (Leius, 1967; Shahjahan, 1974; Lewis and Nordlund, 1985; Whitman, 1988). Plant pollen and nectar increase longevity and fecundity of many parasitoids, resulting in greater herbivore mortality (Whitman, 1988).

Plant produced synomones (long range attractants) have been reported in several systems (Read *et al.*, 1970; Vinson *et al.*, 1975; Powell and Zhang Zhi-Li, 1983; Elzen *et al.*, 1983, 1984, 1986; Martin *et al.*, 1990; Takabayashi and Dicke, 1992). For example, Vinson *et al.* (1975) observed *Cardiochiles nigriceps* Viereck

searching host-free tobacco plants. Martin *et al*. (1990) reported that females of *Eucelatoria bryani* Sabrosky, a parasitoid of *Heliothis* spp., responded to volatiles emanating from 13 out of the 19 fresh plant materials tested, all of which were food sources for *Heliothis* spp.. 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), McAuslane *et al*. (1990, 1991) and Vinson and Williams (1991). Plant cultivars are known to differ in the production of entomophage attracting synomones. For example, genetically similar cotton cultivars produce different quantities of terpenes that are attractive to parasitoids (Elzen *et al*., 1985, 1986; Van Emden, 1986). Naive, *Macrocentus grandii* Goidanich, a larval parasitoid of the European corn borer *Ostrinia nubilalis* Hubner was attracted to numerous plant species, including its host food (maize) (Ding *et al*., 1989). Takabayashi and Dicke (1992) reported similar observations in their work with predatory mites. They observed that *Phytoseiulus persimilis* Athias-Henriot was attracted to volatiles from tomato leaves and lima bean leaves but did not respond to volatiles from cucumber leaves or leaves from *Solanum* spp.

2.1.2 Host location

Host location has been 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). This step of the host selection process involves long distance volatile

compounds and short range chemical stimuli originating from the host habitat and /or hosts. Long range attractants could be released from the host food, the host communication system or by organisms associated with the host. Short range attractants may be released by the host or from host products.

The utilization of the chemical information by natural enemies depends on their detectability and reliability (Vet and Dicke, 1992). Chemicals from the herbivore are the most reliable indicators of the herbivore's presence and identity. However, these chemicals appear to have a low detectability at some distance (Vet and Dicke, 1992). On the other hand, chemicals from the host's food plants are more detectable at a distance because of the larger biomass of plants relative to the herbivores. However, information on the presence of food is generally not very reliable in indicating the presence of herbivores (Vinson, 1981). Thus, natural enemies are confronted with a 'reliability/ detectability' problem (Vet *et al.* 1991, Vet and Dicke 1992). To counter this problem some parasitoids have adopted one of two strategies: response to herbivore-induced plant volatiles (Dicke, 1994), or associative learning in which volatiles emanating from the by-products of herbivore feeding activity act as kairomones (Vet and Dicke, 1992). Natural enemies may also cope with the complexity of the sensory information they receive from the environment by focussing on a limited set of chemicals that, through experience, have been shown to be characteristic of a particular plant-herbivore combination (Van Alphen and Vet, 1986; Lewis and Tumlinson, 1988; Vet and Groenewold, 1990; Turlings *et al.*, 1990a, 1991a).

Response to host food.

Natural enemies of herbivores respond to their host's food, particularly when it is being fed on by the host. Food plants generally release odours that attract the third trophic level. Natural enemies are able to discriminate between odours emitted by uninfested plants and plants infested by their host (herbivore). It has been demonstrated that the chemical stimuli involved are not emitted by the herbivore, but by the plant after being damaged by the herbivore (eg. Roth *et al.*, 1982; Sabelis *et al.*, 1984; Vet, 1985; Dicke and Sabelis, 1988b; Eller *et al.* 1988ab; Dicke *et al.* 1989, 1990a b; Whitman and Eller, 1990; Turlings *et al.*, 1990b, 1991b; Steinberg *et al.* 1992, 1993; Agelopoulos and Keller, 1994a; Mattiaci *et al.*, 1994). Eller *et al.* (1988a,b) reported that volatiles collected from a herbivore-plant complex placed on filter paper elicited positive responses from *Micropletis croceipes* (Cresson). In single and dual choice tests conducted in a flight tunnel, females of the larval parasitoid *Cotesia marginiventris* (Cresson) were mostly attracted by volatiles emitted by plants damaged by its host larvae, the beet armyworm *Spodoptera exigua* (Hubner) (Turlings *et al.*, 1991a). Damaged plants from which larvae and frass had been removed (=damage alone), frass alone, artificially damaged plants or larvae alone were less attractive than the host-plant complex (plant with feeding larvae). Similarly, a flight chamber experiment showed that the larval parasitoid *Cotesia glomerata* (L.) was more attracted to cabbage plants infested with its host, *Pieris brassicae* L. than uninfested plants (Steinberg

et al., 1993). The larvae and their faeces were more attractive than uninfested plants, but far less attractive than artificially damaged and herbivore damaged plants. Uninfested plants were the least attractive. In a Y-tube olfactometer, *Cotesia flavipes* exhibited a preference for maize stems with feeding stemborers over artificially damaged maize stems, larvae alone, frass or uninfested maize stems. Maize stems infested with stemborers and damaged maize stems with stemborers removed (damage alone) were equally attractive (Potting *et al.*, 1995).

Artificially damaged plants have been shown to be more attractive than undamaged plants (Elzen *et al.*, 1983, 1987; Eller *et al.*, 1988a,b; Ding *et al.*, 1989; Turlings *et al.*, 1990b; McAuslane *et al.*, 1991; Steinberg *et al.*, 1993). Loke *et al.* (1983), studying the influence of fall armyworm larvae and corn plant damage on host finding by *Cotesia marginiventris*, reported that this parasitoid was attracted to odours from artificially damaged corn plants. Eller *et al.* (1988a) also reported that artificially damaged leaves elicited positive responses from *M. croceipes*.

Research conducted during the past ten years has demonstrated that chemicals released by artificially damaged plants were not sufficient to attract the third trophic level but that volatiles released during herbivore feeding often increased attractiveness (Dicke *et al.*, 1990a,b; Turlings *et al.*, 1991b; Takabayashi *et al.*, 1991; Turlings and Tumlinson, 1992). Chemical studies on the tritrophic interaction between plants, spider mites and predatory mites revealed that plants infested with mites released several terpenes that were exploited by the predatory mites, and which were not present in mechanically damaged plants (Dicke and

Sabelis 1988b, Dicke *et al.*, 1990a,b; Dicke and Dijkman, 1992). Similarly, damage on maize seedlings caused by larval feeding of *S. exigua* induced the release of relatively large amounts of terpenes and terpenoids that were attractive to *Cotesia marginiventris*, a parasitoid of *S. exigua* (Turlings *et al.*, 1990b, 1991b). The herbivore induced production and/or release of specific synomones are referred to as 'herbivore-induced' synomones.

Emissions of synomones by plants are not limited to damaged sites, but have been shown to be systemic, occurring throughout the plant (Dicke *et al.*, 1990b; Turlings and Tumlinson, 1992; Mattiacci *et al.*, 1994). For example, in olfactometric bioassays, Nadel and van Alphen (1987) showed that cassava plants infested by the cassava mealybug attracted the parasitoid *Epidinocarsis lopezi* (DeSantis), and that infested leaves as well as uninfested leaves of infested plants were more attractive than leaves of uninfested plants.

In some systems, the production of plant induced synomones has been shown to be triggered by a factor present in the spit of the herbivore. Mechanical damage on maize seedlings triggered release of only minor amounts of terpenes. However, when caterpillar regurgitant was applied to artificially damaged sites, maize seedlings released terpenoids in amounts similar to those from seedlings that had been damaged by the herbivore (Turlings *et al.*, 1993). Regurgitant of several species of herbivores induced the same reaction on the maize seedlings, indicating that this might be a general response of the maize plant to insect attack (Turlings *et al.*, 1993). Similarly, Mattiacci *et al.* (1994), working on the Brussels

sprouts-*Pieris brassicae-Cotesia glomerata* system, reported that caterpillar regurgitant applied on artificiallydamaged cabbage leaves induced an increase in the release of attractive volatiles. Also, young maize plants treated with regurgitant of *Chilo partellus* larvae were more attractive to *Cotesia flavipes* in a Y-tube olfactometer than untreated plants, suggesting that volatile emissions from the treated plant materials had changed quantitatively and/or qualitatively (Potting *et al.*, 1995).

Response to host products

During foraging, some insects utilize non- or semi-volatile chemical stimuli provided by behavioural or physiological activities specific to the host. These originate from activities such as the construction of shelter, from waste products of the host (frass, honeydew), or from defense and feeding secretions. Host products are reliable indicators of host presence. Other host products include hairs, scales, and silk.

Host frass has often been cited as a source of kairomonal cues. Sauls *et al.* (1979) reported that diet significantly affected the attractiveness of the frass of *Heliothis zea* (Boddie) to *M. croceipes*, although the diets (plant material and artificial diet) *per se* were not attractive. Elzen *et al.* (1984) reported that *Campoletis sonorensis* (Cameron) responded to frass of *Heliothis virescens* F. when larvae were fed on cotton, but did not respond to frass produced by other larvae fed on an artificial diet without cotton plant material. Takabayashi and

Takahashi (1990) reported that the movement of females of the larval parasitoid *Apanteles kariyai* (Watanabe) was arrested on faecal pellets from *Acantholeucania loreyi* (Duponchel) larvae.

Cotesia flavipes (obtained from Pakistan), which is adapted to stemborers feeding on maize, is poorly attracted to frass of hosts fed on sugarcane (CAB, 1987). Inayatullah (1983) found that *Cotesia flavipes* exhibited a differential response to various stemborer hosts fed on different grass diets. Frass produced by *Chilo partellus*, *Sesamia inferens* (Walker), and *Sesamia uniformis* (Dudgeon) fed on sorghum were the most attractive. Van Leerdam *et al*. (1985) reported that *Cotesia flavipes* presented with fresh frass of *D. saccharalis* or a water soluble substance from fresh frass showed a characteristic host-seeking response (decrease in the rate of locomotion coupled with intense palpation of frass with their antennae). However, volatile stimuli emanating from frass was diet dependent. Similarly, *Cotesia sesamiae* females were more responsive to frass from *Chilo infuscatellus Snellen*, *Emmalocera depressella* (Swinhoe) and *Acigona steniellus* (Hampson) that were fed on maize as compared to frass obtained from the same hosts fed on sugarcane (Shami and Mohyuddin, 1986).

The mandibular gland secretions of the larvae of *Plodia interpunctella* (Hubner) attracted and stimulated oviposition in the parasitoid, *Nemeritis canescens* (Gravenhost), leading to increased parasitization (Mossadegh, 1980). A chemical found in the larval mandibular glands of *Ephestia kuehniella* Zeller was determined to be an oviposition kairomone for the larval parasitoid *N. canescens*

(Mudd and Corbet, 1982). A kairomone in the larval mandibular glands of Chilo *partellus* stimulated oviposition in *Cotesia flavipes* (Mohyuddin *et al.*, 1981; Muzaffar and Inayatullah, 1986). The low parasitism of *A. steniellus* and *Calamotropha lupatus* Meyr in the field was attributed to the inability of *Cotesia flavipes* to locate these hosts due to the lack of production of the mandibular gland kairomone (Muzaffar and Inayatullah, 1986).

Response to host communication system.

Some parasitoids have evolved to exploit the communication system of their host (Vinson, 1991). Cases of parasitoid attraction to sex pheromones are found in some egg parasitoids (Vinson, 1988). For example, *Trichogramma evanescens* Westwood was attracted to the sex pheromone of the adult moths, *Pieris brassicae* and *Mamestra brassicae* L., emanating from oviposition sites (Noldus and Van Lenteren, 1985).

Response to associated organisms.

Parasitoids may also respond to odours from organisms associated with their host. Micro-organisms are known to produce allelochemicals that affect the third trophic level. Parasitoids respond to fermentation products from bacterial and fungal activities in the same media (host food) in which their hosts develop (Greany *et al.*, 1977; Vet *et al.*, 1983, 1984; Dicke *et al.*, 1984). Also, host finding by the fruit fly parasitoid, *Biosteres* (*Opius*) *longicaudatus* Ashmed, was found to involve

attraction to fermentation products emanating from rotting fruit;a probable location of host larvae. Bioassays conducted in a greenhouse with saturated 1-, 2-, and 3-carbon primary alcohols, aldehydes and organic acids (chemicals identified from rotting fruits) indicated that acetaldehyde was the most active agent followed by ethanol and acetic acid. Rotting fruit was attractive irrespective of the presence of the host larvae (Greany *et al.*, 1977).

2.2 Isolation and Identification of attractive allelochemicals

2.2.1 Plant-produced long range attractants (synomones).

The semiochemical complexes involved in tritrophic interactions have been reviewed by Vinson (1991), Tumlinson *et al.* (1992), Takabayashi *et al.* (1994), and Dicke (1994). Plant compounds attractive to some parasitoids have been identified. For example, allyl isothiocyanate, a volatile component of crucifers, attracts *Diaeretiella rapae* (McIntoch), a braconid parasitoid of aphids that feed on crucifers (Read *et al.*, 1970). Elzen *et al.* (1984) reported that the parasitoid, *Campoletis sonorensis* was attracted to sesquiterpenes isolated from cotton essential oils. The sesquiterpenes included α-humulene, γ-bisabolene, β-caryophyllene oxide, spathulenol, β-bisabolol and gossonorol. These compounds were isolated from ethyl ether washes of freshly cut flowers and buds of cotton, which is a food plant of *H. virescens*.

It was demonstrated that parasitoids respond to chemicals from damaged plants. For example, female wasps of *Diadromus pulchellus* Wesmael were

attracted to odours of dipropyl disulphide, S-propyl propanethiosulphonate and S-propyl propanethiosulphinate from damaged leeks during their search for larvae of the moth, *Acrolepiopsis assectella* Zeller (Lecomte and Thibout, 1986).

Some plants are reported to actively produce, when damaged, volatile chemicals in response to a substance produced by the attacking herbivore (Turlings *et al.* 1990b, 1991b). For example, attack by *S. exigua* larvae on corn seedlings resulted in the release of large amounts of volatiles. Eleven compounds were identified, (1). (Z)-3-hexenal, (2). (E)-2-hexenal, (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. The composition of the volatiles varied with larval feeding time. Immediately after the larvae began feeding, green leaf aldehyde and alcohol volatiles were released and continued to be released as long as larvae were actively feeding (Tumlinson *et al.*, 1992). After several hours, 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. Artificially damaged plants treated with regurgitant from larvae, induced the release of similar amounts of compounds 5-11 as were released from infested plants. Attraction to green leaf volatiles (GLVs) was also reported by Whitman and Eller (1990). They indicated that undamaged plants emitted low levels of GLVs, while damaged and artificially damaged plants emitted relatively higher levels of certain GLVs. Females of the braconid parasitoid, *M.*

croceipes, and the ichneumonid, *Netelia heroica* Townes were attracted to individual GLVs in a wind tunnel (Whitman and Eller, 1990). The GLVs tested were the following: hexenal, (E)-2-hexenal, (Z)-3-hexen-1-ol, (Z)-3-hexenyl acetate; (E)-2-hexenyl acetate, (Z)-3-hexenyl propionate; (Z)-3-hexenyl butyrate.

The role of some terpenoids as host induced synomones has been reviewed by Dicke (1994) and Takabayashi *et al*. (1994) for the mite system. Linalool, (E)-β-ocimene, (3E)-4,8-dimethyl-1,3,7-nonatriene and methyl salicylate are compounds that attracted predatory mites. These compounds were identified in volatiles from spider mite-infested lima bean leaves but were not present in the volatiles from uninfested or artificially damaged leaves (Dicke *et al*., 1990b). Takabayashi *et al*. (1991) reported the presence of similar compounds from different plants (apple, cucumber, lima bean). However, there were quantitative differences and a few qualitative differences between the different plant species.

In the tritrophic system consisting of *Cotesia rubecula* Marshall-*Pieris rapae* L. - and cabbage, volatiles emitted by undamaged plants were α-pinene, β-pinene, myrcene, 1,8-cineole, n-hexyl acetate, *cis*-3-hexen-1-yl acetate and dimethyl trisulfide (Agelopoulos and Keller, 1994c). Mechanical damage induced the release of two more compounds, *trans*-2-hexenal and 1-methoxy-3-methylene-2-pentanone. Feeding by larvae *P. rapae* induced the release of all compounds released by the mechanical damage and additionally, 4-methyl-3-pentenal and allyl isothiocyanate (Agelopoulous and Keller, 1994c).

It is likely that the attractive volatiles in the faeces of herbivores are derived

from the plants or other substrates on which the herbivores feed. It is also possible that plant constituents are modified by micro-organisms in the insect gut or faeces to produce long range kairomones, although this has not yet been demonstrated (Tumlinson *et al*. 1992). Eller (1990) reported that the parasitoid *M. croceipes* was attracted to a compound identified as *trans*-phytol and isolated from the hexane extract of the frass from *H. zea* larvae fed on cowpea. It was suggested that long range kairomones produced by herbivores are released from their faeces. However, none has so far been identified (Tumlinson *et al*., 1992).

2.2.2 Host-produced long range attractants (kairomones)

In some cases, parasitoids exploit the pheromonal system of their host during foraging. The parasitoids, *Spathius benefactor* Matthews, *Cheiropachus colon* (L.),*Entedon leucogramma* (Ratzeburg), *Dendrosoter protuberans* (Nees) are 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).

2.2.3 Host-produced short range attractants (kairomones).

During foraging, as a parasitoid approaches its host, it is exposed to chemical cues that are host specific. These chemicals are often found in the host by-products (Tumlinson *et al*., 1992). Long chain hydrocarbons, such as, tricosane and related hydrocarbons from the scales of adult *H. zea*, increase

parasitism by *T. evanescens* (Lewis *et al.*, 1972; Jones *et al.*, 1973). Other types of compounds such as 2,5-dialkyltetrahydrofurans 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). These compounds were also found in the faeces of the larvae of another lepidopteran, *Acantholeucania loreyi* (Duponchel), although this host is not suitable for the parasitoid's development (Takabayashi and Takahashi, 1990). Vinson *et al.* (1975) isolated and identified a component from mandibular gland secretions of *H. virescens* that elicited short range attraction of its parasitoid *C. nigriceps*.

Chemical analysis of similar secretions found in the mandibular glands of *P. interpuntella* indicated the presence of two components with the empirical formulae $C_{24}H_{40}O_4$ and $C_{22}H_{38}O_3$ (Mossadegh, 1980). Extraction of the kairomone from the mandibular glands of *Chilo partellus* using various solvents showed that the methanol extract contained the chemical cues attractive to *Cotesia flavipes*. The molecular weight of the active compounds was determined to be 436.3505, which corresponded to the formula $C_{27}H_{48}O_4$ (CAB, 1987).

2.3 Host acceptability and suitability for the development of parasitoids

2.3.1 Host acceptance

According to Singer (1986), host acceptance is a positive response to an encounter. Chemical cues play a very important role in the acceptance of hosts by parasitoids (Vinson, 1976, 1991). Other factors, such as shape, size, age,

movement, and sound also influence host acceptance (see review by Vinson, 1976).

Chemical stimuli

Chemical cues influence host acceptance behaviour in various ways. For instance, acceptance behaviour of *Campoletis sonorensis* towards *H. zea* is divided into four steps; examination of the host, thrusting of the ovipositor, ovipositor insertion and egg-laying. Non-volatile chemicals associated with a variety of tissues (eg. larval integuments) are implicated in these behavioural responses (Schmidt, 1974). Burks and Nettles (1978) reported that the oviposition stimulant of *Eucelatoria* sp. was a solvent-extractable component in the cuticle extract of *H. virescens* larvae. Odell and Godwin (1984) studying the host selection process of *Blepharipa pratensis* (Meigen), a parasitoid of the gypsy moth, *Lymantria dispar* L., found that damaged leaf edges and leaf exudates arrested parasitoid movement and stimulated searching. When the edge of a leaf eaten by gypsy moth was contacted, oviposition occurred, suggesting the presence of contact kairomone(s) at the edge of the eaten leaf.

Diet influences the composition of the volatiles emanating from the faeces of herbivores, and therefore influences host acceptance. Stemborer larvae reared on artificial diet were less attractive to parasitoids than those fed on a natural diet of maize (Mohyuddin *et al.*, 1981). Similarly, Inayatullah (1983) observed that *Cotesia flavipes* collected from maize in Pakistan were more

attracted to larvae of *Chilo partellus*, and *Sesamia* spp. fed on maize and sorghum than larvae fed on sugarcane.

Other factors

In addition to chemical stimuli, shape, texture, sound, movement, colour, age and size of host also influence host acceptance by parasitoids (Vinson, 1976). *Pimpla instigator* was initially attracted from a distance by odours to a close proximity of the hosts, then to the cylindrical shape, the latter increases acceptance (Carton, 1974). Hairiness provided by secondary setae influenced acceptance of the gypsy moth by *Apanteles melanoscelus* (Ratzeburg) (Weseloh, 1974). Smith (1943) reported that motionless hosts were palpated by female *Microtonus vittatae* and were attacked if movement was induced. The growth stage of the host may also be an important factor in host acceptability (Hendrikse *et al.*, 1980; van Alphen and Drijver, 1982). The parasitoid *Ooencyrtus nezarae* Ishii accepted 0-7 days old eggs of *Riptortus clavus* Thumberg, but the number of eggs laid per host decreased with increasing host age (Takasu and Hirose, 1993). Age and size are usually related, but age has been shown to influence host acceptance irrespective of size (Gutierrez, 1970; Schmidt, 1974). It was also reported that when a larval host, *Trichoplusia ni* Hubner reaches pharate pupal stage it becomes unacceptable to the parasitoid, *Hyposoter exiguae* (Viereck) (Smilowitz, 1974).

2.3.2 Host suitability

Suitability encompasses various aspects of host quality that affect insect performance (Singer, 1986). The suitability of a host for parasitoid development will depend on several factors, including environmental factors, the ability to evade the host's internal defensive system, competition with other parasitoids, the presence of toxins detrimental to the parasitoid eggs or larvae and the host's nutritional adequacy (Vinson and Iwantsch, 1980).

Host defense

Behavioural and physiological changes occur in a parasitized host. The physiological changes mainly involve the immune system of the host, which in the presence of foreign bodies may activate the internal defense system which includes humoral and cellular responses. The humoral system generally refers to biochemical factors within the haemolymph of the organism that exert some effect on the invader or are involved in its recognition. These biochemicals include inducible antibacterial factors, lysozymes, lectins, acid mucopolysaccharides and the phenoloxidase-melanin system (Vinson, 1993). The cellular component involves haemocytes. Depending on the size and the number of foreign bodies encountered, the cellular response may be in the form of any of three processes, phagocytosis, nodule formation and/or encapsulation (Pathak, 1993). Encapsulation is a common multicellular immune reaction mounted by host insects towards invading foreign organisms such as

parasitoid eggs (Kaya and Tanada, 1969; Kaya, 1970; Salt, 1970; Vinson, 1977; Kitano, 1982; Prevost *et al.*, 1990). For example, encapsulation of eggs of *Cotesia flavipes* occurs in *Diatraea saccharalis* and *D. grandiosella* Dyar (Overholt and Smith, 1990a,b; Wiedenmann *et al.*, 1992; Wiedenmann and Smith, 1994).

Reciprocally, parasitoids have evolved mechanisms of evading encapsulation (Vinson, 1977; Kitano and Nakatsuji, 1978; Kitano 1982; Davies *et al.* 1987; Ross and Dunn, 1989; Strand and Noda, 1991; Strand and Wong, 1991; Vinson, 1993). These include:

(1) molecular mimicry: the parasitoid has surface characteristics that are identical or very similar to the surfaces within the host so that the latter fails to respond to the foreign surface because it appears to be "self". For example *Cardiochiles nigriceps* has a thick chorion consisting of a fibrous outer coat which is not recognized as foreign. As long as the fibrous layer remains the eggs are not encapsulated (Davies and Vinson, 1986; Davies *et al.*, 1986). The eggs of *C. nigriceps* are encapsulated in larvae of *Helicoverpa (=Heliothis) zea* (Lepidoptera: Noctuidae), but not in those of *Helicoverpa (=Heliothis) virescens*, due to the presence of the fibrous layer which is removed within 2 hours in *H. zea* and persists for 12-24 hours in *H. virescens* (Lewis and Vinson, 1971).

(2) Target proliferation: cells of the embryonic membrane of the

developing parasitoid (e.g. teratocytes) provide so many targets that the immune system is unable to focus an effective attack (Salt, 1971), for example, the encapsulation of *C. nigriceps* larvae injected into hosts previously injected with teratocytes was reduced (Vinson, 1972).

(3) Destruction and/or suppression of host immunity: In this case parasitoids inject factors that suppress the immune response of the host e.g. venom from ovaries and/or virus particles (Vinson, 1990). Viral particles are found in calyx fluid of female parasitoids. They consist of segmented double-stranded, circular DNA genomes. These viruses have been placed in a new class called Polydnaviridae (Stolz *et al.*, 1984). Mechanisms of polydnavirus suppression of the immune system have been discussed by Davies *et al.* (1987), Davies and Vinson (1988), Tanaka (1987), Stolz and Guzo (1986) (reviewed by Vinson, 1993).

Competition

Many mature female parasitoids are able to discriminate between parasitized and unparasitized hosts because of the marking of the host by ovipositing females. However, some parasitoids are unable to make this distinction and superparasitism may occur (Vinson and Iwantsch, 1980). When larval overcrowding occurs in some species of parasitoids, the total number of hosts yielding parasitoids decreases because all competitors in the superparasitized host often die (Vinson and Sroka, 1979). Beg and Inayatullah

(1980) demonstrated that superparasitism by *Cotesia flavipes* had a detrimental effect on the progeny, the sex ratio was affected, there was also an increase in the number of inviable pupae and the number of larvae which failed to emerge from the host. In other cases, a preferential survival of one sex, usually the male, may occur (Wylie, 1966; Ziser *et al*. 1977). Supernumerary parasitoids may be eliminated by physical or physiological suppression (toxins, anoxia, nutritional depravation) (Vinson and Iwantsch, 1980).

Toxins

The suitability of a host for parasitoid development may depend on the absence of substances innocuous to the host, but toxic to the parasitoid (Vinson and Iwantsch, 1980). Thurston and Fox (1972) found that tobacco hornworm fed on tobacco were suitable for the development of *Apanteles congregatus* larvae, but additional nicotine in the diet, or topical application on host larvae, although it had no effect on the host, prevented the parasitoid's emergence. Other examples of host diets affecting the parasitoid's fitness have been reported (Flanders, 1942; Narrayanan and Subba Rao, 1955; Pimentel, 1966; Cheng, 1970; Zhody, 1976; Blumberg and DeBach, 1979; Bergman and Tingey, 1979; Price *et al*., 1980; Kester and Barbosa, 1991)

Nutritional inadequacy

Nutritional quality of the host has a profound effect on the sex ratio, size, developmental times and longevity of parasitoids (Vinson and Iwantsch, 1980). Host age and/or size may affect the number of parasitoids that emerge (Hendrikse *et al.*, 1980; Van Alphen and Drijver, 1982) the sex ratio (Lawrence *et al.*, 1976) or the size of parasitoids (Liu, 1985). Petitt and Wietlisbach (1993) reported that the parasitoid, *Opius dissicus* Muesebeck produced 1.7 to 3 times more offspring when provided second and third instar leafminers, *Liriomyza sativae* Blanchard, as compared to first instars. The developmental time also increased by about two days when the parasitoids oviposited in first instar larvae of the hosts. Different hosts may show differences in suitability for different parasitoid species depending on whether the hosts and parasitoids have coevolved or not. Tillman *et al.* (1993) reported a difference in suitability of two *Helicoverpa spp.* for the development of one indigenous and two exotic parasitoids species. There was also a difference in the mortality and developmental time of immature parasitoids in the two hosts. Shami and Mohyuddin (1986) found that *Cotesia sesamiae* preferred *Chilo partellus*, *S. inferens*, *A. stenellus*, *E. depressella*, *Chilo infuscatellus*, *Calamotropha lupatus* and *Scirpophaga nivella* (F.) in descending order. Host suitability followed the same pattern, and *Cotesia sesamiae* did not complete development in *E. depressella* and *C. lupatus*. Among the hosts examined, *Chilo partellus* was the most suitable in terms of the number of cocoons per larva, adult

emergence, and sex ratio, although all the hosts tested were new associations. In laboratory studies, although *Cotesia flavipes* oviposited in *Chilo partellus*, *Chilo infuscatellus*, *Tryporyza incertulas* (Walker) and *Ostrinia kasmirica* (Moore), development of their progeny was only completed in *Chilo partellus*, and *Chilo infuscatellus* (Beg and Inayatullah, 1980)

CHAPTER THREE

3 General materials and methods

3.1. Insects

Parasitoids. A colony of *Cotesia flavipes* was initiated from material collected from *Chilo partellus* at Rawalpindi, Pakistan by the International Institute of Biological Control (IIBC). The *Cotesia sesamiae* colony was initiated with material collected from *Chilo partellus* in the coastal zone of Kenya. *Cotesia flavipes* and *Cotesia sesamiae*, were reared on *Chilo partellus* and *S. calamistis* larvae, respectively, according to the method described by Overholt (1993). After parasitization, stemborers were maintained on 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 a 20% honey/water solution as diet.

Hosts. *Chilo partellus*, *S. calamistis* and *Chilo orichalcociliellus* were collected from maize fields from the Kenya coast. A colony of *B. fusca* was initiated with material collected from western Kenya. The *Chilo* spp. larvae were reared on artificial diet as reported by Ochieng *et al.* (1985). The noctuids, *B. fusca* and *S. calamistis*, were reared on media developed at ICIPE (Onyango *et al.*, 1992a).

Maruca testulalis Geyer (Lepidoptera: Pyralidae), the cowpea pod borer

was used in several tests. This insect was supplied by the Insect and Animal Breeding Unit at the ICIPE where it was reared on semi-artificial diet (Onyango *et al.*, 1992b).

3.2 Plants

Maize (*Zea mays* L., (hybrid 5-12)) and sorghum (*Sorghum bicolor* (L.), (Serena)), were grown in twenty litre buckets in a nursery, and in the field at ICIPE; plants were kept under fine mesh cages (400 micron) to protect them from insect attack. Napier grass (*Pennisetum purpureum* Schumach.) was collected from farmers' fields in Nairobi. Cowpea (*Vigna unguiculata* L.) was grown in pots in a nursery at ICIPE.

3.3 Bioassay set-up

3.3.1 Y-tube olfactometer

The Y-tube olfactometer used in this study has been described by Sabelis and Van de Baan (1983) and Steinberg *et al.* (1992). The odour sources were placed in two perspex chambers (30 x 30 x 120 cm) sufficiently large to accommodate whole plants (2-3 months old) (Figure 3.1). The two chambers were connected to the arms of the Y-tube with tygon tubing from the top of the chambers. An inlet, through which clean air entered the chamber, was bored 30 cm from the bottom of the chamber on one side. A vacuum pump (Cole-Parmer Air-Cadet) drew and pushed air through the closed system.

Air was pushed through activated charcoal filter into the two chambers and drawn into the Y-shaped glass tubing of the olfactometer. The airflow was set at 2.5 l/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 more than 15 seconds beyond the finishing line (4 cm past the intersection), it was recorded as a choice. The connections of the odour source chambers to the arms of the olfactometer were reversed after testing 5 insects to rule out any asymmetrical bias in the olfactometer. Tests were replicated at least three times with 20 parasitoids per replicate. Tests were conducted at 23-26 ^{o}C, 65-75% relative humidity and light intensity of 350-450 lux.

3.3.2 T-tube olfactometer

A T-tube olfactometer was constructed from two pieces of 4 mm diameter (ID) glass tubing, joined to the stem of a 7.5 cm diameter funnel to form a 'T'. The arms of the 'T' were 5 cm long and the stem 10 cm long. Test materials were placed in small vials (5 cm long, and 1 cm diameter) behind a fine mesh screen to eliminate visual cues. The vials were placed over the arms of the 'T-tube' (Figure 3.2). Parasitoids were introduced into the olfactometer and the arm chosen by the test insects was recorded. An arm was considered chosen when the parasitoid, reached the end of the arm.

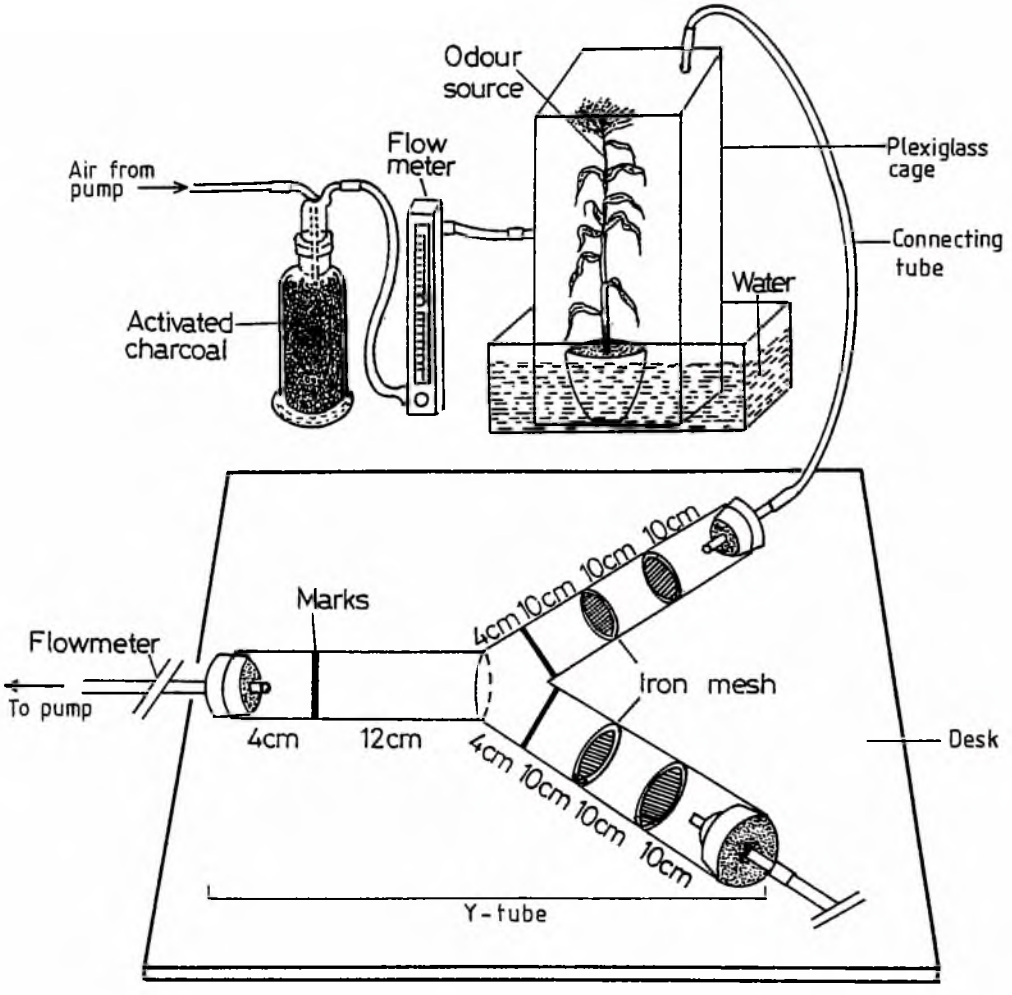

Figure 3.1 A schematic representation of the Y-tube olfactometer

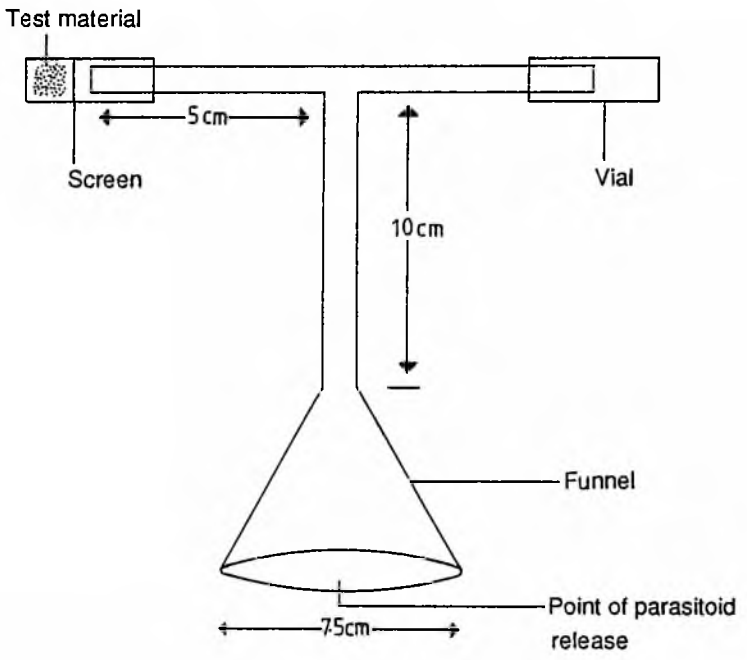

Figure 3.2 A schematic diagram of the T-tube olfactometer

The position of the T-tube was also turned 180° after testing 5 insects to avoid any bias. No time limit was given, but the response typically occurred in less than 3 minutes. Tests were conducted at 23-26 °C, 65-75% relative humidity and light intensity of 350-450 lux.

3.3.3 Glass vial experiments

The acceptability of stemborers for parasitization was tested by offering one stemborer larva to one parasitoid in a clean glass vial. One-day old female parasitoids were introduced into an inverted vial (7.5 x 2.5 cm) placed on a flat surface, and allowed to climb to the top (the bottom of the vial). Once the parasitoid had reached the top, the vial was placed over a stemborer larva. Each female was allowed five minutes in the vial. An observation was terminated when the parasitoid stung the larva, or after five minutes. Ten larvae were used per treatment and this was replicated at least four times. The number of stemborers attacked and the time to oviposition, within five minutes, were recorded.

3.4 Collection and analyses of volatiles

3.4.1 Collection of volatiles

The procedure reported by Torto *et al.* (1994) was used. Volatiles were collected in traps containing activated charcoal (80-100 mesh Chrompack, The Netherlands) as adsorbent. Traps were prepared by packing about 50 mg of

charcoal between two glass wool plugs in glass tubes 6-cm-long x 8-mm ID. Before use, the packed charcoal was cleaned by soxhlet extraction with dichloromethane (Merck) for 48 hours, followed by activation under a stream of nitrogen at 250 °C. A push-pull odour collection system was used for trapping volatiles from untreated and treated 2-3 month old maize plants. A vacuum pump (Cole-Parmer Air-Cadet) pulled and pushed air through the collection apparatus at 526 ml/min. The collection chamber was a rectangular glass box measuring 120 cm x 20 cm x 20 cm, with one of the square ends left open. An inlet was bored at one of the lower sides of the cage while an outlet was made at the top of the box (Figure 3.3). For the system to be airtight, the open end of the glass box was placed over the plants which stood in water held in an aluminium basin. The open end was submerged 15cm below the meniscus. Air drawn through the box was cleaned using a charcoal filter and volatiles were collected on charcoal traps as described above. Collection of odour from test materials lasted 24 hrs. Trapped volatiles were eluted with HPLC grade dichloromethane (Aldrich Ltd., UK; 4ml) and concentrated under a stream of nitrogen at 0°C to 100 μl. Blanks were also trapped to detect impurities in the system.

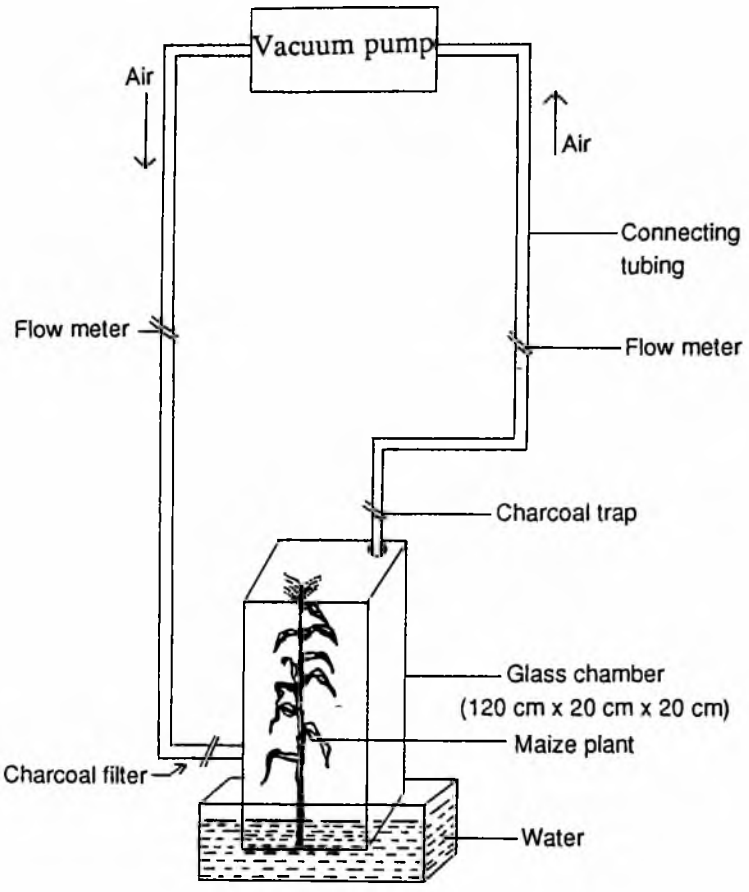

Figure 3.3 Push - pull volatile collection system

Volatiles from small sized test materials (e.g. 10 days old maize seedlings and frass) were collected using a different system: Air from a compressed air cylinder (East Africa Oxygen (EAO), Nairobi) was cleaned through an activated charcoal filter and drawn over test materials (in a one litre or five litre three-necked round bottomed flask or quickfit tubing (see below)) and through the charcoal trap at 70ml/min for four hours at 25 ± 3 °C. Two flowmeters, one placed before the filter and another after the trap regulated the airflow. The two side necks of the flask were fitted with the activated charcoal columns, while the central neck of the flask was closed with a glass stopper (Figure 3.4). Alljoints were sealed with teflon tape to ensure that no leakage occurred. A five litre flask was used for collecting volatiles from 10 days old plants and a one litre flask for collecting volatiles from cut stems. Volatiles were collected from frass confined in a two piece glass tube, 5-cm x 2.8-cm-ID for the male part and 9-cm x 3.4-cm-ID for the female part, with quickfit joints (Figure 3.5).

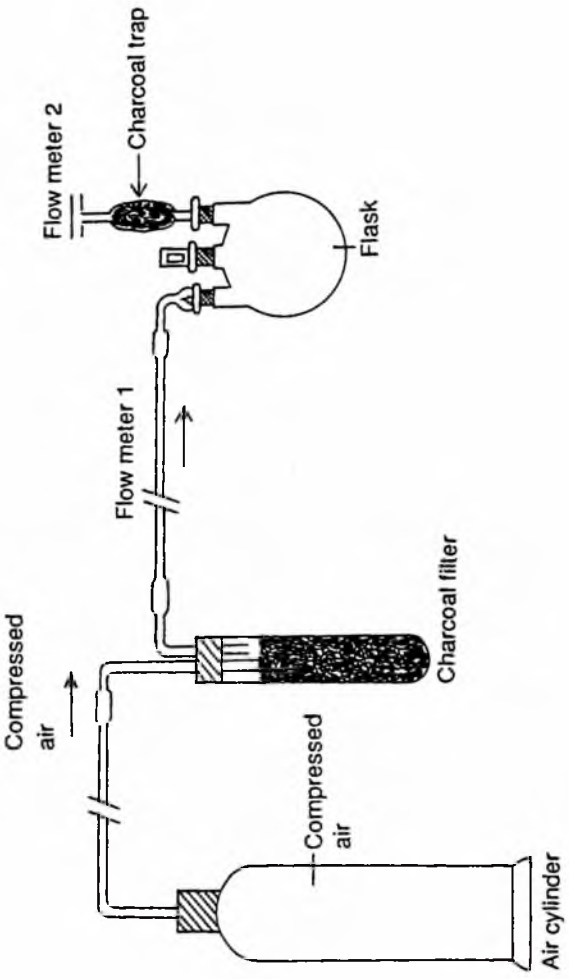

Figure 3.4 Odour collection system using compressed air

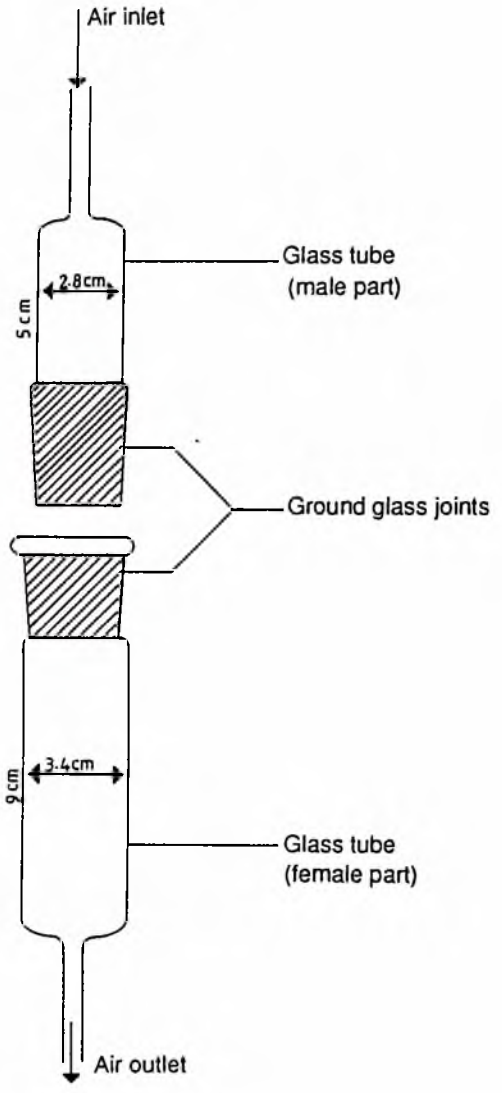

Figure 3.5 Glass chamber for collection of volatiles from frass

3.4.2 Analyses of volatiles

The procedure described by Torto *et al*. (1994) was used. Aliquots of volatile collections were analyzed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). GC analyses were performed on a Hewlett-Packard (HP) 5890 Series II gas chromatograph equipped with a flame ionization detector (FID) and HP capillary column (methyl silicone, 50m x 0.2mm ID x 0.2 μm or Carbowax, 50m x 0.2mm ID X 0.2 μm) using nitrogen as carrier gas at a flow rate of 0.35 ml/min. A different GC programme was used for each column. For the methyl silicone column, the oven temperature was initially set at 40°C for two minutes, then programmed at 5°C/min to 150°C for two minutes and to 220°C at a rate of 10°C/min and isothermal for fifteen minutes. For the Carbowax column, the oven temperature was initially set at 60°C for ten minutes, then programmed at 5°C/min to 180°C for five minutes and to 220°C at a rate of 10°C/min then isothermal for ten minutes. Chromatographic peaks were integrated using a HP Series II 3396 integrator. GC-MS analyses were carried out on a VG Masslab 12-250 mass spectrometer (EI, 70 ev) coupled to a HP 5790 gas chromatograph.

3.4.3 Gas chromatography-electroantennography.

Coupled gas chromatography-electroantennographic detector (GC-EAD) analyses were performed on either the Carbowax or methyl silicone (50m x 0.2mm ID x 0.2 μm) columns and employed the same conditions as for the GC

analyses of volatiles. The column effluent was split 1:1 into two 50-cm-long deactivated fused silica columns connected to the FID and the delivery tube placed near the antenna respectively. A make-up gas (40 ml/min) was added just before the split point to accelerate the effluent through the deactivated columns. The effluents were driven from the chromatograph through a transfer line maintained at 150 ^{o}C by a THC-3 temperature control unit (Syntech, Netherlands) and into a moistened airstream (4.0ml/sec, 90 % Rh, 25 ^{o}C) focussed onto the antennal preparation via a stainless steel tube (5 mm ID). FID and EAD signals were monitored synchronously using a programme on a GC/EAD interface card (Syntech, The Netherlands) installed in a PC (Harvard Professional Computer, American Megatrends Inc.).

Antennal preparation. Electroantennograms (EAGS) were recorded on antennae of one-day old, mated, and fed naive females of *Cotesia flavipes*. Each antenna was cut off from the head capsule at the level of the scape. The cut part of the antenna was inserted into the recording electrode (a glass micropipette containing a saline solution (Hoyle, 1951)). The tip of the antenna was inserted into the indifferent electrode. Both electrodes were appropriately connected to the universal AC/DC UNO5 amplifier (Syntech, The Netherlands). Four microlitres of samples were then injected into the GC for GC-EAD analyses.

3.5 Haemocyte count methods

Host immune experiments were conducted to determine changes in host haemocyte levels as a function of parasitization, using the methods of Stolz and Guzo (1986) and Davies *et al.* (1987). Total haemocyte counts were assayed. Fourth instar larvae of *Chilo partellus* were used in this experiment. Two tests were conducted: (1) Total haemocyte counts were assayed 1-24 hours after parasitization and (2) total haemocyte counts were assayed one to six days after parasitization.

(i) Haemocyte count 1-24 hours after parasitization

Larvae were assayed at six different times after being parasitized: 1, 2, 4, 8, 14 and 24 hours after exposure. Individual larvae were exposed to an individual female parasitoid in a glass vial. Care was taken to ensure that each larva was stung only once. After being parasitized, larvae were transferred to glass vials containing diet and kept in an incubator at 28 ^{o}C. At each of the assay times, five larvae from the parasitized cohort and five from those unparasitized (controls) were randomly selected. Parasitized larvae were subsequently dissected to detect encapsulation. Each test larva was immobilized on ice and haemolymph was quickly squeezed out of a cut proleg on to a sheet of parafilm. Aliquots of 4 μl were diluted in 36 μl of a saline solution (0.15 M NaCl, 5mM KPO4, pH 6.5) to prepare a 1:10 dilution. Aliquots of 10 μl of this sample were then transferred immediately to an improved

Neubaeur haemocytometer slide (Cole-Parmer, Inc., Chicago, USA). Haemocyte counts were made from ten grids. This test was replicated four times. The mean haemocyte counts for the different hours were calculated and comparisons were made using analyses of variance (ANOVA) followed by the Student Newman-Keul mean separation test when the ANOVA was significant ($p < 0.05$). The exposure and assaying schedule is illustrated in Table 3.

Table 3. Treatment schedule for the total haemocyte counts

	Interval after parasitization					
(0h)	(1h)	(2h)	(4h)	(8h)	(14h)	(24h)
Sting 30 larvae	Assay 5 larvae	Assay 5 larvae	Assay 5 larvae	Assay 5 larvae	Assay5 larvae	Assay 5 larvae
30 control larvae	Assay 5 larvae	Assay 5 larvae	Assay 5 larvae	Assay 5 larvae	Assay 5 larvae	Assay 5 larvae

(ii) Haemocyte counts 1-6 days after parasitization

Larvae were assayed daily for six successive days after parasitization. The above procedure was used for the total haemocyte count. A total of 50 larvae was parasitized on day zero. Eight test and eight control larvae were assayed each day. This test was replicated six times. Data were analyzed as indicated above.

CHAPTER FOUR

4. Response of Cotesia flavipes and Cotesia sesamiae to plant-produced and host related allelochemicals.

4.1 Introduction

Successful reproduction by insect parasitoids is dependent on the location of suitable hosts for oviposition. The host selection process is divided into three steps: host habitat location, host location and host acceptance (Vinson, 1976). Allelochemicals are known to play an important role in this process (see review by Nordlund *et al.*, 1988). Chemicals emitted by plants influence parasitoid behaviour (Lewis and Martin, 1990). In addition, females of many insect parasitoids rely on host and host-related chemicals (such as frass, and silk) in their search for hosts (Turlings *et al.*, 1990a,b, 1991a,b; Mc Auslane *et al.*, 1991; Steinberg *et al.*, 1993). The stimuli involved in the searching behaviour of *Cotesia flavipes*, have been studied by several authors (Mohyuddin, 1971; Mohyuddin *et al.*, 1981; Van Leerdam *et al.*, 1985; Potting *et al.*, 1993, 1995). *Cotesia flavipes* was reported 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 stemborers were also very attractive (Potting *et al.*, 1995). A water soluble substance from fresh frass was also reported to attract *Cotesia flavipes* (Van Leerdam *et al.*, 1985). Based on

the short range attraction to host frass, it was reported that there are different strains of *Cotesia flavipes* with different plant preferences (maize or sugarcane) (Mohyuddin *et al.*, 1981; Shami and Mohyuddin, 1992).

Cotesia flavipes, which was collected from *Chilo partellus* on maize, was introduced into Kenya from Pakistan for the control of the introduced pest *Chilo partellus*. This pest is found in gramineous plants with other indigenous noctuid and pyralid stemborer species. Potting *et al.* (1993) reported that *Cotesia flavipes* could not discriminate between *Chilo partellus* infested maize plant and *B. fusca* infested maize plant. However, it is not known whether this parasitoid would prefer any of the different gramineous plants (maize, sorghum, napier grass, which are the food of its hosts) occurring at the area of release, the coast of Kenya. It is also not known whether it has a preference for any of the different stemborer species (*Chilo orichalcocilielius*, *Chilo partellus*, *S. calamistis*) occurring in the coast of Kenya. No information is available on the host selection process of the closely related indigenous parasitoid, *Cotesia sesamiae*. This parasitoid has been unable to regulate *Chilo partellus* population densities at levels acceptable to farmers. A study of its searching behaviour was necessary to explain why this parasitoid is not able to control the pest and the degree of niche overlap between the two *Cotesia* species. The aim of this study was, therefore, to investigate the response of *Cotesia flavipes* and *Cotesia sesamiae* to volatiles from: (1) uninfested maize, sorghum and napier grass; (2) different stemborer-plant complexes that are likely to be found

in the area of release; (3) frass of the different hosts under study; and (4) from the stemborer.

4.2 Materials and methods

Tests were conducted using *Cotesia flavipes* and *Cotesia sesamiae*. These parasitoids were reared on *Chilo partellus* and *S. calamistis*, respectively, as described in Section 3. One-day-old mated naive female parasitoids were used in all experiments. Hosts included fourth instar larvae of *Chilo partellus*, *Chilo orichalcociliellus*, *B. fusca*, *and S. calamistis*. Stemborers were reared on semi-artificial diets (see section 3). Prior to any experiment, stemborer larvae were removed from artificial diet and fed on fresh maize stems for a minimum of 24 hours. The cowpea borer, *M. testulalis*, was used in bioassays involving a non-host insect, and this was reared on a semi-artificial diet described by Onyango *et al*. (1992b) (see section 3).

4.2.1 Response to volatiles from uninfested plants

Uninfested maize, sorghum and napier grass plants of 8-10 weeks old were used in the experiments. All species of plants were tested singly and in dual choice in a Y-tube olfactometer (see section 3.3.1). Tests were conducted at 23-26 ^{o}C, 65-75% relative humidity and light intensity of 350-450 lux.

(i) A first series of experiments was conducted to determine the attractiveness of odours from uninfested plants. Maize, napier grass and

sorghum were tested in single choice experiments. Individual parasitoids were given a choice between odour from a potted plant and air drawn over a pot of soil, or odour from a plant cut above the soil level and air drawn over an empty chamber. Attraction of *Cotesia flavipes* to a non-host plant, cowpea, was also tested; four potted cowpea plants were tested against air drawn over a pot of soil.

(ii) A second series of tests was conducted to determine the parasitoid's preference for odours from different uninfested plant species. Plants were placed in chambers connected to both arms of the olfactometer. Volatiles from maize were compared to volatiles from sorghum, napier grass, and cowpea. When comparing maize to cowpea, two maize plants were tested against four cowpea plants. Different numbers of plants were used because of the difference in plant sizes between the two plant species.

All tests were replicated at least 3 times with 20 parasitoids per replicate. Data were analyzed using G-test (Chi-Square test) (Sokal and Rohlf, 1981). Parasitoids that did respond ('No response'group) were excluded from the analyses.

4.2.2 Response to volatiles from plants infested by different stemborer species

The first series of tests was conducted in a Y-tube olfactometer to determine the parasitoid's preference between plants infested with different

stemborer species (plant-host complex (PHC)) and uninfested plants. The following PHC combinations were tested against the control (uninfested maize):

(1) maize plants infested with *Chilo partellus*

(2) maize plants infested with *Chilo orichalcocilielius*

(3) maize plants infested with *S. calamistis*

Maize plants were infested with two fourth instar stemborer larvae by boring two holes in the maize stem with a 4 mm cork borer and placing one larva in each hole. Larvae were allowed to feed overnight and tests were conducted 18-20 hours after infestation. Plants were transferred into the chambers connected to the Y-tube in the pot in which they were grown (or cut just above the soil) 30 minutes before observations were made (to allow time for volatiles to be released in the chamber). Plants in both arms were treated in the same manner (cut at the base or kept in pots).

A second series of tests was conducted to determine the parasitoids preference between plants infested by different stemborer species. The following combinations were tested:

(1) maize infested with *Chilo partellus* larvae versus maize infested with *Chilo orichalcocilielius* larvae

(2) maize infested with *Chilo partellus* larvae versus maize infested with *S. calamistis* larvae

(3) maize infested with *Chilo partellus* larvae versus maize infested with *B. fusca* larvae (only *Cotesia sesamiae* was used for the test

with *B. fusca*; *Cotesia flavipes* response having already been studied by Potting *et al.*, 1993)

A third series of tests was conducted to determine whether the response of parasitoids was dose dependent. The following number/species combinations of stemborers per plant were compared: (a) two *Chilo partellus* versus ten *S. calamistis*; (b) five *Chilo partellus* versus five *S. calamistis*; (c) ten *Chilo partellus* versus two *S. calamistis*; (d) two *B. fusca* versus ten *Chilo partellus* larvae.

Finally preference of *Cotesia flavipes* for either infested maize (host-plant of *Chilo partellus*) versus infested cowpea (non-host) or infested maize versus uninfested cowpea were tested. Cowpea plants infested with the cowpea borer, *M. testulalis*, were compared to maize infested with *Chilo partellus;* ten second instars of the pod borer were placed on the young pods of the plants to be tested and were allowed to feed overnight. Four cowpea plants were tested each time against two maize plants on which four medium *Chilo partellus* larvae have been feeding overnight. The test plants were cut just above the soil and placed in the olfactometer chambers thirty minutes before the bioassay. As indicated earlier, different numbers of plants were used because of the difference in size between the two plant species.

Similarly, *Cotesia flavipes* was given a choice between two maize plants infested with *Chilo partellus* and four uninfested cowpea plants. All tests were replicated at least three times with 20 parasitoids per replicate. Analyses were

conducted as indicated in section 4.2.1.

4.2.3 Response to host frass

Bioassays were conducted to determine attraction of *Cotesia flavipes* and *Cotesia sesamiae* to frass produced by *Chilo partellus, Chilo orichalcociliellus, S. calamistis* and *B. fusca* fed on maize, sorghum and napier grass. Tests were also conducted to investigate whether the parasitoids preferred frass produced by any of the four stemborer species when these were fed on maize.

Tests were conducted in a 'T-tube' olfactometer (see section 3 for description and bioassay procedure). Stemborers were fed on fresh plant material and the frass produced within the first 24 hours was discarded to avoid contamination. Frass produced in the following 24-48 hours was collected and weighed. Fresh frass (1 g) obtained from each of the four stemborer species fed on maize, sorghum and napier grass were tested against moist cotton wool (control).

To test for preference, frass samples from stemborers fed on maize only were also tested in the following dual choices:

1. *Chilo partellus* frass versus *B. fusca* frass
2. *Chilo partellus* frass versus *Chilo orichalcociliellus* frass
3. *Chilo partellus* frass versus *S. calamistis* frass
4. *B. fusca* frass versus *S. calamistis* frass
5. *B. fusca* frass versus *Chilo orichalcociliellus* frass

6. *Chilo orichalcociliellus* frass versus *S. calamistis* frass

All tests were replicated six times with 8 parasitoids per replicate. Data were analyzed as indicated in section 4.2.1.

4.2.4 Response to stemborer larvae

In this experiment, the presence of attractive odours on the body surface of a stemborer is investigated. The host acceptance of *Cotesia flavipes* and *Cotesia sesamiae* for parasitization of washed and unwashed larvae of *Chilo partellus* was tested by exposing the larvae to parasitoids in clean glass vials (7.5 x 2.5 cm). The bioassay procedure is as described in section 3.3.3. Unwashed larvae were exposed directly after removal from maize stems, while washed larvae were removed from maize stems, washed several times in distilled water, to remove any odour from the surface of the body of the larva, and dried on filter paper before exposure. Ten larvae were used per treatment and this was replicated at least four times. The proportion of larvae attacked was calculated and the time spent by each female until oviposition was recorded for females which oviposited within the five minutes exposure period. Means of time to oviposition were compared using analysis of variance (Proc GLM, SAS Institute, 1988) followed by the Student Newman-Keul mean separation test when the ANOVA was significant at $p < 0.05$. Proportions were transformed to arcsin before being subjected to ANOVA (Snedecor and Cochran, 1967).

4.3 Results

4.3.1 Response to volatiles from uninfested plants

Cotesia flavipes and *Cotesia sesamiae* were more attracted to odours from gramineous plants (maize, sorghum, napier grass), the food plants of their host, than to the control (Figure 4.1). *Cotesia flavipes* did not exhibit a preference between maize and napier grass (Figure 4.2). However, maize was preferred over sorghum by *Cotesia flavipes*, whereas *Cotesia sesamiae* preferred sorghum over maize (Figure 4.2; $X^2=16.71$, $p<0.005$); *Cotesia sesamiae* also preferred napier grass over maize (Figure 4.2).

Cotesia flavipes did not exhibit a greater attraction to uninfested cowpea plant than to the control (Figure 4.3). Similarly, when odours from uninfested cowpea were compared to odours from uninfested maize, no preference for either of the plant species was observed (Figure 4.3).

4.3.2 Response to plants infested by different stemborer species

Parasitoids of both species preferred odours from maize plants infested by *Chilo partellus*, *S. calamistis* or *Chilo orichalcociliellus* over that from uninfested maize plants (Figure 4.4).

The parasitoids did not show a greater preference for odours from plants infested with *Chilo partellus* than odours from plants infested with *Chilo orichalcociliellus* (Figure 4.5). When plants infested with *Chilo partellus* were tested against plants infested with *S. calamistis*, the parasitoids preferred the *S.*

calamistis infested plants (Figure 4.5). However, in dose response tests, *Cotesia flavipes* preferred odours from maize infested with ten *Chilo partellus* larvae to odours from maize infested with two *S. calamistis* (Figure 4.6). *Cotesia flavipes* also preferred odours from plants infested with ten *S. calamistis* over odours from plants infested with two *Chilo partellus*. However, *Cotesia flavipes* did not exhibit a preference when responding to odours from plants infested with five *Chilo partellus* and five *S. calamistis* larvae (Figure 4.6). There was no significant difference in the number of *Cotesia sesamiae* that was attracted to maize plants infested with ten *Chilo partellus* or two *S. calamistis* larvae (Figure 4.7).

Cotesia sesamiae preferred odours from plants infested with *B. fusca* over odours from uninfested maize. It also preferred odours from plants infested with *B. fusca* over odours from those infested with *Chilo partellus* at the same density (two larvae per plant) (Figure 4.8). However, when plants were infested with ten *Chilo partellus* larvae and two *B. fusca* larvae, *Cotesia sesamiae* showed preference for odours emanating from the *Chilo partellus* infested plants (Figure 4.8). *Cotesia flavipes* preferred odours from infested maize plants to odours from uninfested cowpea or cowpea plants infested with *M. testulalis* (Figure 4.9).

4.3.3 Response to frass

Response of *Cotesia flavipes* and *Cotesia sesamiae* to frass odours showed that frass obtained from all borer species fed on maize, sorghum or napier grass were highly attractive (Figure 4.10). In dual choice tests, odour from frass from the two *Chilo* species was more attractive to *Cotesia flavipes* than odours from *B. fusca* frass ($G=15.02$, $p<0.005$ and $G=9.13$, $p<0.01$ for *Chilo orichalcociliellus* and *Chilo partellus*, respectively), but no difference was observed in the attraction between odours from frass in the other combinations (Figure 4.11). For *Cotesia sesamiae*, there was no difference in attraction between odours from the different types of frass exposed (Figure 4.11).

4.3.4 Response to stemborer larvae

Cotesia flavipes and *Cotesia sesamiae* more often oviposited in unwashed hosts than washed hosts. There was no difference in the time taken by the parasitoids to oviposit in washed or unwashed stemborer larvae (Table 4.1 and 4.2).

Table 4.1 Acceptability of washed and unwashed larvae of Chilo partellus for parasitization by Cotesia flavipes

Treatment	N	Mean Percentage of larvae attacked	N	Mean Time to oviposition ± SE (seconds)
Washed larvae	50	56.0 b	28	67.57 ± 11.03a
Unwashed larvae	40	82.5 a	33	48.91 ± 8.83a

Numbers followed by the same letter in the same column are not significantly different (SNK). Percentage of larvae attacked: $F=5.80$; $P=0.0469$; df=1, 7; Time to oviposition: $F=1.78$; $P=0.1867$; df=1, 59.

Table 4.2 Acceptability of washed and unwashed larvae Chilo partellus for parasitization by Cotesia sesamiae

Treatment	N	Percentage of larvae attacked	N	Time to oviposition ± SE (seconds)
Washed larvae	40	25.0 b	10	77.10 ± 14.37a
Unwashed larvae	40	60.0 a	24	91.96 ± 12.61a

Numbers followed by the same letter in the same column are not significantly different (SNK). Percentage of larvae attacked: $F=17.10$; $P=0.0061$; df=1, 6; Time to oviposition: $F=0.47$; $P=0.50$; df=1, 32.

4.4 Discussion

Plant odours play an important role in communication in tritrophic systems. Plant volatiles are commonly found to be long range attractants for parasitoids (Vinson, 1985; Nordlund *et al.*, 1988; Whitman, 1988; Williams *et al.*, 1988; Lewis and Martin, 1990; Lewis *et al.*, 1990). For example, *Campoletis sonorensis* was attracted to uninfested food plants of its hosts (Elzen *et al.*, 1983). *Macrocentrus grandii*, a larval parasitoid of the European corn borer, *Ostrinia nubilalis*, was also attracted to several plant species including the food plant of its host, uninfested maize (Ding *et al.*, 1989). In the present study, *Cotesia flavipes* and *Cotesia sesamiae* were strongly attracted to selected gramineous plants (maize, sorghum and, a wild host, napier grass) which are host plants of stemborers. In all single-choice tests, the gramineous plant was selected over the control, regardless of the plant species. *Cotesia flavipes* showed a preference for maize over sorghum in the dual choice experiments. This parasitoid was imported from Pakistan where it was collected from *Chilo partellus* in maize fields. This preference may, therefore, be a reflection of a genetic adaptation to searching in maize. In contrast, the indigenous parasitoid *Cotesia sesamiae*, was more attracted to sorghum odours, and this may be due to a long coevolutionary history between *Cotesia sesamiae* and sorghum, which has its centre of origin in Africa (Doggett, 1988).

In a single choice test *Cotesia flavipes* was not attracted to uninfested cowpea plants. When it was given a choice between maize and cowpea (both

uninfested) the parasitoid did not show any preference. However, maize infested with *Chilo partellus* was preferred to uninfested cowpea or cowpea infested with *M. testulalis*, indicating that *Cotesia flavipes* was able to distinguish between the infested gramineous plants and a legume. It is difficult to explain why *Cotesia flavipes* did not show a preference for the undamaged maize when this was tested against uninfested cowpea. Except for few questionable records (*Cotesia flavipes* attacking *Diacrisia obliqua* Walker and *Porthesia scintillans* Walker, feeding on castor in India (Muthukrishnan and Senthamizhselvan, 1987; Senthamizhselvan and Muthukrishnan, 1989)), *Cotesia flavipes* and *Cotesia sesamiae* have been collected only from noctuid and pyralid hosts feeding on gramineous plants (Polaszek and Walker, 1991), which further implicates plants in providing important cues for host finding.

Cotesia flavipes and *Cotesia sesamiae* preferred odours from infested plants over odours from uninfested plants, indicating that the parasitoids were able to narrow their search to plants attacked by their hosts. Similar findings have been reported in several tritrophic systems. Dicke *et al*. (1990a,b) reported that lima bean and cucumber plants infested with two spotted spider mites, *Tetranychus urticae* Koch, emitted a blend of volatiles that attracted the predatory mites *Phytoseiulus persimilis*. Turlings *et al*. (1990a,b; 1991a,b) found that plants damaged by the beet armyworm (*Spodoptera exigua* (Hubner)) emitted volatile chemicals that were attractive to the parasitoid *Cotesia marginiventris*.

Odours from plants infested with *S. calamistis* were more attractive to *Cotesia flavipes* and *Cotesia sesamiae* than those infested with *Chilo partellus*, when the same number of plants was infested with an equal number of stemborers. *Sesamia calamistis* is a larger stemborer and feeds more voraciously than *Chilo partellus*, thereby inflicting greater injury to the plant and producing greater quantities of frass. Assuming that the volatiles involved in parasitoid attraction are compounds commonly found in injured gramineous plants, the quantities produced may be proportional to the amount of plant injury, which could explain the greater attraction to plants infested with *S. calamistis*. This hypothesis was supported by the fact that a larger number of hosts on a plant increased attractiveness (Figure 4.6 and Figure 4.7). Similar observations were made when *Cotesia sesamiae* was exposed to odour from plants infested with *B. fusca*, also a large stemborer, and *Chilo partellus* (Figure 4.8). In a related study, Potting *et al.* (1993) also found that odours from plants infested with the noctuid *B. fusca* were as attractive to *Cotesia flavipes* as odours from maize plants infested by the smaller *Chilo partellus*. These results strongly suggest that the two parasitoids are not able to discriminate between maize plants infested by the four herbivore species under study, and that they both seem to use a similar set of stimuli to locate their hosts. In contrast, *Cotesia marginiventris*, a generalist parasitoid, which attacks numerous host species on a wide range of plants, is able to distinguish between odours of two different plants fed upon by the same host and also between two different host

species feeding on the same plant species (Turlings *et al.*, 1989, 1990b). It is suggested that due to different feeding characteristics, different herbivore species may cause a differential release of plant compounds which can be detected by parasitoids after experience (Turlings *et al.*, 1991a). In *Cotesia flavipes*, however, there is no learning (Potting *et al.*, unpublished data), as the insects live for only 2-3 days (Moutia and Courtois, 1952; Gifford and Mann, 1967; Kajita and Drake, 1969). Because of their short longevity, females of *Cotesia flavipes* may search primarily within the habitat where they emerge, and may be adapted to parasitize all lepidopterous stemborers occurring in that habitat.

Once a parasitoid reaches the microhabitat of its host, it may rely on kairomones present in the by-products of the host (e.g. frass) to finally locate its host (Nordlund *et al.*, 1988). The results of the single choice experiments with host frass showed that frass odours from *Chilo partellus*, *Chilo orichalcociliellus*, *S. calamistis* and *B. fusca* fed on three different plants (maize, sorghum, and napier grass) were all attractive to the searching *Cotesia flavipes* and *Cotesia sesamiae* females. In dual choice experiments, results indicated that odours produced by frass from *Chilo* spp. were more attractive to *Cotesia flavipes* than odours from frass of *B. fusca*. *Cotesia sesamiae* was also attracted to frass obtained from the four different hosts under study, but did not discriminate between frass from different species fed on maize. The host diet in this case, maize, sorghum and napier grass, did not seem to influence

attraction of *Cotesia flavipes* and *Cotesia sesamiae*. In contrast, Van Leerdam *et al*. (1985) reported that *Cotesia flavipes* was more attracted to fresh frass of *D. saccharalis* larva from sugarcane than Johnson grass, *Sorghum halepense* (L.) Pers. However, frass from both sources were equally attractive in a no-choice situation. Similarly, Mohyuddin *et al*. (1981) showed that *Cotesia flavipes* strongly responded to frass from *Chilo partellus* larvae that had fed on maize, but weakly to frass from larvae fed on sugarcane. Inayatullah (1983) also reported that, frass produced by *Chilo partellus*, *S. inferens, S. uniformis* fed on sorghum, were highly attractive, but frass from *E. depressela* fed on maize, sorghum, sugarcane, pearl millet, and frass from *Chilo partellus, Chilo infuscatellus, A. stenellus* fed on sugarcane were less attractive. *Cotesia sesamiae* showed a stronger response to frass from *Chilo infuscatellus, E. depressella*, and *A. steniellus* fed on maize when compared to frass obtained from the same hosts fed on sugarcane (Shami and Mohyuddin, 1986).

Host larvae which were removed from plants where they had been feeding for more than 24 hours, were readily attacked by *Cotesia flavipes* and *Cotesia sesamiae*. Washed larvae that had been similarly exposed were also attacked but the proportion attacked was lower. These results suggest that short range attractants and contact kairomones may have been removed by washing. The kairomone(s) responsible for short range attraction and oviposition kairomone(s), if they exist, may be water soluble. However, about 55% of washed larvae were stung by *Cotesia flavipes*, which suggests that

there were other components of the kairomone(s) that were not removed. Alternatively the parasitoid may have responded to secretions from the mouth and volatiles from faeces as they walked near the mouthparts and the tip of the abdomen, since the larva continuously regurgitated fluids and defecated even after being washed. In another study, it was reported that *Cotesia rubecula* is attracted to oral secretions of its host *P. rapae* (Agelopoulos and Keller, 1994a). An oviposition kairomone has been reported from the mandibular gland of *Chilo partellus* (Mohyuddin *et al*. 1981; Muzaffar and Inayatullah, 1986).

The exotic parasitoid, *Cotesia flavipes*, was released at the Kenya coast in 1993 by ICIPE. The target species of this biological control programme is the introduced stemborer *Chilo partellus*. However, two other stemborer species, *S. calamistis* and *Chilo orichalcociliellus*, occur in the same ecosystem. During the two cropping seasons (long rains, April-June; short rains October-December) all three stemborer species coexist in maize fields (Warui and Kuria, 1983) and wild grasses (Mbapila J.C., unpublished data). In periods between cropping seasons, stemborers aestivate in maize stubbles, but remain actively feeding in wild grasses (Mbapila J.C., unpublished data), including napier grass, in the proximity of cultivated areas. The results of this study suggest that *Cotesia flavipes* may be able to search and attack all three stemborer species in cultivated and wild grasses during the cropping seasons, and wild grasses during periods between cropping seasons. Moreover, when maize and sorghum are grown in close proximity, our results suggest that *Cotesia flavipes*

may exhibit a greater propensity for searching in maize. These results also suggest that the niches of the two parasitoids overlap and competition for hosts is likely to occur. Competition between the two parasitoids could lead disappearance of one of the two species. However, due to differences observed in plant preference both parasitoids may persist in the same region.

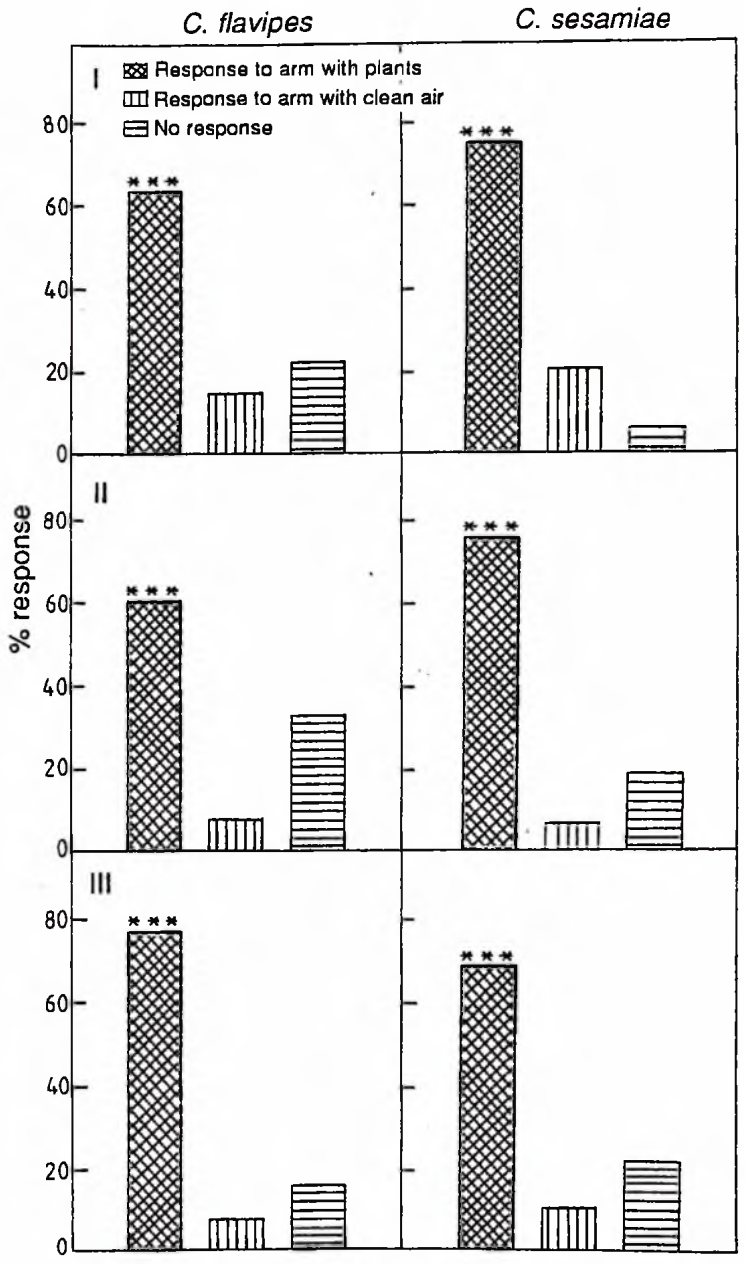

Figure 4.1 Response of *Cotesia flavipes* and *Cotesia sesamiae* to odours from uninfested plants: (I) maize, (II) sorghum, and (III) napier grass in a Y-tube olfactometer. *** = P < 0.005; ** = P < 0.01; * = P < 0.05

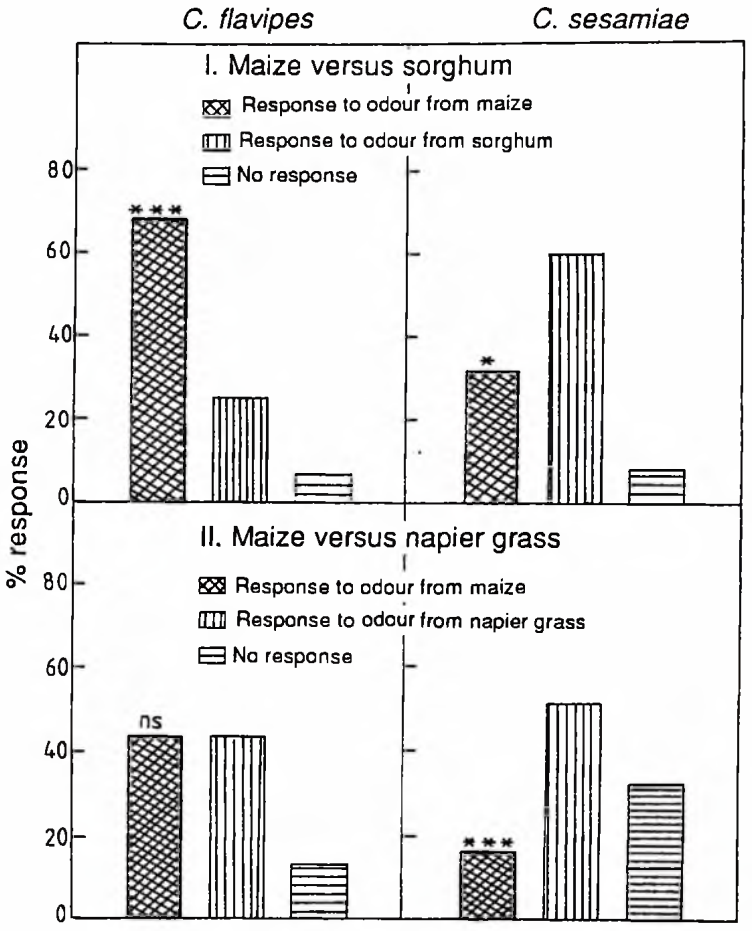

Figure 4.2 Response of *Cotesia flavipes* and *Cotesia sesamiae* to odours from uninfested plants in two choice experiments in the Y-tube olfactometer. (I) Maize versus sorghum, (II) Maize versus napier grass. *** = $P < 0.005$; ** = $P < 0.01$; * = $P < 0.05$; ns = not significant.

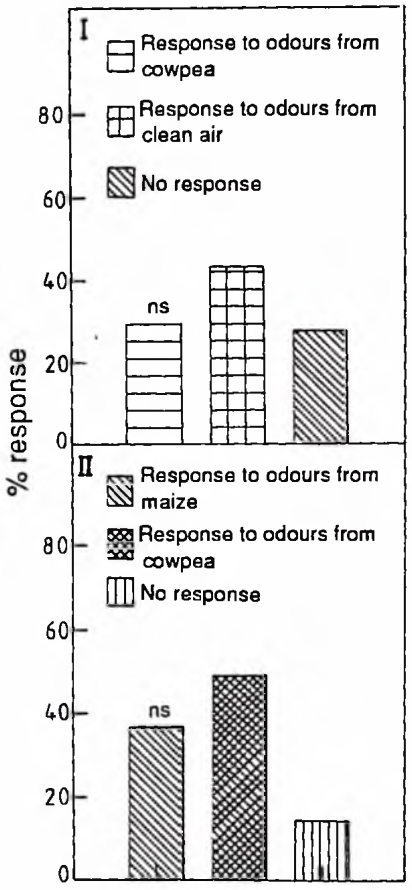

Figure 4.3 Response of *Cotesia flavipes* to odours from uninfested plants in the Y-tube olfactometer. (I) cowpea versus clean air. (II) cowpea versus maize. ns = not significant.

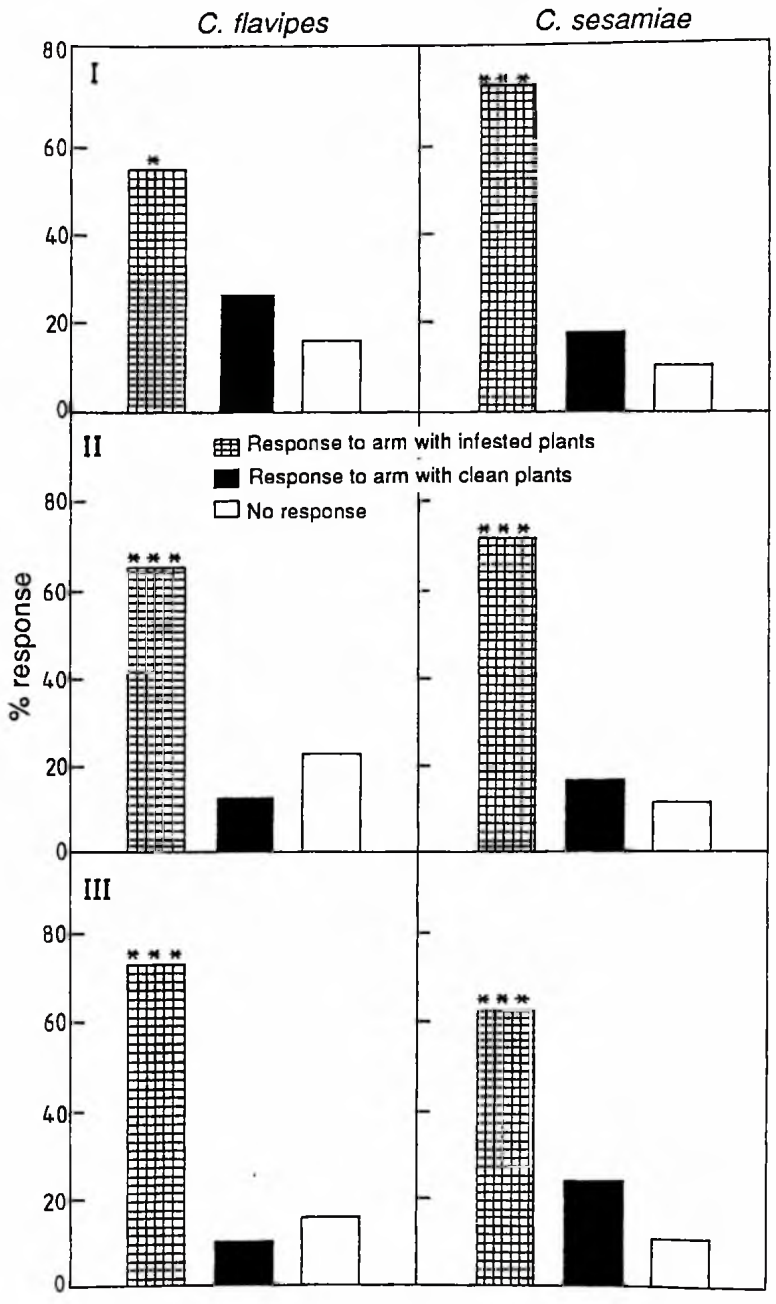

Figure 4.4 Response of *Cotesia flavipes* and *Cotesia sesamiae* to odours from maize plants infested with (I) *Chilo partellus*, (II) *Chilo orichalcociliellus*. (III) *S. calamistis* and uninfested maize plants in the Y-tube olfactometer. *** = P < 0.005; ** = P < 0.01; * = P < 0.05.

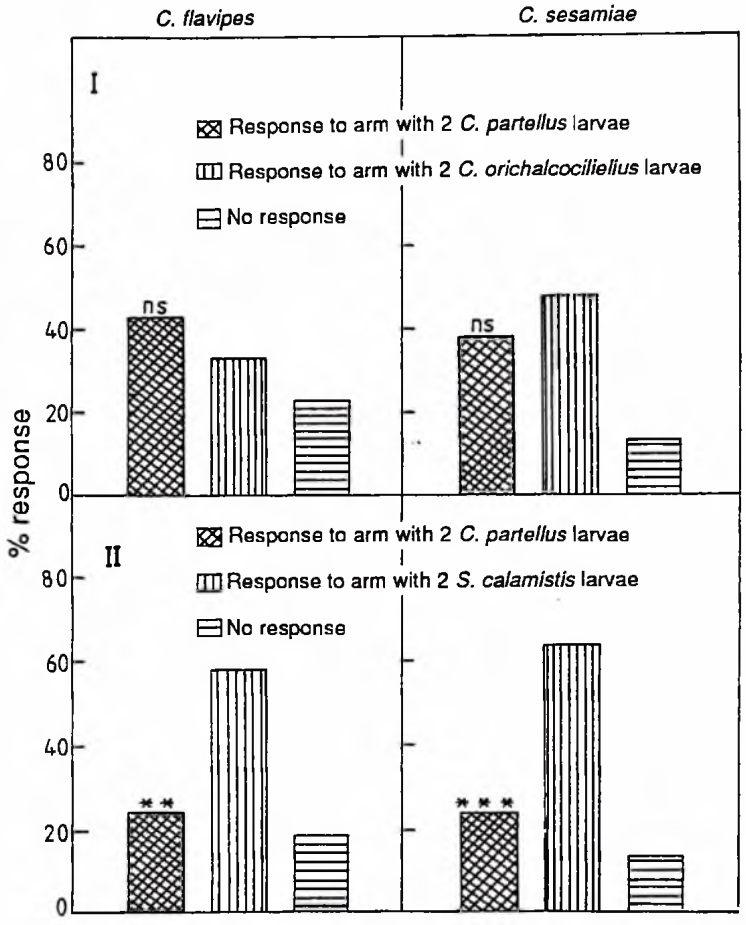

Figure 4.5 Response of *Cotesia flavipes* and *Cotesia sesamiae*, in the Y-tube olfactometer, to odours from infested maize in two choice tests: (I) maize infested with 2 *Chilo partellus* larvae versus maize infested with 2 *Chilo orichalcociliellus* larvae. (II) maize infested with 2 *Chilo partellus* larvae versus maize infested with 2 *S. calamistis* larvae. *** = $P < 0.005$; ** = $P < 0.01$; * = $P < 0.05$; ns = not significant.

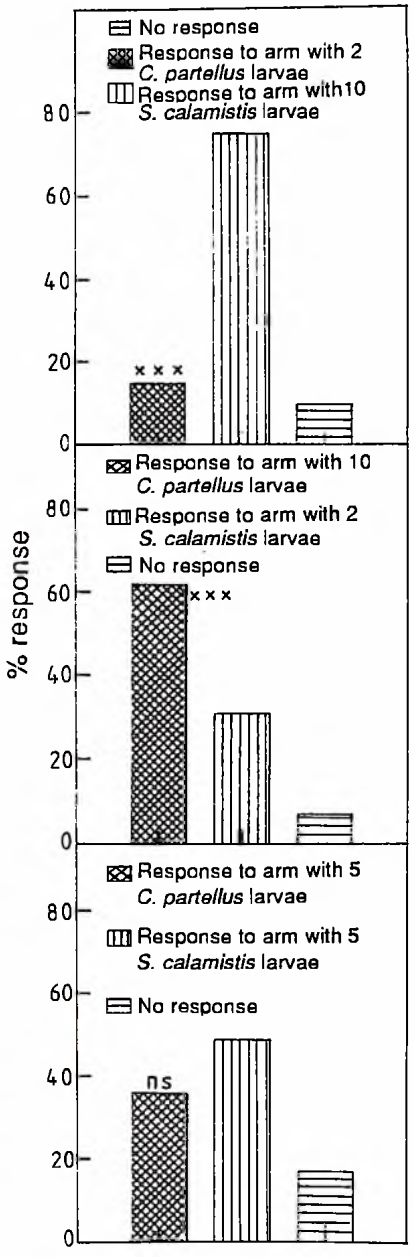

Figure 4.6 Choice by *Cotesia flavipes* in the Y-tube olfactometer between odours from maize plants infested with different stemborers species at different densities. *** = $P < 0.005$; ** = $P < 0.01$; * = $P < 0.05$; ns = not significant.

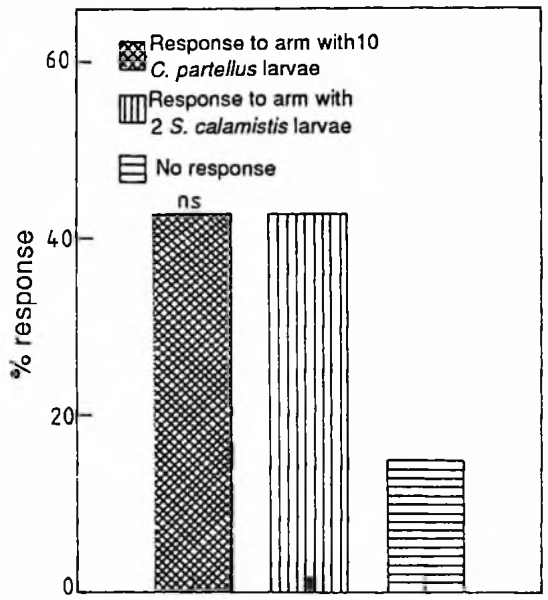

Figure 4.7 Choice by *Cotesia sesamiae* in the Y-tube olfactometer, between odours from maize plants infested by two or ten stemborers. ns = not significant.

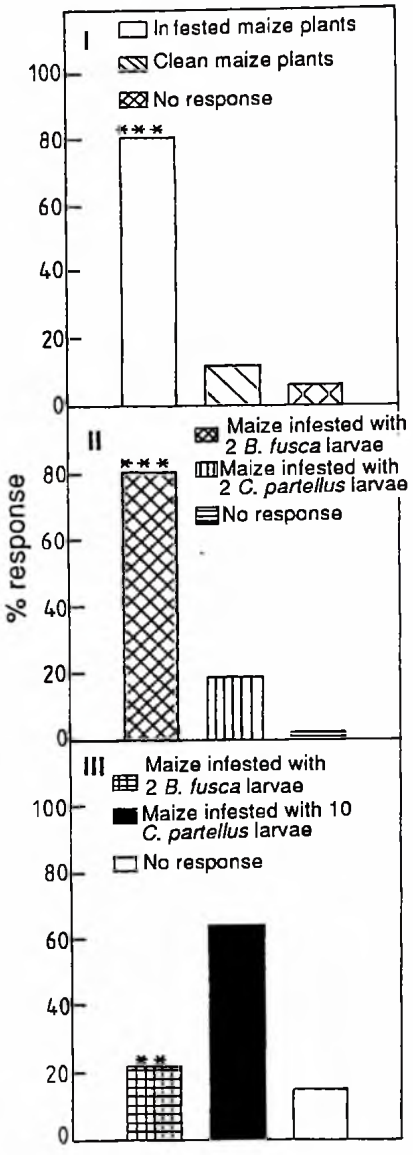

Figure 4.8 Choice by *Cotesia sesamiae* in the Y-tube olfactometer between odours from: (I) maize plants infested with *B. fusca* larvae and uninfested maize; (II) maize plants infested with 2 *Chilo partellus* larvae and 2 *B. fusca* larvae; (III) Maize plants infested with 10 *Chilo partellus* larvae and 2 *B. fusca* larvae. *** = $P < 0.005$; ** = $P < 0.01$; * = $P < 0.05$.

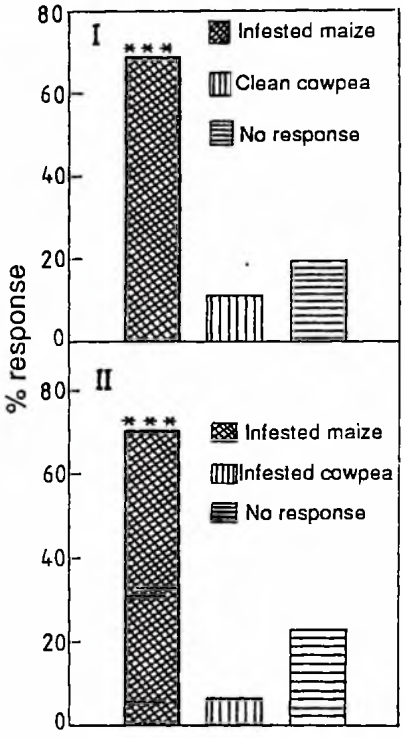

Figure 4.9 Choice by *Cotesia flavipes* in the Y-tube olfactometer between odours from : (I) maize infested with *Chilo partellus* and uninfested cowpea; (II) maize infested with *Chilo partellus* and cowpea infested with *M. testulalis*. *** = $P < 0.005$; ** = $P < 0.01$; * = $P < 0.05$.

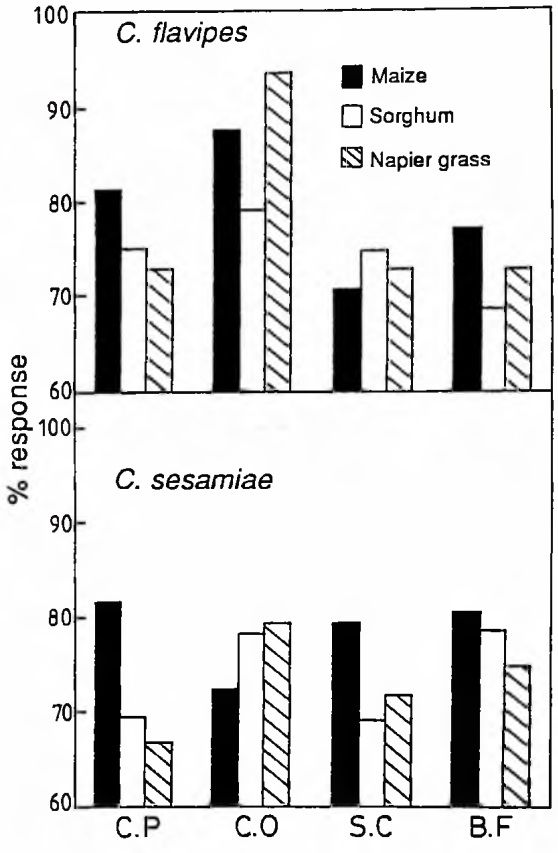

Figure 4.10 Response of *Cotesia flavipes* and *Cotesia sesamiae* to odours from frass produced by different stemborer species fed on maize, sorghum, and napier grass in the T-tube olfactometer. C.P.= *Chilo partellus*; C.O.= *Chilo orichalcociliellus*; S.C.= *S. calamistis*; B.F.= *B. fusca*. All frass were highly attractive.

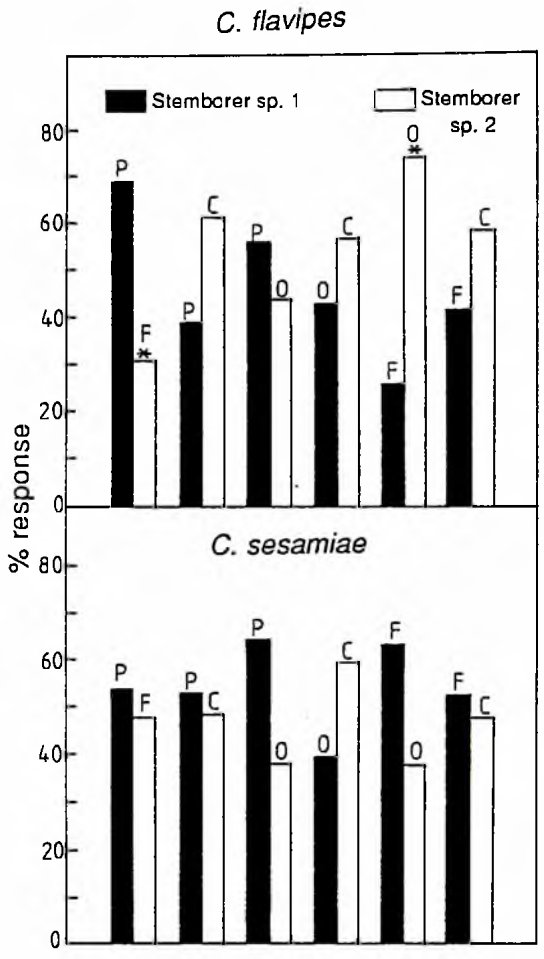

Figure 4.11 Response of *Cotesia flavipes* and *Cotesia sesamiae* to odours from frass produced by different stemborer species fed on maize in the T-tube olfactometer (dual choice). P = *Chilo partellus*; O = *Chilo orichalcociliellus*; C = *S. calamistis*; F = *B. fusca*. * = $P < 0.05$.

CHAPTER FIVE

5. Isolation and Identification of allelochemicals involved in the host selection process of Cotesia flavipes

5.1 Introduction

The bioassay results from Chapter four strongly suggested that volatile compounds originating from maize plants infested with stemborers, or maize plants treated with stemborer regurgitant (Potting *et al.*, 1995), or frass from larvae of different species of stemborers play an important role in the orientation of *Cotesia flavipes*, to the microhabitat of its hosts (See Section 4). The nature of the attractive components of the volatile blend is not known. This information could be useful in understanding the foraging strategies of *Cotesia flavipes* in the new environment where it is introduced and could provide potential for the manipulation of the behaviour of the parasitoids (Whitman, 1988). This study was therefore undertaken with the aim to identify chemicals present in the volatile extracts from maize plants infested with *Chilo partellus* that are attractive to *Cotesia flavipes*, and to compare chemical profiles of odours from infested maize, uninfested maize, maize treated with larval regurgitant, and frass from different host species. Details of the experiments conducted are described in section 5.2.

5.2 Materials and methods

Insects. Parasitoids and herbivores were reared and held as described in section 3.1 *Chilo partellus*, *Chilo orichalcociliellus*, *B. fusca* and *S. calamistis*,

were used in the experiments to infest maize plants (Hybrid 5-12). *Cotesia flavipes* was reared on *Chilo partellus* fed on artificial diet. One-day old, mated, and fed female parasitoids were used in the bioassays.

5.2.1 Volatile collections

Volatiles were collected from different test materials for comparative studies or for the identification of electrophysiologically active components.

(a) Collection of volatiles for comparative studies

(i) Infested versus uninfested plants

Two sets of seedlings of 10 days old maize plants were used: 10 uninfested maize seedlings (UNINFES) and 10 seedlings infested with 10-20 third instar *Chilo partellus* larvae (PHC-L). Before trapping volatiles, plants from both sets were pulled out of the soil and the roots washed and cut off. The cut ends of the individual plants were wrapped in wet cotton wool and then in aluminum foil before being placed in a five litre round bottomed flask for collection of volatiles. Collection of odour from UNINFES and PHC-L lasted four hours. Traps were eluted using HPLC grade dichloromethane (Aldrich Ltd., UK; 4 ml) and concentrated under a stream of nitrogen at 0 ^{o}C to 50 μl. Samples were stored in a freezer at -15 ^{o}C.

(ii) Plants treated with regurgitant versus untreated plants

Two sets of 10 days old maize seedlings were either used uninfested (UNINFES) or treated with regurgitant from *Chilo partellus* (PHC-R). Regurgitation was induced by holding each 4th or 5th instar larva of *Chilo partellus* with a pair of soft forceps and squeezing them gently (Mattiacci *et al.*, 1994). The regurgitant was collected in a 10 μl microcapillary tube positioned close to the mouth of the larva. Ten plants were artificially damaged by making holes in three leaves including the leaf whorl with a pin, and 10 μl of regurgitant was applied per plant on the damaged leaves. Another set of ten undamaged plants served as the control. Both test and control plants were kept for 16-18 hours (overnight) before volatiles were collected. Volatiles were trapped from plants for four hours. Traps were eluted, as indicated above, using HPLC grade dichloromethane (Aldrich Ltd., UK; 4 ml) and concentrated under a stream of nitrogen at 0 ^{o}C to 50 μl. Samples were stored in the freezer at -15 ^{o}C.

(iii) Infested pieces of maize stem versus uninfested maize stems

Tasseling plants were excised from the field and their leaves removed from the stems and discarded. Stems were then cut into small pieces of about 5 cm long. Twenty of these pieces of stem, together with 200 medium size larvae of *Chilo partellus* (which had been feeding on stalks for at least 48 hours) (PHC-S), were placed in a three neck one litre round-bottomed flask and larvae were allowed to feed for 18-20 hrs prior to collection of volatiles. For the control, the

same number of pieces of stem were kept for the same duration without larvae before volatiles were collected (UNINFES-S). Traps were also eluted using HPLC grade dichloromethane (Aldrich Ltd., UK; 4ml), and were concentrated and stored as mentioned above prior to analyses.

(iv) Frass

Frass was produced by medium, 4th or 5th, instar larvae feeding for 48 hours on fresh maize stems from tasseling plants in a glass jar covered with aluminum foil. Frass obtained during the first 24 hours was discarded to avoid contamination from the artificial diet. Frass produced in the next 24 hours was collected, weighed and placed in the trapping chamber. Frass produced by *Chilo partellus*, *S. calamistis* and *B. fusca* was used. Collection of volatiles lasted four hours. Samples were eluted, concentrated, and stored as indicated above.

Volatiles released by the different test materials were collected from chambers of different sizes depending on the test material. The air entrainment method described in section 3.4 was used to trap volatiles. A five litre round bottomed flask was used for collecting volatiles from PHC-L, UNINFES, PHC-R, and a one litre flask for PHC-S and UNINFES-S. Volatiles were collected from frass confined in a two part glass tube with quick fitground glass joints; the male part had 5 cm x 2.6 cm ID and the female part was 9 cm x 3.4 cm ID.

(b) Volatile collection for chemical identification

Volatiles were collected from 40 two to three months old potted maize plants infested with 4th or 5th instar larvae of *Chilo partellus* (PHC-B) at a density of ten larvae per plant. These larvae were placed in the leaf whorl and allowed to eat their way into the stems overnight. Plants were cut just above the soil level and placed in a glass box 10-15 minutes before trapping (see section 3.4.1). The cut ends of plants stood in water. A push-pull odour collection system described in section 3.4.1 was used for trapping volatiles from PHC-B. Collection of odour from these test materials lasted 24 hrs. Trapped volatiles were eluted with HPLC grade dichloromethane (Aldrich Ltd., UK; 4ml). Four volatile extracts obtained in four consecutive days from different sets of plants were merged, concentrated under a stream of nitrogen at 0 ^{o}C to 50 μl and stored as indicated earlier for subsequent analyses.

5.2.2 Analyses of Volatiles

Volatile extracts from PHC-L, UNINFES, PHC-R, PHC-S, UNINFES-S, and frass, were analyzed by GC (Hewlett-Packard (HP) 5890) using either a methyl silicone or Carbowax column, as indicated in section 3.4. 40 ng of an internal standard, dodecane, was added to the sample before it was injected into the GC. The chromatograms obtained from different test materials analyzed on the same column were compared.

Coupled gas chromatography-electroantennographic detector (GC-EAD)

analyses were performed on a Carbowax column (50mm x 0.2mm ID x 0.2 μm) and employed the same conditions as for the GC analyses of volatiles. Electroantennograms (EAGs) were recorded from antennae of one-day old mated and fed naive females of *Cotesia flavipes*. Four to five microlitres of samples (PHC-B) were injected into the GC and EAGs and corresponding GC peaks were monitored.

GC-MS analyses were carried out on a VG Masslab 12-250 mass spectrometer as outlined in section 3.4 for chemical identification of both EAG-active and inactive peaks. Identities of compounds were confirmed by comparison of retention times, and mass spectral data of authentic samples on the Carbowax column.

5.2.3 Behavioural bioassays

Bioassays were conducted on extracts of collected volatiles and synthetic chemicals in the T- and Y-tube olfactometers (See section 3.3.1 for bioassay set-up and procedure).

(a) Bioassays of volatile extracts

(i) *Cotesia flavipes* females were exposed in the Y-tube olfactometer to volatiles collected from PHC-B. Extracts from one volatile collection were brought to 500 μl by adding solvent before it was used. Doses were prepared in volumes and converted into collection minute equivalent (CME). One CME is the amount

of volatiles collected within one minute. Each collection contained 1440 CME (24 hrs). Five different doses; 10, 30, 100, 300, and 1000 CME were tested. Each dose was mixed with 100μl of light paraffin oil (Merk, Germany), then applied to filter paper discs (Whatman No.1, 5.5 cm diameter). Control discs contained similar amounts of solvent and paraffin oil only. Tests for each dose were replicated six times, each with six parasitoids.

(ii) Volatile extracts from PHC-L were tested for activity in the T-tube olfactometer. Extracts were brought to 500 μl before use. The amount of volatile collected was also expressed in collection minute equivalents (CME). Since the results from the previous experiment showed that the parasitoids responded in the same manner to all doses tested, only one dose (80 CME) was used in this experiment. The sample was mixed with 100μl of paraffin oil and then applied onto filter paper discs (Whatman No. 1, 5.5 cm diameter). Control discs contained a similar amount of solvent and paraffin oil only. There were three replicates of 20 parasitoids each. One collection was used for one replicate.

(b) Bioassays of synthetic compounds

(i) Of the compounds which were identified from infested maize plants, three gave antennal responses in the GC-EAD analyses, (E)-β-farnesene (authentic sample supplied by J. A. Pickett, Rothamsted experimentation station, UK), (Z)-3-hexenyl acetate (authentic sample supplied by M. Dicke Wageningen Agricultural

University, The Netherlands) and anisole (authentic sample obtained from Aldrich, UK). Another compound, linalool (authentic sample obtained from Aldrich) also gave antennal response. These four chemicals were tested singly, and/or in combination. Tests were carried out at 250, 500, and 1000 ng. In the experiments involving combinations of compounds, blends were tested as follows: (1). a mixture of (E)-β-farnesene (FAR), (Z)-3-hexenyl acetate (HA) and linalool (LIN) (in a 1:1:1 ratio); (2). the mixture described in (1) minus FAR; (3). the mixture described in (1) minus HA; (4). the mixture described in (1) minus LIN.

(ii) Authentic sample of the ten compounds which were identified from the infested maize, myrcene (Aldrich), 2-heptanone (Roth), 4,8-dimethyl-1,3,7-nonatriene (from J. A. Pickett), (Z)-3-hexenyl acetate (from M. Dicke), anisole (Aldrich), (Z)-3-hexen-1-ol (Aldrich), cyclosativen (ICIPE), α-copaene (Roth), cedrene (Roth), (E)-β-farnesene (from J. A. Pickett), were mixed together in the proportion reflected in the GC results, i.e. 10:0.4:17:100:4:26:20:1:8:21 and tested at two doses (1 and 10 μg). Samples were applied on filterpaper discs (Whatman No.1, 5.5 cm diameter) as described above. The synthetic compounds were mixed with paraffin oil and hexane (E-β-farnesene and 4, 8-dimethyl-1,3,7-nonatriene, were obtained from J. A. Pickett, in hexane). Control discs contained similar amounts of oil and hexane.

(iii) In addition, nine commercially available synthetic chemicals (identified

from maize volatiles by Buttery and Ling, 1984) were tested individually for attraction in the T-tube olfactometer. These included hexanal (Aldrich), (E)-2-hexenal (supplied by M. Dicke), 2-heptanone (Roth), (Z)-3-hexenyl acetate (M. Dicke), (Z)-3-hexen-1-ol (Aldrich), myrcene (Aldrich), linalool (Aldrich), α-copaene (Roth) and nerolidol (supplied by H. J. Williams, Texas A&MUniversity, USA). The compounds were dissolved in dichloromethane and paraffin oil and applied onto filter paper discs (Whatman No. 1, 5.5 cm diameter) as described above. Four different doses were tested: 0.5, 5, 10, and 25 μg. Filter papers impregnated with test material were folded and placed in either arm of the T-tube as described in section 3. The control disc contained equivalent amounts of solvent and paraffin oil only. Thirty parasitoids were observed per replicate and the experiment was replicated 3 times. Vials carrying test and control material were changed after 15 minutes (about ten observations) and new material applied. Synthetic chemicals used in the bioassays were 98% pure by GC analyses.

5.3 Results

5.3.1 Analyses of volatiles

Infested versus uninfested plants. Gas chromatographic analyses of trapped volatiles from PHC-L and UNINFES showed both qualitative and quantitative differences in the profiles (Figure 5.1). Trapped volatiles from UNINFES recorded consistently fewer and smaller peaks than PHC-L. The obvious quantitative increase in chemicals emitted was shown by peaks 8, 9 and 18 of

PHC-L identified as 4,8-dimethyl-1,3,7-nonatriene, anisole, and (E)-β-farnesene respectively (see below). Peaks 9 and 18 were absent in UNINFES.

Plants treated with regurgitant versus untreated plants. The GC profiles of volatiles collected from untreated maize seedlings and those treated with larval regurgitant showed that PHC-R emitted similar compounds as PHC-L but in relatively smaller amounts. However the amount released was still higher than that of UNINFES (Figure 5.2 and Table 5.1). Peak 9 was present only in trace amount in UNINFES.

Infested pieces of maize stem versus uninfested maize stems. The GC profiles from volatiles collected from uninfested maize stem and maize stem infested with *Chilo partellus* larvae also showed quantitative differences (Figure 5.3).

Frass. There was very little qualitative difference in the chemical profiles of frass collected from *Chilo partellus* and *B. fusca* fed on maize (Figure 5.4). However, significant quantitative differences were found between the volatile profiles of *Chilo partellus* and *S. calamistis* as shown by the peak labelled AB (Figure 5.5)

GC-EAD analyses. GC-EAD analyses of volatiles from PHC-B showed that *Cotesia flavipes* antenna responded to about 12 peaks in the volatile blend.

Strong as well as weak responses were recorded (Figure 5.6). Three of the EAG active peaks were identified as anisole, (Z)-3-hexenyl acetate, and (E)-β-farnesene (see GC-MS analyses below). GC-EAD tests with the four authentic compounds (anisole, (Z)-3-hexenyl acetate, (E)-β-farnesene and linalool) showed that they were EAG active and eluted from the GC at the same retention times as the respective peaks of the crude volatile collection (Figure 5.7 and Figure 5.8) thus confirming their identity.

GC-MS analyses. GC-MS analyses of extracts from PHC-B gave the chromatogram shown in Figure 5.9. Eleven compounds were identified (see Table 5.2). Their spectra and structures are given in Figure 5.10a to Figure 5.10j. These included an aldehyde, ketones, aromatic compounds, alcohols terpenes and esters.

5.3.2 Behavioural bioassays

(a) Response of parasitoid to volatile extracts

At all the doses tested, *Cotesia flavipes* was highly attracted to volatiles collected from maize plants infested with *Chilo partellus* in the glass box (PHC-B) when compared to the control (solvent and paraffin oil) (Figure 5.11). Similar results were obtained for volatiles collected from maize seedlings infested with *Chilo partellus* larvae (PHC-L) (Figure 5.12). Odours from uninfested seedlings were also more attractive than the control (Figure 5.12).

(b) Response of parasitoid to synthetic compounds

When parasitoids were exposed to a mixture of the three chemicals that gave antennal responses, ((Z)-3-hexenyl acetate, (E)-β-farnesene and linalool) in a ratio of 1:1:1, significant ($P<0.05$) attraction was observed at 250 and 500 ng (Figure 5.13). The binary mixture in which (Z)-3-hexenyl acetate or (E)-β-farnesene were present were also as attractive. (E)-β- farnesene or (Z)-3-hexenyl acetate were attractive individually (Figure 5.14). Anisole (methoxybenzene) was attractive at all the doses tested (Figure 5.14).

Cotesia flavipes was strongly attracted to 10 μg of the synthetic blend of 10 identified compounds (Figure 5.15). The monoterpene, 4,8-dimethyl-1,3,7-nonatriene was attractive at a dose of 1000 ng (Figure 5.16).

No significant attraction was observed for the nine individual compounds identified from maize volatiles (Buttery and Ling, 1984) which were tested at 4 different doses (Figure 5.17 and Figure 5.18).

5.4 Discussion

Results in section 4 indicated that although *Cotesia flavipes* was attracted to odours from uninfested maize plants, host frass, or artificially damaged plants (cf. Potting *et al.*, 1995); the parasitoid was preferentially attracted to volatiles from maize plants on which stemborers had been feeding (PHC). This was further supported by olfactometric data on tests with volatile extracts from infested and uninfested maize plants which were also differentially attractive to *Cotesia flavipes*,

albeit in a dose-independent manner. This suggests that the different doses tested were within a narrow band of the whole range of attractive doses. Turlings *et al.* (1991b) found that *Cotesia marginiventris* responded to the crude extract of maize seedlings damaged by *S. exigua*; however, this parasitoid responded in a dose related manner.

GC-EAD analyses of the volatile extracts showed that the antennal receptors of females of *Cotesia flavipes* responded to 12 peaks in the crude extracts, three of which were identified as (Z)-3-hexenyl acetate, anisole and (E)-β-farnesene. These three compounds were behaviourally active when tested singly and in binary and tertiary mixtures (Figure 5.14). There is no evidence in the literature that anisole has been identified previously in this system. However, recently Torto *et al.* (1994) identified anisole as one of the EAG-active components of volatiles of the adult desert locust, *Schistocerca gregaria* (Forskal). Although anisole was inactive in aggregation bioassays (Torto B. *et al.* 1994), it has recently been shown to be a component in the blend of volatiles involved in accelerated maturation of the desert locust, *S. gregaria* (Torto B., pers. comm.). The significance of these findings in chemical ecology is that a range of allelochemicals has been demonstrated to play different and often opposite semiochemical roles in different insects (Janzen, 1979; Matsuda and Senbo, 1986; Norris, 1986; Whitman, 1988) reflecting different evolutionary responses of herbivores to chemicals elaborated by plants. The GC-EAD technique has widely and reliably been used for identification of insect pheromones, but only a small number of reports indicate

the use of this technique in characterization of allelochemicals involved in the tritrophic interactions of insects (Struble and Arn, 1984). Identification of anisole as an additional kairomone of *Cotesia flavipes* in the tritrophic system under study shows that this technique can be used for a full characterization of the kairomones involved in host selection by parasitoids. Using GC-EAD recordings Baehrecke *et al.* (1989) demonstrated that *Campoletis sonorensis* antenna responded to chemicals identified from cotton (*Gossypium hirsutum* L.), the habitat of its host *Heliothis virescens*.

Studies involving several tritrophic systems have demonstrated that feeding by the herbivore on the plant induces release of new volatile compounds (herbivore induced synomones) that attract predators and/or parasitoids of the herbivores (Sabelis *et al.*, 1984; Dicke and Sabelis, 1988b; Dicke *et al.*, 1990 b; Turlings *et al.*, 1990 ab, 1991b; Agelopoulos and Keller, 1994 abc; Mattiacci *et al.*, 1994). Chemical data obtained in this study showed that stemborer-damaged plants are a source of compounds attractive to *Cotesia flavipes*. There were qualitative and quantitative differences between the volatile profiles of uninfested maize plants, infested maize and artificially damaged maize plants treated with larval regurgitant. Uninfested plants released only minute quantities of compounds. However, when these plants were fed on by stemborers, a dramatic increase occurred in the number and the proportions of volatiles released (Figure 5.1). In particular, terpenoids and green leaf volatiles (GLVs) were among the chemicals identified from the PHC.

Green leaf volatiles (GLVs) are saturated and unsaturated six carbon alcohols 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) and shown to play a role in host detection by phytophagous insects (Visser, 1986). Recently, synthetic and natural GLVs from damaged cowpea leaves, were reported to attract the braconid parasitoid, *Microplitis croceipes* in a wind-tunnel (Whitman and Eller, 1990). The green leaf volatiles identified in this study included (E)-2-hexenal, (Z)-3-hexen-1-ol, and (Z)-3-hexenyl acetate. These compounds were also identified in a similar study involving maize seedlings, the beet armyworm and the parasitoid *Cotesia margiventris* (Turlings *et al.*, 1991 b).

In addition to GLVs, terpenoids have been implicated in the host searching behaviour of parasitoids (Takabayashi *et al.*, 1994). Terpenoids identified in this study include myrcene, 4,8-dimethyl,1,3,7-nonatriene, cyclosativen, α-copaene, (E)-β-farnesene and cedrene. Other unidentified essential oils were also present in the volatile extracts. More chemical work is needed for a complete identification of all the components of the volatile blend. (E)-β-farnesene was the most abundant terpenoid in the volatile extract from PHC-B but it was absent in the headspace collection of uninfested maize plants, suggesting that it is a host induced synomone. A similar observation was made by Turlings *et al.* (1991b) in the maize - beet armyworm *Cotesia marginiventris* system. This compound was also identified in the volatiles of maize leaves by Buttery and Ling (1984). (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).

In the T-tube olfactometer test, *Cotesia flavipes* responded to 4,8-dimethyl-1,3,7-nonatriene. There was also a significant increase in the amount of 4,8-dimethyl-1,3,7-nonatriene released by damaged plants. However, in a tritrophic system involving the brussels sprouts plants - *Pieris brassicae* larvae - and *Cotesia glomerata*, significant quantitative difference was observed in the amounts of GLVs rather than terpenoids released by infested and uninfested plants when their volatile emissions were compared (Mattiacci *et al*., 1994). The terpenoid 4,8-dimethyl-1,3,7-nonatriene was also 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) and 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 spider mite infestation. The 4,8-dimethyl-1,3,7-nonatriene has been listed among the herbivore-induced synomones that attract natural enemies (reviewed by 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 alcohols, nerolidol and geranyllinalool.

A factor present in the regurgitant of caterpillars has been previously implicated in the production of herbivore-induced synomones (Potting *et al*., 1995).

It was demonstrated that *Cotesia flavipes* preferred leaves of maize plants treated with stemborer regurgitant to leaves of untreated maize plants. In this study, it was noted that the chemical composition of plants treated with regurgitant was qualitatively and quantitatively different from that of undamaged maize plants. These changes in the volatile composition may be the basis of the enhanced attractiveness of volatiles from damaged plants treated with larval regurgitant as reported by Potting *et al*. (1995). Similar observations have been made in other tritrophic systems involving several plant species such as maize, lima bean, cucumber, and cabbage (Turlings *et al*. 1993; Dicke, 1994; Mattiacci *et al*. 1994; Agelopoulos and Keller, 1994a).

The bioassay data showed that compounds previously identified by Buttery and Ling (1984) did not elicit a significant response from *Cotesia flavipes*. It is possible that these compounds may be active at very small doses or in blends as synergists. Further work is necessary to evaluate the efficiency of these compounds in host detection by *Cotesia flavipes*.

The GC profiles of volatiles from frass obtained from *Chilo partellus*, and *B. fusca* larvae fed on maize stems did not show any major differences. This may explain the lack of preference of *Cotesia flavipes* for volatiles from frass of the four stemborer species fed on maize (see section 4). However, the only conspicuous quantitative difference between frass volatiles of *Chilo partellus* and those of *S. calamistis* was in peak AB (Figure 5.5). Whether this difference influences the behaviour of *Cotesia flavipes* or *Cotesia sesamiae* is yet to be elucidated.

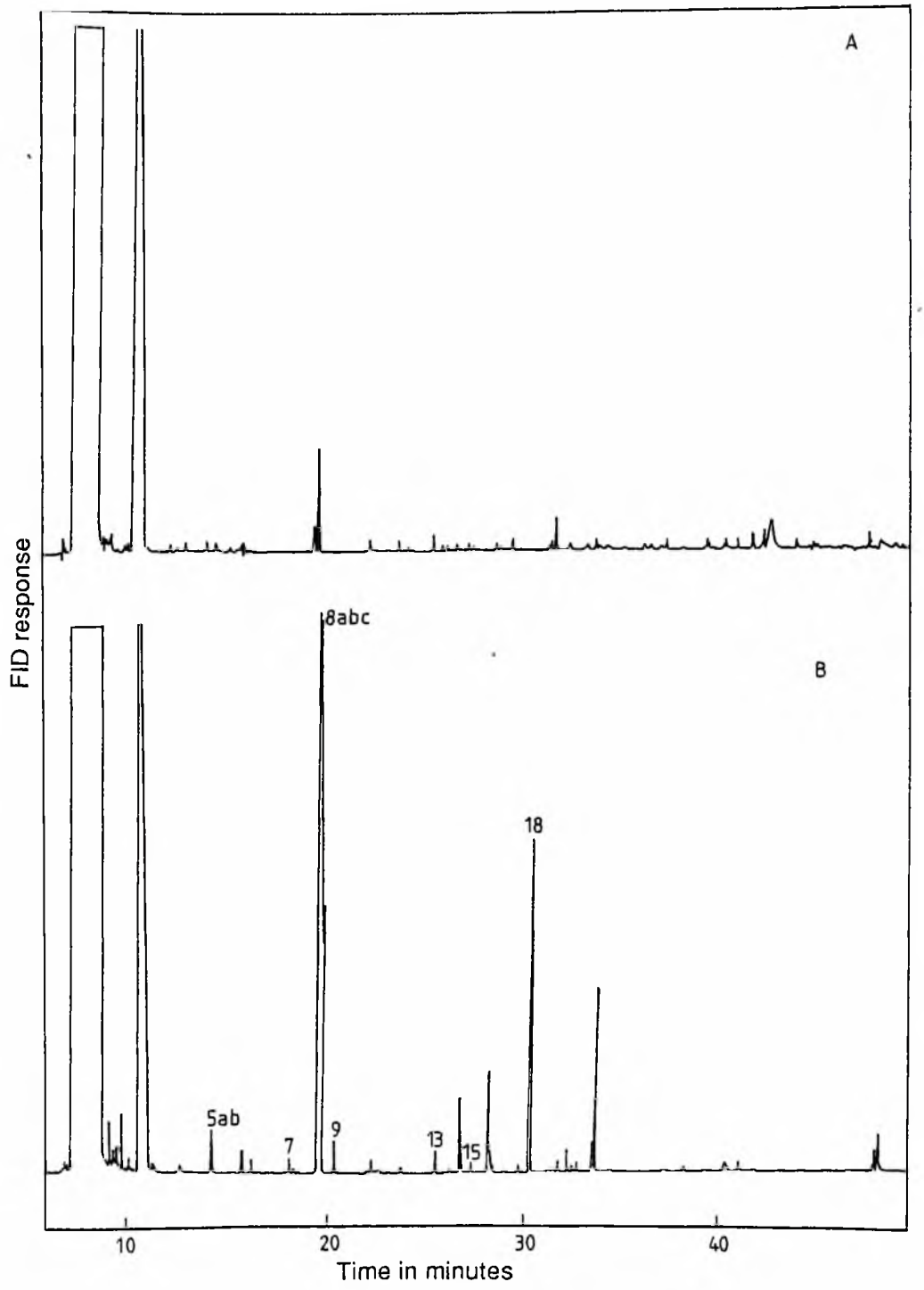

Figure 5.1 Gas chromatograms of volatiles released by: (A) uninfested maize seedlings (UNINFES); (B) maize seedlings infested with *Chilo partellus* (PHC-L). Chromatograms were obtained by analyses of volatiles on a 50 m Carbowax column after 4 hours of collection. Peak numbers correspond to compounds in Table 5.2.

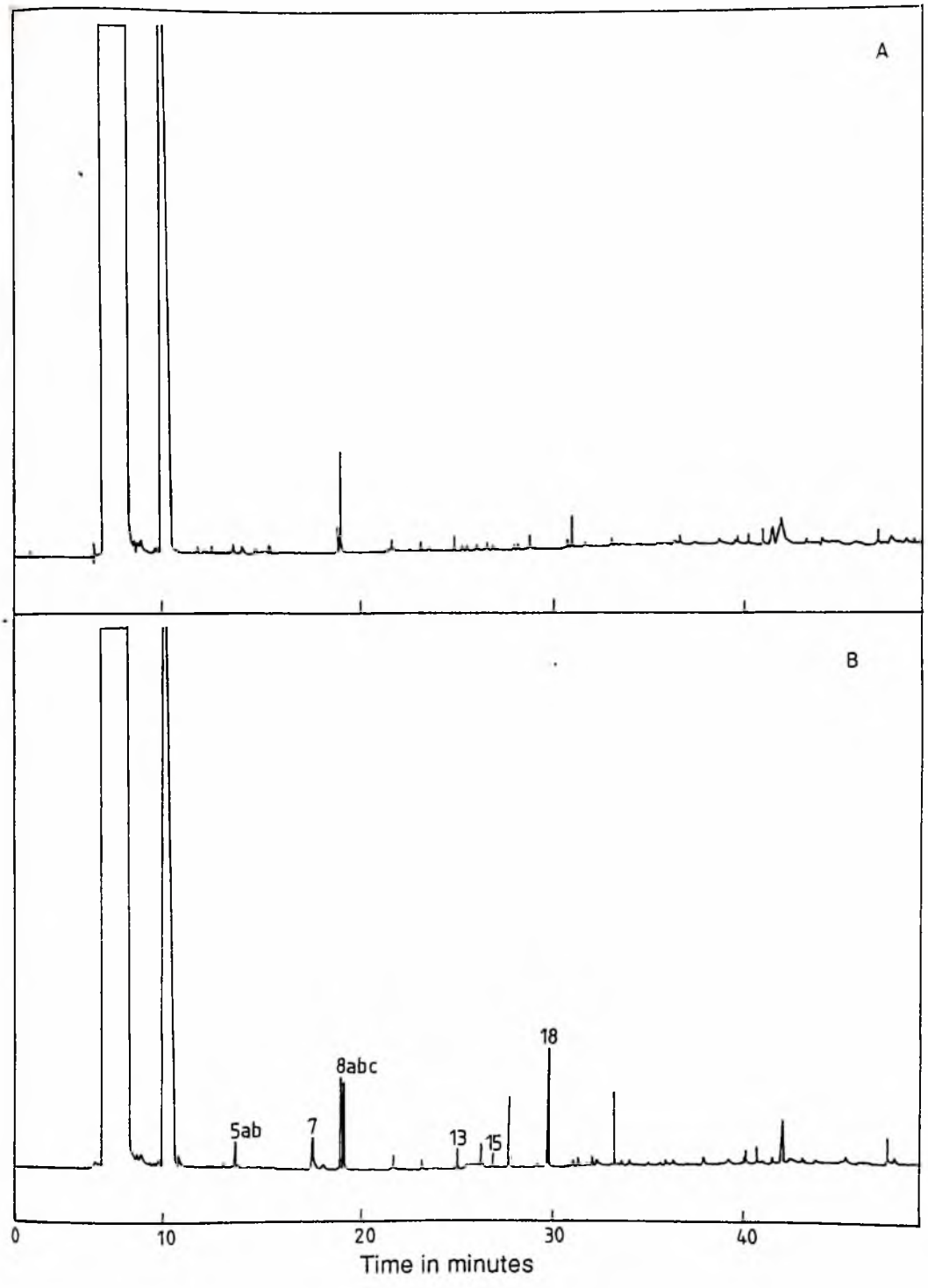

Figure 5.2 Gas chromatograms of volatiles released by: (A) undamaged maize seedlings (UNINFES); (B) maize seedlings treated with regurgitant of *Chilo partellus* (PHC-R). Chromatograms were obtained by analyses of volatiles on a 50 m Carbowax column after 4 hours of collection.

Table 5.1 Compounds identified in the headspace of maize plant in different treatments.

Compounds	Treatments			
	UNINFES	PHC-B	PHC-L	PHC-R
myrcene	+	+	+	+
2-heptanone	(+)	+	(+)	(+)
4,8-dimethyl-1,3,7-nonatriene	(+)	+	(+)	(+)
(Z)-3-hexenyl-acetate	+	+	+	+
(Z)-2-hexenal	+	+	+	+
anisole		+	+	~
(Z)-3-hexen-1-ol		+		
cyclosativen	+	+	+	+
α-copaene	+	+	+	+
cedrene	+	+	+	+
(E)-*β*-farnesene		+	+	+

UNINFES = uninfested maize seedlings; PHC-B = 2-3 months old maize plants infested with *C. partellus* larvae; PHC-L = maize seedlings infested with *C. partellus* larvae; PHC-R = maize seedlings treated with *C. partellus* regurgitant. + Present; (+) Not always present; - Absent; ~ Trace amounts;

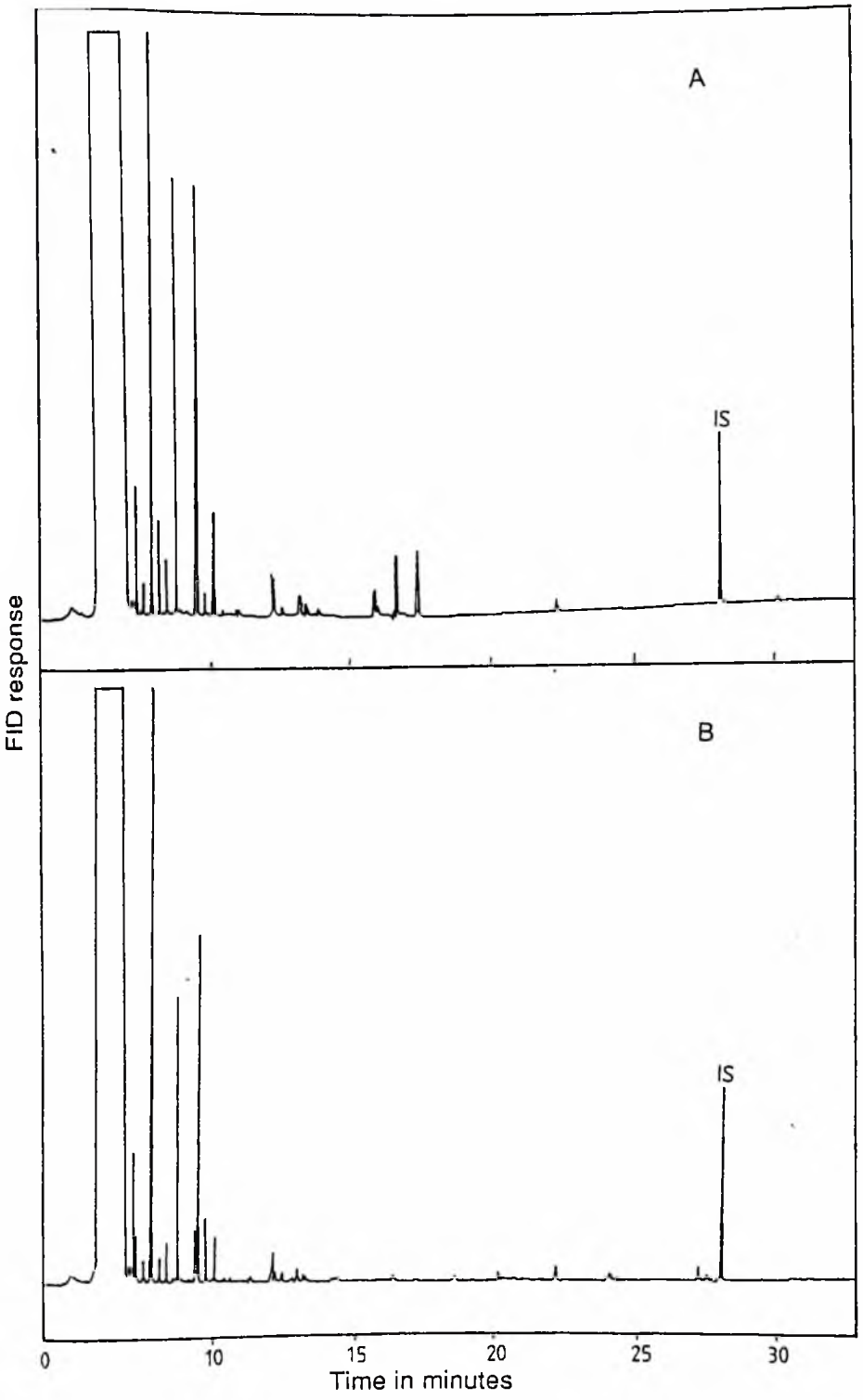

Figure 5.3 Gas chromatograms of volatiles released by (A) maize stem infested with *Chilo partellus* larvae (PHC-S); (B) uninfested maize stem (UNINFES-S). Chromatograms were obtained by analyses of volatiles on a 50 m Methyl silicone column, after 4 hours of collection. IS = internal standard, dodecane.

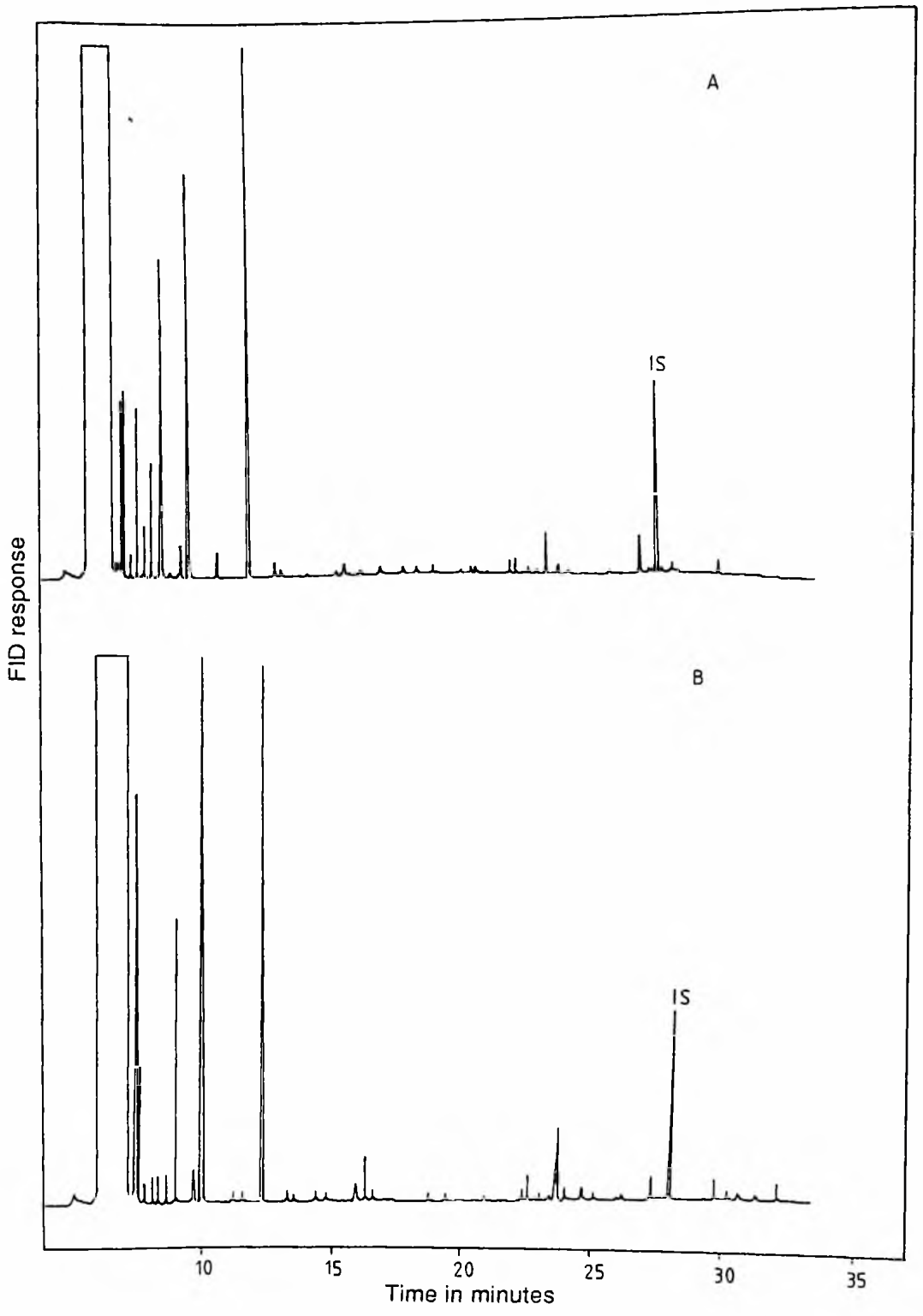

Figure 5.4 Gas chromatograms of volatiles released by frass produced by (A) *Chilo partellus* fed on maize stem; (B) *B. fusca* fed on maize stem. Analyses were done after 4 hours of collection on a 50 m methyl silicone column. IS = internal standard, dodecane.

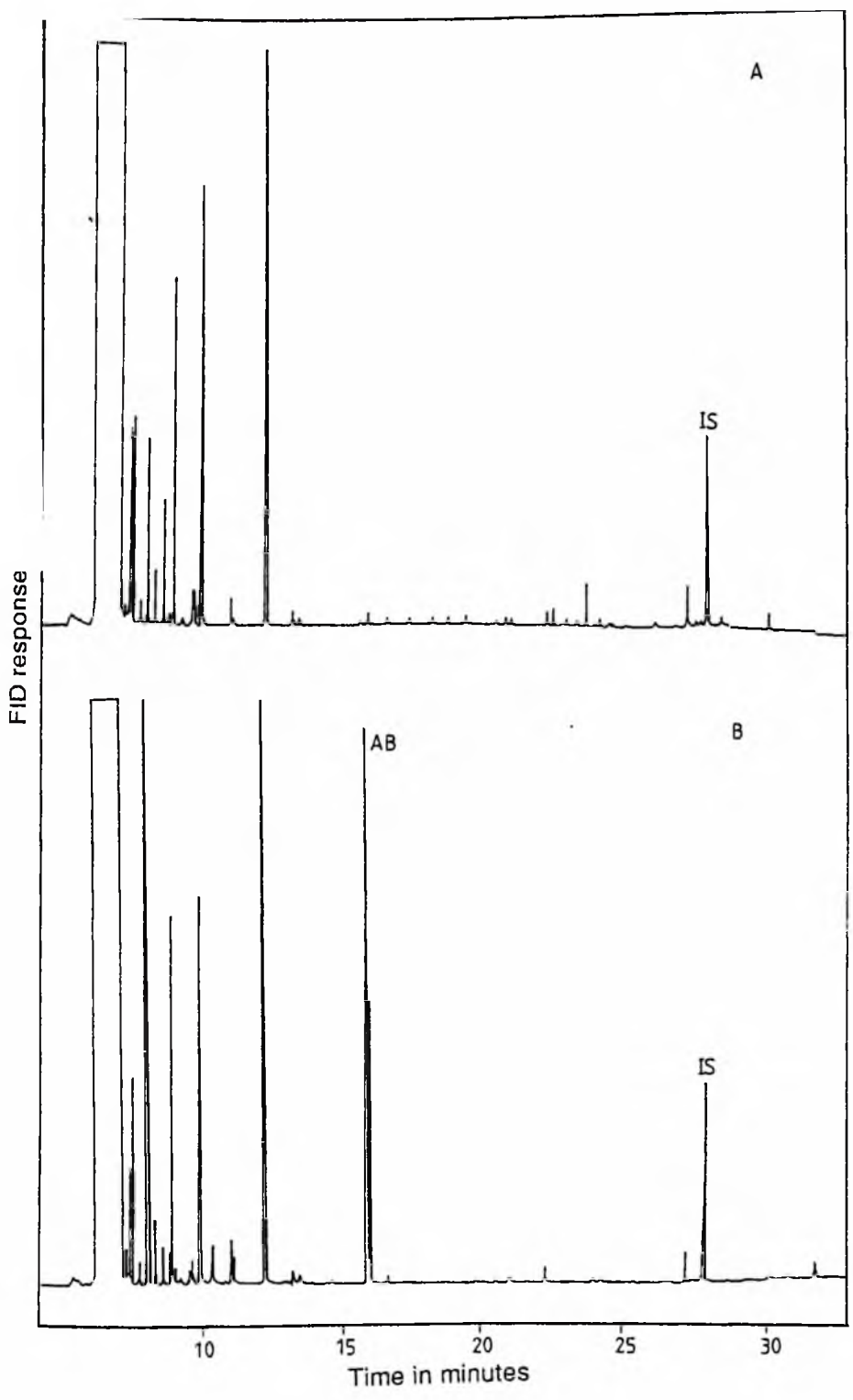

Figure 5.5 Gas chromatograms of volatiles released by frass produced by (A) *Chilo partellus* larvae fed on maize stem; (B) *S. calamistis* larvae fed on maize. Analyses were done after 4 hours of collection on a 50 m methyl silicone column. AB = peak only present in large quantities in frass of S. calamistis. IS = internal standard, dodecane.

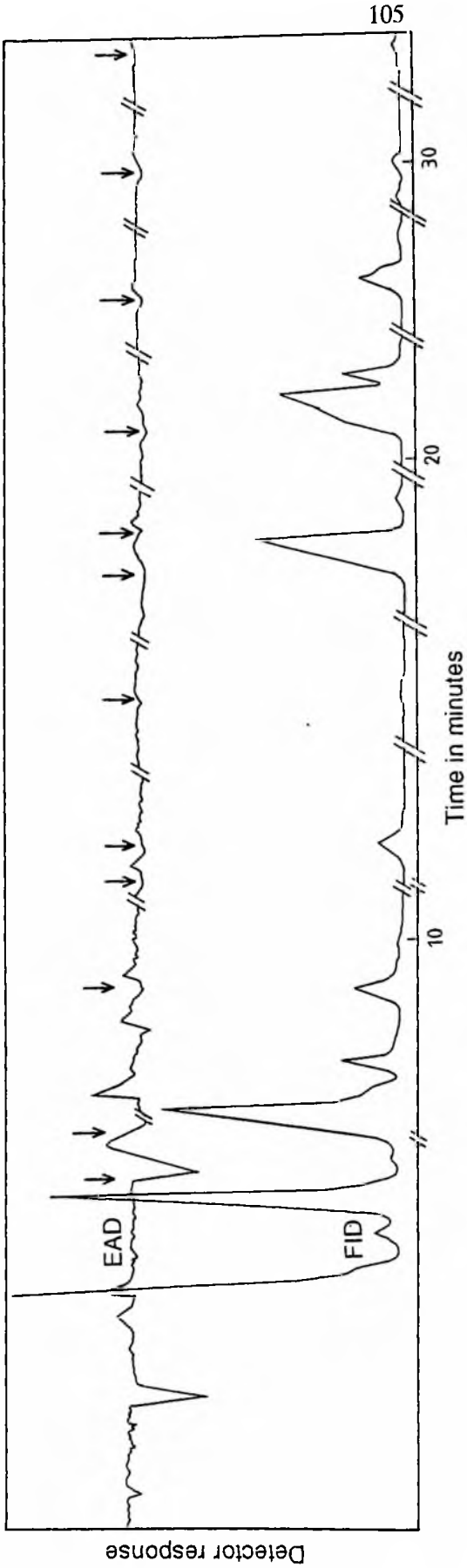

Figure 5.6 GC-EAD trace showing EAG responses of a *Cotesia flavipes* antenna to corresponding gas chromatogram of volatile components from 2-3 month old maize infested with *Chilo partellus* larvae. Analyses done on a 50 m Carbowax column after 24 hours of collection.

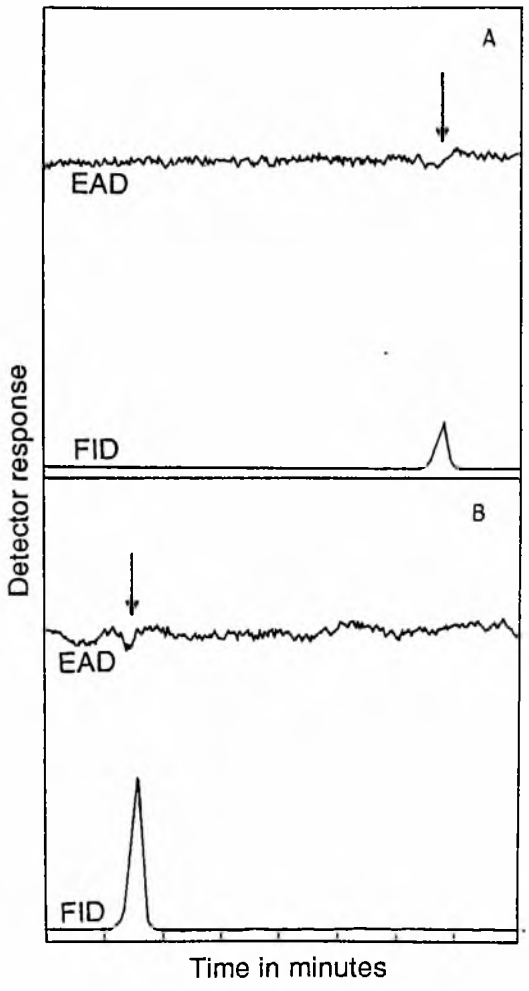

Figure 5.7 EAG response (arrow) to synthetic chemicals: (A) (Z)-3-hexenyl acetate; (B) anisole in GC-EAD recording

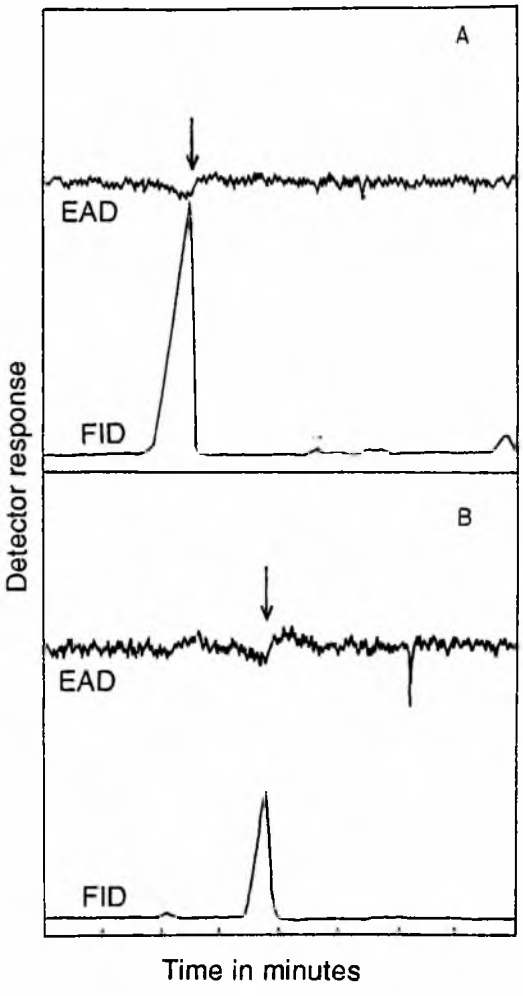

Figure 5.8 EAG response to synthetic chemicals: (A) (E)- *β*-farnesene; (B) linalool in GC - EAD recording

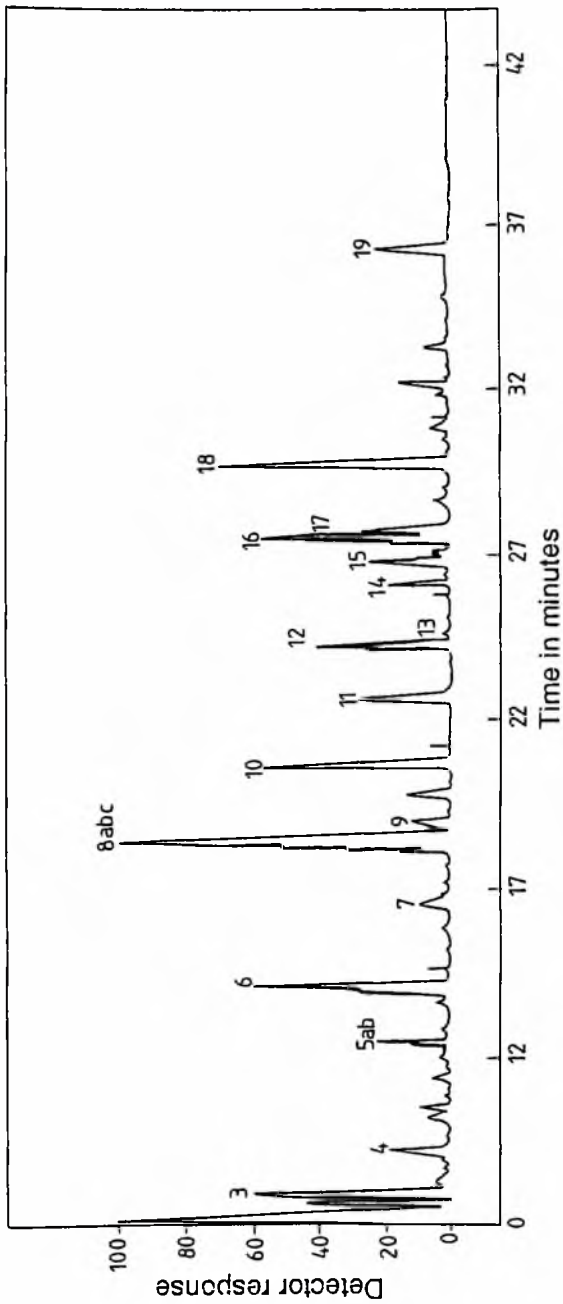

Figure 5.9 GC chromatogram and MS (Figures 5.10a-5.10j) of volatiles from maize plants infested with *Chilo partellus*. Peak numbers correspond to numbers given in Table 5.2.

Table 5.2. Compounds identified in volatiles trapped from 2-3 month old maize plants infested with Chilo partellus larvae.

Peak No.	Compounds	Relative %[1]	Retentin time (in min.)
5a	myrcene	0.53	15.14
5b	2-heptanone	0.02	15.34
8a	4,8-dimethyl-1,3,7-nonatriene	0.90	21.03
8b	(Z)-2-hexenal		
8c	(Z)-3-hexenyl acetate[2]	5.44	21.35
9	anisole[2]		22.00
10	(Z)-3-hexen-1-ol	1.41	23.51
12	cyclosativen	1.10	27.46
13	α-copaene	0.06	27.63
15	cedrene	0.17	29.76
18	(E)-β-farnesene[2]	1.13	32.91
	p-xylene[3]		
	o-xylene[3]		
	m-xylene[3]		

[1] based on the integration of peaks in the GC. [2] Compounds giving an antennal response. [3] Best identified using the methyl silicone column.

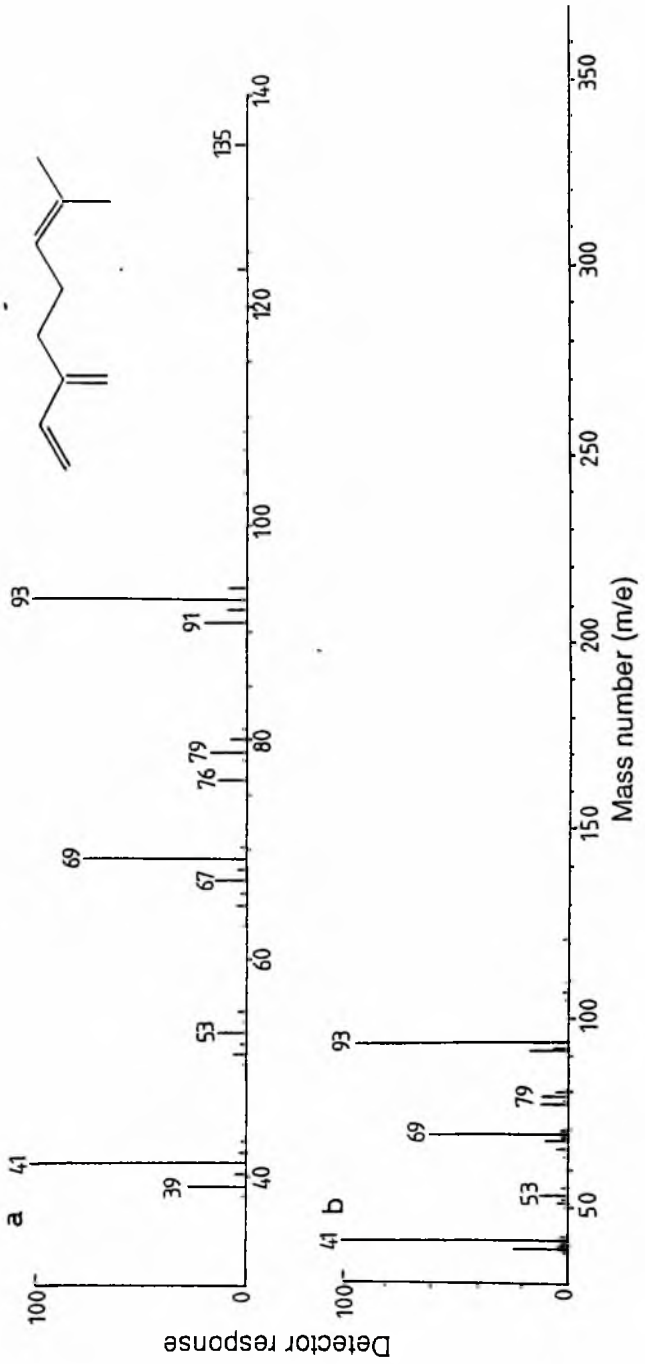

Figure 5.10a Mass spectra of myrcene: (a) authentic sample (MW = 136); (b) compound from volatiles collected from maize infested with *C. partellus* (PHC - B).

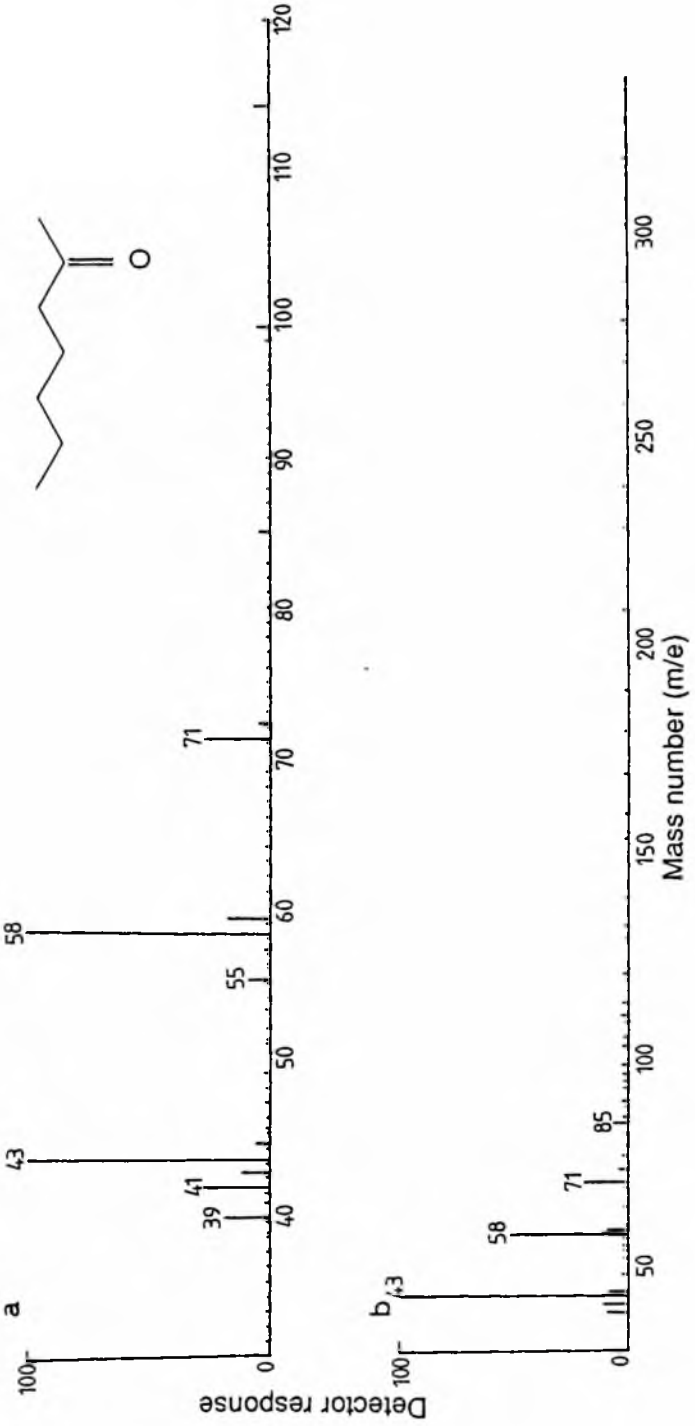

Figure 5.10b Mass spectra of2-heptanone: (a) authentic sample (MW = 114) ; (b) compound from volatiles collected from maize infested with *C.partellus* (PHC - B).

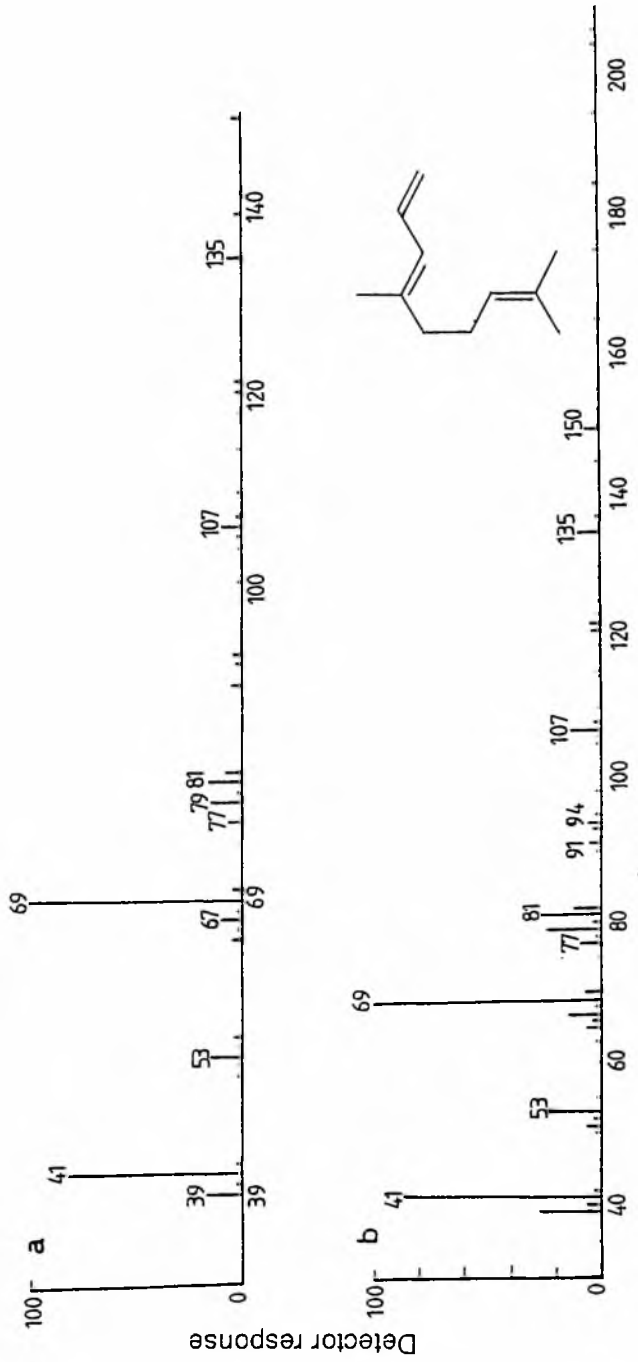

Figure 5.10c Mass spectra of 4, 8-dimethyl-1,3, 7-nonatriene: (a) authentic sample; (b) compound from volatiles collected from maize infested with *C.partellus* (PHC - B).

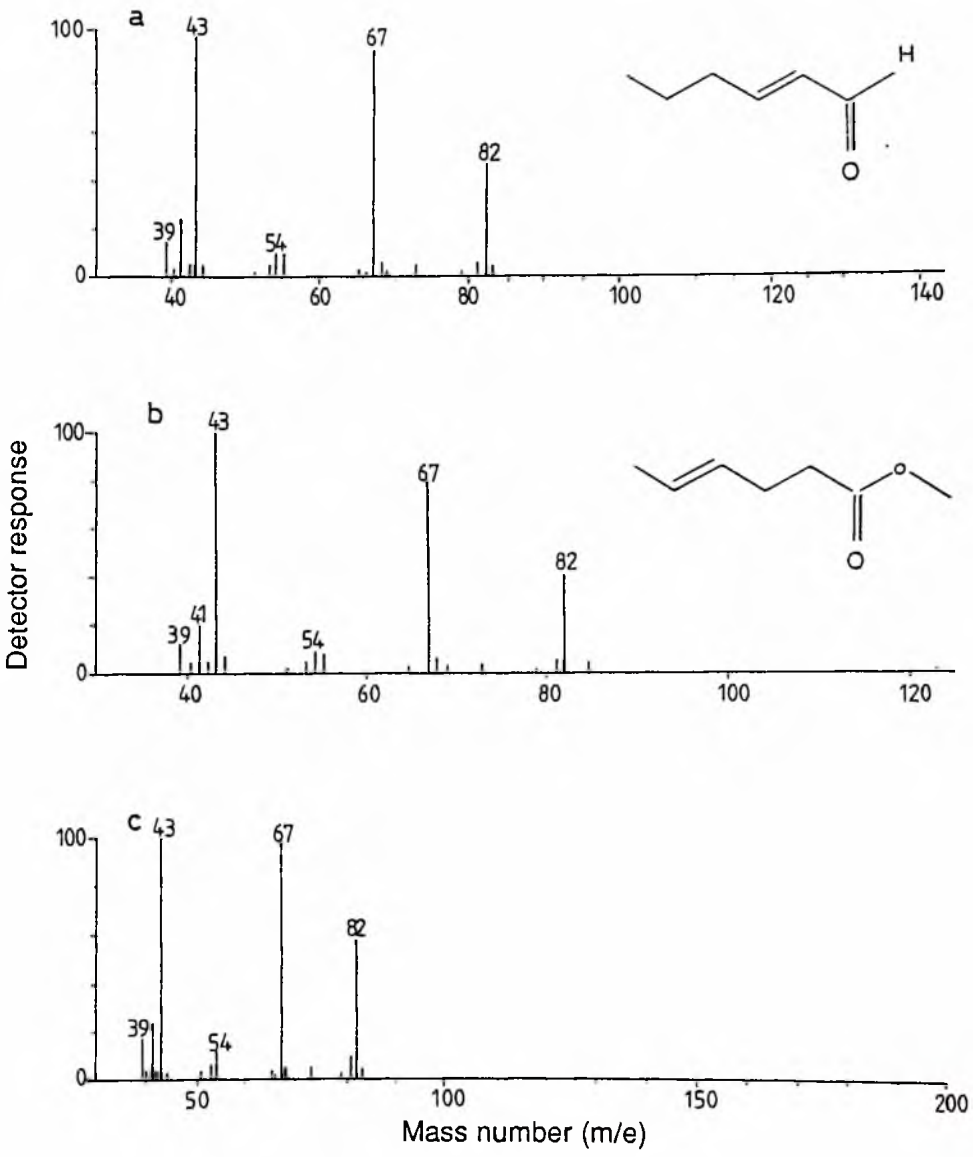

Figure 5.10d Mas spectra of authentic samples of (a) (E)-2-hexenal; (b) (Z)-3-hexenyl acetate; and (c) compound from volatiles collected from maize infested with *C. partellus* (PHC - B)

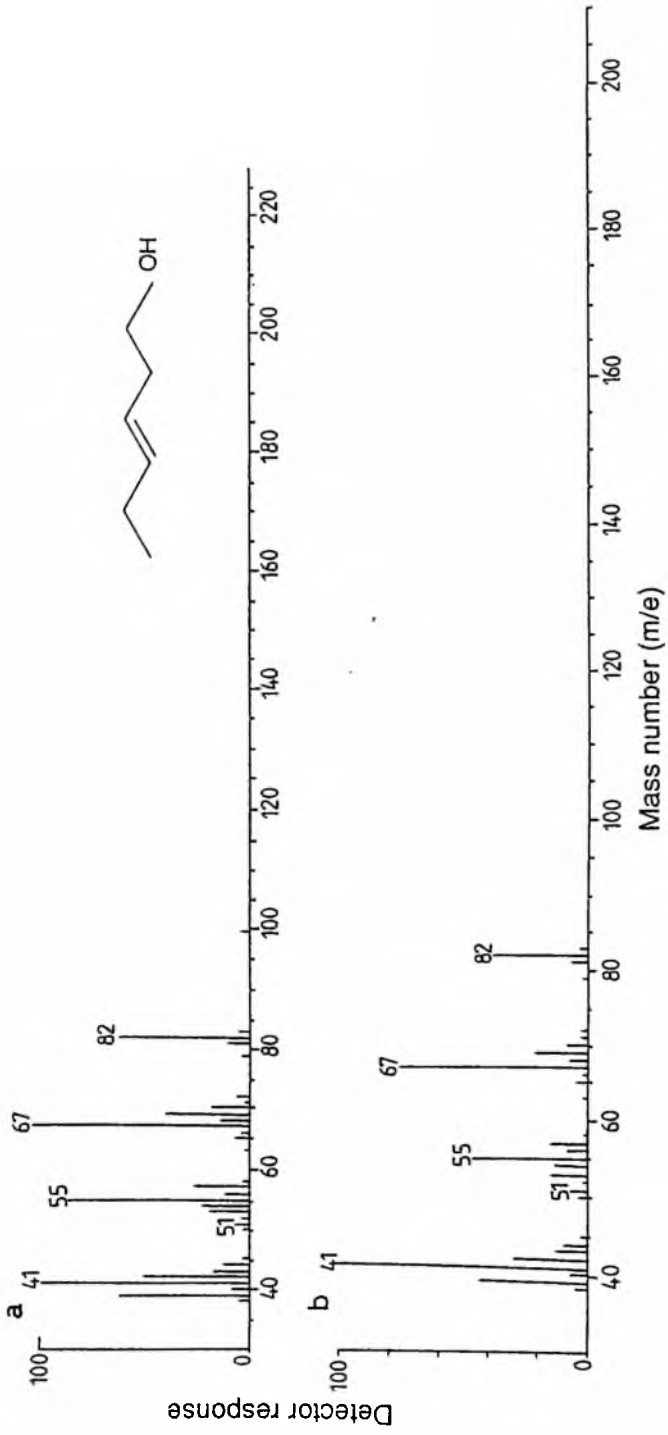

Figure 5.10e Mass spectra of (Z)-3-hexen-1-ol: (a) authentic sample; (b) compound from volatiles collected from maize infested with *C. partellus* (PHC - B).

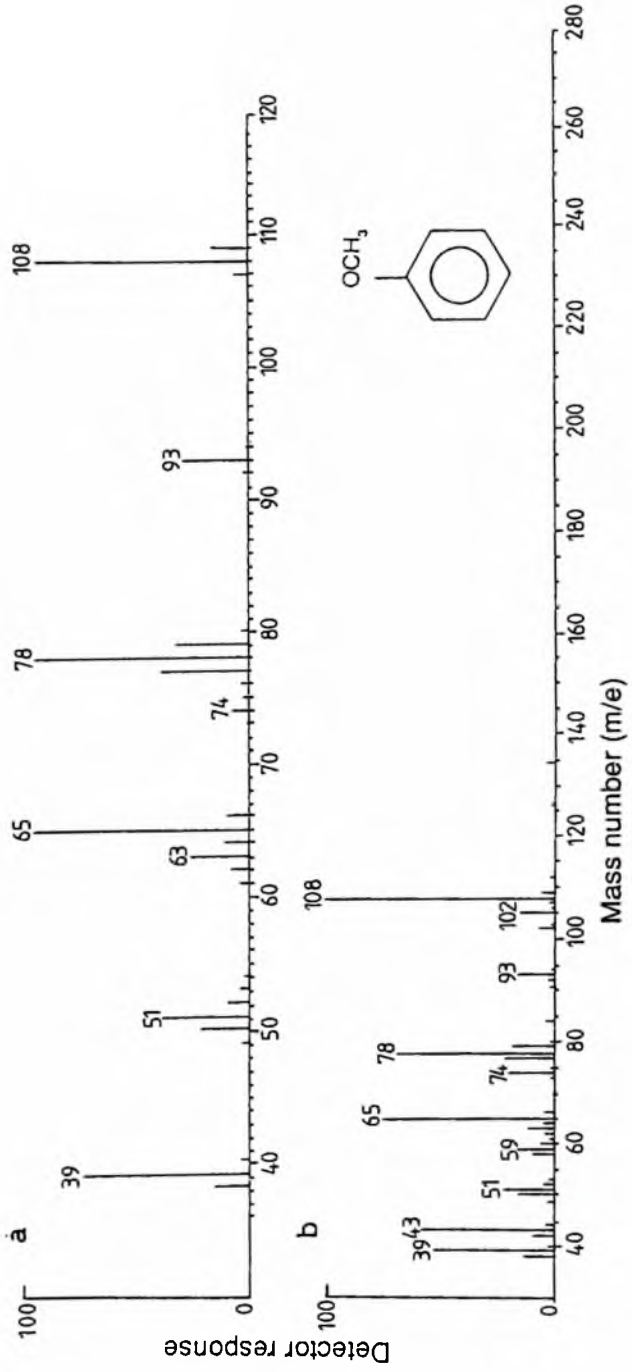

Figure 5.10f Mass spectra of anisole: (a) authentic sample; (b) compound from volatiles collected from maize infested with *C. partellus* (PHC - B).

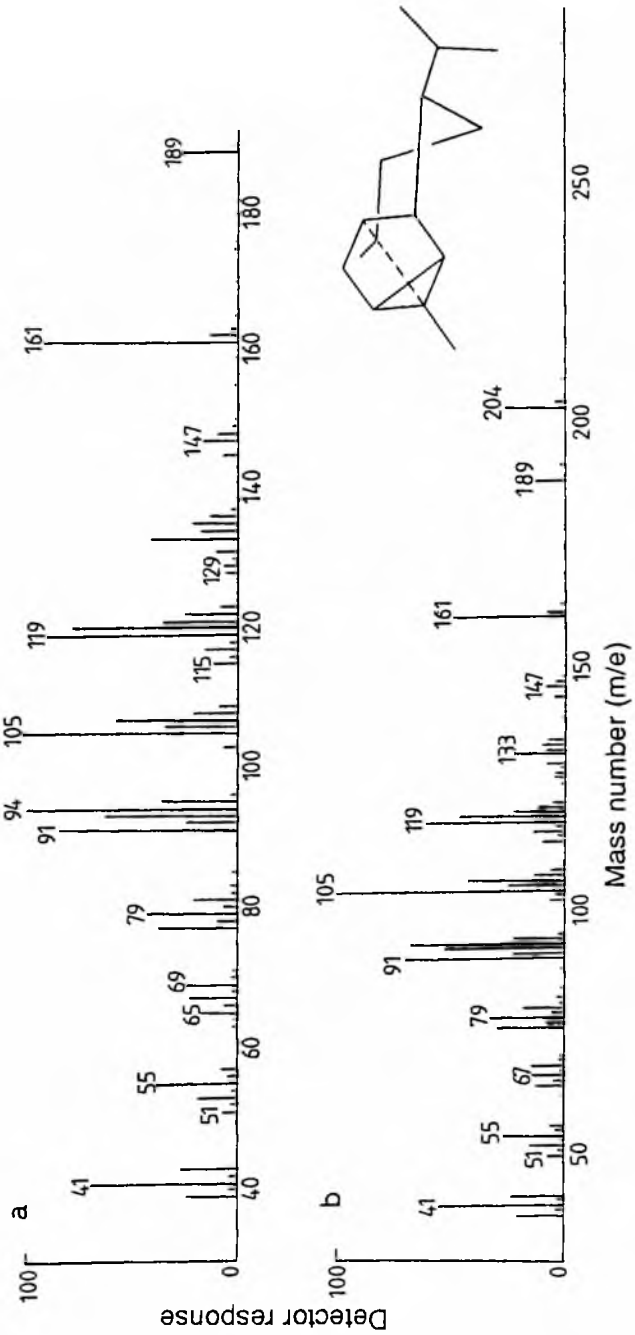

Figure 5.10g Mass spectra of cyclosativen: (a) authentic sample; (b) compound fromvolatiles collected from maize infested with *C. partellus* (PHC-B)

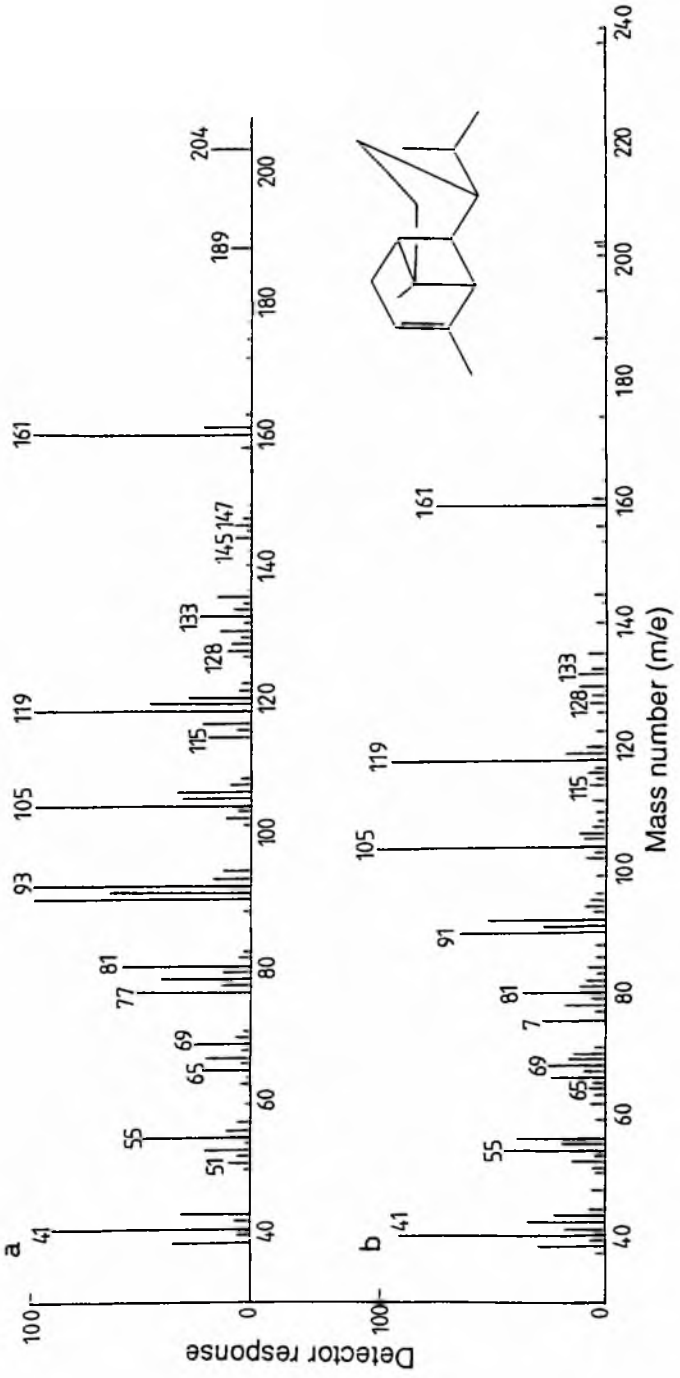

Figure 5.10h Mass spectra of α-copaene: (a) authentic sample; (b) compound from volatiles collected from maize infested with *C. partellus* (PHC-B)

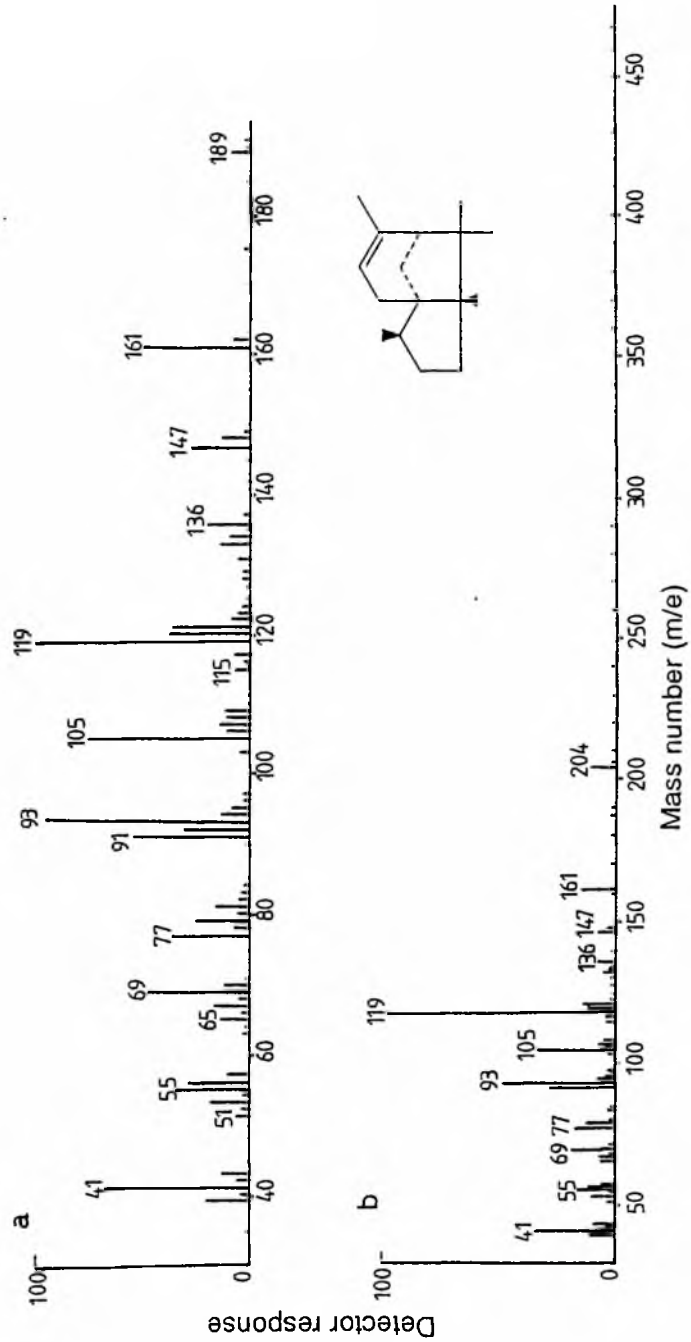

Figure 5.10i Mass spectra of cedrene: (a) authentic sample; (b) compound from volatile collected from maize infested with *C.partellus* (PHC - B).

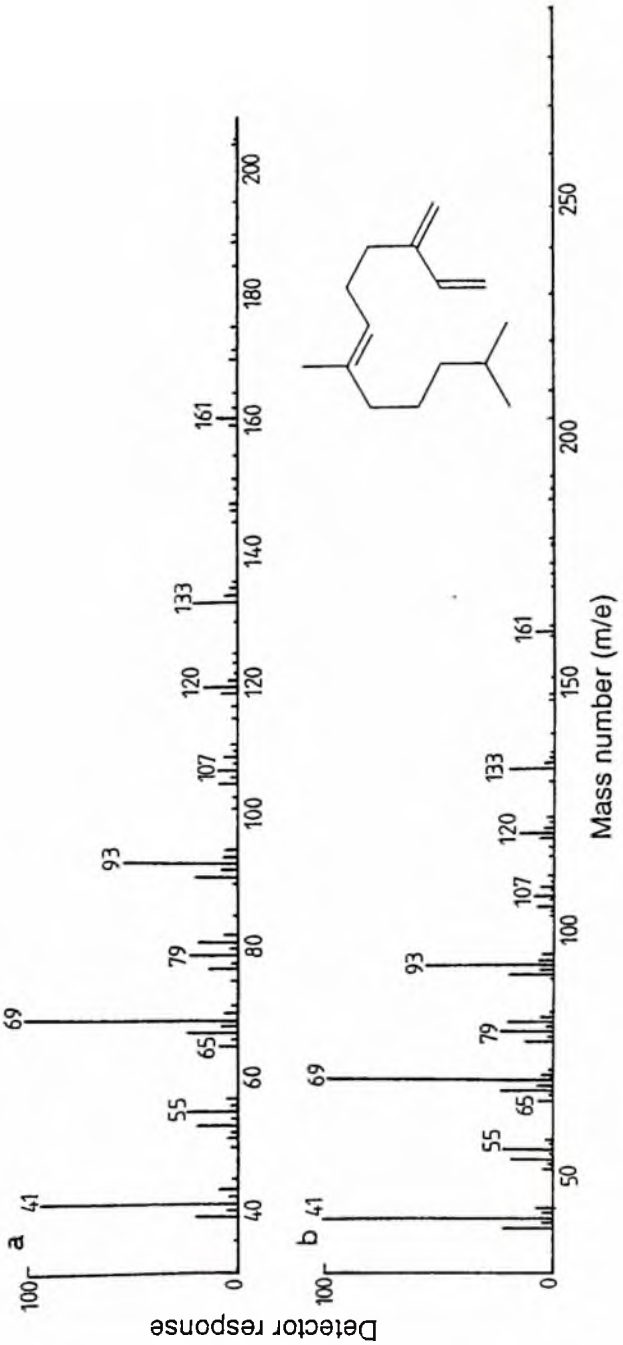

Figure 5.10j Mass spectra of(E)-β-farnesene: (a) authentic sample; (b) compound from volatiles collected from maize infested with *C.partellus* (PHC - B).

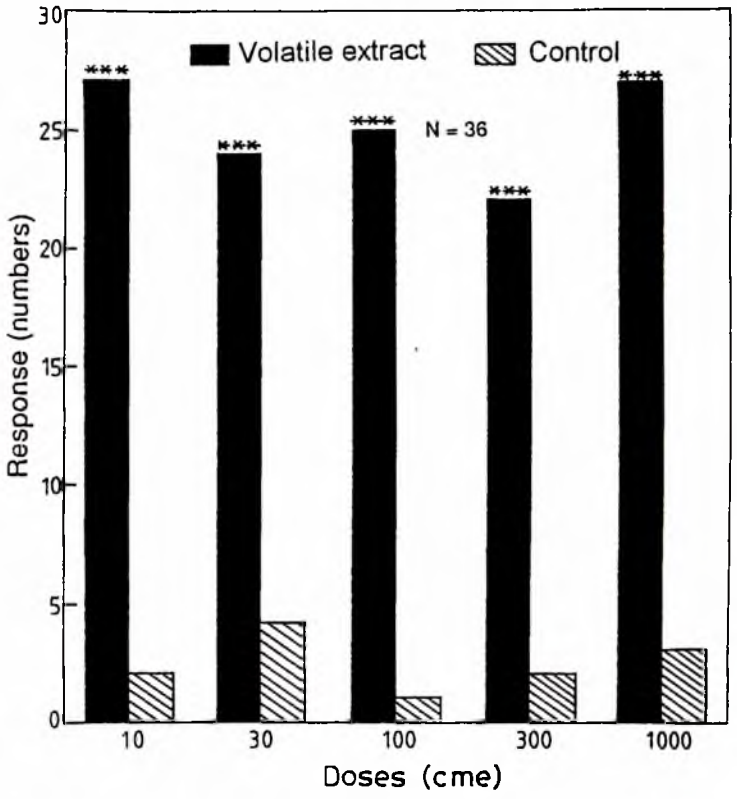

Figure 5.11 Response of *Cotesia flavipes* in the Y-tube olfactometer to volatiles collected from 2-3 month old maize infested with *Chilo partellus* larvae.
*** = $P < 0.005$.

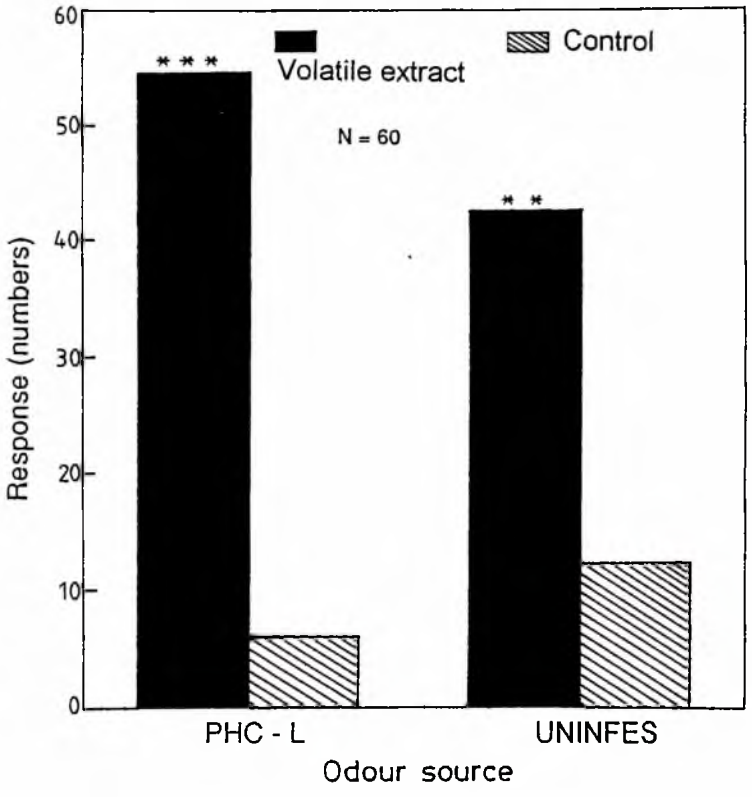

Figure 5.12 Response of *Cotesia flavipes* in the T-tube olfactometer to volatiles collected from maize seedlings infested with larvae of *Chilo partellus* (PHC-L) and uninfested maize seedlings (UNINFES). *** = $P < 0.005$; ** = $P < 0.01$.

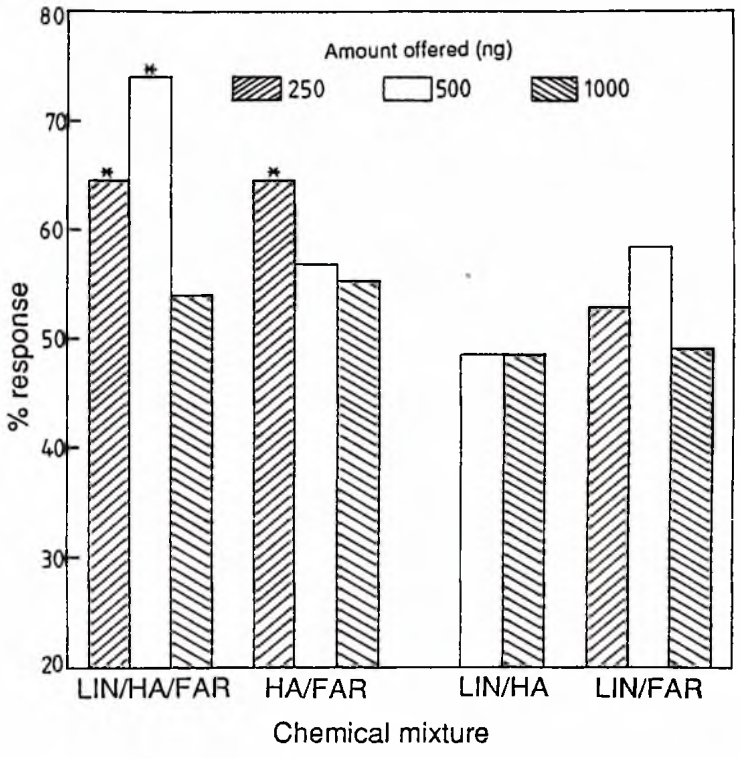

Figure 5.13 Response of *Cotesia flavipes*, in the T-tube olfactometer, to mixtures of chemicals. FAR = (E)-β-farnesene; HA = (Z)-3-hexenyl acetate; LIN = linalool. * = $P < 0.05$.

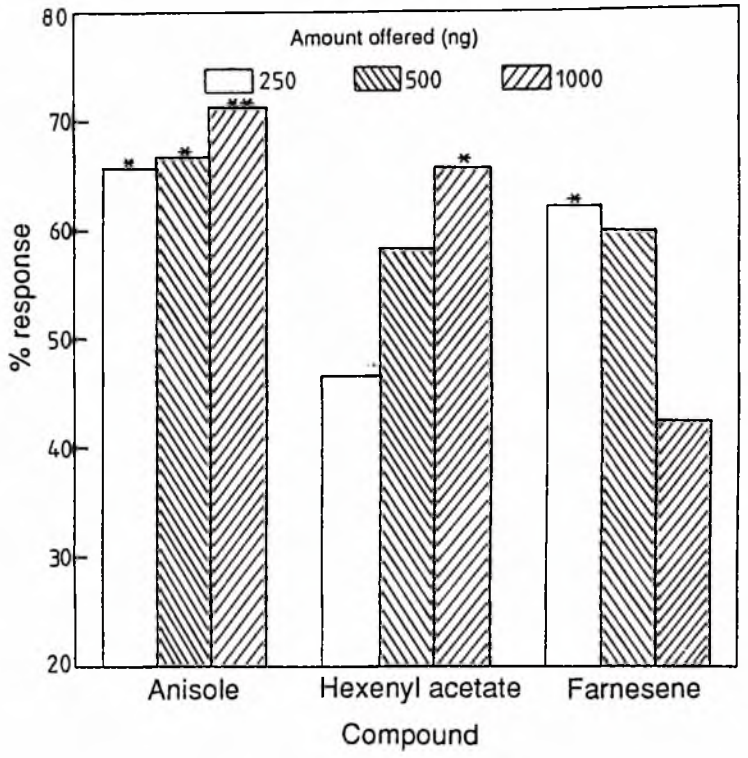

Figure 5.14 Response of *Cotesia flavipes* to three synthetic compounds viz., (Z)-3-hexenyl acetate, anisole, and (E)-farnesene, in the T-tube olfactometer. ** = $P < 0.01$; * = $P < 0.05$.

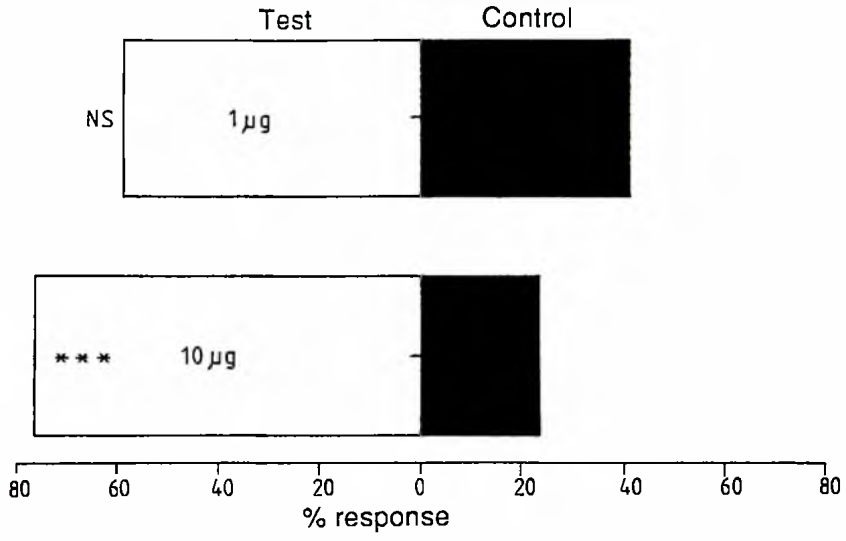

Figure 5.15 Response of *Cotesia flavipes*, in the T-tube olfactometer, to mixtures of 10 chemicals (myrcene, 2-heptanone, 4,8-dimethyl-1,3,7-nonatriene, (Z)-3-hexenyl acetate, anisole, (Z)-3-hexen-1-ol, cyclosativen, α-copaene, cedrene, and (E)-farnesene) at two doses 1.0 and 10 µg. *** = $P < 0.005$; ns = not significant.

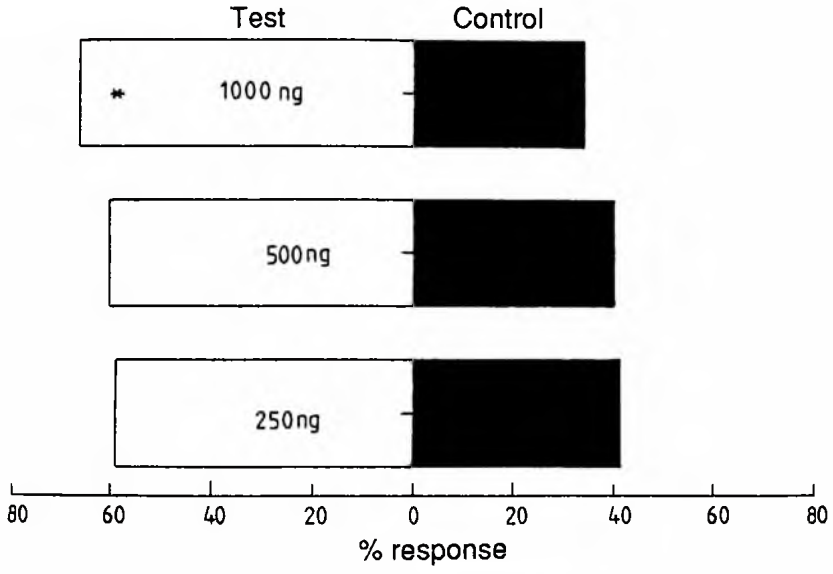

Figure 5.16 Response of *Cotesia flavipes*, in the T-tube olfactometer, to synthetic 4,8-dimethyl-1,3,7-nonatriene at 250, 500, and 1000 ng doses. * = $P < 0.05$.

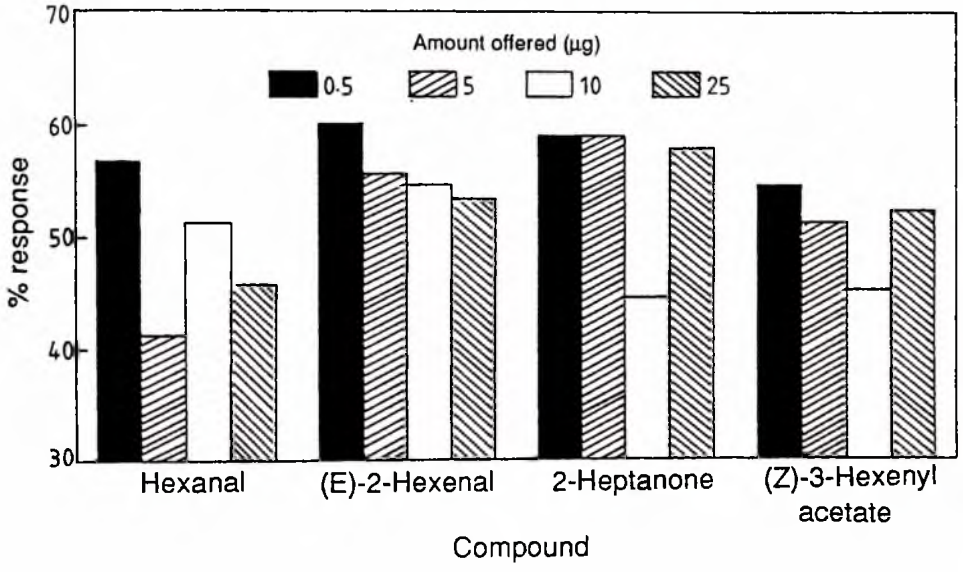

Figure 5.17 Response of *Cotesia flavipes*, in the T-tube olfactometer, to selected synthetic chemicals at doses of 0.5, 5, 10, and 25 µg.

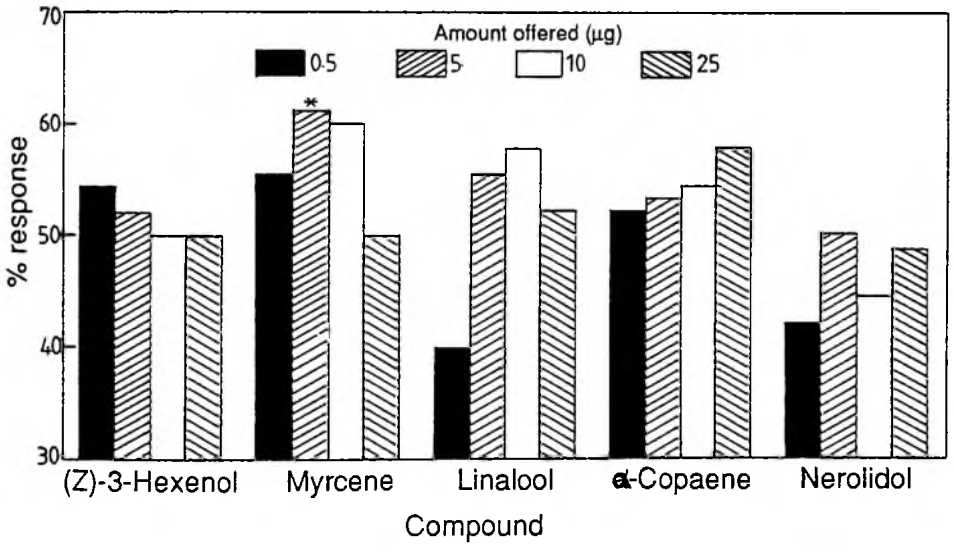

Figure 5.18 Response of *Cotesia flavipes*, in the T-tube olfactometer, to selected synthetic chemicals at doses of 0.5, 5, 10, and 25 µg. * = $P < 0.05$.

CHAPTER SIX

6 Acceptability and suitability of selected African stemborers for the development of *Cotesia flavipes* and *Cotesia sesamiae*.

6.1 Introduction

Several species of lepidopteran stemborers of gramineous plants occur in Africa, and their geographic distributions often overlap. With the exception of *Chilo partellus*, they are thought to be indigenous (Nye, 1960). At the Kenya coast, three stemborers, *Chilo partellus*, *Chilo orichalcociliellus* and *S. calamistis*, are typically found attacking maize and sorghum simultaneously in space and time (Overholt *et al.*, 1994a). A third species, *B. fusca*, is the predominant stemborer in many other maize growing areas in Kenya. Among the three species found at the coast, *Chilo partellus* is the most abundant, and there is evidence that it may be displacing the indigenous stemborers (Overholt *et al.*, 1994a). The most common larval parasitoid of *Chilo partellus* at the Kenya coast is *Cotesia sesamiae*, but mortality due to *Cotesia sesamiae* is generally quite low (Overholt *et al.*, 1994b).

Cotesia flavipes, was imported from Pakistan and released at the Kenya coast in 1993 to suppress populations of *Chilo partellus* and the indigenous stemborers. Several unsuccessful attempts have been made to establish *Cotesia flavipes* in a number of continental African countries, including South Africa (Skoroszewski and Van Hamburg, 1987), Ivory Coast (Breniere and Bordat, 1982),

Ghana (Scheibelreiter, 1980), and the East African countries of Uganda, Kenya, and Tanzania (CIBC, 1968-72). With the exception of South Africa, where failure to establish was thought to be due to climate, there is little information available on the reasons why *Cotesia flavipes* did not establish.

The biology of *Cotesia flavipes* has been investigated on several host species (Gifford and Mann, 1967; Kajita and Drake, 1969; Van Leerdam *et al.*, 1985; Inayatullah, 1987; Shami, 1990; Wiedenmann *et al.* 1992). Mohyuddin (1971) found that the native African stemborer, *B. fusca*, was not a suitable host for the development of *Cotesia flavipes*. Furthermore, Mohyuddin (1971) reported that *Cotesia flavipes* only parasitized fifth to seventh-instar larvae of *Chilo partellus*. In contrast, Skoroszewski and Van Hamburg (1987) recovered *Cotesia flavipes* from *B. fusca* in South Africa. In host suitability studies Skoroszewski and Van Hamburg (1987) found that *Cotesia flavipes* parasitized third and later instars of *Chilo partellus* larvae.

Considering the lack of understanding of the reasons underlying the previous failures to establish *Cotesia flavipes* in Africa, it became necessary to investigate the biology and the behavior of *Cotesia flavipes* in the context of the target ecosystem at the Kenya coast. The specific objective of the present study was to examine the suitability of the African stemborers (indigenous and exotic) for oviposition and development of *Cotesia flavipes,* and to compare the performance of *Cotesia flavipes* with the indigenous *Cotesia sesamiae*. For the purpose of mass production, the suitability of third, fourth and fifth instars of *Chilo partellus* for

the development of *Cotesia flavipes* was also examined to identify the optimal stage to be used for multiplication.

6.2 Materials and Methods

Insects. *Cotesia flavipes* and *Cotesia sesamiae* were used in these experiments. They were reared as described in section 3.1. Fourth-instar larvae of the four stemborers, *Chilo partellus*, *B. fusca*, *S. calamistis* and *Chilo orichalcociliellus* were used in the experiments. However, when measuring the suitability of different larval stages of *C. partellus*, third, fourth, fifthand sixth instars were used. Allstemborers were also reared as indicated in section 3.1.

Host Acceptability. The acceptability of the four stemborers for parasitization by *Cotesia flavipes* and *Cotesia sesamiae* was tested by offering one host larva to one parasitoid in a clean glass vial. The procedure is described in section 3.3.3. A minimum of 80 larvae per stemborer species were exposed in four to eight replicates. Individual parasitoids and stemborer larvae were used only once. The percentage of stemborer larvae attacked was calculated. The time spent by each female until oviposition was recorded for females which oviposited within the five minutes exposure period.

Host Suitability. Stemborer larvae that were stung in the above experiment and several others were placed individually on their respective artificial media after exposure and maintained in an incubator at 28°C, 65-80% RH, and 12:12 (L:D) photoperiod. Host larvae were inspected daily for mortality or parasitoid

emergence. The developmental time, the proportion of host larvae dying or pupating before egression of parasitoids, the proportion of hosts that produced cocoons, the proportion of hosts that did not produce cocoons but were still alive 20 days after parasitization, the total progeny produced per parasitized host, and the proportion of female progeny (female progeny/total progeny) were recorded. The mortality of larval parasitoids was determined by dissecting host larvae 20 days after parasitization if no parasitoids had emerged. Host larvae from which parasitoids emerged were dissected three days after parasitoid egression. Additionally, the number of parasitoid larvae that exited the host but failed to pupate were counted.

Age Suitability. Third to sixth instars of *Chilo partellus* were exposed to female *Cotesia flavipes* as described above. Instars were determined by measuring the head capsule (Ampofo, 1988). The same parameters specified in the host suitability study were recorded.

Host immune Response. Total haemocyte counts of parasitized and unparasitized *Chilo partellus* were assayed. Eighteen days old larvae were used in the tests. As indicated in section 3.5, haemocytes were counted 1, 2, 4, 8, 14, and 24 hours after parasitization in the first test and 1, 2, 3, 4, 5, and 6 days after parasitization in the second test (see section 3.5 for the procedures).

Data Analyses. Analysis of variance (ANOVA) PROC GLM, SAS Institute (1988) was performed to compare the time spent by a female until oviposition on the different stemborer species. Means were separated by Student-Newman-

Keul's (SNK) multiple range test when the ANOVA was significant ($P<0.05$). Parasitoid developmental time, parasitoid mortality, host mortality, host pupation, the proportion of exposed hosts producing cocoons, progeny produced per oviposition, and the proportion of female progeny were also subjected to ANOVA followed by the SNK means separation procedure when the ANOVAwas significant ($P<0.05$). Proportions were transformed to arcsin before being subjected to ANOVA.

6.3 Results

Host Acceptability. All stemborer species were equally accepted for oviposition by *Cotesia flavipes*. There were no differences in the percentage of hosts attacked or the time spent by a female parasitoid until oviposition (Table 6.1). In the case of *Cotesia sesamiae*, the proportion of hosts attacked varied significanly among stemborer species, although there was no significant difference between the two *Chilo* species. No difference was detected in the time spent by a female *Cotesia sesamiae* before ovipositing in different hosts (Table 6.2).

When comparing the two parasitoid species, no difference was found in the time spent before oviposition on *Chilo partellus*, *S. calamistis* or *B. fusca* ($F=0.46$; $df=1,264$; $P=0.50$). However, oviposition by *Cotesia flavipes* on *Chilo orichalcociliellus* occurred more rapidly than oviposition by *Cotesia sesamiae* on the same host ($F=4.07$; $df=1,133$; $P=0.05$). The proportions of *Chilo partellus*, *S. calamistis* and *B. fusca* larvae attacked by *Cotesia sesamiae* were less than the

proportions attacked by *Cotesia flavipes*. There was, however no difference in the proportion of *S. calamistis* larvae attacked by the two parasitoids.

Host Suitability. *Cotesia flavipes* did not develop on *B. fusca*. Dissections revealed that the majority of *B. fusca* larvae stung by *Cotesia flavipes* encapsulated the parasitoid eggs (Plate 1). There was no difference in the number of parasitoid progeny per female among *Chilo partellus*, *Chilo orichalcociliellus* and *S. calamistis* (Table 6.3). The developmental periods (egg-adult) of *Cotesia flavipes* on *Chilo partellus* and *S. calamistis* were not significantly different. However, the developmental period on *Chilo orichalcociliellus* was longer than on the other two suitable host species (Table 6.3). Excluding *B. fusca*, the highest number of immature stages of *Cotesia flavipes* dying (larval-pupal mortality) was observed on *S. calamistis*, followed by *Chilo orichalcociliellus* and *Chilo partellus*. The proportion of female progeny per host was not significantly different between hosts (Table 6.3).

The proportion of individuals successfully parasitized was significantly high for *Chilo partellus* and significantly low for *S. calamistis*; however, the difference between *Chilo partellus* and *Chilo orichalcociliellus* or *Chilo orichalcociliellus* and *S. calamistis* was no significant (Table 6.4). The proportion of stemborer larvae that were alive (as larvae or pupae) 20 days after parasitism by *Cotesia flavipes* was highest for *B. fusca*, but not significantly different among the three other host species. There was no difference in the proportion of hosts dying before egression of the parasitoids among the four stemborer species (Table 6.4).

Cotesia sesamiae successfully parasitized all the stemborer species except *B. fusca* (Table 6.5). As with *Cotesia flavipes*, dissections of *B. fusca* revealed that parasitoid eggs were encapsulated. The mean number of progeny obtained per host was greatest for *S. calamistis*, intermediate for *Chilo partellus* and least for *Chilo orichalcociliellus* (Table 6.5). The developmental time of *Cotesia sesamiae* was shortest on *S. calamistis*, but not significantly different between *Chilo partellus* and *Chilo orichalcocilielius*. The highest number of immature parasitoids dying was observed on *Chilo orichalcociliellus* and *Chilo partellus*, followed by *S. calamistis*. The proportion of female progeny was not significanly different among the three successfully parasitized species (Table 6.5).

The difference in the proportion of hosts successfully parasitized between *Chilo partellus*, *Chilo orichalcociliellus*, and *S. calamistis*, or in the proportion of hosts dying before egression of parasitoids (Table 6.6) was no significant. A higher proportion of *B. fusca* larvae pupated or were still alive twenty days after parasitization, as compared to the other stemborer species.

When the two parasitoid species were compared, no significant difference was observed in the mean number of progeny produced or the mortality of the immature parasitoids. More *Cotesia flavipes* progeny were obtained from *Chilo partellus* and *Chilo orichalcociliellus* than progeny of *Cotesia sesamiae*, and a greater number of *Cotesia sesamiae* larvae and pupae died in these hosts than those of *C. flavipes*. On *S. calamistis*, *Cotesia sesamiae* mortality was lower than *Cotesia flavipes* mortality. The developmental period of *Cotesia sesamiae* was

slightly shorter than *Cotesia flavipes* on the three suitable hosts. More of the *S. calamistis* larvae parasitized by *Cotesia sesamiae* produced cocoons than those parasitized by *Cotesia flavipes*. There was no significant difference in the sex ratio of the two parasitoids among the stemborer species that were successfully parasitized ($F=0.49$; $df=1, 102$; $p=0.48$).

Age Suitability. The mean number of progeny obtained per female *Cotesia flavipes* was lowest in third instars, not different between fourth and fifthinstars and highest in sixth instars. The developmental time was significantly different for the four stages. Parasitoid mortality was highest in third instar larvae, not different in fourth and fifth instar larvae, and lowest in sixth instar larvae. The proportion of female parasitoid progeny obtained from the four larval stages were not different (Table 6.7).

Fewer third instar larvae of *Chilo partellus* parasitized by *Cotesia flavipes* produced cocoons than fourth, fifth or sixth instar larvae (Table 6.8). Mortality of *Chilo partellus* parasitized by *Cotesia flavipes* was lowest in sixth instar larvae, and was not different in fourth and fifth instar larvae, and highest in third instar larvae.

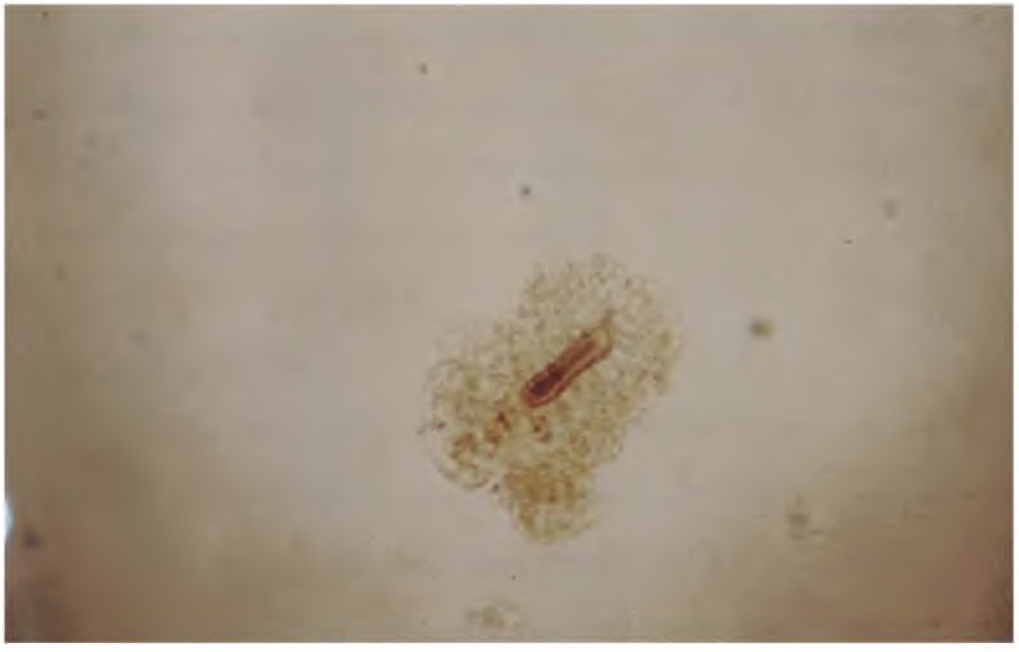

Plate 1 Egg of *Cotesia flavipes* encapsulated in *B. fusca* larva

Host immune response to parasitism. *Chilo partellus* larvae did not encapsulate *Cotesia flavipes* eggs. There was no difference in the total haemocyte counts at 1, 2, 8, 14, and 24 hours after parasitization between parasitized and unparasitized larvae (Figure 6.1, Appendix 6.1). However, at four hours after parasitization, the total haemocyte count was higher in parasitized than unparasitized larvae ($P=0.0364$). From the second to the sixth day, the total haemocyte count was significantly higher in parasitized than unparasitized larvae (Figure 6.2, Appendix 18).

6.4 DISCUSSION

The four stemborer species exposed to *Cotesia flavipes* were accepted for oviposition with over 80% of exposed larvae being stung by the parasitoids within five minutes. Many studies have demonstrated that *Cotesia flavipes* will sting a wide variety of stemborer larvae in an artificial laboratory environment, but only successfully complete development in some hosts (Mohyuddin, 1971; Beg and Inayatullah, 1980). In laboratory studies, Beg and Inayatullah (1980) reported that *Cotesia flavipes* stung *Chilo partellus*, *Chilo infuscatellus*, *Tryporyza incertulas* and *Ostrinia kasmirica*, but completed development only in *Chilo partellus* and *Chilo infuscatellus*.

In the present study, *Cotesia flavipes* developed on the three stemborer species that occur on the Kenya coast: *Chilo partellus*, *Chilo orichalcociliellus* and *S. calamistis*. However, the suitability of these stemborers varied. The coevolved

host, *Chilo partellus*, was the most suitable, followed by *Chilo orichalcociliellus*. The noctuid, *S. calamistis*, was less suitable than the *Chilo* species due to the high mortality of the immature parasitoids within the *S. calamistis* larvae. It is of interest to note that 42% of *S. calamistis* larvae exposed to *Cotesia flavipes* produced cocoons in the present study, as compared to 3% reported by Mohyuddin (1971). In Pakistan and India, *Cotesia flavipes* attacks *Sesamia inferens*, which could explain the partial suitability of *S. calamistis* for the development of *Cotesia flavipes*.

The developmental time of *Cotesia flavipes* was longer in *Chilo orichalcociliellus* than in *Chilo partellus*. In related studies, it was shown that the larval development of *Chilo orichalcociliellus* was longer than that of *Chilo partellus* (Overholt, unpublished data). Furthermore, *Chilo orichalcociliellus* is a smaller host than *Chilo partellus* with average pupal weights from our colonies of 86 mg and 126 mg, respectively (Overholt, unpublished). These two interrelated factors may explain the slower development of the parasitoids in *Chilo orichalcociliellus*.

Mohyuddin (1971) reported that *Cotesia flavipes* did not develop on *B. fusca*. The present results confirm this finding, as all *Cotesia flavipes* eggs were encapsulated in *B. fusca*. The genus *Busseola* Thuran is restricted to Africa, and thus, *Cotesia flavipes* has been under no selection pressure to evade the immune system of *B. fusca*. The reported recoveries of *Cotesia flavipes* from *B. fusca* in South Africa (Skoroszewski and Van Hamburg, 1987) may probably have been

based on misidentifications (*Cotesia flavipes* and *Cotesia sesamiae* are morphologically very similar). Alternatively, *Cotesia flavipes* may have developed in *B. fusca* which were already invaded by other natural enemies resulting in a weakened immune system. During this study, it was observed that *Cotesia flavipes* can develop past the egg stage in *B. fusca* larvae infected with entomogenous nematodes (unpublished observations). A third possibility is that there may be a variation in the immune system between the populations of *B. fusca* from South Africa and Kenya, or that the strain of *Cotesia flavipes* released in South Africa might have been able to evade the immune system of *B. fusca*.

An unexpected result in this study was that *Cotesia sesamiae* could not successfully complete development on *B. fusca*. *Cotesia sesamiae* has been reared from *B. fusca* in western Kenya (Mohyuddin and Greathead, 1970; Overholt, unpublished data), and is reported to be one of the most common larval parasitoids in several countries, including South Africa (Ullyett, 1935; Skoroszewski and Van Hamburg, 1987; Van Rensburg *et al.*, 1988), Uganda (Ingram, 1958), and Ghana (Scheilbelreiter, 1980). Mohyuddin (1971) reported that progeny production of *Cotesia sesamiae* was higher in *B. fusca* than in *Chilo partellus*. These contrasting results suggest that different populations of *Cotesia sesamiae* may express differential abilities to evade the immune system of *B. fusca*. *Cotesia sesamiae* from the Kenya coast, where *B. fusca* does not occur (Overholt *et al.*, 1994a), cannot successfully develop in *B. fusca*, while in the areas where *Cotesia sesamiae* and *B. fusca* occur sympatrically, *Cotesia sesamiae* is able to

successfully parasitize *B. fusca*.

Cotesia sesamiae exhibited greater discrimination in host acceptance than *Cotesia flavipes*. *Sesamia calamistis* was the most acceptable host followed by *Chilo orichalcociliellus* and *Chilo partellus*. In Mauritius, *Cotesia sesamiae* that was introduced from Kenya has successfully established on *S. calamistis* in maize (Greathead, 1971), but does not successfully parasitize *Chilo sacchariphagus* on sugarcane (Rajabalee and Govendasamy, 1988). Mohyuddin (1971) reported that *Cotesia sesamiae* preferred *Chilo partellus* to *S. calamistis*. Shami and Mohyuddin (1986), studying host preference and suitability of *Cotesia sesamiae* on stemborers occurring in Pakistan, found that the most preferred host was *Chilo partellus*, followed by *S. inferens*, *Acigona steniellus*, *Emmalocera depressella*, *Chilo infuscatellus*, *Calamotropha lupatus* and *Scirpophaga nivella*, in descending order. They also found that *Cotesia sesamiae* completed development in *Chilo partellus*, *Chilo infuscatellus*, *A. steniellus*, *S. nivella* and *S. inferens*. The parasitoid did not complete development in *E. depressella* or *Calomotropha lupatus*. Among the hosts examined, *Chilo partellus* was the most suitable as measured by the number of cocoons per larva, adult progeny production, and the sex ratio of the parasitoids.

Third to sixth instar larvae of *Chilo partellus* were acceptable and suitable for parasitization by *Cotesia flavipes*. However, in the third instars, there was higher mortality of host larvae, as well as immature parasitoid stages. Kajita and Drake (1969) showed that the developmental time of *Cotesia chilonis* (Munakata)

in *Chilo suppressalis* Walker decreased progressively as the host instar increased. It was found that the developmental time in *Cotesia flavipes* was longest in third instars and shortest in sixth instars. In contrast, Wiedenmann and Smith (1994) found that the developmental time of *Cotesia flavipes* was not different in third to sixth instars of *D. saccharalis*.

This study revealed that physiological changes took place in stemborer larvae after they were parasitized. *Busseola fusca* responded to *Cotesia flavipes* attacks by encapsulating the parasitoid's eggs, while *Chilo partellus*, which is a suitable host, did not encapsulate the parasitoid's eggs. However, there is evidence of an immune reaction in *Chilo partellus* as, from the second to the sixth day after parasitization, there was a net increase in the total haemocyte count in the parasitized larva (Figure 6.2). A few studies have investigated the different mechanisms by which parasitoids evade the host immune system (Ross and Dunn, 1989; Strand and Noda, 1991; Strand and Wong, 1991; Vinson, 1993), but none has yet examined the immunological interaction between *Cotesia flavipes* and *Chilo partellus*.

The results of this study suggest that the coastal population of *Cotesia sesamiae* willprimarily attack and develop on *S. calamistis*, and to a lesser extent on *Chilo* spp., whereas *Cotesia flavipes* willsuccessfully develop on *Chilo partellus* and *Chilo orichalcociliellus*, and to a lesser extent on *S. calamistis*. Thus, both parasitoids may be complementary in regulating the stemborer species complex in the region.

Table 6.1. Acceptability of four stemborer species for parasitization by C. flavipes

Host species	N	Percentage of host larvae attacked	N	Time to oviposition ± SE (sec)
C. partellus	212	94.8a	199	52.6 ± 3.7a
B. fusca	80	83.8a	67	45.2 ± 6.5a
S. calamistis	78	94.9a	74	53.1 ± 6.0a
C. orichalcociliellus	76	97.4a	74	39.1 ± 3.0a

Numbers followed by the same letter in a column are not significantly different (SNK). Percentage of larvae attacked: $F=1.78$; $P=0.18$; df=3,25. Time to oviposition: $F=1.68$; $P=0.17$; df=3,410.

Table 6.2. Acceptability of four stemborer species for parasitization by *Cotesia sesamiae*

Host species	N	Percentage of host larvae attacked	N	Time to oviposition ± SE (sec)
C. partellus	100	67.0b	67	57.8 ± 7.3a
B. fusca	80	48.8c	39	67.8 ± 10.6a
S. calamistis	78	96.2a	75	56.4 ± 6.2a
C. orichalcociliellus	80	77.5b	61	50.6 ± 5.1a

Numbers followed by the same letter in the same column are not significantly different (SNK). Percentage of larvae attacked: $F = 19.49$; $P = 0.0001$; df = 3,13. Time to oviposition: $F = 0.80$; $P = 0.50$; df = 3,238.

Table 6.3. Host suitability of various stemborers for the development of *Cotesia flavipes*

Host species	N	No. of parasitoid progeny per female (mean ± SE)	N	Developmental time (days ± SE)	N	No. of parasitoid larvae and pupae dying (± SE)	N	Proportion of female progeny (95% CL) (%)
C. partellus	183	36.5 ± 1.2a	180	17.9 ± 0.9b	184	7.6 ± 0.9b	180	60.6a (54.9-66.1)
B. fusca	100	0.0 ± 0.0 b		-		-		-
S. calamistis	36	34.0 ± 3.9a	36	17.8 ± 0.5b	39	14.3 ± 3.9a	36	65.9 a (53.3-77.5)
C. orichalcociliellus	61	32.8 ± 2.1a	64	20.5 ± 0.5a	61	10.8 ± 1.9ab	61	63.9 a (54.1-73.1)

Numbers followed by the same letter in the same column are not significantly different (SNK). Number of parasitoid progeny per female: $F = 1.21$; $P = 0.3$; df = 2,277. Developmental time: $F = 19.40$; $P = 0.0001$; df = 2,277. Number of parasitoid larvae and pupae dying: $F = 9.47$; $P = 0.0001$; df = 3,332. Proportion of female progeny: $F = 0.38$; $P = 0.68$; df = 2,274.

Table 6.4. Host fate after parasitization by *Cotesia flavipes*

Host species	N	[1]Proportion of hosts successfully parasitized (%)	[2]Proportion of host larvae alive (%)	[3]Proportion of host larvae dying (%)	Proportion of host pupating (%)
C. partellus	252	74.6a	5.2b	16.3a	4.0b
B. fusca	93	0.0c	48.4a	23.7a	28.0a
S. calamistis	85	42.4b	9.4b	42.4a	5.9b
C. orichalcociliellus	106	63.2ab	1.2b	31.1a	3.8b

Numbers followed by the same letter in the same column are not significantly different (SNK). Proportion successfully parasitized host: $F = 19.57$; $P = 0.0001$; df = 3,20. Proportion of host larvae alive: $F = 8.81$; $P = 0.0006$; df = 3,20. Proportion of hosts dying: $F = 1.23$; $P = 0.32$; df = 3,20. Proportion of host pupating: $F = 7.46$; $P = 0.002$; df = 3,20. [1]Proportion of host larvae that produced cocoons. [2]Proportion of host that did not produce cocoons and were still alive 20 days after parasitization. [3]Proportion of host larvae that died after parasitization and before egression of parasitoids.

Table 6.5. Host suitability of various stemborer species for the development of *Cotesia sesamiae*

Host species	N	No. of parasitoid progeny per female (mean ± SE)	N	Developmental time (days ± SE)	N	No. of parasitoid larvae and pupae dying (± SE)	N	Proportion of female progeny (95% C.L.) (%)
C.partellus	93	20.5 ± 1.6b	94	18.0 ± 0.2a	99	14.2 ± 1.2a	97	68.0a (60.2-75.3)
B. fusca	60	0.0 ± 0.0c		-	66	0.0 ± 0.0c		-
S.calamistis	68	35.2 ± 1.3a	68	16.1 ± 0.1b	77	10.4 ± 0.9b	77	64.5a (54.5-73.9)
C. orichalcociliellus	77	22.9 ± 1.3b	63	17.9 ± 0.3a	89	14.4 ± 1.0a	87	60.6a (51.1-69.6)

Numbers followed by the same letter in the same column are not significantly different (SNK). Number of parasitoid progeny per female: $F = 25.70$; $P = 0.0001$; df = 2,217. Developmental time: $F = 28.29$; $P = 0.0001$; df = 2,222. Number of parasitoid larvae and pupae dying: $F = 20.51$; $P = 0.0001$; df = 3,285. Proportion of female progeny: $F = 0.75$; $P = 0.48$; df = 2,217.

Table 6.6. Host fate after parasitization by *Cotesia sesamiae*

Host Species	N	Proportion of hosts successfully parasitized (%)	Proportion of host larvae alive (%)	Proportion of host larvae dying (%)	Proportion of host pupating (%)
C. partellus	46	73.3a	3.7ab	12.6a	10.4b
B. fusca	90	0.0b	15.2a	28.3a	56.5a
S. calamistis	90	76.7a	0.0b	10.0a	13.3b
C. orichalcociciliellus	118	58.3a	3.4ab	26.3a	11.0b

Numbers followed by the same letter in the same column are not significantly different (SNK). Proportion successfully parasitized host: $F=47.96$; $P=0.0001$; df=3,21. Proportion of host larvae alive: $F=2.34$; $P=0.10$; df=3,21. Proportion of hosts dying: $F=2.07$; $P=0.14$; df=3,21. Proportion of host pupating: $F=11.25$; $P=0.0001$; df=3,21.

Table 6.7. Age suitability for the development of *Cotesia flavipes* on *Chilo partellus*

Larval Instar	N	No. of parasitoid progeny per female (mean ± SE)	N	Developmental time (days ± SE)	N	No. of parasitoid larvae and pupae dying (± SE)	N	Proportion of female progeny (95% C.L.)
3rd instar	44	25.3 ± 2.0c	47	24.0 ± 0.5a	62	15.6 ± 1.6a	44	66.0a (57.4-74.1)
4th instar	119	37.8 ± 1.2b	117	20.5 ± 0.3b	111	6.9 ± 0.9b	119	72.6a (67.7-77.3)
5th instar	101	37.6 ± 1.5b	104	16.8 ± 0.1c	91	7.7 ± 1.0b	101	75.2a (69.9-80.0)
6th instar	75	48.8 ± 1.6a	76	16.2 ± 0.1d	75	4.1 ± 0.8c	75	67.3a (60.8-73.5)

Numbers followed by the same letter in the same column are not significantly different (SNK). Number of parasitoid progeny per female: $F = 26.70$; $P = 0.0001$; df = 3,335. Developmental time: $F = 162.07$; $P = 0.0001$; df = 3,340. Number of parasitoid larvae and pupae dying: $F = 16.83$; $P = 0.0001$; df = 3,335. Proportion of female progeny: $F = 1.86$; $P = 0.14$; df = 3,335.

Table 6.8: Host fate after parasitization by *Cotesia flavipes*

Larval instar	N	Proportion of hosts successfully parasitized (%)	Proportion of host larvae alive (%)	Proportion of host larvae dying (%)	Proportion of host larvae pupating (%)
3rd	138	43.5b	8.7a	42.8a	5.1a
4th	156	78.9a	0.6b	17.9ab	2.6a
5th	138	77.5a	0.7b	15.2ab	6.5a
6th	92	82.6a	1.1b	5.4b	10.9a

Numbers followed by the same letter in the same column are not significantly different (SNK). Proportion successfully parasitized host: $F=7.59$; $P=0.005$; df = 3,11. Proportion of host larvae alive: $F=5.51$; $P=0.015$; df = 3,11. Proportion of hosts dying: $F=6.38$; $P=0.009$; df = 3,11. Proportion of host pupating: $F=1.06$; $P=0.41$; df = 3,11.

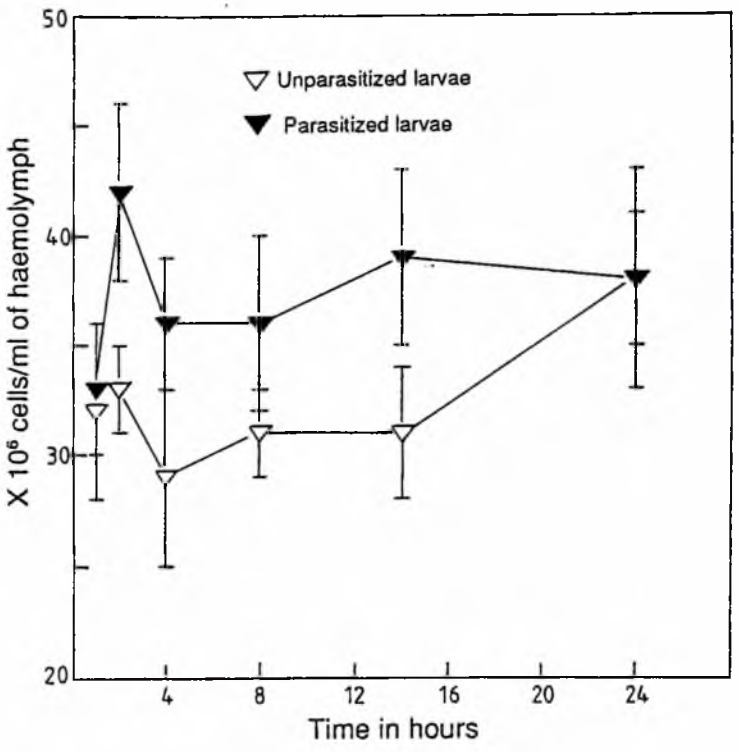

Figure 6.1 Total haemocyte count of parasitized and unparasitized larvae of *Chilo partellus* different times after parasitization within 24 hrs.

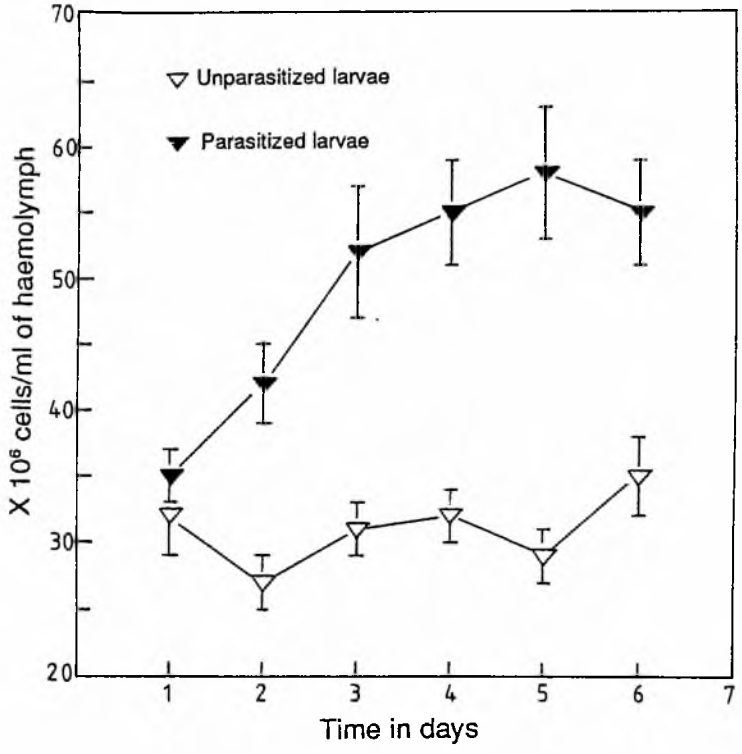

Figure 6.2 Total haemocyte count of parasitized and unparasitized larvae of *Chilo partellus* different days after parasitization.

CHAPTER SEVEN

7 General discussion and conclusions

The introduced lepidopteran stemborer, *Chilo partellus,* is a major constraint to maize and sorghum production in Kenya. The most common indigenous larval parasitoid of *Chilo partellus*, *Cotesia sesamiae*, is unable to maintain the population of this stemborer at a level acceptable to farmers. A programme has been initiated to introduce the exotic parasitoid *Cotesia flavipes* from Pakistan to reduce damage due to this pest. Studies reported here have examined the searching behaviour of both parasitoids with reference to host habitat location, host location, host acceptance and suitability, and the semiochemicals involved.

The success of biological control agents have been attributed partly to the high searching efficiency of the natural enemies for their hosts. Increasing evidence suggests that the host plant is the main provider of airborne chemicals utilized by natural enemies to locate their hosts or prey (Vinson, 1975; Dicke *et al.*, 1990a,b; Turlings *et al.*, 1990b, 1991a,b; Whitman and Eller, 1990; Dicke and Takabayashi, 1991). The tritrophic system in this study included the host-plants, maize, sorghum and napier grass at the first trophic level; the stemborers, *Chilo partellus, Chilo orichalcociliellus, S. calamistis* and *B. fusca* at the second trophic level; and the parasitoids, *Cotesia flavipes* and *Cotesia sesamiae* at the third trophic level. Results showed that plant odours played an important role in attracting parasitoids. Both parasitoids were attracted to uninfested maize,

sorghum and napier grass. In dual choice experiments, the exotic parasitoid, *Cotesia flavipes*, showed a preference for maize over sorghum, but maize and napier grass were equally attractive. *Cotesia flavipes* was obtained in Pakistan from *Chilo partellus* feeding on maize. In contrast, *Cotesia sesamiae* preferred sorghum and napier grass to maize. Maize was introduced into East Africa about four hundred years ago, but only became widely grown during the last hundred years (Purseglove, 1972), while sorghum and napier grass are indigenous to Africa (Dogget, 1988). A legume plant, cowpea, did not attract the parasitoids. These results may reflect an adaptation to a long association between the parasitoids and plants that are the habitat of their hosts.

Previous studies have shown that plants under attack by herbivores may actually initiate the release of chemicals that indicate the presence of prey or hosts to natural enemies (Dicke and Sabelis, 1988b; Dicke *et al.*, 1990a,b; Turlings *et al.* 1990b, 1991a,b; Turlings and Tumlinson, 1991; Mattiacci *et al.*, 1994; Agelopoulos and Keller, 1994c). Results of this work confirm the above findings. Maize, sorghum and napier grass were highly attractive when they were fed upon by four different species of stemborers, *Chilo partellus*, *Chilo orichalcociliellus*, *S. calamistis* and *B. fusca*. *Cotesia flavipes* and *Cotesia sesamiae* showed no special preference for odours from maize plants infested by a particular stemborer species. However, the parasitoids could detect quantitative differences in odours released by infested maize plants. For example, when the same densities of stemborers were introduced into a maize plant, the parasitoid preferred plants

infested with large/older stemborers, such as *S. calamistis*, which caused more damage and induced a greater release of volatiles.

Attractive odours from maize infested with *Chilo partellus* have been identified. Green leaf volatiles, terpenoids, aromatic compounds and an aldehyde were present in the volatile blends. Interestingly, as reported by Turlings *et al.* (1991b), the terpenoids, (E)-β-farnesene and 4,8-dimethyl-1,3,7-nonatriene, were present in large amounts in the volatile blend of infested plants, but were absent in the volatiles collected from uninfested maize, suggesting that the release of these compounds was induced by stemborer feeding, and that these compounds may have been responsible for the enhanced attractiveness of infested maize. Terpenoids have been reported to be important for prey and host location by predators and parasitoids (Takabayashi *et al.*, 1994; Dicke, 1994). However, the release of these compounds is likely to be a direct defense against herbivores and pathogens that invade injured plants (Turlings and Tumlinson, 1991).

It was shown that the release of the volatiles attractive to parasitoids could be induced in maize with an elicitor present in the regurgitant of the herbivores (Turlings *et al.*, 1993; Potting *et al.*, 1995; this work). The eliciting factors are common to lepidopterous larvae, and may occur in a variety of herbivorous insects, such as grasshoppers, suggesting that the induced chemicals are common natural compounds (Turlings *et al.*, 1993). This supports the idea that plants provide parasitoids with highly detectable cues, but these cues may not always be reliable indicators of the presence of suitable hosts (Vet *et al.*, 1991; Vet

and Dicke, 1992).

Compounds that gave antennal response in the GC-EAD analyses were found to be behaviourally active, indicating that this method was reliable for identification of semiochemicals involved in parasitoid attraction. The EAD active chemicals included a terpene (E)-β-farnesene, and ester (Z)-3-hexenyl acetate and a phenolic compound, anisole. Anisole was also identified from the volatile collection from infested maize but was absent from uninfested maize. Synthetic anisole attracted *Cotesia flavipes* in a T-tube olfactometer. There are no reports known to the writer of the involvement of anisole in tritrophic interactions although, some other aromatic compounds such as toluene, m-xylene, o-xylene and p-xylene have been identified from volatile emissions from maize and sorghum (Lwande and Bentley, 1987).

Once in the habitat of their host, parasitoids may narrow their search by using more specific chemical stimuli which are host derived. *Cotesia flavipes* and *Cotesia sesamiae* used odours from host frass as cues and they responded to odours from frass produced by the four stemborer species under study. *Cotesia flavipes* was more attracted to from frass from *Chilo* spp. than frass from *B. fusca* when both host were fed on maize (Figure 4.11).

More stemborer larvae were stung when they were directly removed from the plant diet than when they were washed, indicating that the host recognition kairomones might be present in the substances on the insect surface. These substances may be of plant origin, or from the insect's oral secretions or/and the

insect body surface. Other studies have reported the presence of an oviposition kairomone in the mandibular gland secretions or oral secretions of herbivorous insects (Mohyuddin *et al.* 1981, Mudd and Corbet, 1982; Muzzafar and Inayatullah, 1986; Agelopoulos and Keller, 1994a). Additional research is needed to pinpoint the source of these kairomones and their identity.

Once the parasitoid finds the host, the next step is either to accept or reject it. Parasitization is successful only if the accepted host is suitable for development. *Cotesia flavipes* accepted all the four stemborer species used in this study. However, it completed development only in *Chilo partellus*, *Chilo orichalcociliellus* and *S. calamistis*. *Busseola fusca* was unsuitable and eggs of the parasitoid were encapsulated in this host. *Sesamia calamistis* was only partially suitable as a large number of immature *Cotesia flavipes* died within this host. In contrast, *Cotesia sesamiae* showed variability in host acceptance. The most accepted host, *S. calamistis*, was also the most suitable one, followed by the *Chilo* spp. Surprisingly, *B. fusca* was also unsuitable for the development of *Cotesia sesamiae*. *Cotesia sesamiae* has been recovered from *B. fusca* in several African countries, including South Africa (Van Rensburg *et al.*, 1989), Uganda (Ingram, 1958), Kenya (Nye, 1960; Mohyuddin, 1971) and is often cited as being the most common larval parasitoids of *B. fusca* (Polaszek and Walker, 1991). Since *Cotesia sesamiae* used in this study was collected from the coastal zone of Kenya where *B. fusca* does not occur, it is hypothesized that there are at least two distinct populations of *Cotesia sesamiae* within Kenya; one that has evolved with *B. fusca* and has

developed a means to escape the immune system of this host, and one that cannot evade the *B. fusca* immune defense system. It will be interesting to examine in more detail the immune defense mechanisms involved in both cases. This study also suggests that if *Cotesia flavipes* is release in areas where *B. fusca* is the most predominant stemborer species, this parasitoid willhave little chances to establish.

Conclusions

1. *Cotesia flavipes* and *Cotesia sesamiae* responded to similar cues when foraging, including odours from uninfested maize, sorghum and napier grass.
2. Both parasitoids were preferentially attracted to the habitat of their aboriginal hosts (maize for *Cotesia flavipes* and sorghum for *Cotesia sesamiae*).
3. The parasitoids were more attracted to infested plants than uninfested ones, but did not discriminate between plants infested by the different stemborers under study.
4. Odours from host frass were highly attractive regardless of host species or diet (maize, sorghum, napier grass) in a single choice situation.
5. *Cotesia flavipes* was more attracted to frass produced by *Chilo* species that those produced by *B. fusca* in dual choice tests. However, in other combinations, parasitoids did not discriminate between host species.

6. The difference in the chemicals released by infested and uninfested maize plants in this study has also been reported in several other studies involving other herbivore insect species. The production of these chemicals may be a general response to insect attack. However, more chemical work (quantitative and qualitative) is needed to identify of all compounds involved in the tritrophic interactions in the grasses/stemborers/*Cotesia* spp. system.
7. Identification of attractive compounds present in odours from frass is needed to fully understand their origin.
8. More research is also needed to determine the origin and understand the role of anisole in the system.
9. Additional studies of the immune defense of the different hosts will provide greater insight into host suitability.
10. The results suggest that the niches of *Cotesia flavipes* and *Cotesia sesamiae* may overlap, and therefore, they are likely to compete. However, due to the differences observed in host-plant preference and host suitability, both species are likely to survive in the same region.
11. The success of *Cotesia flavipes* as a biological control agent depend on its ability to attack stemborer in wild grasses throughout the year, and move to cultivated gramineous plants, during cropping seasons.

REFERENCES

Agelopoulos, N. G., and M.A. Keller, (1994a). Plant-natural enemy association in tritrophic system, *Cotesia rubecula-Pieris rapae-* Brassicaceae (Cruciferae). I. Sources of infochemicals. J. Chem. Ecol. 20: 1725-1734.

Agelopoulos, N. G., and M.A. Keller, (1994b). Plant-natural enemy association in tritrophic system, *Cotesia rubecula-Pieris rapae-* Brassicaceae (Cruciferae). II. Preference of *C. rubecula* for landing and searching. J. Chem. Ecol. 20: 1735-1748.

Agelopoulos, N. G., and M.A. Keller, (1994c). Plant-natural enemy association in tritrophic system, *Cotesia rubecula-Pieris rapae-* Brassicaceae (Cruciferae). III: Collection and Identification of plant and frass volatiles. J. Chem. Ecol. 20(8): 1955-1967.

Alam, M. M., F. D. Bennett and K. P. Carl. (1971). Biological control of *Diatraea saccharalis* (F.) in Barbados by *Apanteles flavipes* Cam. and *Lixophaga diatraeae* T.T. Entomophaga 16: 151-158.

Alam M.M., Beg M.N. and Ghani M.A. (1972). Introduction of *Apanteles* spp. against graminaceous borers into Pakistan. Tech. Bull. Commonw. Inst. Biol. Control. 15: 1-10.

Alphen J.J.M. van and R.A.B. Drijver (1982). Host selection by *Asobara tabida* Nees (Braconidae: Alysiinae) a larval parasitoid of fruit inhabiting *Drosophila* sp. I. Host stage selection with *Drosophila melanogaster* as host

species. Neth. J. Zool. 32: 215-231.

Altieri, M.A., W.J. Lewis, D.A. Nordlund, R.G. Gueldner, and J.W. Todd (1981). Chemical interactions between plants and *Trichogramma* spp. wasps in Georgia soybean fields. Protect. Ecol. 3: 635-648.

Ampofo, J.K. (1986). Maize stalk borer damage and plant resistance. Environ. Entomol. 15: 1124-1129.

Ampofo, J.K.O. (1988). Some observations on *Chilo partellus* (Lepidoptera: Pyralidae) developmental biology under field and laboratory conditions. Insect Sci. Applic. 9: 271-274.

Appert, J. M., M. Betbeder-Matibet, and H. Ranaivosa (1969). Vingt années de lutte biologique à Madagascar. Agron. Trop. 24: 555-572.

Arthur, A.P. (1981). Host acceptance by parasitoids. In Semiochemicals: Their role in Pest Control. D.A.Nordlund, R.L.Jones, and W.J. Lewis (eds.). John Wiley and Sons, New York pp. 97-120.

Attique M. R., A. I. Mohyuddin, C. Inayatullah, A. A. Goraya and M. Mushtaque (1980). The present status of biological control of *Chilo partellus* (Swinh.) (Lep: Pyralidae) by *Apanteles flavipes* (Cam.) (Hym.: Braconidae) in Pakistan. Proceedings of First Pakistan Congress of Zoology (B): 301-305.

Ayquipa A. G. , C.M.A. Cueva and R. J. Sirlopu (1979). Introduction of *Apanteles flavipes* (Cameron) (Hym:Braconidae) for biological control of sugarcane borer *Diatraea saccharalis* F. (Lep.: Crambidae) in Peru.

Entomol. Newslet. 7: 8.

Baehrecke E.H., H.J. Williams and S.B. Vinson (1989). Electroantennogram responses of *Campoletis sonorensis* (Hymenoptera: Ichneumonidae) to chemicals in cotton (*Gossypium hirsutum* L.). J. Chem. Ecol. 15: 37-45.

Baehrecke, E.H., S.B. Vinson, and H.J. Williams (1990). Foraging behavior of *Campoletis sonorensis* in response to *Heliothis virescens* and cotton plants. Entomol. Exp. Appl., 55: 47-58.

Bahana, J.W. (1985). *Dentichasmias busseolae*, the pupal parasite of *Chilo partellus*. Annual Report of the International Centre of Insect Physiology and Ecology (ICIPE) p. 16.

Bahana, J.W. (1987). Behavioural studies on *Dentichasmias busseolae* Henrich (Hymenoptera: Ichneumonidae). Fifteenth Annual Report of the International Centre of Insect Physiology and Ecology (ICIPE) p. 17.

Beg M. N., and C. Inayatullah (1980). Studies on *Apanteles flavipes* a parasite of graminaceous borers. Pakistan J. Agric. 1 (1): 50-53.

Bennett, F.D. (1993). Do introduced parasitoids displace native ones? Florida Entomologist 76: 54-63.

Bergman, J.M. and W.M Tingey (1979). Aspects of interaction between plant genotypes and biological control. Bull. Entomol. Soc. Am. 25: 275-279.

Betbeder-Matibet, M. and P. Malinge (1968). Un succès de la lutte biologique: contrôle de *Proceras sacchariphagus* Boj. Borer ponctué de la canne à sucre à Madagascar par un parasite introduit: *Apanteles flavipes* Cam.

Agron. Trop. 22: 1196-1220.

Bleszynski, S. (1970). A revision of the world species of *Chilo* Zincken (Lepidoptera: Pyralidae). Bulletin of the British Museum of Natural History, Entomology 25: 101-195.

Blumberg, D. and P. Debach (1979). Development of *Habroleptis rouxi* Compere (Hymenoptera: Encyrtidae) in two armored host (Homoptera: Daspididae) and parasite egg encapsulation by California red scale. Ecol. entomol. 4: 299-306.

Bordat, D. (1983). Mise au point de l'élevage de masse d'*Apanteles chilonis* Matsumura, 1912 et d'*Apanteles flavipes* (Cameron, 1891) (Hymenoptères: Braconidae) sur trois lépidoptères Pyralidae foreurs des graminées (*Chilo zacconius* Bleszynski, 1970; *Chilo partellus* (Swinhoe, 1884) et *Diatraea saccharalis* (Fabricius, 1794) dans un objectif de lutte. Thèse, Université des Sciences et Technique du Languedoc; Academie de Montpellier.

Bosque-Perez, N.A. and Mareck, J.H. (1990). Distribution and composition of lepidopterous maize borers in southern Nigeria. Bull. Entomol. Res. 80: 363-368.

Bowers, W.S., L.R. Nault, R.E. Webb, and S.R. Dutky (1972). Aphid alarm pheromone: isolation, identification, synthesis. Science 177:1121-1122.

Breniere, J. and D. Bordat (1982). Intérêt et difficultés de la lutte biologique par introduction d'entomophages: cas des foreurs du riz en Afrique. Agron. Trop. 37: 203-206.

Breniere, J. D. Bordat, B. Vercambre, H. Hamza and M. Renand (1985). Les opérations de lutte biologique contre le foreur du maïs *Chilo partellus* (Swinhoe), Lepidoptera, dans l'ilede Ngazidja. Agron. Trop. 40: 157-166.

Brownbridge, M. (1991). Natural *Bacillus thuringiensis* isolates for the management of Lepidopteran cereal pests. Insect Sci. Applic. 12: 57-61.

Burks, M.L. and W.C.Jr. Nettles (1978). *Encelatoria* sp. effects of cuticular extracts from *Heliothis virescens* and other factors on oviposition. Enviro. Entomol. 7: 897-900.

Buttery, R.G. and L.C. Ling (1984). Corn leaf volatiles: Identification using Tenax trapping for possible insect attractants. J. Agric. Chem. 32: 1104-1106.

C.A.B. International Institute of Biological control Pakistan Report (1987). On development of procedure for using *Apanteles flavipes* in periodic releases for control of stalk borers feeding on sugarcane and other graminaceous plants. December 1980-March 1986.

Carl, K. P. 1962. Graminaceous moth-borers in west Pakistan. Tech. Bull. Commonw. Inst. Biol. Contrl. 2: 29-76.

Carl, K. P. (1982). Biological control of native pests by introduced natural enemies. Biological control News and Information 3: 191-200.

Carton, Y. (1974). Biologie de *Pimpla instigator* III. Analyse experimentale du processus de reconnaissance de l'hôte. Chrysolida. Entomol. Exp. Appl. 17: 265-278.

Chacko, M.J. and K. Ogedah (1990). Role of *Trichogramma* sp. nr. *Mwanzai* and predators in the control of *Chilo partellus*. Annual Report of the International Centre of Insect Physiology and Ecology (ICIPE) p 30.

Cheng, L. (1970). Timing of attack by *Lypha dubia* Vall. (Diptera; Tachinidae) on the winter moth, *Operophthera brumata* (Lepidoptera: Geometridae) as a factor affecting parasitoid success. J. Anim. Ecol. 39: 313-329.

CIBC (1968-72). Reports of work carried out, Commonwealth Institute of Biological Control.

CIMMYT (1992). 1991-92 CIMMYT world maize facts and trends: maize research investment and impacts in developing countries. Mexico; CIMMYT,57 pp.

Conlong, D.E. (1990). A study of pest-parasitoid relationships in natural habitats:an aid towards the biological control of *Eldana saccharina* (Lepidoptera: Pyralidae) in sugarcane. Proceedings of the South African Sugar Technologists' Association 64: 111-115.

Davies, D.H. and S.B. Vinson (1986). Passive evasion by eggs of braconid parasitoid *Cardiochiles nigriceps* of encapsulaition in vitro by haemocytes of host *Heliothis virescens*. Possible role of fibrous layer in immunity. J. Ins. Physiol. 32: 1003-1010.

Davies, D.H. and S.B. Vinson (1988). Interference with functions of plasmatocytes of *Heliothis virescens* in vivo by calyx fluid of the parasitoid *Campoletis sonorensis*. Cell tissue Res. 251: 467-475.

Davies, D.H., R.L. Burghardt and S.B. Vinson (1986). Oogenesis of *Cardiochiles nigripes* Vierick (Hymenoptera: Braconidae); Histochemistry and development of the chorion with special reference to the fibrous layer. Int. J. Ins. Morphol. 15: 363-365.

Davies, D.H., M.R. Strand and S.B. Vinson (1987). Changes in differential haemocyte count and in vitro behavior of plasmatocytes from *Heliothis virescens* caused by *Campoletis sosnorensis* polydnavirus. J. Ins. Physiol. 33: 143-153.

DeBach, P. and D. Rosen (1991). Biological control by natural enemies. Second Edition. Cambridge University Press, Cambridge 440 pp.

Delobel, A. (1975). *Chilo orichalcociliellus* Strand (Lepidoptera: Pyralidae), Foreur des tiges du sorgho et du mais a Madagascar II. Premiere donnees biologiques. ORSTOM Series biologie 10: 11-16.

Denno, R.F., and M.S. McClure (1983). Variable plants and herbivores in natural and managed systems. Academic Press, New York.

Dicke, M. (1994). Local and systemic production of volatile herbivore-induced terpenoids: their role in plant-carnivore mutualism. J. Plant Physiol. (In Press).

Dicke, M., and M.W. Sabelis (1988a). Terminology of chemicals involved in interactions between individual organisms: Should it be based on cost-benefit analysis rather than on origin of compounds? Funct. Ecol. 2: 131-139.

Dicke,M., and M.W. Sabelis (1988b). How plants obtain predatory mites as body guards. Neth. J. Zool. 38: 148-165.

Dicke, M., and H. Dijkman (1992). Induced defence in detached uninfested leaves: effects on behaviour of herbivores and their predators. Oecologia 91: 554-560.

Dicke, M., and J. Takabayashi (1991). Specificity of induced indirect defence of plants against herbivores. Redia 17(3): 105-113.

Dicke, M., J.C. Van Lenteren, G.J.F. Boskamp and E. Van Dongen-van Leewen (1984). Chemical stimutli in host-habitat location by *Leptopilina heterotoma* (Thomson), a parasite of *Drosophila*. J. Chem. Ecol. 16: 381-396.

Dicke, M., M. De Jong, M.P.T. Alers, F.C.T. Stelder, R. Wunderink, and J. Post (1989). Quality control of mass reared arthropods: Nutritional effects on performance of predatory mites. J. Appl. Entomol. 108: 462-475.

Dicke M., Beek T. A Van.,M.A. Posthumus, N. Ben Dom, H. Van Bokhoven and A. De Groot (1990a). Isolation and identification of volatile kairomones that affect acarine predator-prey interactions. Involvement of host plant in its production. J. Chem. Ecol.16: 381-396.

Dicke M., M.W. Sabelis, J. Takabayashi, J. Bruin and M.A. Posthumus (1990b). Plant strategies of manipulating predator-prey interactions through allelochemicals: prospects for application in pest control. J. Chem. Ecol. 16(11): 3091-3118.

Ding , D., P.D. Swedenborg and R.L. Jones (1989). Chemical stimuli in host-seeking behavior of *Macrocentus grandii* (Hymenoptera: Ichneumonidae). Ann. Entomol. Soc. Am. 82: 232-236.

Doggett, H. (1988). The origin of the sorghum crop. In Sorghum. pp. 40-43. Second edition, Tropical agriculture series. Longman.

Doutt, R.L. (1964). Biological characteristics of entomophagous adults. In Biological Control of Insect Pests and Weeds. P. Debach, ed. Chapman and Hall, London. pp145-167.

Duerdon, J.C. (1953). Stem borers of cereal crops at Kongwa, Tanganyika, 1950-52. East Africa Agricultural and Forestry Journal 19: 105-119.

Dwumfour, E.F., and M.J. Chacko (1990). Compatibility of intercropping with varietal difference for biological control of *Chilo partellus* by egg parasitoid *Trichogramma* sp. nr. *Mwanzai*. Annual Report of the International Centre of Insect Physiology and Ecology (ICIPE) p 30.

Edwards, L.J., J.B. Siddall, L.L. Dunham, P. Uden, and C.J. Kislow (1973). Trans-β-farnesene alarm pheromone of the green peach aphid, *Myzus persicae* Sulzer. Nature 241: 126-127.

Eller, F.J. (1990). Foraging behavior of *Microplitis croceipes* (Cresson) (Hymenoptera:Braconidae) A parasitoid of Heliothis (Lepidoptera: Noctuidae) species. PhD Dissertation, University of Florida, Gainesville.

Eller, F.J., J.H. Tumlinson, and W.J. Lewis (1988a). Beneficial arthropod behavior mediated by airborne semiochemicals: Source of volatiles

mediating the host-location flight behavior of *Micropletis croceipes* (Cresson) (Hymenoptera: Braconidae), a parasitoid of *Heliothis zea* Boddie (Lepidoptera: Noctuidae). Environ. Entomol. 17: 745-763.

Eller, F.J., J.H. Tumlinson, and W.J. Lewis (1988b). Beneficial arthropod behavior mediated by airborne semiochemicals II. Olfactometric study of host location by the parasitoid *Micropletis croceipes* (Cresson) (Hymenoptera: Braconidae). J. Chem. Ecol. 14: 425-434.

Elzen, G.W., H.J. Williams, and S.B. Vinson (1983). Response by the parasitoid *Campoletis sonorensis* to chemicals (synomones) in Plants.: Implication for habitat location. Environ. Entomol., 12: 1872-1

Elzen, G.W., H.J. Williams, and S.B. Vinson (1984). Role of diet in host selection of *Heliothis virescens* by parasitoid *Campoletis sonorensis* (Hymenoptera: Ichneumonidae). J. Chem Ecol. 10: 1535-1541.

Elzen, G.W., H.J. Williams, and S.B. Vinson (1986). Wind tunnel flight responses by hymenopterous parasitoid *Campoletis sonorensis* to cotton cultivars and lines. Entomol. Exp. Appl. 42: 285-290.

Elzen, G.W., H.J. Williams, A.A. Bell, R.D. Stipanovic, and S.B. Vinson (1985). Quantification of volatile terpenes of glanded and glandless *Gossypium hirsutum* L. cultivars and lines by gas chromatography. J. Agr. Food Chem. 33: 1079-1082.

Elzen, G.W., H.J. Williams, and S.B. Vinson, and J.E. Powell (1987). Comparative flight behavior of parasitoids *Campoletis sonorensis* and

Microplitis croceipes. Entomol. Exp. Appl. 45: 175-180.

FAO (1991). Yearbook vol. 45, 85 pp.

Flanders, S.E. (1942). Sex differenciation in the polyembryonic proclivity of the Hymenoptera. J. Econ. Entomol. 35: 108

Flanders, S.E. (1953). Variations in susceptibility of citrus-infesting coccids to parasitization. J. Econ. Entomol., 46: 266-269.

Fuchs, T. W, F. R. Huffman and J. W. Smith, Jr. (1979). Introduction and establishment of *Apanteles flavipes* (Hym:Braconidae) on *Diatraea saccharalis* (Lep:Pyralidae) in Texas. Entomophaga 24: 109-114.

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

Godfray, H.C.J. (1994). In Parasitoids: Behavioral and Evolutionary Ecology. Monograph in behavior and ecology. J.R. Krebs and T. Clutton-Brock (eds). Princeton University Press, Princeton, New Jersey.

Gounou, S., F. Schulthess, T. Shanower, W.N.O. Hammond, H. Braima, A.R. Cudjoe, R. Adjakloe, K.K. Antwi and I. Olaleye (1993). Plant Health Management Research Monograph No. 3. International Institute of Tropical Agriculture, 31 pp.

Greany P.D., J.H. Tumlinson, D.L. Chambers, and G.M. Boush (1977). Chemically mediated host finding by *Biosteres* (Opius) *longicaudatus*, a parasitoid of tephrid fruit fly larvae. J. Chem. Ecol. 3: 189-195.

Greathead, D. J. (1971). A review of biological control in the Ethiopian region. Tech. Commun. Commonw. Inst. Biol. Control, No 5, 168 pp.

Gutierrez, A.P. (1970). Studies on host selection and host specificity of the aphid hyperparasite *Charips vietrix* IIIHost suitability studies. Ann. Entomol. Soc. Am. 63: 1485-1491.

Harris, K.M. (1990). Bioecology and *Chilo* species. Insect Sci. Applic. 11: 467-477.

Hatanaka, A. (1993). The biogeneration of green odour by green leaves. Phytochemistry, 34: 1201-1208.

Hedin, P.A. (1976). Seasonal variations in the emission of volatiles by cotton plants growing in the field. Environ. Entomol., 5(6): 1234-1238.

Hendrikse, A., R. zucchi, J.C. Van Lenteren and J. Woets (1980). *Dacnusa sibiria* Telenga and *Opius pallipes* Wesmael (Hymenoptera: Braconidae) in the control of the tomato leaf miner *Liriomyza bryonidae*. In: Proc. work. Group Integrated control Glasshouses, Vantaa, Finland. Bull. Int. Org. Biol. Control/West Paleact. Reg. sect. III/3.

Hill, D.S. (1983). In Agricultural Insect Pests of the Tropics and their Control. Second edition. Cambridge University Press. pp. 50-54.

Hoyle, G. (1951). Potassium ions and insect nerve muscle. J. Exp. Biol. 30: 121-135.

IAPSC (1985). *Chilo partellus* (Swinhoe). Distribution maps of major crop pests and diseases in Africa No. 199. Yaounde, Cameroon; Inter-African

Phytosanitary Council, 35 pp.

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

Inayatullah, C. (1987). Development and survival of *Apanteles flavipes* (Cam.) (Hymenoptera:Braconidae) on *Chilo partellus* (Swinh.) (Lepidoptera:Pyralidae) larvae reared on gram-flour diet. Insect Sci. Applic. 8: 95-97.

Ingram, W. R. (1958). The lepidopterous stalk borers associated with Graminae in Uganda. Bull. Ent. Res. 49: 67-83.

Ingram, W. R. (1983). Biological control of graminaceous stem-borers associated with gramineae in Uganda. Insect Sci. Applic. 4: 205-209

Janzen, D.H. (1979). New horizon in the biology of plant defenses. In: Herbivore: their interaction with secondary plant metabolites. G. Rosenthal and D. H. Janzen (eds). Academic Press, New York. pp 331-350.

Jepson, W.F. (1954). A critical review of the world literature on the lepidopterous stalk borers of tropical graminaceous crops. Commonwealth Institute of Entomology. 127 pp.

Jones, R.L. (1981). Chemistry of semiochemicals involved in parasitoid-host and predators-prey realtionships, In Semiochemicals: Their Role in Pest Control. D.A.Nordlund, R.L.Jones, and W.J. Lewis (eds.). John Wiley and Sons, New York 239-250 pp.

Jones, R.L., W.J. Lewis, M. Beroza, B.A. Bierl and A.N. Sparks (1973). Host-seeking stimulants (Kairomones) for the egg parasite, *Trichogramma evanescens*. Environ. Entomol. 2: 593-596.

Kainoh, Y. (1990). Chemicals controlling the the behavior of parasitoids and predators. In The use of Natural Enemies to Control Agricultural Pests. O. Mochida (ed.). Food Fertilizer Technology Center. Book series No. 40, Taipei, Taiwan.

Kaiser, R. (1987). Night-scented flowers not only attractive to moths. Abstrats EUCHEM conference semiochemicals in plant and animal kingdoms. October 12-16 1987, Angers, France.

Kajita, H. and F. E. Drake (1969). Biology of *Apanteles chilonis* and *A. flavipes* (Hymenoptera: Braconidae), parasites of *Chilo suppressalis*. Mushi 42: 163-179.

Kamm, J.A., and R.G. Buttery (1983). Response of the alfalfa seed chalcid *Bruchophagus roddi*, to alfalfa volatiles. Entomol. Exp. Appl. 33: 129-134.

Kaya, H.K., and Y. Tanada (1969). Responses to high temperature of the parasite *Apanteles militaris* and of its host, the armyworm, *Pseudoleta unipuncta*. Ann. Entomol. Soc. Am. 62: 1303-1306.

Kaya, H.K. (1970). Toxic factor produced by a granulosis virus in armyworm larvae. Effect on *Apanteles militaris*. Science. 168: 251-253.

Kennedy, B.H. (1984). Effect of multilure and its components on parasites of *Scolytus multistriatus* (Coleoptera: Scolytidae). J. Chem. Ecol. 10: 373-385.

Kester, K.M. and P. Barbosa (1991). Behavioral and ecological constraints imposed by plants on insect parasitoids: Implications for biological control. Bio. con. 1: 94-106.

Kfir, R. (1994). Attempts at biological control of the stem borer *Chilo partellus* (Swinhoe) (Lepidoptera: Pyralidae)in South Africa. African Entomology. 2: 67-68.

Kitano, H. (1982). Effect of venom of the gregarious parasitoid *Apanteles glomeratus* on its haemocytic encapsulation by the host, *Pieris rapae crucivora*. J. Invert. Pathol. 40: 61-67.

Kitano, H. and N. Nakatsuji (1978). Resistance of *Apanteles* eggs to the hemocytic encapsulation by their habitual host, *Pieris*. J. Ins. Physiol. 24: 261-271.

Lawrence, P.O., R.M. Baranowski, and P.D. Greany (1976). Effect of host age on development of *Biosteres longicaudatus* a parasitoid of the Caribbean fruit fly, *Anastrepha suspensa*. Fla. Entomol. 59: 33-39.

Lecomte, C. and E. Thibout (1986). Analyse, dans deux types d'olfactometre, du comportement de quête des femelles de *Diadromus puchellus* en presence d'odeur du phytophase hôte et du vegetal attaque ou non. Entomophaga, 31: 69-79.

Leerdam Van, M. B., J. W. Smith, Jr. and T. W. Fuchs (1985). Frass-mediated, host finding behavior of *Cotesia flavipes*, a braconid parasite of *Diatraea saccharalis* (Lepidoptera: Pyralidae). Ann. Entomol. Soc. Am. 78: 647-650

Leius, K. (1967). Influence of wild flowers on parasitism of tent caterpillar and codling moth. Can. Entomol. 99: 444-446.

Leuschner, K., S.L. Tanaja, and H.C. Sharma (1985). The role of host plant resistance in pest management in sorghum in India. Insect Sci Applic. 6: 453-460.

Lewis, W.J. and S.B. Vinson (1971). Suitability of certain *Heliothis* spp. as hosts for the parasite *Cardiochiles nigripes*. Ann. Entomol. Soc. Am. 64: 970-973.

Lewis, W.J., and D.A. Nordlund (1985). Behaviour modifying chemicals to enhance natural enemies effectiveness. In: Biological Control in Agriculture IPM Systems. M. A. Hoy and D.C. Herzog (eds.), pp 89-101. Academic Press, New York.

Lewis, W.J., and J.H. Tumlinson (1988). Host detection by chemically mediated associative learning in parasitic wasps. Nature 331: 257-259.

Lewis, W.J. and W.R.Jr. Martin (1990). Semiochemicals for use with parasitoids: Status and future. J. Chem. Ecol. 16(11): 177-181.

Lewis, W.J., R.L. Jones and A.N. Sparks (1972). A host-seeking stimulant for the egg parasite *Trichogramma evanescens*: its source and a demonstration of its field and laboratory activity. Ann. Entomol. Soc. Amer. 65: 1087-1089.

Lewis, W.J., L.E.M. Vet, J.H. Tumlinson, J.C. Van Lenteren and D.R. Papaj (1990). Variations in parasitoid foraging behavior. Essential element of a

Leius, K. (1967). Influence of wild flowers on parasitism of tent caterpillar and codling moth. Can. Entomol. 99: 444-446.

Leuschner, K., S.L. Tanaja, and H.C. Sharma (1985). The role of host plant resistance in pest management in sorghum in India. Insect Sci Applic. 6: 453-460.

Lewis, W.J. and S.B. Vinson (1971). Suitability of certain *Heliothis* spp. as hosts for the parasite *Cardiochiles nigripes*. Ann. Entomol. Soc. Am. 64: 970-973.

Lewis, W.J., and D.A. Nordlund (1985). Behaviour modifying chemicals to enhance natural enemies effectiveness. In: Biological Control in Agriculture IPM Systems. M. A. Hoy and D.C. Herzog (eds.), pp 89-101. Academic Press, New York.

Lewis, W.J., and J.H. Tumlinson (1988). Host detection by chemically mediated associative learning in parasitic wasps. Nature 331: 257-259.

Lewis, W.J. and W.R.Jr. Martin (1990). Semiochemicals for use with parasitoids: Status and future. J. Chem. Ecol. 16(11): 177-181.

Lewis, W.J., R.L. Jones and A.N. Sparks (1972). A host-seeking stimulant for the egg parasite *Trichogramma evanescens*: its source and a demonstration of its field and laboratory activity. Ann. Entomol. Soc. Amer. 65: 1087-1089.

Lewis, W.J., L.E.M. Vet, J.H. Tumlinson, J.C. Van Lenteren and D.R. Papaj (1990). Variations in parasitoid foraging behavior. Essential element of a

sound biological control theory. Environ. Entomol. 19(5): 1183-1193.

Li, Lien-Chang (1965). Studies on the apple pierid (*Aporia crataegi* L.). In: Shansi. Acta Entomol. Sin. 14: 545-551.

Liu, S.S. (1985). Development, adult size and fecundity of *Aphidius sonchi* reared in two instars of its aphid host, *Hyperomyzus lactucae*. Entomol. Exp. appl. 37: 41-48.

Loke W.H., T.R. Ashley and R.I. Sailer (1983). Influence of fall army; *Spodoptera frugiperda* (Lepidoptera: Noctuidae) larvae and corn plant damage on host finding in *Apanteles marginiventris* (Hymenoptera: Braconidae). Environ. Entomol. 12: 911-915.

Lu, Q.G. (1988). *Trichogramma mwanzai* for biological control of crop borers. Annual Report of the International Centre of Insect Physiology and Ecology (ICIPE).

Lwande, W. and M.D. Bentley (1987). Volatiles of *Sorghum bicolor* seedlings. J. Nat. Prod. 50(5): 950-952.

Macedo N., A. F. Mendonca Filho, J. A. Moreno, and A. H. Pinazza (1984). Evaluation of the economic advantages of 10 years of biological control of *Diatraea* spp. through *Apanteles flavipes* (Cameron) in the state of Alagoas (Brazil). Entomol. Newslet. 16: 9-10.

Maniania, N.K. (1991). Potential of some fungal pathogens for the control of pests in the tropics. Insect Sci. Applic. 12: 63-70.

Martin, W.R.Jr, D.A. Nordlund and W.C.Jr Nettles (1990). Response of

parasitoid *Encelatoria bryani* to selected plant material in an olfactometer. J. Chem. Ecol. 16(2): 489-508.

Mathez, F.C. (1972). *Chilo partellus* Swinh., *C. orichalcociliella* Strand (Lep. Crambidae) and *Sesamiae calamistis* Hmps. (Lep.: Noctuidae) on maize in the coast province of Kenya. Mitt. Schwei. Entomol. Gesell. 45: 267-289.

Matsuda, K., and S. Senbo (1986). Chlorogenic acid as a feeding deterrent for Salilaceae-feeding leaf beetle, *Lochmaeae capreae cribrata* (Coleoptera: Chrysomelidae) and other species of leaf beetles. Appl. Entomol. Zool. 21: 411-416.

Mattiacci, L., M. Dicke, and M.A. Posthumus (1994). Induction of parasitoid attracting synomone in brussels sprouts plants by feeding *Pieris brassicae* larvae: role of mechanical damage and herbivore elicitor. J. Chem. Ecol. 20(9): 2229-2247.

Maurer, B., A. Hauser and J.C. Froidevaux (1986). (E)-4,8,Dimethyl-1,3,7-nonatriene and (E,E)-4,8,12-trimethyl-1,3,7,11-tridecatetraene, two unusual hydrocarbons from cardamom oil. Tetrahedron Lett. 27: 2111-2121.

McAuslane, H.J., S.B. Vinson, and H.J. Williams (1990). Influence of Host plant on mate location by the parasitoid *Campoletis sonorensis* (Hymenoptera:Ichneumonidae). Environ. Entomol. 19: 26-31.

McAuslane, H.J., S.B. Vinson, and H.J. Williams (1991). Stimuli influencing host microhabitat location in the parasitoid *Campoletis sonorensis*. Entomol. Exp. Appl., 58: 267-277.

Minja, E.M. (1990). Management of *Chilo* spp. infesting cereals in eastern Africa. Insect Sci. Applic. 11: 489-499.

Mohyuddin, A.I. (1970). Notes on the distribution and biology of *Pediobus furvus* (Gah.) (Hym.: Eulophidae), a parasite of graminaceous stem-borers. Bull. Entomol. Res. 59: 681-689.

Mohyuddin, A. I. (1971). Comparative biology and ecology of *Apanteles flavipes* and *A. sesamiae* Cam. as parasites of graminaceous borers. Bull. Entomol. Res. 61: 33-39.

Mohyuddin, A.I. (1978). The scope of biological control of sugarcane in Pakistan. In Proceedings of Pakistan Society of Sugar Technologist, Plant Protection Seminars, Lahore, 1978: 29-39.

Mohyuddin, A.I. (1989). Utilization of natural enemies in the control of insect pests on sugarcane. In Proceedings of Second International Conference on Tropica Entomology, Nairobi, Kenya.

Mohyuddin, A.I. (1990). Biological control of *Chilo* spp. in maize, sorghum and millet. Insect Sci. Applic. 11: 721-732.

Mohyuddin, A. I., and D. J. Greathead (1970). An annoted list of the parasites of graminaceous stem-borers in East Africa with the discussion of their potential in biological control. Entomophaga 15: 241-274.

Mohyuddin, A. I., C. Inayatullah and E. G. King (1981). Host selection and strain occurrence in *Apanteles flavipes* (Cameron) (Hymenoptera: Braconidae) and its bearing in biological control of graminaceous

stemborers (Lepidoptera: Pyralidae). Bull. Entomol. Res. 71: 575-581.

Mossadegh, M.S. (1980). Inter- and Intraspecific effects of the mandibular gland secretion of larvae of the indian-meal moth *Plodia interpunctella*. Physiol. Entomol. 5: 165-173.

Moutia, L.A. and C.M. Courtois (1952). Parasites of the moth-borers of sugar-cane in Mauritius. Bull. Entomol. Res. 43: 325-359.

Mudd A., and S.A. Corbet (1982). Response of the ichneumonid parasite *Nemeritis canescens* to kairomone from the flour moth, *Ephestia kuehniella*. J. Chem. Ecol. 8: 843-850.

Muthukrishnan, J., and M. Senthamizhselvan (1987). Effect of parasitization by *Apanteles flavipes* on the biochemical composition of *Diacrisia obliqua*. Insect Sci. Applic. 8(2): 235-238.

Muzaffar, N. and C. Inayatullah (1986). Role of salivary glands of pyralid borer larvae in host selection by *Apanteles flavipes* (Cameron) (Hym: Braconidae). J. Agric. Res. 203-205.

Nadel, H. and J.J.M. Van Alphen (1987). The role of host and host-plant odours in the attraction of the parasitoid *Epidinocarcis lopezi* to the habitat of its host, the cassava mealybug, *Phenacoccus manihoti*. Entomol. Exp. Appl. 45: 181-186.

Narayanan, E.S. and B.R. Subba Rao (1955). Studies in insect parasitism I-III:The effect of different hosts on the physiology, on the development and behavior and on the sex ratio of *Microbracon gelechiae* Ashmead. Beitr.

Entomol. 5: 36-60.

Noldus, L.P.J.J. and J.C. Van Lenteren (1985). Kairomones for the egg parasite *Trichogramma evanescens* Westwood IIEffect of contact chemicals produced by two of its hosts, *Pieris brassicae* L. and *Pieris rapae* L. J. Chem. Ecol. 11(6): 793-800.

Nordlund, D.A. (1981). Semiochemicals: a review of the terminology. In: Semiochemicals: Their Role in Pest Control. D.A.Nordlund, R.L.Jones, and W.J. Lewis (eds.). John Wiley and Sons, New York pp. 13-28.

Nordlund, D.A, and W.J. Lewis (1976). Terminology of chemicals releasing stimuli in intraspecific interactions. J. Chem. Ecol., 2: 211-220.

Nordlund, D. A., W.J. Lewis and M.A. Altieri (1988). Influences of plant-produced allelochemicals on host/prey selection behavior of entomophagous insects. P. Barbosa and D. K. Letourneau (eds). In: Novel Aspects of Insect-Plant Interactions. John Wiley, New York pp 65-90.

Norris, O.M. (ed) (1986). Antifeeding compounds, In Sterol Biosynthesis, Inhibitors and antifeeding compounds. Springer-Verlag, Berlin pp. 96-146.

Nwanze, K.F., and O. Youm (1994). Integrated pest management of sorghum and pearl millet. Proceedings of the Third International Conference on Tropical Entomology, 30th October-4th November 1994, Nairobi, Kenya.

Nye, I.W.B. (1960). The insect pests of graminaceous crops in East Africa. Colon. Research Study number 31. London: Her Magesty's Stationery

Office, 48 pp.

Ochiel, G.R.S. (1989). Biology of *Trichogramma* species near *exiguum* Pinto & Plantner (Hymenoptera: Trichogrammatidae) on some Lepidopterous hosts in South Nyanza, Kenya. M. Phil thesis submitted at Rivers State University of Science and Technology, Port Harcourt, Nigeria.

Ochieng, R. S., F. O. Onyango and M.D.O. Bungu (1985). Improvement of techniques for mass culture of *Chilo partellus* (Swinhoe). Insect Sci. Applic. 6: 425-428.

Odell, T.M. and P.A.Godwin (1984). Host selection by *Blepharipa pratensis* (Meigen) a tachinid parasite of the gypsi moth, *Lymantria dispar* L. J. Chem. Ecol. 10(2): 311-320.

Odindo, M.O. (1991). Management of cereals stemborers, especially *Chilo partellus* using Microsporidia. Insect Sci. Applic. 12: 51-55.

Oloo, G.W. (1989). The role of local natural enemies in population dynamics of *Chilo partellus* (Swinh.) (Pyralidae) under subsistence farming systems in Kenya. Insect Sci. Applic. 10 (2): 243-251.

Oloo, G.W., and K. Ogeda (1990). The incidence of *Chilo partellus* and the contribution of natural enemies to its mortality under intercropping system in Kenya. Trop. Pest Man. 36(3): 244-248.

Onyango, F.O., A.G. Nyangwara, B.O. Owino, D.J. Okode and J.P.R. Ochieng-Odero (1992a). Prodecure for sustained rearing of *Busseola fusca* on semi-synthetic diet. Annual Report of the International Centre of

Insect Physiology and Ecology (ICIPE) pp. 110-111.

Onyango, F.O., P.O. Wagara, A.G. Nyangwara, and J.P.R. Ochieng-Odero (1992b). Prodecures for artificial rearing of *Maruca testulalis*. Annual Report of the International Centre of Insect Physiology and Ecology (ICIPE) p. 110.

Overholt, W. A. (1993). Laboratory rearing procedure for *Cotesia flavipes*. In: W.A. Overholt (ed.). Proceedings of Group Training Course on Identification of *Cotesia* spp. Stemborer Parasitoids. ICIPE Science Press, Nairobi. 42 pp.

Overholt, W. A. (1994). A review of classical biological control of gramineous stemborers in Africa. In Proceedings of group training course on 'Cereal Stemborers in Africa: Taxonomy, Natural Enemies and Control '. 8-19 August 1994, Nairobi, Kenya.

Overholt W.A. and J.W.Jr. Smith (1990a). Comparative evaluation of three exotic insect parasites (Hymenoptera: Braconidae) against the southwestern corn borer (Lepidoptera: Pyralidae) in corn. Environ. Entomol. 19: 1155-1162.

Overholt, W.A. and J.W. Jr Smith (1990b). Colonization of six exotic parasites (Hymenoptera) against *Diatraea grandiosella* (Lepidoptera: Pyralidae) in corn. Environ. Entomol. 19: 1889-1902.

Overholt, W. A., K. Ogeda and P. M. Lammers (1994a). Distribution and sampling of *Chilo partellus* (Swinhoe) (Lepidoptera: Pyralidae) in maize and

sorghum on the Kenya coast. Bull. Entomol. Res. 84: 367-378.

Overholt, W. A., A. J. Ngi-Song, S. K. Kimani, J. C. Mbapila, P. M. Lammers, and E. N. Kioko (1994b). Environmental considerations and the introduction of *Cotesia flavipes* Cameron (Hymenoptera: Braconidae) for biological of *Chilo partellus* (Swinhoe) (Lepidoptera: Pyralidae), an introduced gramineous stemborer in Africa. Biocontrol News and Information. 15: 19-24

Pathak, J.P.N. (1993). Cell-mediated defense reaction in insects. In: Insect immunity. J.P.N. Pathak. Oxford & IBH Publishing Co. PVT LTD. India. 48-67 pp.

Pats, P. (1994). Intercropping and crop residue management in the control of *Chilo partellus* in maize. Proceedings of the Third International Conference on Tropical Entomology, 30th October-4th November 1994, Nairobi, Kenya.

Pettit, F.L. and D.O. Wietlisbach (1993). Effects of host instar and size on parasitization efficiency and life history parameters of *Opius dissitus*. Entomol. Exp. Appl. 66: 227-236.

Pimentel, D. (1966). Wasp parasite *Nasonia vitripennis* survival on its fly host *Musca domestica* reared on various food. Ann. Entomol. Soc Am. 59: 1031-1038.

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

Potting R.P.J., F. Osae-Danso, W.A. Overholt and A.J. Ngi-Song (1993). Host selection in *Cotesia flavipes*, parasitoid of tropical stemborers. Proc. Exper. Appl. Entomol., N.E.V. Amsterdam 4: 47-52.

Potting, R.P.J., L.E.M. Vet and M. Dicke (1995). Host microhabitat location by stemborer parasitoid *Cotesia flavipes*: the role of herbivore volatiles and locally and systemically induced plant volatiles. J. Chem. Ecol. (In Press).

Powell, W. and Zhang Zhi-li (1983). the reaction of two cereal aphid parasitoids, *Aphidius Uzbekistanicus* and *A. ervi* to host aphids and their food-plants. Physiol. Entomol. 8: 439-443.

Prevost, G. D.H. Davies and S.B. Vinson (1990). Evasion of encapsulation by parasitoid correlated with the extent of host hemocyte pathology. Entomol. Exp. Appl. 55: 1-10.

Price, P.W., C.E. Bouton, P. Gross, B.A. McPherson, J.N. Thompson and A.E. Weis (1980). Interactions among three trophic levels; influence of plants and interactions between insect herbivores and natural enemies. Ann. Rev. Ecol. Syst. 11: 41-65.

Purseglove, J.W. (1972). Tropical crops-monocotyledons. London; Longman, 607 pp.

Rajabalee, M. A. and M. Govendasamy (1988). Host specificity of *Apanteles flavipes* (Cam.) and *Apanteles sesamiae* (Cam.) (Hymenoptera: Braconidae) parasites of sugarcane moth borers in Mauritius. Rev. Agric.

et Sucr.. 67: 78-80.

Read, D.P., P.P. Fenny, and R.B. Root (1970). Habitat selection by the aphid parasite *Diaeretiella rapae* (Hymenoptera: Braconidae) and hyperparasite *Charips brassicae* (Hymenoptera: Cynipidae). Can. Entomol., 102: 1567-1578.

Rensburg, J.B.J. Van, M. C. Walters and J. H. Giliomee 1988). Mortality in natural populations of the maize stalk borer, *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) in South Africa. Phytophylactica. 20: 17-19.

Ross D.R. and P.E. Dunn (1989). Effect of parasitism by *Cotesia congregata* on the susceptibility of *Manduca sexta* larvae to bacterial infection. Develop. Comp. Immunol. 13: 205-216.

Roth, J.P., E.G. King, and S.D. Hensley (1982). Plant, host and parasite interactions in the host selection sequence of the tachinid *Lixophaga diatraeae*. Environ. Entomol. 11: 273-277.

Sabelis,M.W. and Van De Baan (1983). Location of distant spider mite colonies by phytoseiid predators: demonstration of specific kairomones emitted by *Tetranychus urticae* and *Panonychus ulmi*. Entomol. Exp. Appl. 33: 303-314.

Sabelis, M.W., B.P. Afman and P.J. Slim (1984). Location of distant spider mite colonies *Phytoseiulus persimilis*: location and extraction of a kairomone. Acarology. VI(1): 431-440.

Salt, G. (1935). Experimental studies in insect parasitism. III. Host selection. Proc. Roy. Soc. London Ser. B, 117: 413-435.

Salt, G. (1970). The cellular defence reactions of insects. Cambridge monograph in Experimental Biology. Cambridge Univ. Press, London.

SAS Institute (1988). Users' Guide: Statistics. SAS Institute, Release 6.03 edition. Cary, N.C. USA. 1028 pp.

Sauls, C.E., D.A. Nordlund, and W.J. Lewis (1979). Kairomones and their use for management of entomophagous insects. VIIIEffect of diet on the kairomonal activity of frass from *Heliothis zea* (Boddie) larvae for *Microplitis croceipes* (Cresson). J. Chem. Ecol. 5: 363-369.

Saxena, K.N., A.M. Nour, F.D.O. Odawa and S.M. Otieno (1992). Components and mechanism of resistance in sorghum to stemborers. Annual Report of the International Centre of Insect Physiology and Ecology (ICIPE) pp. 42-45.

Scheibelreiter, G. K. (1980). Sugarcane stemborers (Lepidoptera: Noctuidae and Pyralidae) in Ghana. J. Appl. Entomol. 89: 87-99.

Schmidt, G.T. (1974). Host-acceptance behavior of *Campoletis sonorensis* (Hym: Ichneum) towards *Heliothis zea* (Lepidoptera: Noctuidae). Ann. Entomol. Soc. Amer. 67: 835-844.

Schulthess, F., N.A. Bosque-Perez, and S. Gounou (1991). Sampling lepidopterous pests on maize in West Africa. Bull. Entomol. Res. 81: 297-301.

Senthamizhselvan M., and J. Muthukrishnan (1989). Bioenergetics of *Apanteles flavipes* Cameron (Hymenoptera: Braconidae), a parasitoid of *Porthesia scintillans* Walker (Lepidoptera: Lymantridae). Insect Sci. Applic. 10(3): 295-299.

Seshu Reddy, K.V. (1983). Sorghum stem borers in eastern Africa. Insect Sc. Applic. 4: 33-39.

Seshu Reddy, K.V. (1988). Assessment of on-farm yield losses in sorghum due to insect pests. Insect Sci. Applic. 9: 679-685

Seshu Reddy, K.V. (1989). Sorghum stem borers in eastern Africa. Internatonal Workshop on Sorghum Stemborers. Patancheru, India 17-20 November 1987. pp 33-40.

Seshu Reddy, K.V. (1991). Insect pests of sorghum in Africa. Insect Sci. Applic. 12: 653-657.

Seshu Reddy, K.V and J.C. Davies (1979). Pest of sorghum and pearl millet and their parasites and predators recorded at ICRISAT centre, India up to August 1979. Cereal Entomolgy Department Progress Report 2 ICRISAT Patancheru, A.P., India. pp 23..

Seshu Reddy, K.V. and Walker, P.T. (1990). A review of the yield losses in graminaceous crops caused by *Chilo* spp. Insect Sci. Applic. 11: 563-569.

Shahjahan, M. (1974). *Erigeron* flowers as a food and attractive odor source for *Peristenus pseudopallipes* (Hymenoptera: Braconidae), a parasitoid of the tarnished plant bug. Environ. Entomol. 2: 921-925.

Shami, S. K. (1990). Suitability of three strains of *Apanteles flavipes* (Cameron) and one of *A. sesamiae* Cameron for biological control of graminaceous borers in Pakistan and effects of feeding of *A. flavipes* larvae on the development of its hosts. Ph.D. Dissertation, University of Punjab, Lahore, India.

Shami, S., and Mohyuddin A. I. (1986). Suitability of *Apanteles sesamiae* Cameron as biological control agent for graminaceous borers found in Pakistan. pp 289-294 Proc. 22nd Annual Convention, Pakistan Society of Sugar Technologists, Peshawar 19-21 September 1986.

Shami, S. and A.I. Mohyuddin (1992). Studies of host plant preference of *Cotesia flavipes* and important parasitoid of graminaceous stalk borers. Pakistan J. Zool. 24(4): 373-376.

Shanower, T., F. Schulthess, S. Gounou (1991). Distribution and abundance of some stem and cob borers in Benin. Plant Health Management Research Monograph No.1 , International Institute of Tropical Agriculture, Ibadan, Nigeria, 18 pp.

Simmonds, F. J. (1969). Report of work carried out during 1969. Commonw. Inst. Biol. Contrl. 103 pp.

Simmonds, F. J. (1974). Second brief resume of successes achieved. Comm onw. Inst. Biol. Contrl. 31 pp.

Singer, M.C. (1986). The definition and measurement of oviposition preference in plant-feeding insects. In Insect-Plant Interactions. J.R. Miller

and T.A. Miller (eds). Springer-Verlag, New York pp 65-94.

Sithole, S.Z. (1989). Sorghum stem borers in southern Africa. Proceedings of the International Workshop on Sorghum Stemborers, ICRISAT Centre, Patancheru, India, 17-20 November 1987, pp. 41-47.

Skoroszewski, R. W., and H. Van Hamburg (1987). The release of *Apanteles flavipes* (Cameron) against stalk borers of maize and grain sorghum in South Africa. J. Ent. Soc. South Afr. 50 (1): 249-255.

Smilowitz, Z. (1974). Relationships between the parasitoid *Hyposoter exiguae* (Viereek) and cabbage looper, *Trichoplusia ni* (Hubner): evidence for endocrine involvement in successful parasitism. Ann. Entomol. Soc. Am. 67: 317-320.

Smith, H.O. (1943). Laboratory rearing of *Microbracon vesticida* Vier. on the bean weevil, with notes on the life history of the parasite. J. econ. Entomol. 36: 101-104.

Snedecor, G.W., and W.G. Cochran (1967). Statistical methods. Second Edition. The Iowa State University Press, USA. pp 327-328.

Sokal, R.R., and F.J. Rohlf (1981). Replicated tests of goodness of fit. In Biometry. The principles and practice of statistics in biological research. Second edition. Freeman and Company. New York. pp.721-730.

Steinberg, S., Dicke M., and L.E.M. Vet (1993). Relative importance of infochemicals from first and second trophic level in long-range host location by larval parasitoid *Cotesia glomerata*. J. Chem. Ecol. 19: 47-59.

Steinberg, S. Dicke M.,Vet L.E.M. and R. Wanningen (1992). Response of braconid *Cotesia* (= *Apanteles*) *glomerata* to volatiles infochemicals: effect of bioassay set-up, parasitoid age experience and barometric flux. Entomol. Exp. Appl. 63: 163-175.

Stolz, D.B. and D. Guzo (1986). Apparent haemocytic transformation associated with parasitoid induced inhibition of immunity in *Malacosoma disstria* larvae. J. Ins. Physiol. 32: 377-388.

Stolz, D.B., P. Krell, M.D. Summer, and S.B. Vinson (1984). Polyviridae: a proposed family of insect viruses with segmented, double-stranded, circular DNA genome. Intervirology 21: 1-4.

Strand, M.R. and T. Noda (1991). Alterations in the haemocytes of *Pseudoplusia includeus* after parasitism by *Microplitis demolitor*. J. Ins. Physiol. 37: 839-858.

Strand, M.R. and E.A. Wong (1991). The growth and role of *Microplitis demolitor* teratocytes in parasitism of *Pseudoplusia includeus*. J. Ins. Physiol. 37: 503-515.

Struble, D.L. and H. Arn (1984). Combined gas Chromatography and Electroantennogram recording of insect olfactory responses. In: Techniques in pheromone research. H.E. Hummel and T.A. Miller (Eds). Springer-Verlag, New York. pp. 161-178.

Subba Rao, B.R., R.N. Singh, J.D. Saxena and A.K. Sharma (1969). Bionomics of *Apanteles flavipes* (Cameron) a parasite of *Chilo zonellus*

(Swinhoe) at Dehli with special reference to the mode of overwintering of the parasite. Ind. J. Entomol. 31: 7-12.

Swaine, G. (1957). The maize and sorghum stalk borer *Busseola fusca* Fuller in peasant agriculture in Tanganyaka territory. Bull. Entomol. Res. 48: 711-722.

Takabayashi, J. and S. Takahashi (1986). Effect of pheromone in the host searching behavior of *Apanteles kariyai* Watanabe (Hymenoptera: Braconidae) a parasitoid of the common armyworm *Pseudoletia separata* Walker (Lepidoptera: Noctuidae) II isolation and identification of arrestants produced by host larvae. Appl. Entomol. zool. 21: 114-118.

Takabayashi, J. and S. Takahashi (1990). An allelochemical elicits arrestment in *Apanteles Kariyai* in feces of nonhost larvae *Acantholecania loreyi*. J. Chem. Ecol. 16(6): 2009-2017.

Takabayashi, J., and M. Dicke (1992). Response of predatory mites with different rearing histories to volatiles of uninfested plants. Emtomol. Exp. Appl. 64: 187-193

Takabayashi, J., M. Dicke , and M.A. Posthumus (1991). Induction of indirect defence against spider-mites in uninfested Lima bean leaves. Phytochemistry. 30: 1459-1462.

Takabayashi,J., M. Dicke, and M.A. Posthumus (1994). Volatile herbivore-induced Terpenoids in plant-mite interactions: variation caused by biotic and abiotic factors. J. Chem. Ecol. 20(6): 1329-1354.

Takasu, K. and Y. Hirose (1993). Host acceptance behavior by the host-feeding egg parasitoid *Oenocyrtus nearae* (Hymenoptera: Encyrtidae) host age effects. Ann. Entomol. Soc. Am. 86(1): 117-121.

Tams, W.H.T. (1932). New species of African Heterocera. Entomologist, 65: 1241-1249.

Tanaka, T. (1987). Morphological changes in the haemocytes of the host, *Pseudoletia separata* parasitized by *Microplitis mediator* or *Apanteles kanyai*. Dev. Comp. immunol. 11: 57-63.

Thurston, R. and P.M. Fox (1972). Inhibition by nicotine of emergence of *Apanteles congregatus* from its host, the tobacco hornworm. Ann. Entomol. Soc. Am. 65: 547-550.

Tillman, P.G., M.L. Laster, and J.E. Powell (1993). Development of the endoparasitoids *Microplitis croceipes, Microplitis demolitor* and *Cotesia kazak* (Hymenoptera: Braconidae) on *Helicoverpa zea* and *H. armigera* (Lepidoptera: Noctuidae). J. Econ. Entomol. 86(2): 360-362.

Torto, B., D. Obeng-Ofori, P.G.N. Njagi, A. Hassanali and H. Amiani (1994). Aggregation pheromone system of adult gregarious desert locust *Schistocerca gregaria* (Forskal). J. Chem. Ecol. 20(7): 1749-1762.

Tumlinson, J.H., T.C.J. Turlings and W.J. Lewis (1992). The semiochemical complexes that mediate insect parasitoid foraging. Agric. zool. Rev 5: 221-252.

Turlings , T.C.J., and J.H. Tumlinson (1991). Do parasitoids use herbivore-

induced plant chemical defenses to locate hosts? Fla. Entmol. 74: 24-50.

Turlings, T.C.J., and J.H. Tumlinson (1992). Systemic release of chemical signals by herbivore-injured corn. Proc. Natl. Acad. sci. 89: 83-99

Turlings, T.C.J., J.H. Tumlinson, W.J. Lewis and L.E.M. Vet (1989). Beneficial arthropod behavior mediated by airbone semiochemicals. VIII Learning of host related odors induced by a brief contact experience with host-by-products in *Cotesia marginiventris* (Cresson), a generalist larval parasitoid. J. Insect behav. 2: 217-225.

Turlings, T. C. J., Tumlinson J. H. and Lewis W. J. (1990b). Exploitation of herbivore induced plant odors by host-seeking parasitic wasps. Science. 250: 1251-1253

Turlings, T.C.J., J.W.A. Scheepmaker, L.E.M. Vet, J.H. Tumlinson, and J.W. Lewis (1990a). How contact foraging experiences affect preferences for host-related odors in larval parasitoid *Cotesia marginiventris* (Cresson) (Hymenoptera: Braconidae). J. Chem. Ecol. 16: 1577-1589.

Turlings, T. C. J., Tumlinson J. H., F. J. Eller and Lewis, W.J. (1991a). Larval-damaged plants: source of volatile synomone that guide the parasitoid *Cotesia marginiventris* to the micro-habitat of its hosts. Entomol. Exp. Appl. 58: 75-82.

Turlings, T. C. J., J.H. Tumlinson, R.R. Heath, A.T. Proveaus, and R.E. Doolittle (1991b). Isolation and identification of allelochemicals that attract the larval parasitoid, *Cotesia marginiventris* (Cresson) to the microhabitat of

one of its hosts. J. Chem. Ecol. 17: 2235-2251.

Turlings, T.C.J., P.J. McCall, H.T. Alborn, and J.H. Tumlinson, (1993). An elicitor in caterpillar oral secretions that induces corn seedlings to emit chemical signals attractive to parasitic wasps. J. Chem. Ecol. 19(3): 411-425.

Ullyett, G. C. (1935). Notes on *Apanteles sesamiae* Cam., a parasite of the maize stalk-borer (*Busseola fusca* Fuller) in South Africa. S. Afri. Bull. Entomol. Res. 26: 253-262.

Van Alphen, J.J.M., and L.E.M. Vet (1986). An evolutionary approach to host finding and selection. In: Insect Parasitoids. J. Waage and D. Greathead (eds.). Academic Press, new York. pp. 23-61.

Van Emden H.F. (1986). The interaction of plant resistance and natural enemies: Effects on populations of sucking insects. In: Interactions of the plant resistance and parasitoids and predators of insects. D.J. Boethel and R.D. Eikenbary (eds). Ellis Horwood, Chichester. pp. 138-150.

Vet, L.E.M. (1985). Olfactory microhabitat location in some eucoilid and alysiine species (Hymenoptera), larval parasitoid of Diptera. Neth. J. Zool. 35: 720-730.

Vet, L.E.M., and A.W. Groenewold (1990). Semiochemical and learning in parasitoids. J. Chem Ecol. 16: 3119-3135.

Vet, L.E.M., and M. Dicke (1992). Ecology of infochemical use by natural enemies in a tritrophic context. Ann. Rev. Entomol. 37: 141-172.

Vet, L.E.M., F.L. Wackers and M. Dicke (1991). How to hunt for hiding hosts: the reliability-detectability problem in foraging parasitoids. Neth. J. Zool. 41: 202-213.

Vet, L.E.M., J.C. Van Lenteren, M. Heymans and E. Meelis (1983). An air flow olfactometer for measuring olfactory responses of hymenopterous parasitoids and other small insects. Physiol. Entomol. 8: 97-106.

Vet, L.E.M., C.J. Janse, C. Van Achterberg, and J.J.M. van Alphen (1984). Microhabitat location and niche segregation in two sibling species of drosophilid parasitoids; *Asobara tabida* (Nees) and *A. eufescens* (Foerster). Oecologia, 61: 182-188.

Vinson, S.B. (1972). Factors involved in successful attack on *Heliothis virescens* by the parasitoid *Cardiochiles nigriceps*. J. Invert. Pathol. 20: 118-123.

Vinson, S.B. (1975). Source of material in the tobacco budworm involved in host recognition by the egg-larval parasitoid, *Chelonus texanus*. Ann. ent. Soc. Amer., 68: 381-384.

Vinson, S.B. (1976). Habitat selection by insect parasitoids. Ann. Rev. Entomol. 21: 109-134.

Vinson, S.B. (1977). *Microplitis croceipes* Inhibition of the *Heliothis zea* defense reaction to *Cardiochiles nigriceps*. Exp. Parasitol. 41: 112-117.

Vinson, S.B. (1981). Habitat location. In Semiochemicals: Their Role in Pest Control. D.A.Nordlund, R.L.Jones, and W.J. Lewis (eds.). John Wiley and Sons, New York 306pp.

Vinson, D.B. (1985). The behaviour of parasitoids. In Comprehensive insect physiology, biochemistry, and pharmacology. G.A. Kerkut and L.I. Gilbert eds. Pergamon Press. New York. pp 417-469.

Vinson, S.B. (1988). Comparison of host characteristics that elicit host recognition behavior of parasitoid hymenoptera In: Advances in parasitic hymenoptera reseach. V. Gupta (ed.) E.J. Bull. Pub., Amsterdam. pp.285-29.

Vinson, S.B. (1990). How parasitoid eal with the immune system of their host. An overview. Arch. Ins. Biochem. physiol. 13: 3-27.

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

Vinson, S.B. (1993). Suppression of the insect immune system by parasitic hymenoptera. In: Insect immunity. J.P.N. Pathak (ed.). Oxford & IBH Publishing CO. PVT. LTD. India. pp.171-187.

Vinson, S.B. and P. Srota (1979). Effects of superparasitism by a solitary endoparasitoid on the host, parasitoid and field sampling. Southwest Entomol. 3: 299-303.

Vinson, S.B. and G.F. Iwantsch (1980). Host suitability for insect parasitoids. Ann. Rev. Entomol. 25: 397-419.

Vinson, S.B., and H.J. Williams (1991). Host selection behavior of *Campoletis sonorensis*: a model system. Bio. cont. 1: 107-117.

Vinson, S.B., R.L. Jones, P. Sonnet, B.A. Beire, and M. Beroza (1975). Isolation, identification and synthesis of host seeking stimulants for *Cardiochiles nigriceps*, a parasitoid of the tobacco budworm. Entomol.

Exp. Appl., 18: 443-450.

Visser, J.H. (1986). Host odor perception in phytophagous insects. Ann. Rev. Entomol. 31: 121-144.

Visser, J.H., S.V. Straten and H. Maarse (1979). Isolation and identification of volatiles in the foliage of potato, *Solanum tuberosum*, a host plant of the Colorado beetle *Leptinotarsa decemlineata*. J. Chem. Ecol. 5(1): 13-25.

Waage, J. (1990). Ecological theory and the selection of biological control agents. In: Crital issues in biological control. M. Mackauer, L.E. Ehler and J. Roland (eds). Intercept, Andover Hants.

Walker, P.T. (1961). Insecticide studies on the maize stalk borer, *Busseola fusca* Fuller, in East Africa. Bull. Entomol. Res. 51: 321-351.

Warui, C. M., and J. N. Kuria (1983). Population incidence and the control of maize stalk-borers *Chilo partellus* (Swinh.) *C. Orichalcociliellus* Strand and *Sesamia calamistis* Hmps. in coast province Kenya. Insect Sci. Appl. 4(12): 11-18.

Weseloh, R.M. (1974). Host-related microhabitat preferences of the gypsy moth larval parasitoid, *Parasetigena agilis*. Environ. Entomol. 3: 363-364.

Weseloh, R.M. (1981). Host location by parasitoids. In Semiochemicals: Their Role in Pest Control. D.A. Nordlund, R.L. Jones, and W.J. Lewis (eds.). John Wiley and Sons, New York pp. 79-95.

Whealey, P.E. (1961). The insect pests of agriculture in the Coast province Kenya. V. maize and sorghum. E. Afr. Agr. For. Jour. 27: 105-107.

Whitman, D.W. (1988). Allelochemicals interactions among plants, herbivores and their predators. In Novel aspects of Insect-Plant Interactions. Barbosa P. and Letourneau D.K. (eds.). John Riley. New York, pp 11-64.

Whitman, D.W., and F.J. Eller (1990). Parasitic wasps orient to green leaf volatiles. Chemoecology 1: 69-75.

Whittaker, R.N. (1970). Communities ecosystems. MacMillan, New York.

Wiedenmann, R. N., and J. W. Smith Jr. (1994). Parasitization of *Diatraea saccharalis* by *Cotesia chilonis* and *Cotesia flavipes*. Entomol. Exp. Appl. (In press).

Wiedenmann, R. N., J. W. Smith, Jr. and P.O. Darnell (1992). Laboratory rearing and biology of the parasite *Cotesia flavipes* (Hymenoptera: Braconidae) using *Diatraea saccharalis* (Lepidoptera:Pyralidae) as a host. Environ. Entomol. 21: 1160-1167.

Williams, H.J., G.W. Elzen and S.B. Vinson (1988). Parasitoid host-plant interactions, emphasizing cotton (Gossypium). In: Novel aspect of insect-plant interactions. P. Barbosa and O.K. Letourneau (eds). John Wiley, New York. pp. 171-200.

Wohlers, P. (1981). Effects of the alarm pheromone (E)-β-farnesene on the behavior of the pea aphid *Acyrthosiphon pisum*. Entomol. Exp. Appl. 9: 117-124.

Wylie, H.G. (1966). Some mechanisms that affect the sex ratio of *Nasonia vitripennis* (Walk.) reared from superparasitized house fly pupae. Can.

Entomol. 98: 645-653.

Youm, O., H.W. Browning and E.F. Gilstrap (1988). Population dynamics of stalk borers attacking corn and sorghum in the Texas Rio Grande Valley. Southwest. Entomol. 13: 199-204.

Young, W.R., and G.L. Teetes (1977). Sorghum entomology. Ann. Rev. Entomol. 22: 193-218.

Zhody, N. (1976). On the effect of the food of *Myzus persicae* sulz. On the hymenopterous parasite, *Aphelinus asychis* Walker. Oecologia. 26: 185-191.

Ziser, S.W., J.A. Wojtowicz, and C.Jr. Nettles (1977). The effects of the number of maggots per host on length of development, puparial weight and adult emergence of *Encelatoria* sp. Ann. Entomol. Soc. Am. 70: 733-736.

APPENDICES

Appendix 1 Response of C. flavipes odours from uninfested plant in a Y-tube olfactometer

Odour source	N	Choice	No choice	G-value
Maize Clean air	60	38 9	13	19.05***
Sorghum Clean air	80	48 6	26	36.85***
Napier grass Clean air	90	69 7	14	58.25***

*** = P<0.005; ** = P<0.01; * = P<0.05; ns = not significant.

Appendix 2 Response of *C. sesamiae* to odours from uninfested plant in a Y-tube olfactometer

Odour source	N	Choice	No choice	G-value
Maize Clean air	65	48 13	4	21.19***
Sorghum Clean air	60	45 4	11	39.81***
Napier grass Clean air	60	41 6	13	28.95***

*** = P<0.005; ** = P<0.01; * = P<0.05; ns = not significant

Appendix 3 Response of *C. flavipes* to uninfested plants in a two choice experiment

Odour source	N	Choice	No choice	G-value
Maize Sorghum	60	41 15	4	12.44***
Maize Napier grass	90	39 39	12	0.00ns

*** = P<0.005; ** = P<0.01; * = P<0.05; ns = not significant

Appendix 4 Response of *C. sesamiae* to uninfested plants in a two choice experiment

Odour source	N	Choice	No choice	G-value
Maize Sorghum	60	19 36	5	5.29*
Maize Napier grass	90	13 41	26	15.11***

*** = P<0.005; ** = P<0.01; * = P<0.05; ns = not significant

Appendix 5 **Response of *C. flavipes* in the Y-tube olfactometer to cowpea plant**

Odour source	N	Choice	No choice	G-value
Cowpea Clean air	58	17 25	16	1.52ns
Cowpea Maize	57	28 21	8	0.99ns

*** = P<0.005; ** = P<0.01; * = P<0.05; ns = not significant.

Appendix 6 **Response of *C. flavipes* to infested plants versus uninfested maize (control)**

Odour source	N	Choice	No choice	G-value
Maize with *C. partellus* Control	60	34 16	10	6.56*
Maize with *C. orichalcociliellus* Control	80	52 10	18	30.92***
Maize with *S. calamistis* Control	80	59 8	13	43.55***

*** = P<0.005; ** = P<0.01; * = P<0.05; ns = not significant.

Appendix 7 Response of *C. sesamiae* to infested plants versus uninfested maize (control)

Odour source	N	Choice	No choice	G-value
Maize with *C. partellus* Control	60	44 10	6	22.90***
Maize with *C. orichalcociliellus* Control	60	43 10	7	21.93***
Maize with *S. calamistis* Control	60	38 15	7	10.22***
Maize with *B. fusca* Control	60	49 7	4	35.12***

*** = P<0.005; ** = P<0.01; * = P<0.05; ns = not significant.

Appendix 8 Response of *C. flavipes* to infested versus infested maize

Odour source	N	Choice	No choice	G-value
Maize with *C. partellus* Maize with *C. orichalcociliellus*	90	39 30	21	1.17ns
Maize with *C. partellus* Maize with *S. calamistis*	76	18 44	14	11.16***

*** = P<0.005; ** = P<0.01; * = P<0.05; ns = not significant.

Appendix 9 **Response of *C. sesamiae* to infested versus infested maize**

Odour source	N	Choice	No choice	G-value
Maize with *C. partellus* Maize with *C. orichalcociliellus*	60	23 29	8	0.69ns
Maize with *C. partellus* Maize with *S. calamistis*	60	14 38	8	11.40***

*** = P<0.005; ** = P<0.01; * = P<0.05; ns = not significant.

Appendix 10 **Response of *C. flavipes* to maize plants infested with different stemborer densities**

Odour source	N	Choice	No choice	G-value
2 *C. partellus* 10 *S. calamistis*	60	9 45	6	25.96***
10 *C. partellus* 2 *S. calamistis*	100	62 31	7	10.48***
5 *C. partellus* 5 *S. calamistis*	130	46 63	21	2.65ns

*** = P<0.005; ** = P<0.01; * = P<0.05; ns = not significant.

Appendix 11 **Response of *C. sesamiae* to maize plants infested with different stemborer densities**

Odour source	N	Choice	No choice	G-value
2 *C. partellus* 2 *B. fusca*	55	10 44	1	22.90***
10 *C. partellus* 2 *B. fusca*	60	38 13	9	12.68***
10 *C. partellus* 2 *S. calamistis*	54	23 23	8	0.00ns

*** = P<0.005; ** = P<0.01; * = P<0.05; ns = not significant.

Appendix 12 **Response of *C. flavipes* to infested cowpea plants versus infested maize**

Odour source	N	Choice	No choice	G-value
Infested maize Uninfested cowpea	60	41 7	12	26.39***
Infested maize Infested cowpea	60	42 4	14	36.20***

*** = P<0.005; ** = P<0.01; * = P<0.05; ns = not significant.

Appendix 13 ***C. flavipes*** **response to frass obtained from different stemborer species fed on maize, sorghum, and Napier grass.**

odour 1	Odour 2	N	Choice 1	Choice 2	G-value
FMCP	FMBF	59	41	18	9.13
FMCP	FMSC	59	23	36	2.86
FMCP	FMCO	66	37	29	0.97
FMCO	FMSC	60	26	34	1.06
FMBF	FMCO	62	16	46	15.02
FMBF	FMSC	60	25	35	1.67
FMSC	AIR	48	34	14	8.50
FNSC	AIR	48	35	13	10.36
FSSC	AIR	48	36	12	12.42
FMCP	AIR	48	39	9	20.01
FNCP	AIR	48	35	13	10.36
FSCP	AIR	48	36	12	12.43
FMCO	AIR	48	42	6	30.06
FNCO	AIR	48	45	3	43.64
FSCO	AIR	48	38	10	17.24
FMBF	AIR	48	37	11	14.72
FNBF	AIR	48	35	13	10.36
FSBF	AIR	48	33	15	6.85

*** = P<0.005; ** = P<0.01; * = P<0.05; ns= not significant.

FMSC: frass obtained from *S. calamistis* fed on maize

FNSC: frass obtained from *S. calamistis* fed on napier grass

FSSC: frass obtained from *S. calamistis* fed on sorghum

FMCP: frass obtained from *C. partellus* fed on maize

FNCP: frass obtained from *C. partellus* fed on napier grass

FSCP: frass obtained from *C. partellus* fed on sorghum

FMCO: frass obtained from *C. orichalcociliellus* fed on maize

FNCO: frass obtained from *C. orichalcociliellus* fed on napier grass

FSCO: frass obtained from *C. orichalcociliellus* fed on sorghum

FMBF: frass obtained from *B. fusca* fed on maize

FNBF: frass obtained from *B. fusca* fed on napier grass

FSBF: frass obtained from *B. fusca* fed on sorghum

Appendix 14 ***C. sesamiae*** **response to frass obtained from different stemborer species fed on maize, sorghum, and Napier grass.**

odour 1	Odour 2	N	Choice 1	Choice 2	G-value
FMCP	FMBF	40	21	19	0.10
FMCP	FMSC	40	19	21	0.10
FMCP	FMCO	37	25	15	2.50
FMCO	FMSC	40	15	22	1.32
FMBF	FMCO	40	25	15	2.50
FMBF	FMSC	40	21	19	0.10
FMSC	AIR	39	31	8	14.49
FNSC	AIR	39	28	11	7.57
FSSC	AIR	39	27	12	5.85
FMCP	AIR	38	31	7	16.16
FNCP	AIR	36	24	12	4.02
FSCP	AIR	36	25	11	5.51
FMCO	AIR	40	29	11	8.30
FNCO	AIR	39	31	8	14.30
FSCO	AIR	37	29	8	12.49
FMBF	AIR	52	23	10	20.97
FNBF	AIR	52	39	13	13.48
FSBF	AIR	52	41	11	18.25

*** = P<0.005; ** = P<0.01; * = P<0.05; ns = not significant.

Appendix 15 ***C. flavipes*** **response to odours collected from maize seedlings uninfested (UNINFES) and infested (PHC-L) with** ***C. partellus*** **larvae in a T-tube olfactometer.**

	Positive response	Negative response	G-Values
PHC-L	54	6	43.80 **
UNDAM	42	18	9.79 *

*** = P<0.005; ** = P<0.01; * = P<0.05; ns = not significant.

Appendix 16 ***C. flavipes*** **response to odours collected from two to three months old maize plants infested with** ***C. partellus*** **larvae in a Y-tube olfactometer.**

DOSE IN CME	POSITIVE RESPONSE	NEGATIVE RESPONSE	NO RESPONSE	G-VALUES
10	27	2	7	25.21**
30	24	4	8	15.57**
100	25	1	10	27.05**
300	22	2	12	19.10**
1000	27	3	6	21.72**

*** = P<0.005; ** = P<0.01; * = P<0.05; ns = not significant.

Appendix 17 **Total haemocyte count of parasitized and unparasitized larvae 1, 2, 4, 8, 14, 24 hours after parasitization**

		Mean counts (± se) x 10^6 cell/ml					
Hours	N	Unparasitized larvae	N	Parasitized larvae	DF	F-value	P
1	20	32.3 ± 3.7	19	32.6 ± 3.1	4,34	0.15	0.70
2	23	33.1 ± 2.3	24	42.2 ± 3.8	5,41	3.18	0.06
4	24	28.7 ± 3.6	26	36.3 ± 3.1	5,44	4.66	0.04*
8	18	30.1 ± 2.3	19	35.8 ± 4.2	4,34	0.43	0.52
14	19	31.3 ± 2.9	20	39.1 ± 3.8	4,34	2.53	0.12
24	20	38.1 ± 4.5	19	38.1 ± 2.9	4,34	0.26	0.61

Appendix 18 **Total haemocytes count of parasitized and unparasitized larvae one to six days after parasitization**

	Mean counts (± se) x 10^6 cell/ml					
Days	N	Unparasitized larvae	N	Parasitized larvae	DF	F-value	P
1	40	32.3 ± 2.6	41	35.3 ± 2.3	6,74	1.91	0.17 ns
2	39	27.4 ± 2.1	41	41.6 ± 2.8	6,73	19.02	0.0001
3	42	30.8 ± 2.3	44	51.8 ± 4.5	6,79	29.70	0.0001
4	45	32.1 ± 2.4	42	55.3 ± 4.2	6,80	21.05	0.0001
5	45	28.9 ± 2.4	40	57.7 ± 4.7	6,78	28.82	0.0001
6	35	35.4 ± 2.6	31	54.8 ± 4.4	5,60	13.29	0.0006

ns = not significant