Enhancing foraging behaviour of stemborer parasitoids: role of a non-host plant *Melinis minutiflora*

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Enhancing foraging behaviour of stemborer parasitoids: role of a non-host plant *Melinis minutiflora*

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Contents

Abstract

Stemborers are major pests of maize and sorghum in tropical Africa. Various management approaches to stemborer control are being sought with emphasis on those that are environmentally sound, sustainable and economically feasible. Studies reported in this thesis were conducted to explore the potential of employing habitat management techniques to enhance the effectiveness of stemborer parasitoids. Emphasis was placed on the chemical and behavioural ecology of stemborer parasitoids in a diversified system that consisted of the stemborer host plants, maize and sorghum, stemborers and a non-host plant, molasses grass (*Melinis minutiflora*).

Olfactometric studies on the stimuli involved in long-range foraging behaviour of the parasitoids *Cotesia sesamiae* and *Dentichasmias busseolae* revealed that host plant volatiles play a major role in guiding searching parasitoids to habitats that harbour their hosts. Volatiles from herbivore-injured plants were the most attractive. Sorghum was more attractive to the parasitoids than maize. Molasses grass volatiles were attractive to *C. sesamiae* but repellent to *D. busseolae*. Combining host plants and molasses grass did not have an additive effect of increasing the response of the parasitoids. Local growth conditions influenced the volatile blend produced by molasses grass grown in two different locations in Kenya, Thika and Mbita. This was manifested by their differential attractiveness to *C. sesamiae*. *Dentichasmias busseolae* did not discriminate between host species at a distance based on the volatiles released by infested host plants. Headspace analyses of volatiles emitted by uninfested and infested host plants and molasses grass grown at Thika and Mbita, revealed qualitative differences in their compositions. Infested host plants released a richer volatile blend than the uninfested host plants. Most of the compounds identified in the herbivore-injured plants were previously reported to play a role in parasitoid recruitment. Molasses grass from Thika had a number of its identified compounds that were similar to some in the blend of infested host plants. These compounds might have played a role in attracting *C. sesamiae*. Molasses grass from Mbita had very few compounds common to the other plants.

Semi-field studies on the close-range searching behaviour of *C. sesamiae* showed that the parasitoid was not arrested by molasses grass although the grass had previously been demonstrated to be attractive to this parasitoid. The presence of molasses grass in a patch with host plants did not interfere with the foraging behaviour of *D. busseolae*. Both parasitoids recognized host plants at close range and were arrested on infested host plants where they spent most of their time engaged in host searching activities. Field studies showed that stemborer densities were lower in the intercrop than in the monocrop, while diversifying the habitat with molasses grass had no effect on stemborer parasitism. Larval and pupal parasitism was 2.1 and 11.0% in the monocrop compared to 2.0 and 9.8% in the intercrop.

The main conclusion of this thesis is that intercropping maize or sorghum with molasses grass does not enhance the foraging behaviour of stemborer parasitoids. Molasses grass seems to be of more importance to the herbivore than the parasitoids. Hence, to clearly understand why stemborer densities were lower in the intercrop than the monocrop, future studies should focus on the interrelations between molasses grass, stemborers and host plants.

Preface

This PhD research project focused on enhancing biological control of stemborers. It was supported by funds from MHO/NUFFIC of the Netherlands under a collaborative project between Wageningen University and Moi University, Kenya for staff development and capacity building. I acknowledge many individuals and institutions that helped in various ways, without whom this thesis would never have come to fruition.

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Linnet S. Gohole　　Wageningen, March 2003

Many-a-time did I have to prematurely terminate a maize-harvesting session on my parents' 'shamba' because of encountering a stemborer (dudu), little did I know they would be the center of my tertiary academics.

1

General Introduction

Chapter 1

General introduction

Background

In tropical Africa, many agricultural systems are based on cereals such as maize, sorghum, wheat, rice and millet. These are the staple food crops for the majority of the human populations living in this area (FAO, 2002). The crops are almost exclusively grown by smallholder farmers. Full production of these crops is not realised because of various constraining factors. Of considerable concern is damage by lepidopteran stemborers. Infestation by this pest is throughout the crops' growth, from the seedling stage to maturity. Estimates of cereal yield losses due to stemborers vary greatly, but are generally between 20 - 40% of the potential yield (Seshu Reddy & Walker, 1990). A complex of 21 stemborer species in three families, Noctuidae, Pyralidae and Crambidae, attack cereal crops in various parts of Africa (Maes, 1998). Some of the economically important borers include the spotted stemborer, *Chilo partellus* (Swinhoe), the African maize stemborer, *Busseola fusca* Fuller, the pink stemborer, *Sesamia calamistis* Hampson and the sugarcane stemborer, *Eldana saccharina* (Walker).

Pest management

The four general management approaches to stemborer control include chemical control, biological control, cultural methods and host plant resistance (Mugo et al., 2001). Chemical control is effective, but application needs precise timing before larvae bore into the stem and escape exposure to the chemicals. The chemicals also pose a health risk to the users due to lack of appropriate dispensing facilities and limited training. Moreover, the use of chemicals is rarely justified in smallholder systems because of high costs (Abate et al., 2000). Host plant resistance holds promise (Mugo et al., 2001). However, the challenge faced may be in the production of maize and sorghum varieties that combine high yielding ability, drought tolerance, and resistance/tolerance to insect pests and disease. In addition, it is quite a task to produce varieties that perform well over the wide variation in agroecologies in which these crops are grown. Transgenic (*Bt*) maize with resistant factors against herbivores is widely grown in many parts of the world, especially the United States of America. Research on development of *Bt* maize stemborers in Africa is still in its formative stages but the approach might be widely adopted with time based on the findings of detailed research (Mugo et al., 2001). Classical biological control is also receiving some attention, however it most often works against introduced pests and *C. partellus* is the only exotic stemborer in Africa. Historically only about 25% of all attempts to introduce natural enemies have been successful (Waage, 1990). Cultural practices such as destruction of crop residues, planting date manipulation, destruction of volunteer and alternative plants and intercropping are known to lessen the severity of stemborer damage on crops. They are the most relevant and economical methods of stemborer control among resource-poor farmers. However, these practices are not widely practised due to labour constraints and also to the many alternative uses of the crop residues such as use of stems as firewood or for fencing purposes (Kfir, et al., 2002). Integrated pest management (IPM) seems to be the best option to combat the pests and has received research attention during the past three decades (Zethner, 1995). The arena is still open for improvement and development of other novel techniques in the fight against stemborers.

Indigenous natural enemies

Stemborers in Africa are attacked by a diverse group of natural enemies (Mohyuddin & Greathead, 1970; Bonhof, 1997; Kfir, 1997; Polaszek, 1998). Indigenous predators are reported to play a minor role in keeping stemborer populations below economic injury levels (Bonhof, 2000). Records of nematodes and pathogens affecting African stemborers have recently been summarised (Poinar & Polaszek, 1998). Their impact under field conditions is generally low (Odindo et al., 1989). The few detailed and comprehensive surveys on stemborer parasitoids, point out the importance of these parasitoids in borer regulation but their effectiveness in keeping borer populations below acceptable injury thresholds is low (Oloo & Ogeda, 1990, Overholt, et al., 1994). Habitat management has been postulated as one way to enhance the activity of such parasitoids. Habitat management is an ecologically based control approach in which the agroecosystem is selectively manipulated to create environmental conditions that reduce pest pressure on crops and enhances effectiveness of natural enemies (Landis et al., 2000). Research on habitat management techniques to reduce stemborer populations is still in its infancy, but is gaining momentum in several areas in Africa (Khan et al., 2000).

Habitat management

One practise of habitat management is vegetational diversification of the habitat. This can be achieved through intercropping, undersowing or border cropping (Coll, 1998). The mechanisms involved in pest suppression in diversified habitats remain obscure, but studies have commonly, though not universally, found that populations of herbivorous insects reach higher levels in simple than in diversified agroecosystems (Risch et al., 1983, Altieri & Letourneau, 1984, Russel, 1989, Vandermeer, 1989, Andow, 1991). Root (1973) formulated two hypotheses to explain the difference in herbivore populations in simple and diverse habitats. The 'resource concentration hypothesis' states that herbivores, particularly specialists, more easily locate and remain in taxonomically pure plant assemblages than in diverse ones. The 'natural enemies hypothesis' states that there is a greater abundance and diversity of natural enemies in diversified habitats, which consequently lower herbivore populations. Another theory that was recently put forward to explain lower herbivore load in diverse habitats, is the 'appropriate/inappropriate landings' theory (Finch and Collier, 2000). This theory states that non-host plants in close proximity to host plants disrupt the host finding and acceptance behaviour of insects.

Increased vegetational diversity within agricultural fields augments natural enemies primarily through provision of supplemental resources such as pollen, nectar, refuges, favourable microclimates, alternative prey (Barbosa & Benrey, 1998) and allelochemical attraction (Verkerk et al., 1998). The resources must be integrated into the agroecosystem in a way that it is spatially and temporally favourable to natural enemies. There are several studies that have investigated the effects of intercropping on the abundance and effectiveness of natural enemies. Baggen & Gurr (1998) showed that the availability of nearby sources of nectar for the egg parasitoid *Copidosoma koehleri* Blanchard (Hymenoptera: Encyrtidae) translated to higher parasitism rates of its host, the potato moth *Phthorimaea operculella* Zeller (Lepidoptera: Gelechiidae). Girma et al. (2000) recorded higher catches of parasitic wasps in plots with hedgerows than those without. They attributed the observation to the provision of favourable microclimate (shade). Murphy et al. (1998) showed that diversification of vineyards with prune trees provided refuge for overwintering populations of the parasitoid *Anagrus epos* (Girault) (Hymenoptera: Mymaridae), favouring enhancement of parasitism in the grape leafhopper *Erythroneura elegantula* (Osborn) (Homoptera: Cicadellidae). A selected summary of studies that tested the effect of vegetation diversity in agroecosystems on abundance of natural enemies and parasitism/predation is provided in Table 1.

Plant - parasitoid relationships in diversified habitats

Considering the small size of both parasitoids and their hosts and also the structural complexity of the environments inhabited by most parasitoids, finding a suitable host appears to be a formidable task, more so in a system that has been diversified. An example is that of stemborer parasitoids whose hosts live a concealed life style (Smith et al., 1993). Parasitoids engage visual, acoustic or olfactory cues to locate their potential hosts' patches. Olfactory cues, also termed infochemicals, a subcategory of semiochemicals, are considered to be the most important (Vet & Dicke, 1992; van Alphen & Jervis, 1996). They can originate from the herbivore, from its food or from interactions between the herbivore and its food. Stimuli arising from the herbivore are the most reliable in indicating presence, suitability and accessibility of the host. However, they are not easily detected at long distances. Host plants provide more detectable cues because of their large biomass but these stimuli are far less reliable for herbivore location. This is the reliability-detectability dilemma that searching parasitoids have to solve (Vet et al., 1991; Dicke & Vet, 1999). Parasitoids go about this problem by responding to special types of plant volatiles induced after herbivory known as herbivore-induced synomones (Vet & Dicke, 1992; Dicke, 1994). Attraction to these herbivore-induced plant volatiles has been reported in several studies (see Dicke, 1999 for review). In some plant species, these volatiles have a unique odour blend whose chemical profile is different from uninfested or even mechanically damaged plants (Geervliet et al., 1994, Turlings et al., 1995). Several chemical compounds in these plant volatiles have been identified as playing a significant role in parasitoid recruitment by herbivore-injured plants (Du et al., 1998, Ngi-Song et al., 2000)

More interestingly, cases have been reported in which non-host plants play a role in producing volatiles that attract parasitoids that attack pests on host plants. Altieri et al. (1981) observed greater parasitism of *Heliothis zea* Boddie (Lepidoptera: Noctuidae) eggs by *Trichogramma* sp. (Hymenoptera: Trichogrammatidae) in soybean plots sprayed with extracts of a common weed *Amaranthus* sp. L. (Amaranthaceae) than in the control plots. It was postulated that the application of these extracts reinforced

Table 1. Examples of studies on effects of agroecosystem diversification on abundance and parasitism/predation by natural enemies of herbivorous insects.

Cropping system	Abundance or parasitism	Parasitism/predation level[a]	Likely causative mechanism	Reference
Corn/soybean (intercropping)	Parasitoid abundance	Higher	Favourable microclimate, alternative food resource	Tonhasca, 1993
Cabbage/white clover (undersowing)	Larval parasitism	Same	Not mentioned	Wiech and Jankowska, 1999
Cowpea/sorghum (intercropping)	Predator abundance	Lower	Not mentioned	Nampala et al., 1999
Maize/cowpea (intercropping)	Egg parasitism	Higher	Not mentioned	Päts et al., 1997
Soybean/grass (stripcropping)	Larval parasitism	Same	Not mentioned	Pavuk and Barret, 1993
Maize/leucaena (hedgerows)	Egg parasitism	Same/lower	Not mentioned	Ogol et al., 1998
Grape vineyards/prune trees (hedgerows)	Egg parasitism	Higher	Overwintering sites	Murphy et al., 1998
Alfalfa/grass (intercropping)	Predator abundance	Lower	Not mentioned	Degooyer et al., 1999
Cabbage/clover (intercropping)	Predation	Higher	Not mentioned	Booij et al., 1997

Rice bean/pigeon peas (intercropping)	Natural enemy abundance	Higher	Not mentioned	Satyanarayan et al., 1998
Maize/molasses grass (intercropping)	Larval parasitism	Higher	Allelochemical attraction	Khan et al., 1997a,b
Soybean/sorghum (intercropping)	Larval parasitism	Same	Not mentioned	Williams et al., 1995
Cotton/rape (relay intercropping)	Predator abundance	Higher	Refugia/reservoir	Parajulee et al., 1997
Vineyards/flowers (covercropping)	Egg parasitism Predation	Same Higher	Food resources Food resources	Nicholls et al., 2000
Cabbage/white clover (covercropping)	Pupal parasitism	Lower	Not mentioned	Langer, 1996
Bean/maize (intercropping)	Larval parasitism	Higher	Alternative prey, food resources	Abate, 1991
Potato/flowers (margin planting)	Egg-larval parasitism	Higher	Food resources	Gurr et al., 1998
Broccoli/clover (covercropping)	Aphid parasitism	Lower	Allelochemical interference	Costello and Altieri, 1995
Tomato/cabbage (intercropping)	Larval parasitism	Higher	Not mentioned	Bach and Tabashnik, 1990

[a] In intercrop compared to monocrop.

the host searching behaviour of the parasitoid, improving their retention and/or activity in soybean fields. In another study, it was observed that the sugarbeet aphid pest, *Myzus persicae* Sulzer (Homoptera: Aphididae), was heavily parasitized by its parasitoid *Diaeretiella rapae* Mc'Intosh (Hymenoptera: Braconidae) in sugar beet when grown next to collard plants Read et al. (1970). Apparently, the collard plants produced certain chemical volatiles that attracted the parasitoid. The parasitoids consequently moved across and attacked aphids on the beet. The phenomenon of non-host plants attracting parasitoids is intriguing but has not received much research attention.

Intercropping as a means of controlling stemborers

Traditional farming systems in Africa have been based on intercropping or mixed cropping (forms of habitat management) as opposed to monocropping (Nwanze, 1997, Abate et al., 2000). The advantages of this practice are better food security, optimal use of soil and space, maintenance of soil fertility, better erosion control, less weeding and pest control (Zethner, 1995). In line with this, intercropping as a means to control stemborers has been studied by many authors, with positive results (Oloo & Ogeda 1990, Omolo et al., 1993, Ampong-Nyarko et al., 1994, Skovgård & Päts 1997). Possible explanations put forward by these authors for the reduced pest incidence in intercrops include impeded movement of both gravid moths and dispersing larvae, disruption in the host plant location process and increased parasitoid and predator activity. However, the real underlying mechanisms responsible for the reductions were not well determined.

More recently, investigations on utilization of habitat management to control stemborers using a 'push-pull' or stimulo-detterent diversionary strategy (SDDS) (Pickett et al, 1997, Agelopoulos et al., 1999), were conducted. A non-host plant of stemborers, molasses grass, intercropped with maize pushed gravid stemborer moths away from intercrop plots. The moths were in turn pulled or attracted to Napier grass or Sudan grass planted at the border. The stemborer densities were therefore lower in the intercrop than in the monocrop (Khan et al., 1997a,b). Another interesting observation noted in this investigation was the significant increase in stemborer parasitism by the larval parasitoid *Cotesia sesamiae* (Cameron) (Hymenoptera: Braconidae) in intercrop plots. The attraction was attributed to molasses grass producing volatile components similar to those produced by herbivore-damaged maize plants. The compound (E)-4,8-dimethyl-1,3,7-nonatriene was particularly singled out. This is a case of a companion crop aiding in pest control by attracting parasitoids of pests attacking the major crop, maize in this case.

The system studied

In order to design agroecosystems to enhance biological control by natural enemies, a proper mechanistic understanding of the influence of plant diversity on natural enemies is essential. This allows for the making of useful predictions about multitrophic level interactions in diverse habitats. The research presented in this thesis aims at investigating the role of habitat diversification in the functioning of multitrophic interactions involved in a cereal agroecosystem with particular interest on behavioural aspects of parasitoids. The system consisted of host plants (maize and sorghum), herbivores (cereal stemborers), a larval parasitoid, *Cotesia sesamiae* and a pupal parasitoid, *Dentichasmias busseolae*, and the non-host plant of stemborers, molasses grass (*Melinis minutiflora*). The various components of the system under study are described.

Host plants- Maize and sorghum

Maize (*Zea mays* L) and sorghum (*Sorghum bicolor* L) belong to the family Poaceae. Maize is a recent introduction into Africa dating back to some 300 years ago (Acland, 1971). Since its introduction, native stemborers have quickly adapted to it. Indigenous parasitoids have also followed their hosts to maize. However, the thick stems of maize might be a hindrance to effective parasitization of stemborers especially by parasitoids that employ a 'drill and sting' strategy (Nwanze, 1997). In contrast, sorghum is a native African cereal with origins from around the Ethiopian region (Dogget, 1988). Since it shares a long evolutionary history with indigenous stemborer parasitoids, it is most likely to be more attractive to these parasitoids than maize.

Non-host plant - Molasses grass (*Melinis minutiflora*)

Melinis minutiflora Beauv. (Poaceae) is a non-host plant of stemborers (Khan et al., 2000). It is a perennial grass found mainly growing in grasslands, shady places and on hilly slopes. It is confined to an altitude range of 930-2250m. It grows as a tuft to an approximate height of one metre. It is originally from Africa, but has spread to other tropical areas in the world where it is well known as a valuable pasture and hay grass, especially in South America (Edwards & Bogdan, 1951). In East Africa, it has been of great value in re-vegetating overcropped land, since it is easy to establish. It is widely sown on relatively unproductive land in Latin America, South East Asia and Australia, doing well on steep hillsides, thus protecting the soil from erosion (Boonman, 1993). As its common name suggests, *M. minutiflora* has a distinctive sweet smell associated with a sticky excretion from the glandular hairs found on its slender leaves. It is a livestock feed, preferred when young and tender, that results in an increase in live-weight and milk production (Boonman, 1993). It is a valuable grazing grass, especially during the dry season because of its drought resistance characteristics (Fröman & Persson, 1974). Other advantages of *M. minutiflora* include anti-tick properties (Thompson et al., 1978, Mwangi et al., 1995), insecticidal properties, repellency to tsetse flies and mosquitoes (Ibrahim & Kabuye, 1987) and for thatching houses (Chiera et al., 1977).

Biology and damage by stemborers

With a few specific exceptions, the life cycles of the stemborer species are generally similar (Girling 1978; Harris, 1989; Harris, 1990; Päts, 1992). Adult moths lay eggs in clusters on the host plants. Eggs hatch into first instar larvae after 4-8 days of incubation. The larval period lasts for 25-45 days depending on the prevailing weather conditions and there are 6-8 larval instars. Early instar larvae typically migrate to the whorl of the plant where they feed on succulent young tissue. Older larvae bore tunnels in the stem where they remain until adulthood. Many of the larvae maintain relatively clean tunnels by depositing frass outside the entrance of the tunnel. Just before pupation, the mature larva constructs a pupal chamber at one end of the feeding tunnel, and chews an exit hole through the stem, leaving a thin layer of epidermal tissue for egress of the adult moth. The pupal period lasts for 7-10 days. Adults have a lifespan of 4-14 days depending on the species, during which they mate and lay eggs.

Young stemborer larvae feed on the leaves and the leaf whorls producing characteristic 'window panes' and 'pinholes'. The older larvae bore into the stem and may kill the growing point resulting into a 'deadheart'. As the larvae feed, they make tunnels inside the stem, which in turn restricts water and nutrient translocation and weakens the stem, at times leading to lodging of plants. Opportunistic microorganisms also take advantage of the tunnelling and attack the plants. Later generations of the borers attack cobs and heads, contributing to yield reduction.

Biology and host searching behaviour of parasitoids

Cotesia sesamiae (Cameron) (Hymenoptera: Braconidae)

Cotesia sesamiae is the most abundant and widespread indigenous larval parasitoid of several stemborer species in Africa (Mohyuddin 1990, Polaszek & Walker, 1991, Kfir, 1992, Overholt et al., 1994). It is a gregarious endoparasitoid that attacks mid to late instar stemborer larvae. Adults start mating immediately after emergence (sib-mating) especially in bright light. Males emerge first and wait near the cocoons for the females to emerge (Ullyet, 1935). A female's clutch size is 40-60 eggs (Potting et al., 1999, Mochiah et al., 2001). The fully developed larvae emerge from the host larva and spin white cocoons, which are loosely held together by strands of fine silk. The average egg to adult developmental time is 18 days at 25ºC (Ngi-Song et al., 1995, Mochiah et al., 2001). Longevity of the adult wasps ranges from 2 to 10 days depending on availability and type of food (Sathe et al., 2001). *Cotesia sesamiae* is attracted to its host habitat by volatiles emanating from gramineous host plants. Herbivore damage increases attractiveness of host plants to the wasp (Ngi-Song et al., 1996, Rutlegde & Wiedemann, 1999). Frass plays a role in the short-range host location (Ngi-Song & Overholt, 1997, Potting et al., 1999).

Dentichasmias busseolae Heinrich (Hymenoptera: Ichneumonidae)

Dentichasmias busseolae is a solitary pupal endoparasitoid of stemborers. Adults are about 7 – 11.5 mm long. In females, the ovipositor slightly protrudes beyond the gaster, a characteristic used to distinguish females from males. The species derives its name from two small teeth found on the apical margin of the clypeus (Zwart, 1998). Although it has been collected from several stemborer hosts, *D. busseolae* is primarily a parasitoid of *C. partellus* (Mohyddin, 1972, Oloo & Ogeda, 1990, Kfir, 1992). This is counter-intuitive as the parasitoid is African whereas *C. partellus* is originally from Asia. *Dentichasmias busseolae* may have been a parasitoid of African *Chilo* species that quickly adapted to the exotic species (Zhou et al., 2003). The life cycle of *D. busseolae* has been described by Mohyddin (1972) and Bahana (1989). Mating occurs immediately after emergence of the female and lasts for about 3 minutes. The mating behaviour of the male is controlled by a pheromone produced by a female just before emergence (Bahana, 1989). On perceiving the pheromone, males vibrate their wings rapidly and attempt to mount the pupa even before the female emerges (Bahana, 1989, Gohole personal observations). Newly emerged females are more attractive to the males than old ones. A single egg is laid in a 1-2 day old pupa, which develops through five larval instars before pupation. The adult emerges from the host pupal case by biting a circular hole at the anterior end. Developmental period takes about 16-18 days at 25ºC. Adult females live on average 36-40 days during which they can lay about 80 eggs. The sex ratio of progeny is female biased. Bahana (1989) studied some aspects of foraging behaviour of *D. busseolae*. He reported that chemical stimuli from frass played a role in short-range host location by the wasp. However, information on the cues involved in long-range attraction of this parasitoid is lacking.

Thesis aims and outline

Problem definition

The relationship between crop and non-crop plants, herbivores, natural enemies and their physical environment is receiving increasing research attention. The emerging discipline in this area is called conservation biological control. In conservation biological control, improved understanding of the multitrophic relationships is required in order to manipulate systems to enhance natural enemy activity. (Gurr et al., 1998). However, many of the habitat manipulation attempts have been intuitive rather than based on careful preliminary research. At the same time it is impossible to study all possible combinations of crop, herbivore, natural enemy and cultural practise. Therefore, there is a need for identification and careful selection of important elements of diversity that enhance natural enemies rather than encouraging vegetational diversity per se. This approach might be the key to enhancing the effect of indigenous natural enemies in the war against stemborers.

Although a number of indigenous parasitoids have been reported to attack African stemborers, their impact on pest regulation is unsatisfactory (Oloo & Ogeda, 1990, Kfir, 1992, Overholt et al., 1997). In studies to control stemborers using the 'push-pull' strategy, Khan et al. (1997a,b) recorded a decrease in the stemborer population in managed habitats. Higher stemborer parasitism by *C. sesamiae* in a maize-molasses grass intercrop was also noted. The higher parasitism was hypothesized to be due to the attraction of more parasitoids in the intercrop by volatiles from the companion plant. However, the underlying mechanisms are still not well elucidated. An understanding of the behaviours of the parasitoids that may be altered by the manipulation of the habitat is key to success of any habitat management strategy. Therefore, the present study is conducted to further investigate the hypothesis that allelochemical attraction by the non-host plant, molasses grass, was playing a role in the recruitment of parasitoids to the diversified agroecosystem. The relationship between stemborer parasitoids, particularly *C. sesamiae* and *D. busseolae***,** their herbivore hosts, herbivore host plants and the effect of adding a herbivore non-host plant in the system on parasitoid foraging behaviour is investigated.

Information on the chemical ecology of host finding of African stemborer parasitoids is limited. Most of the information available is on larval parasitoids (Table 2). The long-range cues involved in the foraging behaviour of especially pupal parasitoids are

Table 2. Cases of infochemical use by African stemborer parasitoids to locate their hosts. (Adapted from Gohole and Ngi-Song, 2001.)

Parasitoid species	Host	Host plant	Infochemical type	Reference
Egg parasitoids				
Trichogramma sp.	Bf, Co, Cp	Maize, sorghum	Host sex pheromone (kairomone)	No known example
			Moth scales (kairomone)	Ochiel, 1989
Larval parasitoids				
Cotesia flavipes	Cp, Co, Sc, Es	Maize, sorghum, Napier grass	Herbivore induced synomones, frass kairomones	Ngi-Song et al., 1996, Ngi-Song and Overholt, 1997, Potting et al., 1993, Potting et al., 1995, Rutledge and Wiedenmann,
Cotesia sesamiae	Cp, Bf, Sc, Es	Maize, sorghum, Napier grass, sugar cane	Herbivore-induced synomones, frass kairomones	Ngi-Song et al., 1996, Ngi-Song and Overholt, 1997, Rutledge and Wiedenmann, 1999, Potting et al., 1999
Sturmiopsis parasitica	Cp, Bf	Maize, sorghum	Larval frass (kairomones)	Smith et al., 1993
Pupal parasitoids				
Dentichasmias busseolae	Cp	Maize, sorghum	Larval frass (kairomone)	Mohyuddin 1972, Bahana, 1989
Xanthopimpla stemmator	Cp, Es	Maize, sorghum	Larval frass, host odours (kairomones)	Hailemichael et al., 1994

Bf, *Busseola fusca*; Co, *Chilo orichalcociliellus*; Cp, *Chilo partellus*; Es, *Eldana saccharina*; Sc, *Sesamia calamistis*.

not well understood. The close-range behaviour of stemborer parasitoids in a diversified system is an area that has not been exploited. The impact of diversification on parasitism and pest population reduction is an indicator of the performance of the habitat management strategy and needs to be investigated. Finally, knowledge of chemical compounds involved in the system would give a clearer picture of the chemical linkages of the multitrophic relationships in the diversified system.

Research objectives

The objectives of the study were:

1) To investigate the searching behaviour and stimuli used by the stemborer parasitoids, *C. sesamiae* and *D. busseolae*, and role played by a non-host plant, *Melinis minutiflora*, during parasitoid foraging in a plant diversified system.
2) To determine the headspace composition of volatile compounds of host plants and the non-host plant, molasses grass.
3) To determine the composition and abundance of stemborers and their parasitoids in a diversified system in comparison to a monocrop.

Outline of thesis

In chapter **1**, the economic importance of cereal stemborers is highlighted and the need to search for environmentally rational and sustainable control measures is emphasized. The research problem and objectives of the study are defined. The host plant chemical stimuli and the role of volatiles from a non-host plant molasses grass on the foraging behaviour of stemborer parasitoids during long-range foraging are investigated in chapters **2** and **3**. Chapter **2** outlines the foraging behaviour of *Cotesia sesamiae*, a larval parasitoid of stemborers, while in chapter **3**, the response of a stemborer pupal parasitoid, *Dentichasmias busseolae* to host and non-host plant volatiles is investigated. The headspace composition of volatiles from host and non-host plants used in the studies in the previous chapters is described in chapter **4**. This gives an indication as to which chemical compounds could be playing a role in the multitrophic relationships in the system under study. A comparison is made of the close-range behaviour of the parasitoids in the diversified systems of sorghum-molasses grass and maize-molasses grass, and sorghum and maize monocrops. The investigations are described in chapter **5**. A link between laboratory and field studies is outlined in this chapter. Field studies conducted to investigate the effect of intercropping sorghum and molasses grass on the composition and abundance of stemborers and their parasitoids in comparison to a sorghum monocrop are described in chapter **6**. A synthesis of previous chapters is made in chapter **7**. The ecological implications of the use of intercropping in enhancing parasitoid effectiveness are discussed.

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2

Effects of molasses grass, *Melinis minutiflora* volatiles on the foraging behaviour of the cereal stemborer parasitoid, *Cotesia sesamiae*

Chapter 2

Effects of molasses grass, *Melinis minutiflora* volatiles on the foraging behaviour of the cereal stemborer parasitoid, *Cotesia sesamiae*.

Abstract

Olfactory responses of the cereal stemborer parasitoid *Cotesia sesamiae* (Cameron) (Hymenoptera: Braconidae) to volatiles emitted by gramineous host and non-host plants of the stemborers were studied in a Y-tube olfactometer. The host plants from the family Poaceae were maize (*Zea mays* L) and sorghum (*Sorghum bicolor* L), while the non-host plant was molasses grass (*Melinis minutiflora* Beauv.). In single-choice tests, females of *C. sesamiae* chose volatiles from infested and uninfested host plants and molasses grass over volatiles from the control (soil). In dual-choice tests, the wasp preferred volatiles from infested host plants to those from uninfested host plants. There was no discrimination between molasses grass volatiles and those of uninfested maize, uninfested sorghum or infested maize. The wasp preferred sorghum volatiles to maize ones. Combining uninfested maize or sorghum with molasses grass did not make volatiles from the combination more attractive as compared to the sole uninfested host plants. Infested maize alone was as attractive as when combined with molasses grass. Infested sorghum was preferred over its combination with molasses grass. Local growth conditions of the molasses grasses influenced attractiveness to the parasitoids. Volatiles from Thika molasses grass were attractive while those from Mbita molasses grass were not. Growing the Thika molasses grass in Mbita rendered it unattractive and vice versa with the Mbita molasses grass. This is a case of the same genotype expressing different phenotypes due to environmental factors.

Introduction

Habitat management approaches to pest control attempt to divert insect pests away from a crop, and increase natural enemy activity (Landis et al., 2000). One component of this approach is diversification of the agroecosystem, through multiple cropping, intercropping, selective retention of weeds within the crop or conservation of wild plants at field margins (Price et al., 1980; Verkerk et al., 1998; Letourneau & Altieri, 1999). The conditions within the diversified habitat must match the behavioural and nutritional requirements of the natural enemy for effective biological control (Langer, 1996; Altieri et al., 1997; Ogol et al., 1998; Rutledge & Wiedenmann, 1999). As a result, it is important to have information on the interactions involved at the multitrophic level.

Cotesia sesamiae (Cameron) (Hymenoptera: Braconidae) is one of the common larval parasitoids of lepidopterous cereal stemborers (Noctuidae, Crambidae) in eastern

Africa (Mohyuddin & Greathead, 1970; Omwega & Overholt, 1997), but parasitism is typically low. The wasp accounted for only 0.5-3% generational mortality in the spotted stemborer, *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) larvae (Overholt et al., 1994; Oloo, 1989; Songa, 1999). Therefore, to exploit the potential of this parasitoid as a natural control agent, habitat management strategies are being developed to increase parasitism in cereals. Khan et al. (1997a, b) reported that when maize is intercropped with molasses grass, *Melinis minutiflora* Beauv. (Poaceae), a non-host plant for stemborers, not only is there a significant decrease in stemborer infestation levels in the main crop, but also an increase in stemborer parasitism by *C. sesamiae*. They hypothesized that volatile chemicals produced by the intact molasses grass plant attracted the foraging parasitoids. They particularly singled out the compound (E)-4,8-dimethyl-1,3,7- nonatriene and demonstrated its attractiveness to *C. sesamiae*. This compound is known to be released by plants under stress of herbivory and to recruit several species of predators and parasitoids (Dicke, 1994). Molasses grass is a native perennial grass with various beneficial attributes. It is well known as a drought resistant pasture and hay grass and is also used to thatch houses (Fröman & Persson, 1974; Chiera et al., 1977). Furthermore, it has been shown to be repellent to ticks, tsetse flies and mosquitoes (Thompson et al., 1978; Barros & Evans, 1991; Prates et al., 1993; Mwangi et al., 1995; Ibrahim & Kabuye, 1987).

The attraction of parasitoids to a molasses grass intercrop presents an opportunity for the exploitation of this plant to enhance the foraging activities of *C. sesamiae*. No studies on the foraging behaviour of this parasitoid in a diversified system have been reported. Therefore the aim of the present study was to investigate the response of *C. sesamiae* females to volatile cues from molasses grass and uninfested as well as infested sorghum and maize. This would provide information on whether molasses grass has potential for increasing parasitism of stemborers in cereal-based farming systems if intercropped with the cereals.

Materials and methods

Plants

The host plants sorghum (*Sorghum bicolor* (L), cv Seredo) and maize (*Zea mays* L. H-511) (Poaceae) were planted in plastic pots (ø 20 cm). The non-host plant molasses grass (*Melinis minutiflora*), derived from two different locations, Mbita in western Kenya (34° 10' E, 0° 25' S) and Thika in central Kenya (37° 5' E, 1° 3' S), was also planted in plastic pots (ø 25 cm) from root splits. The plants were kept at the International Centre of Insect Physiology and Ecology (ICIPE) in Nairobi, under cages covered with 400-micron mesh netting to protect them from natural insect attack. Sorghum was thinned to 2 plants and maize to one plant per pot at 7 days after emergence (DAE). This was to obtain approximately similar amounts of biomass in tests that compared the two plant species.

Insects

Busseola fusca Fuller (Lepidoptera: Noctuidae) eggs were obtained from a colony maintained at ICIPE-Nairobi and incubated at 25°C until they hatched. After hatching, larvae were starved for one day to eliminate weak individuals and also stimulate voracious feeding in the remaining larvae, which were used for infestation. Third instar *B. fusca* larvae were also obtained from the ICIPE colony reared on an artificial diet (Onyango & Ochieng'-Odero, 1994).

Cotesia sesamiae parasitoids were obtained from a laboratory colony maintained at ICIPE, which had been initiated with individuals collected from *B. fusca* in Kitale, western Kenya. They were reared on the pink stemborer, *Sesamia calamistis* Hampson (Lepidoptera: Noctuidae) according to the method described by Overholt et al. (1994). After parasitization, the stung stemborer larvae were maintained on an artificial diet (Onyango & Ochieng'-Odero, 1994). Parasitoid cocoons were collected in glass vials and kept in clean Perspex cages for emergence. After emergence, adult wasps were provided with a 20% honey/water solution as diet and were allowed to mate during a 24h period. Females used in the bioassays were 1 to 2-days old and naïve with regards to ovipositional experience.

Bioassay

Behavioural responses of *C. sesamiae* females to volatile compounds emitted by different plant sources were determined in a Y-tube olfactometer described by Ngi-Song et al. (1996). Each odour chamber made of Perspex measured 30 × 30 × 120 cm, with an opening drilled at the lower end through which clean charcoal-filtered air was pumped into the chamber. Another opening at the top of the chamber was connected to one of the Y-tube arms by Tygon tubing. Potted plants were placed in large plastic troughs with water and the chambers placed over them. The water was to make the system air-tight (Figure 1). Air was drawn through the closed system using one vacuum pump (Cole-Palmer Air-Cadet). Airflow was set at two liters per minute. The Y-tube was illuminated from above by artificial light consisting of two fluorescent tubes (Philips TLD 32 W/84HF) enclosed in a box made of translucent glass. The airflow was set at 2.5 liters/min in each arm. The parasitoids were introduced individually into the stem of the Y-tube and observed for a period of 5 minutes. When they crossed the 'choice line' (4 cm past the intersection) and remained there for at least 15 seconds, it was recorded as a choice. After 5 parasitoids were tested, the Y-tube was turned 180° (interchange of positions of the arms) to eliminate the effect of any bias for one of the arms. Between different tests, the whole Y-tube apparatus was dismantled and washed thoroughly to remove any possible contaminants from the previous assay. The tests were conducted at 25°C ± 2°C, 60-75% relative humidity and a light intensity of 1200-1300 lux. Trials were conducted between 10.00 and 15.00 hours.

Plant treatment

The sorghum plants used in experiments 1 and 2, were infested at 21 DAE with 3 neonate larvae per plant of the African maize stemborer, *B. fusca* by introducing the larvae in the whorl of the plants. For the other experiments (3 and 4), 5-6 weeks old sorghum plants were used, while in all experiments where maize was the host plant, 5-6 weeks old plants were used. These were infested with 2 third instar *B. fusca* larvae per plant by introducing a larva each into two holes bored in the stem of the plant, 24-36 hours prior to conducting the bioassays.

Plant volatile sources

1) Single-choice tests. The response of females of *C. sesamiae* to a plant volatile source versus a pot of soil (control) was observed for the following: I) uninfested sorghum, II) infested sorghum, III) uninfested maize, IV) infested maize, and V) molasses grass. The molasses grass used in this and subsequent assays (2 and 3), was originally collected from Thika.

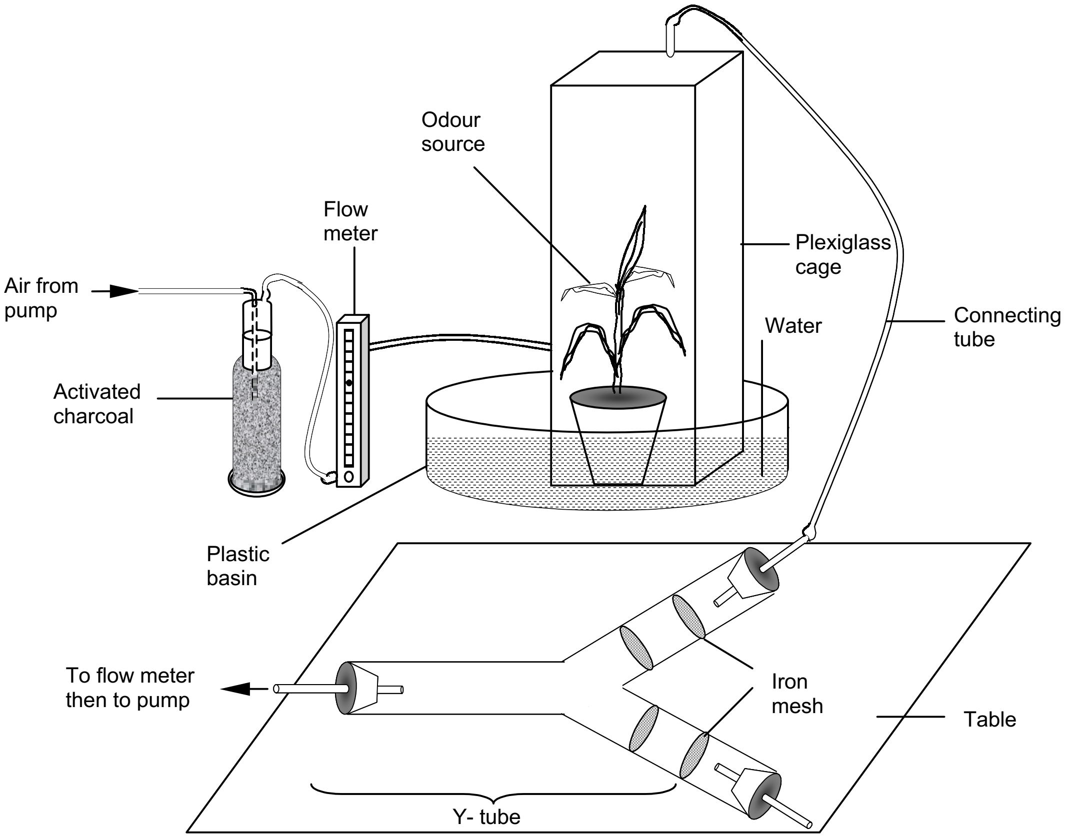

Figure 1. Schematic diagram of Y-tube olfactometer set-up.

2) Dual-choice tests. Experiments were conducted using the following plant volatile sources: I) uninfested sorghum versus molasses grass, II) infested sorghum versus molasses grass, III) uninfested sorghum versus infested sorghum, IV) uninfested maize versus molasses grass, V) infested maize versus molasses grass, and VI) uninfested maize versus infested maize.

For experiments 1 and 2 with sorghum as host plant, 35-50 parasitoids were tested over 5 consecutive days per week, with 7-10 wasps per day per treatment. This was done to control for day effects. The bioassays were repeated each week with the same plants from one week after larval infestation, until 5 weeks after infestation. The aim of conducting the experiments in this manner was to find out if response to the different plant odors changes with time, assuming that with advance in time, the plants will also be growing, having more biomass and thus producing more volatiles. At the same time, the larvae in the host plants will also grow and as they get bigger inflict more damage leading to production of more volatiles. All bioassays using maize were only conducted once at 24-36 hours after infestation.

3) Volatile combinations. The potted test plants were combined as follows: I) molasses grass + one uninfested sorghum versus two uninfested sorghum, II) molasses grass + one uninfested sorghum versus two molasses grass, III) molasses grass + one infested sorghum versus two molasses grass, and IV) molasses grass + one infested sorghum versus two infested sorghum.

A second series of experiments was conducted using maize as the host plant instead of sorghum. Depending on the insect supply, 10-15 parasitoids were tested per day for each treatment and the trials were conducted over several days for a total of 55-90 parasitoids per treatment.

4) Host plants. Response of the parasitoids to volatiles from host plants was investigated. These included: I) uninfested maize versus uninfested sorghum, II) uninfested maize versus infested sorghum, III) infested maize versus uninfested sorghum, and IV) infested maize versus infested sorghum.

5) Molasses grass. Preliminary observations suggested that the attractiveness of molasses grass to *C. sesamiae* varied, depending on the source population of the plants. Thus, experiments were carried out to test the response of the *C. sesamiae* females to odors of molasses grass from two locations, Thika and Mbita. The following plant volatile sources were tested I) Thika molasses grass versus soil, II) Mbita molasses grass versus soil, and III) Thika molasses grass versus Mbita molasses grass.

6) Interchanged molasses grass plants. To test whether the environment where molasses grass was grown had an effect on production of volatiles, potted molasses grass plants from Thika were transported to Mbita and maintained there for a period of 7-8 months (called Thika-Mbita molasses grass). They were transported back to ICIPE-Nairobi for use in bioassays. Likewise, potted molasses grass plants from Mbita were transported to ICIPE-Nairobi and tested after 7-8 months (called Mbita-Thika molasses grass). Responses of *C. sesamiae* to volatiles from these interchanged plants were investigated in a Y-tube olfactometer. The following plant volatile sources were tested I) Thika-Mbita molasses grass versus soil, II) Mbita-Thika molasses grass versus soil, and III) Thika-Mbita molasses grass versus Mbita-Thika molasses grass.

Data analysis

To examine whether there was consistency in response by the parasitoid to volatiles produced by sorghum and molasses grass plants in experiments 1 and 2 over the experimental time period, the logistic regression analysis was performed (GenStat 5.0 for Windows). The other data were analyzed using the log likelihood ratio test (G-test) for goodness of fit with William's correction (Sokal & Rohlf, 1995), to test for significant preferences for an odor.

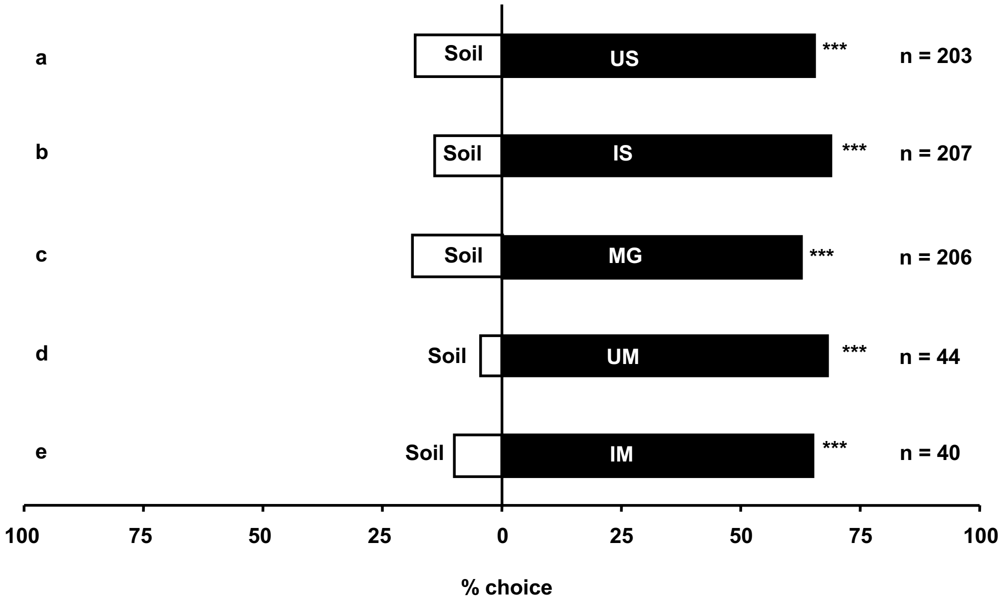

Figure 2. Response of naïve *Cotesia sesamiae* in Y-tube olfactometer single choice tests to volatiles from uninfested sorghum (US), infested sorghum (IS), uninfested maize (UM), infested maize (IM) and molasses grass (MG) versus control (soil). n = number of parasitoids tested. Numbers next to bars indicate percentages of parasitoids making a choice for one of the presented volatile sources. Asterisks indicate significant differences within a choice test. *P< 0.05, **P< 0.01, ***P< 0.001, Gtest for Goodness of Fit. Number of wasps not responding was not included in the analysis.

Results

The logistic regression analysis revealed that responses of the parasitoid to volatiles from sorghum, infested sorghum and molasses grass in experiments 1 and 2 were consistent over time (p> 0.05). The data were therefore pooled and the G-test performed to determine significant preferences for an odour.

Single-choice tests: Naïve *C. sesamiae* females consistently exhibited a preference for uninfested sorghum and infested sorghum, over the control of a pot of soil (Figure 2a & b). Molasses grass was attractive to the wasp (Figure 2c). Volatiles from both uninfested and infested maize were also preferred over the control (Figure 2d & e).

Dual-choice tests: *C. sesamiae* did not discriminate between volatiles emitted by either uninfested sorghum plants or molasses grass (Figure 3a). However, the parasitoids showed a preference for volatiles from infested sorghum over those from molasses grass (Figure 3b). Infested sorghum also attracted parasitoids more than uninfested sorghum (Figure 3c). The wasps did not discriminate between volatiles from uninfested maize and molasses grass or infested maize and molasses grass

(Figure 3d & e). Volatiles from infested maize were more attractive than those from uninfested maize (Figure 3f).

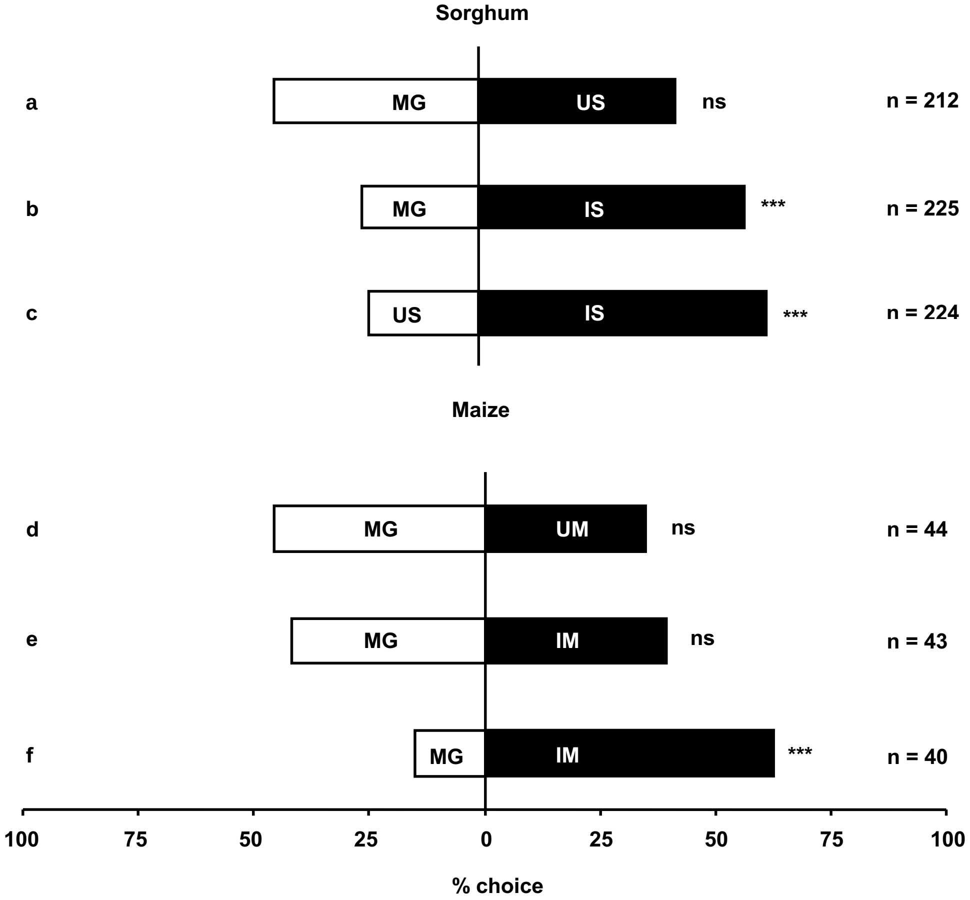

Figure 3. Response of naïve *Cotesia sesamiae* in Y-tube olfactometer dual choice tests to volatiles from uninfested sorghum (US), infested soghum (US), uninfested maize (UM), infested maize (IM) and molasses grass (MG). n = number of parasitoids tested. Numbers next to bars indicate percentages of parasitoids making a choice for one of the presented volatile sources. Asterisks indicate significant differences within a choice test. *P< 0.05, **P< 0.01, ***P< 0.001, G-test for Goodness of Fit. Number of wasps not responding was not included in the analysis.

Plant volatile combinations: No significant difference was found between the response of *C. sesamiae* to uninfested sorghum versus a combination of molasses grass + uninfested sorghum (Figure 4a). The same observation was made for this combination against molasses grass plants (Figure 4b). The wasps showed a clear preference for infested sorghum over the molasses grass + infested sorghum combination (Figure 4c). When the same combination was presented with molasses grass, the parasitoid preferred the combination (Figure 4d). In the bioassays where

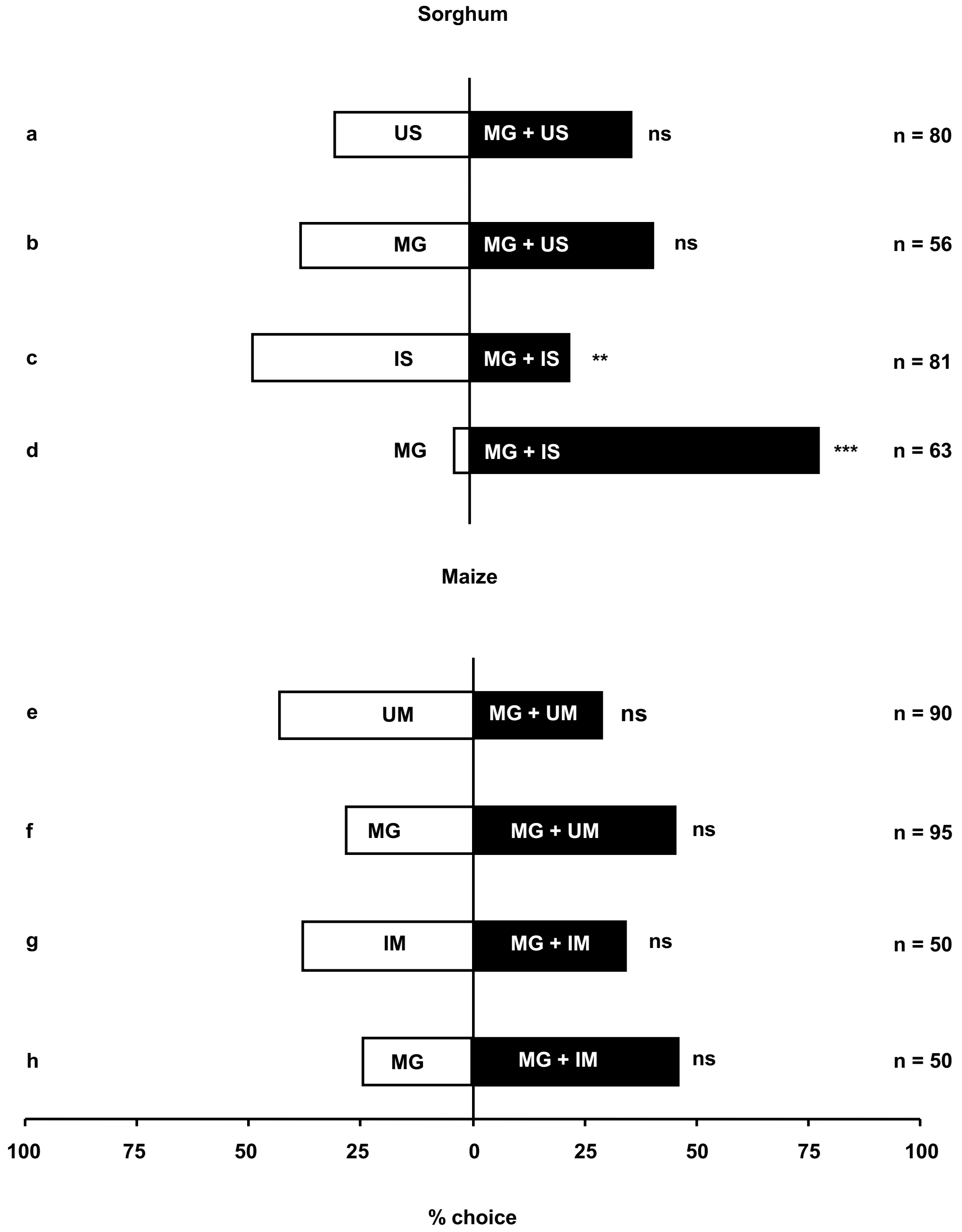

Figure 4. Response of naïve *Cotesia sesamiae* in a Y-tube olfactometer to combinations of volatile sources from uninfested sorghum (US), infested sorghum (IS), uninfested maize (UM), infested maize (IM) and molasses grass (MG). n = number of parasitoids tested. Numbers next to bars indicate percentages of parasitoids making a choice for one of the presented volatile sources. Asterisks indicate significant differences within a choice test. *P< 0.05, **P< 0.01, ***P< 0.001, G -test for Goodness of Fit. Number of wasps not responding was not included in the analysis.

uninfested maize or molasses grass were presented versus a combination of molasses grass + uninfested maize, no clear preference was shown for either of the odor sources (Figure 4e & f). The wasp did not discriminate between infested maize or molasses grass when offered versus a combination of molasses grass + infested maize (Figure 4g & h).

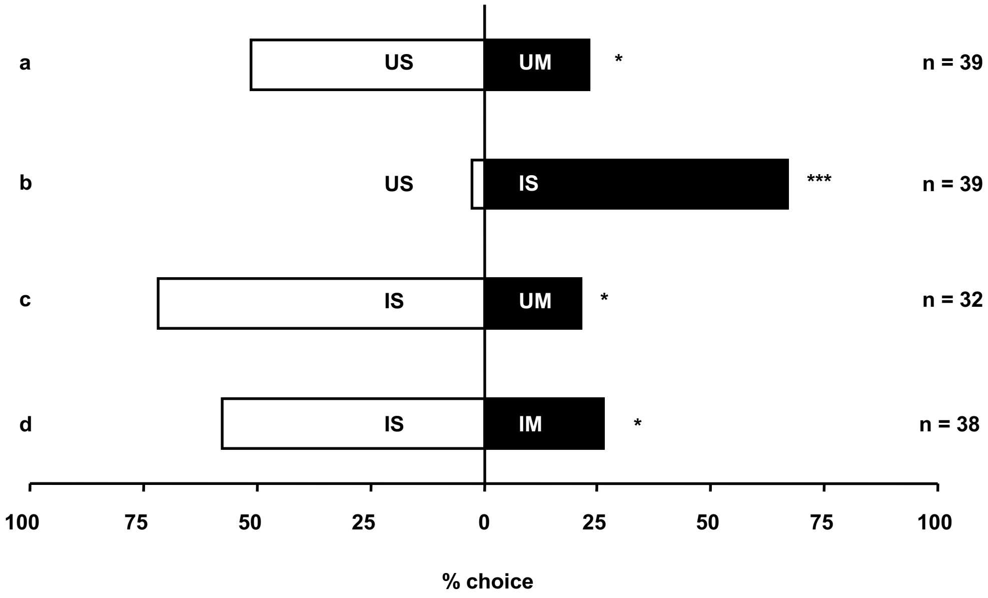

Figure 5. Response of naïve *Cotesia sesamiae* in a Y-tube olfactometer to volatiles from stemborer host plants, uninfested sorghum (US), infested sorghum (IS), uninfested maize (UM) and infested maize (IM). n = number of parasitoids tested. Numbers next to bars indicate percentages of parasitoids making a choice for one of the presented volatile sources. Asterisks indicate significant differences within a choice test. *P< 0.05, **P< 0.01, ***P< 0.001, G -test for Goodness of Fit. Number of wasps not responding was not included in the analysis.

Host plants: When offered uninfested sorghum and uninfested maize, the wasps preferred sorghum (Figure 5a). Infested plants were preferred when presented with uninfested plants (Figure 5b & c). Volatiles from infested sorghum were more attractive than those from infested maize (Figure 5d).

Molasses grass: The wasps exhibited a preference for the Thika molasses grass over the control (pot of soil) (Figure 6a). However, when offered a choice between Mbita molasses grass and soil, they preferred the soil volatiles (Figure 6b). When *C. sesamiae* individuals were offered a choice between the two populations of molasses grass, they showed a clear preference for the Thika population (Figure 6c), which was used in all the previous experiments.

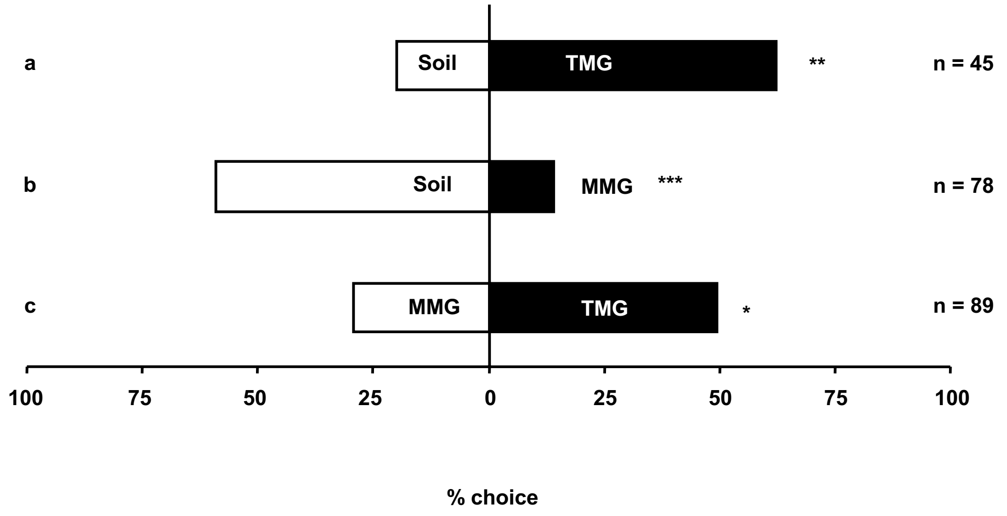

Figure 6. Response of naïve *Cotesia sesamiae* in a Y-tube olfactometer to volatiles from molasses grass plants from two different locations in Kenya namely Thika molasses grass (TMG) and Mbita molasses grass (MMG). n = number of parasitoids tested. Numbers next to bars indicate percentages of parasitoids making a choice for one of the presented volatile sources. Asterisks indicate significant differences within a choice test. *P< 0.05, **P< 0.01, ***P< 0.001, G -test for Goodness of Fit. Number of wasps not responding was not included in the analysis.

The attractiveness of the Thika molasses grass disappeared when these plants had grown in Mbita. Volatiles from Thika -Mbita molasses grass plants were less chosen than soil volatiles (Figure 7a). When the non -attractive Mbita molasses grass was grown in Nairobi, it became attractive. The Mbita -Thika molasses grass was preferred over both the control and the Thika -Mbita molasses grass (Figure 7b & c).

Discussion

Stemborer parasitoids are attracted to volatiles emanating from gramineous plants, which are the main host plants for their herbivore hosts (Potting et al., 1995; Ngi - Song et al., 1996; Smith & Wiedenmann, 1997; Rutledge & Wiedenmann, 1999). The same observation was made in the present study, as *C. sesamiae* was attracted to volatiles from the host plants sorghum and maize. Our results also confirm the attractiveness of some collections of molasses grass to *C. sesamiae*. Findings by

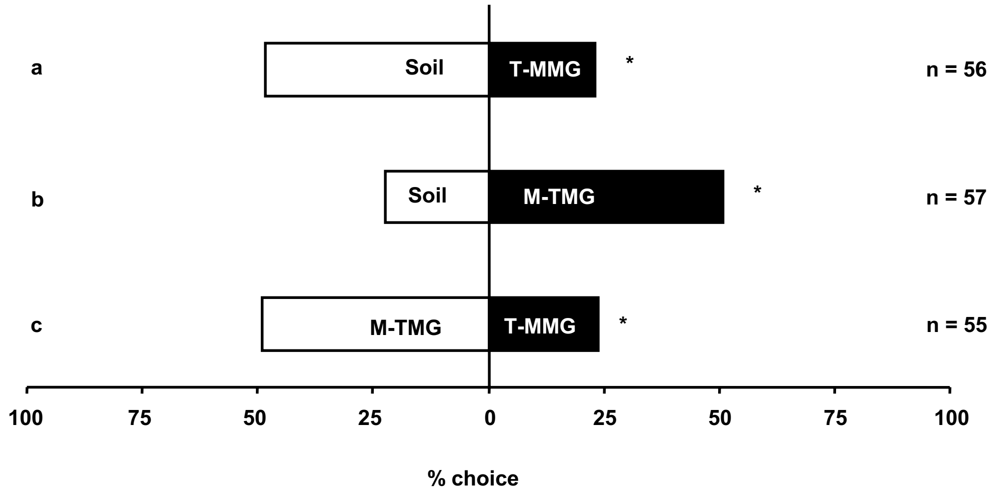

Figure 7. Response of naïve *Cotesia sesamiae* in a Y-tube olfactometer to volatiles from Thika and Mbita molasses grass plants grown in interchanged locations, Thika molasses grass grown in Mbita (T-MMG) and Mbita molasses grass grown in Nairobi (MTMG). n = number of parasitoids tested. Numbers next to bars indicate percentages of parasitoids making a choice for one of the presented volatile sources. Asterisks indicate significant differences within a choice test. *P< 0.05, **P< 0.01, ***P< 0.001, Gtest for Goodness of Fit. Number of wasps not responding was not included in the analysis.

Takabayashi et al. (1991) showed that the parasitoid *Cotesia kariyai* Watanabe (Hymenoptera: Braconidae) not only responded to volatiles from uninfested host plants, but also to volatiles from non-host food plant species. They attributed this attraction to a volatile compound (Z)-3-hexenol, which is commonly produced by many green plants. Khan et al. (1997a) observed attraction of *C. sesamiae* to the non-host plant, molasses grass. The grass produced the compound (E)-4,8-dimethyl-1,3,7-nonatriene, which they demonstrated was attractive to the parasitoid.

We confirmed that volatiles released by herbivore-infested maize and sorghum plants are potent attractors of parasitoids when compared to uninfested plants. This is in agreement with works by Potting et al. (1995), Ngi-Song et al. (1996), Rutledge & Wiedenmann, 1999). These chemicals termed herbivore-induced synomones (Dicke & Sabelis, 1988) are reliable indicators of herbivore presence. Studies have shown that some wasps are more sensitive to these chemicals than they are to those from host and host by-products (Steinberg et al., 1993; Potting et al. 1995; Du et al. 1996).

It was expected that with the advance in time, the plants would gain more biomass, thus producing more volatiles that would be more attractive to the parasitoids. At the same time, the larvae in the infested plants would grow, causing more damage, and thus stimulating the plants to produce greater quantities of herbivore-induced volatiles. On the contrary, responses of the wasps to the different plant odors were consistent over time, whether the plants were young or old or infested by young or older larvae. While investigating attraction of *C. flavipes* and *C. sesamiae* to maize infested with different stemborer species at the same density, Ngi-Song et al (1996) found that the plants infested by larger stemborers were more attractive. They hypothesized that the attractiveness could be attributed to the larger stemborers feeding more voraciously, inflicting more injury to the plant thus increasing quantities of volatiles produced. However, to appropriately compare the attractiveness of plants at different ages or those infested by herbivores of different ages, then dual choice tests of the same would be required.

Based on the assumption by Khan et al. (1997a) that increased parasitism of stemborers by *C. sesamiae* in maize-molasses grass intercrops was due to attractive volatiles produced by the molasses grass, we expected the combinations of the molasses grass and the uninfested host plants maize and sorghum to attract more parasitoids than the sole uninfested host plants, but the field and the present laboratory results do not agree. It should also be noted that there was no difference in attraction to the host plants and molasses grass in dual choice tests except for infested sorghum (Figure 3). It seems there is no interaction or synergistic effect between the host plant and molasses grass volatiles in the combinations. Presence of the molasses grass had a neutral effect on the uninfested host plant volatiles with concerns to attraction of the parasitoid. A possible explanation for the contradiction between the present laboratory results and reported field ones with maize as the host plant could be, the molasses grass may affect long-range host finding which would be an important factor in the field but not in the small cages used in the laboratory experiments. Additionally, Khan et al. (1997a) reported that stemborers were repelled by molasses grass. The higher parasitism found in the maize-molasses grass intercrop may have been due to a lower stemborer density rather than recruitment of parasitoids into the field. Infested sorghum alone was preferred than the molasses grass + infested sorghum combination. Though the combination was more attractive than the molasses grass alone. Infested sorghum must be producing some volatile compounds, which are strong signals to the parasitoids and can be picked out easily despite the presence of the molasses grass. It should be noted that studies on effects of intercropped plants on foraging behavior of parasitoids by testing of such plants in combinations in laboratory experiments are uncommon and this study, to our knowledge, is the first attempt on this aspect.

When offered infested sorghum versus infested maize, *C. sesamiae* showed preference for infested sorghum. While investigating the response of stemborer parasitoids to volatiles of some grasses, Ngi-Song et al. (1996) and Rutledge & Wiedenmann (1999), reported preference of *C. sesamiae* for sorghum over maize volatiles. Both sorghum and *C. sesamiae* are indigenous to Africa (Dogget, 1988; van Achterberg & Walker, 1998), while maize is a relatively recent introduction (Acland, 1971). The parasitoid might have a genetic adaptation to search in sorghum with which it shares a longer evolutionary history thus the reported observations.

The molasses grass from Thika consistently attracted the wasp while plants from Mbita did not. Mbita is located in a hot moist mid-altitude region, while Thika is in a cooler and drier mid-altitude zone. Climatic differences in the two geographical zones had an effect on the growth conditions of the molasses grasses, which influenced the type of volatiles produced leading to the differences in response to these grasses. This observation was confirmed by the bioassay on the interchanged molasses grass plants whereby the molasses grass from Thika when grown in Mbita was no longer attractive, while the one from Mbita grown in Nairobi was now attractive. This finding demonstrates how the environment can influence a plant genotype causing it to express different phenotypes. This information is important because it could form a basis on which to partially answer why some biological agents are successful in some regions and completely fail in others. Several other studies have also reported chemical variations in volatiles brought about by the geographic origin of the plant (Adams 1994; Rafii et al. 1996; Halitschke et al., 2000), but these differences were not confirmed by use of bioassays as described in our study, but by chemical analytical means. Chemical analyses are needed to confirm whether there is a qualitative or quantitative difference in the odor bouquet of the two grass populations.

The study established that *C. sesamiae* was attracted to volatiles from its uninfested and infested host plants and the non-host plant, Thika molasses grass, but not to those from Mbita molasses grass. Based on the results obtained, a rank order on the attractiveness of the plants tested in the bioassays can be determined as follows: soil= Mbita molasses grass< uninfested maize< uninfested sorghum< infested maize< infested sorghum. Thika molasses grass seems to be at the same level of attractiveness as uninfested maize, uninfested sorghum and infested maize but less attractive than infested sorghum.

In conclusion, data from the Y-tube experiments are not sufficient enough to explain parasitoid behaviour in the field situation. The most plausible way to find out is to either carry out semi-field or field experiments. However, the experiments conducted in this study can be used as preliminary indicators as to whether certain plants can be used in combination to enhance foraging of parasitoids. In this case, it would not be recommended to use sorghum with molasses grass because infested sorghum by itself, is more attractive than when combined with molasses grass. Additionally, the environment may influence the attractiveness of plants as shown by the variable response of the parasitoids to molasses grass from two locations. Therefore caution should be taken such that the approach is not generalized for all crops or cropping areas. Trials should be carried out first in prospective crops or zones to validate the approach. Finally, for a better understanding of the different behavioural responses reported in the present study, comparative headspace analyses of volatiles of both the host and non-host plants used in the study are necessary and are reported in chapter 4.

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3

Role of volatiles emitted by host and non-host plants in the foraging behaviour of *Dentichasmias busseolae*, a pupal parasitoid of the spotted stemborer *Chilo partellus*

Chapter 3

Role of volatiles emitted by host and non-host plants in the foraging behaviour of *Dentichasmias busseolae*, a pupal parasitoid of the spotted stemborer *Chilo partellus*.

Abstract

The role of volatiles from stemborer host and non-host plants in the host finding process of a pupal parasitoid *Dentichasmias busseolae* Heinrich (Hymenoptera: Ichneumonidae), of *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae), was studied. The non-host plant, molasses grass (*Melinis minutiflora* Beauv. (Poaceae)), is reported to produce some volatile compounds known to be attractive to some parasitoid species. The studies were conducted to explore the possibility of intercropping stemborer host plants with molasses grass for the enhancement of the foraging activity of *D. busseolae* in such a diversified agro-ecosystem. Olfactometric bioassays showed that volatiles from the host plants maize, *Zea mays* L and sorghum, *Sorghum bicolor* (L) (Poaceae), were attractive to the parasitoid. Infested host plants were most attractive. Volatiles from molasses grass were repellent to the parasitoid. Further tests showed that volatiles from infested and uninfested host plants alone were preferred over those from infested and uninfested host plants combined with the non-host plant, molasses grass. In dual choice tests, the parasitoid did not discriminate between volatiles from maize infested by either of the two herbivore species, *C. partellus* and *Busseola fusca* Fuller (Lepidoptera: Noctuidae). Volatiles from sorghum infested by *C. partellus* were preferred over those from *C. partellus*-infested maize. The study shows that the pupal parasitoid *D. busseolae* uses plant volatiles during foraging with those from the plant-herbivore complex being the most attractive. The fact that volatiles from molasses grass are deterrent to the parasitoid suggests that intercropping maize or sorghum with molasses grass is not likely to enhance foraging behaviour of *D. busseolae*. Volatiles from the molasses grass may hinder *D. busseolae*'s host location efficiency.

Introduction

Plant volatiles play an important role in the interaction between host plants, herbivores and parasitoids. Upon herbivory, plants are induced to produce volatiles, which can be exploited by parasitoids in their foraging process (Vet & Dicke, 1992; Takabayashi & Dicke, 1996, for reviews). A wealth of knowledge is available on the role of chemical cues used by parasitoids attacking larval stages of herbivores (Dicke & Vet, 1999), but negligible information is available on those used by pupal parasitoids (Vet et al., 1995; Rostás et al., 1998). In their review on chemical ecology of African stemborer parasitoids, Gohole & Ngi-Song (2001) emphasized the need to generate information on the long-range volatiles used by pupal parasitoids to locate host habitats, considering the recent interest in utilizing pupal parasitoids in biological control of stemborers. The present study was therefore conducted to fill in this gap with information on the role of plant chemical stimuli on the foraging behaviour of *Dentichasmias busseolae*, a pupal parasitoid of the spotted stemborer *Chilo partellus,* one of the most damaging insect pests of maize and sorghum in east and southern Africa (Overholt et al., 1997).

Although discrepancies exist in the literature about *D. busseolae*'s host range, it is clear that it is primarily a parasitoid of *C. partellus* (Zwart, 1998). This has been confirmed by field collections that show the parasitoid was solely recovered from *C. partellus* even in regions where the stemborers *Busseola fusca* Fuller and *Sesamia calamistis* Hampson (Lepidoptera: Noctuidae) are abundant, (Bahana, 1990, Zhou et al., 2003). Studies showed that *D. busseolae* parasitised *B. fusca* pupae when placed in *C. partellus* tunnels but not when they are in their own tunnels (Mohyuddin (1972). The observation was attributed to an oviposition factor present in *C. partellus* frass. This means that at close range, the parasitoid can discriminate between host species. However, it is not known whether *D. busseolae* can also discriminate between plants infested by different herbivore species on the basis of volatile plant information.

Habitat management is an alternative method being sought in the fight against insect pests. One component of the strategy is diversification of the agroecosystem to improve availability of resources required by parasitoids for optimal performance (Landis et al., 2000). Investigations on effects of habitat diversity on parasitism rates by parasitoids have produced mixed results (Sheehan, 1986; Letourneau, 1987; Päts et al., 1997). It is important to understand how parasitoids function as part of a diversified ecosystem in a bid to promote their effectiveness using habitat management (Lewis et al., 1997). In their attempts to control cereal stemborers, Khan et al. (1997a,b), reported that intercropping maize with molasses grass, had the effect of not only repelling stemborers from the intercrop, but also attracting the larval parasitoid *Cotesia sesamiae* (Cameron) (Hymenoptera: Braconidae) and increasing parasitism levels. The attraction of the parasitoid was attributed to the grass releasing a volatile chemical compound (3E)-4,8-dimethyl-1,3,7-nonatriene, known to be part of the volatile blend that plays a role in recruitment of several species of predators and parasitoids (Weissbecker et al., 1999; Ngi-Song et al., 2000; Hoballah et al., 2002). This finding presents an opportunity to assess whether this type of habitat management can also be exploited to enhance the pupal parasitoid, *D. busseolae*'s host searching ability.

Although *D. busseolae* is an important parasitoid in many maize and sorghum growing areas, it has not received much attention in terms of research. *Dentichasmias busseolae* is widespread in the eastern and southern African countries (Mohyuddin & Greathead, 1970, Oloo, 1989, Kfir, 1990), with parasitism levels ranging between 0-43 % and varying with location and season (Oloo & Ogeda, 1990; Skovgård & Päts, 1996). The few studies done so far on its biology and ecology are those of Mohyuddin (1972) and Bahana (1989; 1990). As a result of their hidden lifestyle, *C. partellus* pupae reduce their accessibility to parasitoids. Coupled with the fact that host chemical cues are hardly detectable from a distance, host plant volatiles could play a role in helping these parasitoids locate their hosts. In a study on short-range cues used by *D. busseolae* to locate hosts, Bahana (1989) reported that sorghum stem pieces damaged by fifth instar larvae of *C. partellus* larvae and their frass were attractive to the parasitoid. This, to our knowledge, is the only attempt ever conducted to investigate chemical cues involved in *D. busseolae*'s host searching behaviour. Here we report investigations on the role of plant volatiles in the host habitat selection process of *D. busseolae*. In laboratory assays we investigated the effects of molasses grass volatiles in combination with host plant volatiles on the foraging behaviour of the parasitoid.

Materials and methods

Plants

Host plants sorghum (cv Seredo) and maize (H-511) were planted in plastic pots (ø 20 cm). The non-host plant, molasses grass, derived from Thika in central Kenya (37° 5'E, 1° 3'S), was also planted in plastic pots (ø 25 cm) from root splits. Maize and sorghum were thinned to one and two plants per pot respectively at seven days after emergence, to have approximately similar amounts of biomass in experiments that tested maize against sorghum. The plants were kept at the International Centre of Insect Physiology and Ecology (ICIPE) at Nairobi, in large cages covered with 400-micron mesh netting to protect them from natural insect attack.

Insects

A laboratory culture of *D. busseolae* was established from adults (seven males and five females) reared from *C. partellus* pupae collected from Mbita in western Kenya (34° 10'E, 0° 25'S). The parasitoids were reared using a slightly modified version of Mohyuddin's (1972) method. Maize stems of 15 cm length were first split longitudinally into two. Then three depressions, 3 cm apart, were scooped out of one of the longitudinal sections. Holes (resembling exit holes) were bored through the other section of the stem at locations corresponding to the depressions. The depressions (approximately 2 cm long and 1 cm deep), were filled with frass from fifth instar *C. partellus* larvae but ensuring there was space for the parasitoid to manoeuvre its way around the pupae. Two one-day old *C. partellus* pupae were placed in each depression. The longitudinal sections were rejoined and secured in place using rubber bands at each end. The holes were lightly sealed with frass to simulate an exit hole. The stem pieces containing the pupae were offered to the *D. busseolae* females in Perspex cages (30 × 30 × 30 cm) at a ratio of one female: two pupae. These were presented in an upright position by placing them in plasticine. Pupae were removed from the stems after 24 h and placed on moistened filter paper in Petri dishes. On emergence, both male and female parasitoids were released in a rearing cage and allowed a 48 h mating period. The females were afterwards placed in separate cages to avoid further mating. The parasitoids were provided with a diet of 20% honey solution, supplied in Petri dishes containing cotton wool pads. The pads were renewed every two days to prevent fermentation. The parasitoids also had access to water supplied on wet cotton wool pads.

Fifth instar *C. partellus* and *B. fusca* larvae used to infest host plants and the *C. partellus* pupae used to rear *D. busseolae* were obtained from a colony maintained at ICIPE-Nairobi. *Chilo partellus* was reared on an artificial diet developed by Ochieng et al. (1985), while *B. fusca* was reared on an artificial diet according to the method of Onyango & Ochieng-Odero (1994).

Bioassay

Behavioural responses of *D. busseolae* females to volatile compounds emitted by different sources were determined in a Y-tube olfactometer. For details of the olfactometer see Chapter 2. Parasitoids were introduced individually into the base of the Y-tube and observed for a period of five minutes. When a parasitoid crossed the 'choice line' (4 cm past the intersection) and remained there for at least fifteen seconds, it was recorded as a choice. The Y-tube was turned 180° (interchange of positions of the arms), after each three parasitoids to eliminate the effect of any bias for one of the arms. The tests were conducted at 25°C ± 2°C, 60-75% relative humidity and light intensity of 1200-1300 lux. Trials were conducted between 11.00 and 15.00 hours.

Odour sources

Uninfested sorghum (US) and uninfested maize (UM) plants were six to eight weeks old potted plants. Infested sorghum (IS) and infested maize (IM) were obtained by boring two holes in the stem of uninfested plants with a cork borer and placing one 5th instar *C. partellus* larva in each hole. For *B. fusca* infestation, a hole was bored in the stem of the host plant and one, fifth instar larva introduced into it. It was assumed that two *C. partellus* larvae would do as much damage as one *B. fusca* larva (derived from a study by Ngi-Song et al., 1996). Infested plants consisted of the complete plant-herbivore complex together with herbivore by-products. Bioassays were conducted 96-120 h after infestation. This period was found to be adequate for the larvae to feed and eventually pupate. We used the presence of an exit window chewed by the borer on the stem as an indicator of pupation. The molasses grasses used in the experiments, were seven to nine weeks old They were in the form of a tuft and had a biomass approximately similar to the host plants. The control consisted of a pot of soil.

Experimental design

The following volatile sources were tested with the plants treated as previously described. Except where it is clearly stated that *B. fusca*-infested plants were used, all the infested plants used were the *C. partellus*-infested ones.

1) Response to volatiles from host and non-host plants.

a) Single-choice tests. These series of experiments were conducted to determine attractiveness of volatiles from host and non-host plants. The response of *D. busseolae* females to the following volatile sources versus a pot of soil (control) were observed: I) uninfested sorghum, II) infested sorghum, III) uninfested maize, IV) infested maize, and V) molasses grass.

b) Dual-choice tests. The second series of bioassays were conducted to determine the parasitoid's preference for volatiles from uninfested and infested host plants and the non-host plant. Bioassays were conducted using the following odour sources: I) uninfested sorghum versus molasses grass, II) infested sorghum versus molasses grass, III) uninfested maize versus molasses grass, IV) infested maize versus molasses grass, V) uninfested sorghum versus infested sorghum, and VI) uninfested maize versus infested maize.

2) Response to volatiles from host and non-host plant combinations. To investigate whether adding a non-host plant to the system would enhance attraction of *D. busseolae*, molasses grass was combined with both infested and uninfested host plants in the following fashion:
a) Sorghum: I) molasses grass + two uninfested sorghum versus four uninfested sorghum plants, II) molasses grass + two uninfested sorghum versus two molasses grass plants, III) molasses grass + two infested sorghum versus four infested sorghum plants, and IV) molasses grass + two infested sorghum versus two molasses grass plants.
b) Maize: I) molasses grass + one uninfested maize versus two uninfested maize plants, II) molasses grass + one uninfested maize versus two molasses grass plants, III) molasses grass + one infested maize versus two infested maize plants, and IV) molasses grass + one infested maize versus two molasses grass plants.

3) Herbivore and host plant species discrimination: To investigate whether *D. busseolae* was able to distinguish between volatiles from maize plants attacked by different stemborer species, one *B. fusca*-infested maize was presented versus one *C. partellus*-infested maize (see section on odour sources). To investigate whether *D. busseolae* could discriminate between different host plant species, one *C. partellus*-infested maize was presented versus two *C. partellus*-infested sorghum plants (see section on plants under materials and methods).
For each treatment in all the experiments (1-3), twenty 5-7 day old, naïve (no host or plant experience), mated female parasitoids were used. Each trial was conducted over four days with five parasitoids per treatment each day. Due to difficulty in rearing *D. busseolae*, the number of parasitoids used per treatment could not be increased. Different plants were used on each of the experimental days.

Data analysis

Data for replicates in each treatment were first subjected to the heterogeneity G-test. If not significant, they were pooled and analysed using the log likelihood ratio test (G-test) for goodness of fit (Sokal and Rohlf, 1995).

Results

Heterogeneity test showed that the outcomes of the replicates were homogenous. Results are based on analysis of pooled data.

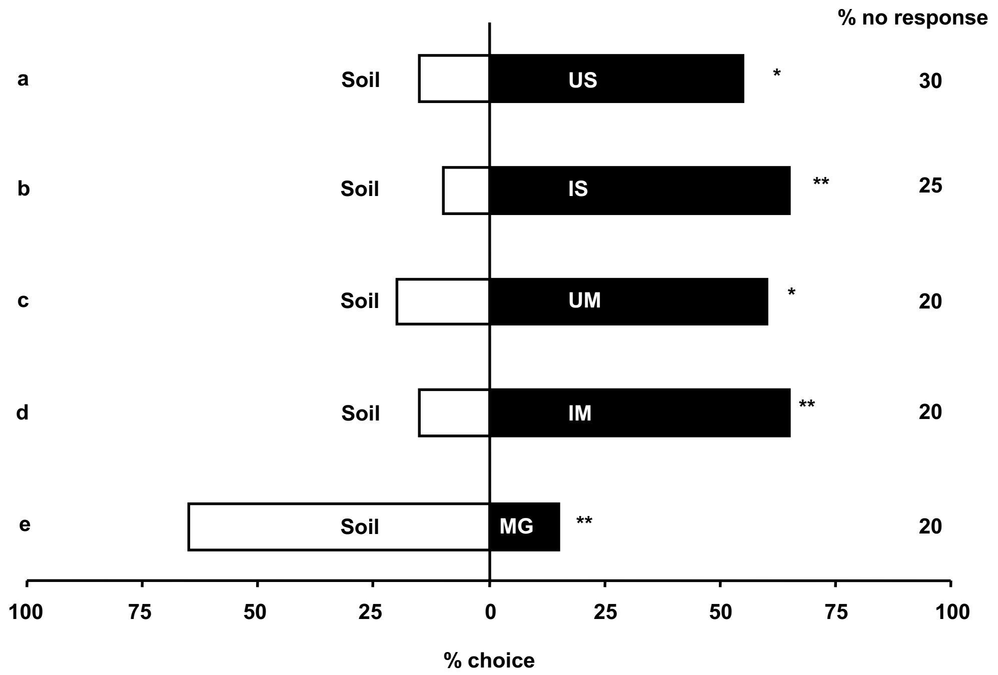

Figure 1. Response of naïve *Dentichasmias busseolae* in Y-tube olfactometer single choice tests to odours from uninfested sorghum (US), infested sorghum (IS), uninfested maize (UM), infested maize (IM) and molasses grass (MG) versus control (soil). n = 20. Numbers next to bars indicate percentages of parasitoids making a choice for one of the two presented odour situations. Asterisks indicate significant differences within the choice test. *P< 0.05, **P< 0.01, ***P< 0.001, G-test for Goodness of Fit. Non-responding wasps were not includedin the analysis.

Response to volatiles from host and non-host plants.

Single choice tests: When offered the host plants versus the control (pot of soil), *D. busseolae* preferred volatiles from uninfested sorghum, infested sorghum, uninfested maize and infested maize to the control (Figures 1a, b, c & d). However, molasses grass volatiles repelled the parasitoids (Figure 1e).

Dual choice tests: The parasitoids were attracted more to volatiles from both uninfested and infested sorghum than to volatiles from molasses grass (Figures 2a & b). Uninfested and infested maize plants were more attractive to the female parasitoids than the molasses grass (Figures 2c & d). Infested host plants were preferred over their uninfested counterparts (Figure 2e & f).

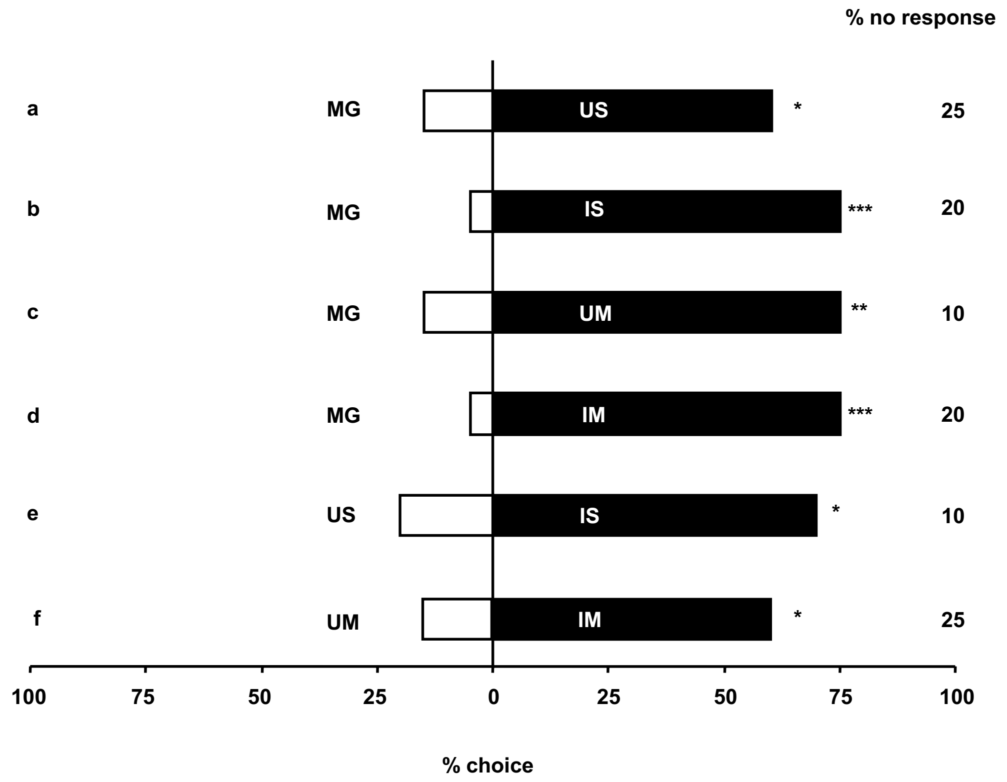

Figure 2. Response of naïve *Dentichasmias busseolae* in Y-tube olfactometer dual choice tests to odours from uninfested sorghum (US), infested sorghum (IS), uninfested maize (UM), infested maize (IM) and molasses grass (MG). n = 20. Numbers next to bars indicate percentages of parasitoids making a choice for one of the two presented odour situations. Asterisks indicate significant differences within the choice test. *P< 0.05, **P< 0.01, ***P< 0.001, G-test for Goodness of Fit. Non-responding wasps were not included in the analysis.

Response to volatiles from host and non-host plant combinations

Sorghum: In both cases where sorghum and molasses grass were combined and presented versus sorghum, the parasitoids preferred volatiles from the sorghum plants, whether infested or uninfested over those from the combination (Figure 3a & c). The parasitoid showed no clear preference when molasses grass was offered versus a combination of uninfested sorghum and molasses grass (Figure 3b). The combination of infested sorghum with molasses grass was more attractive than molasses grass alone (Figure 3d).

Maize: Both the uninfested and infested maize were more attractive than their respective combinations with molasses grass (Figure 3e & g). *Dentichasmias busseolae* did not discriminate between molasses grass and a combination of maize and molasses grass (Figure 3f). When molasses grass was offered against a combination of infested maize and molasses grass, the parasitoid preferred the combination (Figure 3h).

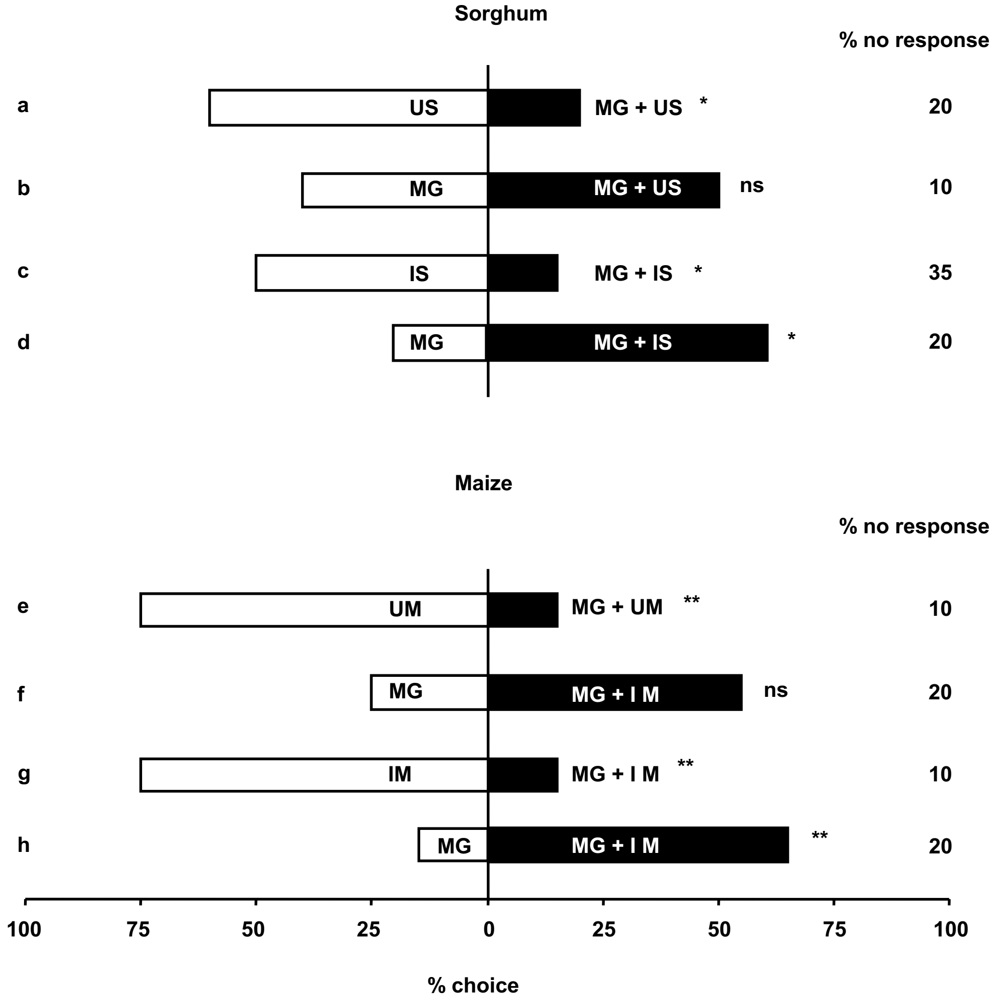

Figure 3. Response of naïve *Dentichasmias busseolae* in a Y-tube olfactometer to combinations of odour sources from uninfested sorghum (US), infested sorghum (IS), uninfested maize (UM), infested maize (IM) and molasses grass (MG). n = 20. Numbers next to bars indicate percentages of parasitoids making a choice for one of the two presented odour situations. Asterisks indicate significant differences within the choice test. *P< 0.05, **P< 0.01, ***P< 0.001, G-test for Goodness of Fit. Non-responding wasps were not included in the analysis.

Herbivore and host plant species discrimination: *Dentichasmias busseolae* did not discriminate between volatiles from *B. fusca* infested- and *C. partellus* infested-maize (Figure 4a). When given a choice between volatiles from infested sorghum and infested maize, the parasitoid preferred volatiles from infested sorghum to those of infested maize (Figure 4b).

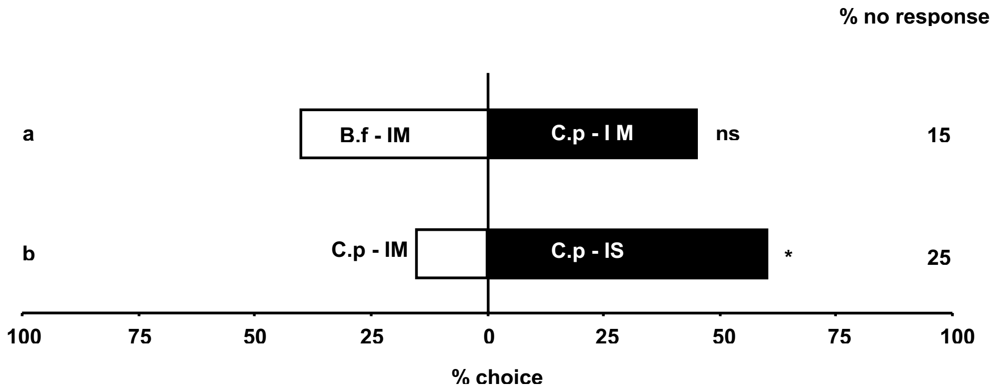

Figure 4. Response of naïve *Dentichasmias busseolae* in a Y-tube olfactometer to odours from maize infested by different stemborer species and to odours from different infested host plants. B.f - IM - *Busseola fusca* infested maize, C.p - IM - *Chilo partellus* infested maize, C.p -IM - *Chilo partellus* infested maize, C.p -IS – *Chilo partellus* infested sorghum. n = 20. Numbers next to bars indicate percentages of parasitoids making a choice for one of the two presented odour situations. Asterisks indicate significant differences within the choice test. *P< 0.05, **P< 0.01, ***P< 0.001, G-test for Goodness of Fit. Non-responding wasps were not included in the analysis.

Discussion

Our results show that the stemborer pupal parasitoid, *D. busseolae* uses plant volatiles during host location. Volatiles from the uninfested host plants, maize and sorghum were attractive to *D. busseolae*. These volatiles must be playing a role in guiding the parasitoid to habitats that most likely harbour hosts. Several other studies have demonstrated the attractiveness of volatiles from uninfested plants to parasitoids (Ngi-Song et al., 1996; Takabayashi et al., 1998; Rutledge & Wiedenmann. 1999; Reddy, 2002). Herbivore-damaged plants, which include host and host-derived by-products, were the most attractive to the parasitoids. Attraction of pupal parasitoids to volatiles of infested host plants has rarely been demonstrated (Sullivan et al., 2000). The present study supports the infochemical detour hypothesis that postulates that parasitoids which attack non-feeding host stages like pupae, resort to information from activities of other stages of the host (Vet & Dicke, 1992). In our case, these chemical volatiles are produced as a result of the feeding activity of the larval stage.

The infested plant together with host by-products might still produce attractive volatiles even after the larvae have pupated. The host pupae do not produce volatiles that attract the parasitoid (Bahana, 1989), though we cannot rule out the possibility that pupae provide other non-chemical close-range cues, like acoustic or mechanical cues, which enable parasitoids to locate them.

Volatiles from the non-host plant molasses grass were deterrent to *D. busseolae*. One of the compounds produced by molasses grass is (3E)-4,8-dimethyl-1,3,7-nonatriene, known to play a role in recruitment of several species of foraging parasitoids like *Cotesia sesamiae* Cameron (Hymenoptera: Braconidae) (Khan et al., 1997a). With this in mind, we hypothesized that the grass would also be attractive to *D. busseolae*. However, our results suggest the contrary. The parasitoid was repelled by molasses grass (Figure 1E). The repellence of molasses grass to *D. busseolae* could be due to yet unknown volatile compounds.

In the choice between volatiles from a combination of the infested host plants and molasses grass versus infested host plants, the wasps preferred volatiles from the infested host plants to those from the combination. Molasses grass in the combination is repellent, therefore the parasitoid goes for the infested plants whose volatiles are attractive and provide information on the presence of a host. In cases where the combination with infested host plants was offered versus molasses grass, the wasps preferred the combination (Figure 3). Despite of the presence of molasses grass, the wasps apparently still perceive the attractive odours from the infested host plants. What is not known is how the host location ability of the parasitoids in the intercrop is influenced by the deterrent cues from the molasses grass. To answer this, it is necessary to conduct experiments in which the foraging behaviour of the wasps in an intercrop is continuously recorded and compared to that in a monocrop.

In this study, *D. busseolae* did not discriminate between maize plants infested by two different stemborer species, *C. partellus* and *B. fusca*. Similar results were reported by Ngi-Song et al. (1996) while working with the parasitoid *Cotesia flavipes* and Geervliet et al. (1996) with naïve *Cotesia glomerata* and *C. rubecula*. Apparently the volatile cues produced do not provide the wasp with specific information on which herbivore species is feeding on the plant. The parasitoid could be using a general set of odour blends produced after herbivore damage to locate the host habitat. Some plants emit qualitatively similar chemical blends irrespective of the herbivore species feeding on them (Mattiacci et al., 1994; Geervliet et al., 1997). Such an odour blend is not supplying the parasitoid information on herbivore identity, which is a likely constraint on the optimization of foraging (Dicke & Vet, 1999; Vet, 1999). Alternatively, the parasitoids used in this study, being naïve, were not in an appropriate condition to demonstrate discrimination. Some parasitoids such as *Cotesia glomerata*, need experience to do so (Geervliet et al., 1998). In contrast, some carnivorous arthropods are able to make this distinction while naïve (Du et al., 1996; Turlings et al., 1998; de Moraes et al. 1998). This ability is attributed to either qualitative or quantitative differences in the odour blends produced due to feeding by the different herbivores (Dicke, 1999, Vet 1999). *Dentichasmias busseolae* is known to attack specific stemborers.

If it does not learn to distinguish between plants infested with different stemborers from a distance, it must be using reliable short range-cues to discriminate between host species after locating an infested plant.

When offered a choice between different species of infested host plants, *D. busseolae* preferred volatiles from infested sorghum to infested maize. Other reports of parasitoids discriminating between plants of different species that are infested by herbivores of the same species are those of McCall et al. (1993), Takabayashi et al. (1994) and Potting et al. (1997). Both *D. busseolae* and sorghum are indigenous to Africa (Dogget, 1988; Zwart, 1998) and as such share a long evolutionary history. Maize is a relatively recent introduction to Africa (Acland, 1971). We would therefore expect a genetic adaptation for the parasitoid to respond and search in sorghum more than in maize. The parent parasitoids used to initiate the *D. busseolae* colony originated from Mbita in the western part of Kenya, an area that has predominantly been more under sorghum cultivation than maize. In his studies on field parasitism by *D. busseolae*, Bahana (1989) noted that the parasitoid was more abundant in sorghum than in maize. Sorghum also harboured a higher stemborer host population.

The present study investigated an aspect of habitat diversification and how it can be utilized to enhance parasitization rates. Results indicate that molasses grass does not attract *D. busseolae*. Therefore any future research activities on diversification of cereal agroecosystems to enhance biological control by this pupal parasitoid should focus on other plants. We propose the use of plants that could provide resources such as food, refugia or alternative hosts. A good candidate would be Sudan grass (*Sorghum vulgare sudanense* (Pers.) (Poaceae), which has been shown to not only act as a stemborer trap plant, but which also increases natural enemy abundance and efficiency (Khan et al., 1997b). The grass acted as a reservoir of parasitoids, which attacked stemborers in the adjacent crop fields.

The relevance of habitat manipulation for biological control depends not only on the nature of plants involved, the herbivores and parasitoids but also on the relationships among these trophic levels. It is therefore of utmost importance to understand these tritrophic interactions and have insight into the foraging behaviour of the parasitoid involved before recommending a habitat management strategy to enhance biological control.

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4

Analysis of headspace volatiles of stemborer host and non-host plants

Chapter 4

Analysis of headspace volatiles of stemborer host and non-host plants

Abstract

Parasitoids use plant volatiles as long-range cues during their search for hosts. Host plants of their herbivore hosts are normally the most attractive but non-host plants may also attract them. Previous olfactometric bioassays showed that *Cotesia sesamiae* (Cameron) (Hymenoptera: Braconidae) and *Dentichasmias busseolae* Heinrich (Hymenoptera: Ichneumonidae), parasitoids of cereal stemborers, are attracted to volatiles of stemborer host plants, maize and sorghum, with volatiles from infested plants being most attractive. Volatiles emitted by a non-host plant molasses grass were attractive to *C. sesamiae*, but repellent to *D. busseolae*. In the present study, headspace collections of volatiles from infested and uninfested host plants and molasses grass were analysed. The analysis revealed qualitative differences between the volatile blends of the plants. Herbivore-infested plants emitted a larger number of compounds than uninfested plants. Differences were also noted between host plant species. Compounds positively identified from molasses grass were few and were also present in the volatile blend of host plants. The results of this study are discussed in relation to behavioural observations on the parasitoids under study.

Introduction

Plant volatiles are known to play a major role in the orientation of parasitoids to habitats that most likely harbour their hosts (Vinson, 1977). Some of these volatile compounds may be constitutive plant products that are produced whether plants are damaged or not. Others may be released after mechanical damage or after damage by particular herbivore species (Turlings et al., 1995; Dicke & Vet, 1999). The advantage of plant-derived signals to parasitoids is that they are released in much larger quantities than host-derived ones and are easier to detect (Vet & Dicke, 1992). The composition of these volatiles can vary substantially due to biotic or abiotic factors that influence the plant. Relevant factors include plant species, plant genotype, attack by herbivorous insects, growth condition of the plant, time of the day (Loughrin et al., 1994; Halistchke et al., 2000; de Moraes et al., 2001; Hoballah et al., 2002; Gouinguené & Turlings, 2002). Volatile chemical composition varies most among plant species and least within a species. The differences are quantitative as well as qualitative in nature (Takabayashi & Dicke, 1996; Geervliet et al., 1997; Hoballah et al., 2002).

Several studies have demonstrated the attractiveness of volatiles emanating from uninfested and mechanically damaged host plants to parasitoids (Williams, 1988; Ngi-Song et al., 1996; Benrey et al., 1997; Rao et al., 1999; Kalberer et al., 2001). However, volatiles emitted by infested plants are the most attractive. These herbivore-induced volatiles could differ from those of intact plants either in quantity, in that there is a change in the amounts of same chemical components or in quality, by release of novel compounds (Dicke & Vet, 1999; Turlings & Fritzsche, 1999; Dicke & van Loon, 2000). There are also reports of volatiles from non-host plants being attractive to foraging parasitoids (Takabayashi et al., 1991; Khan, 1997a; Gohole et al., 2003). Several chemical compounds could be involved in this attraction and one, (*Z*)-3-hexenol has been particularly singled out as being released by many green plants (Hoballah et al., 2002).

In field experiments to control stemborer pests using habitat management methods, Khan et al. (1997a,b) reported higher parasitism rates of stemborers by the larval parasitoid *Cotesia sesamiae* (Cameron) (Hymenoptera: Braconidae) in maize plots intercropped with molasses grass. They attributed this observation to production of volatiles by molasses grass that were similar to herbivore-induced ones and responsible for recruiting parasitoids. They particularly singled out the compound (*E*)-4,8-dimethyl-1,3,7-nonatriene. Based on these results, we conducted several laboratory-based bioassays to investigate further on the potential of molasses grass as a candidate plant for use in a habitat management system designed to enhance the effectiveness of stemborer parasitoids in a cereal based system. The system under study consisted of the host plants maize and sorghum, the non-host plant, molasses grass, the stemborer parasitoids *C. sesamiae* and *Dentichasmias busseolae* Heinrich (Hymenoptera: Ichneumonidae). In olfactometric studies, *C. sesamiae* responded favourably to volatiles from all the plants (Chapter 2). However, the pupal parasitoid *D. busseolae* was repelled by volatiles from molasses grass (Chapter 3). In semi-field tests, the parasitoids discriminated host plants from the non-host plant, molasses grass (Chapter 5). *Cotesia sesamiae* was also reported to respond differently to volatiles emitted by the same molasses grass cultivar grown in two different geographic locations in Kenya. In order to understand the parasitoid behaviour exhibited in earlier studies of this system, it is necessary to determine the type of chemical compounds that the parasitoids most likely face. Analyses of the headspace volatiles of the plants involved in the system were conducted in the present study.

Materials and methods

Plants

The host plants sorghum, *Sorghum bicolor* (L), cv Seredo and maize, *Zea mays* L. H-511 (Poaceae) were planted in plastic pots (ø 20 cm). The non-host plant molasses grass *Melinis minutiflora* Beauv. (Poaceae), derived from two different locations, Mbita Point in western Kenya (34° 10' E, 0° 25' S) and Thika (37° 5' E, 1° 3' S) situated 35 km from Nairobi, was also planted in plastic pots (ø 25 cm) from root splits. The plants were kept at the International Centre of Insect Physiology and Ecology (ICIPE) in Nairobi, under cages covered with 400-micron mesh netting to protect them from natural insect attack. Sorghum was thinned to 2 plants and maize to one plant per pot at 7 days after emergence (DAE). This was to obtain approximately similar amounts of biomass in tests that compared the two plant species.

Insects

Third instar *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) larvae were obtained from laboratory colonies reared on artificial diet (Ochieng et al., 1985) and maintained at ICIPE.

Treatment of plants

Infested host plants (5 weeks after emergence) were obtained by introducing two third-instar *C. partellus* larvae into the whorl of the plant. These were allowed to feed and tunnel into the stem of the plant during a period of approximately 36 hours after which the plants were used in volatile collection. Volatiles were collected from the following plants and each treatment replicated 4 times; uninfested maize (UM), infested maize (IM), uninfested sorghum (US), infested sorghum (IS), Thika molasses grass (TMG) and Mbita molasses grass (MMG)

Collection and analysis of volatiles

The collecting chamber was a simplified version of one used by Turlings et al (1998). It basically consisted of a glass chamber (20 × 20 × 50 cm), with two openings, one at the top to let air into the system and one 7.5 cm from the bottom which allowed for attachment of the collection traps (Figure 1). The aerial part of the plant was placed in the glass chamber, while the aluminium foil-covered soil and the pot were isolated from the system by a glass plate consisting of two halves with a hole of diameter 3-cm in the centre for the stem of the plant. Any gap between the stem and the hole was sealed with clean cotton wool.

Compressed air was pushed through a charcoal filter for purification, at a rate of 1 l/min and passed over the potted plant. Air was pulled from the system though the collection trap at a rate of 0.8 l/min using a vacuum pump. A collection trap consisted of a glass tube (5 cm long and 0.5 cm diameter) containing 80 mg of Porapack Q (Chrompack, 80-100 mesh). The rest of the air vented out through the opening in the glass plate at the bottom. Trapping was done for 8 hours during the photophase. The trapped volatiles were eluted from the adsorbent with 2 ml of HPLC grade Dichloromethane (DCM) and the extracts stored in the freezer at –20°C until used.

Analyses were done with a Hewlett Packard model HP 5890 gas chromatograph (GC) equipped with an on-column injection system and a flame ionisation detector (FID). Aliquots of 2µl of each sample were injected and two columns of different stationary phases were used: HP1 (cross-linked methyl silicone, 50m, 0.32mm ID, 0.52µm film thickness) and DB-WAX (polyethylene glycol, 30m, 0.32 mm ID, 0.5µm film thickness). Hydrogen was used as the carrier gas at a rate of 2.5ml/min. Following injection, column temperature was maintained at 40° C for one minute then programmed to 10° C per minute to 240° C. Tentative identification of compounds was based on calculation of Kovats indices and comparing them with those in a database developed at the Biological Chemistry Division of Rothamsted Research, UK.

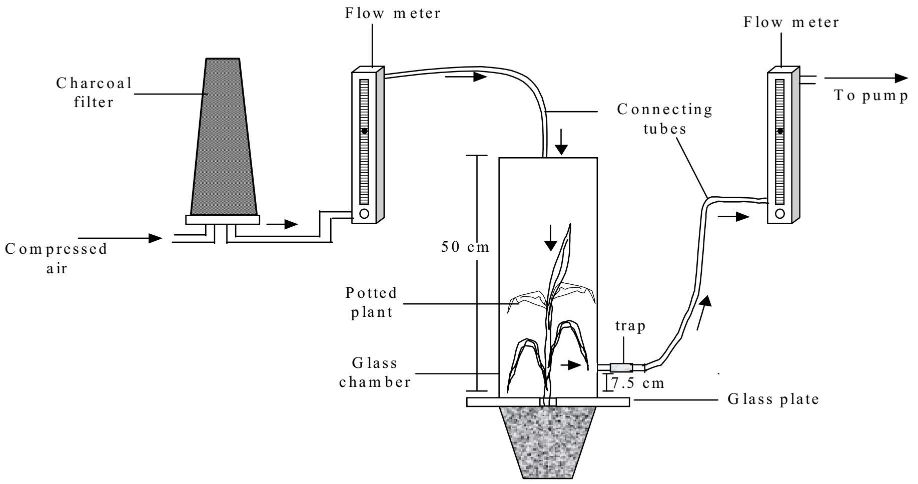

Figure 1. Schematic diagram of apparatus for collection of plant volatiles.

For further identification, 2µl of each extract was analysed using the Hewlett Packard model HP5890 gas chromatograph (GC) on a HP-1 (cross-linked methyl silicone) column (50 m × 0.32 mm i.d) directly coupled to a VG Autospace mass spectrometer (MS). The optic programmed temperature vapourisation (PTV) injector was rapidly heated (20 to 220° C at 16/s) upon injection. The temperature programme was: 30° C for 5 minutes, increasing by 5° C per minute up to 250° C. The MS was used in the electron-impact mode (70 eV). Compounds were identified by comparison of the mass spectra with those in the standard database NIST (1990).

Results

The headspace compositions of stemborer host and non-host plants identified by mass spectometry are given in Table 1. Qualitative differences in compound composition were found between plants species and between infested and uninfested host plants. The following four compounds were common among the host plants, 2-butoxyethanol, naphathalene, α-cedrene and an unidentified sesquiterpene. The infested host plants produced novel compounds when compared to their uninfested counterparts (Table 1) most of which were sesquiterpenes.

Table 1. Chemical compounds identified from headspace volatiles of stemborer host and non-host plants.

Compound	IM	UM	IS	US	TMG	MMG
2-butoxyethanol	√	√	√	√	-	-
nonanal	√	-	√	√	-	-
naphthalene	√	√	√	√	√	√
decanal	√	-	√	√	-	-
limonene	-	-	√	-	-	-
1,3,8-*para*-menthatriene	-	-	√	-	-	-
α-cedrene	√	√	√	√	√	√
(*E*)-β-farnesene	√	-	√	-	√	-
dehydrobisabolene	√	-	-	-	-	-
β-bisabolene	√	-	-	-	-	-
α-humulene	√	√	-	-	√	√
unidentified sesquiterpene	√	√	√	√	√	√
culmorin	-	-	√	-	-	-
sesquiterpene alcohol	-	-	√	-	-	-
α-santalene	√	-	√	-	-	-

IM-infested maize, UM-uninfested maize, IS-infested sorghum, US- uninfested sorghum, TMG- Thika molasses grass, MMG- Mbita molasses grass.

Tentative identification of chemical compounds in the plant volatile blends using Kovats index calculations, revealed additional compounds in all samples (Table 2). (*Z*)-3-hexen-1-yl acetate was produced by the infested host plants and Thika molasses grass while (*E*)-ocimene was unique to the infested host plants. Benzaldehyde, myrcene, (*R*)(-)-phellandrene, acetophenone and nonanal were common occurrences in the headspace of the uninfested host plants and Thika molasses grass. Mbita molasses grass produced few identifiable compounds.

Discussion

Differences in plant volatile emissions among plant species have been demonstrated in several studies (Geervliet et al, 1997; Kalberer et al., 2001; Gouinguené et al., 2001). The present study confirms variations in volatile blend of the plant species. This could account for the differences in response to volatiles of different plants noted in bioassays conducted earlier (Chapters 2 & 3). A compound that was commonly produced by all plant samples under study was α-cedrene. Naphthalene was common to all host plants whether infested or uninfested. Naphthalene is highly

Table 2. Relative amounts of chemical compounds (% of the total peak area) tentatively identified by calculations of Kovats indices of the eluted compounds of headspace volatiles of stemborer host and non-host plants. (Calculations are based on peak areas extrapolated from a known amount of 50ng of a C8 alkane).

Compound	IM	UM	IS	US	TMG	MMG
(*Z*)-hexen-1-ol	-	-	-	-	3.36	-
1-octen-3-one	-	-	3.78	-	-	-
(*Z*)-hexen-1-yl acetate	5.33	-	4.74	-	5.07	-
(*E*)-ocimene	7.34	-	4.14	-	-	-
benzaldehyde	-	4.12	-	2.03	2.66	-
myrcene	-	3.17	-	3.75	4.28	-
(*R*)(-)-α-phellandrene	-	2.04	-	1.20	2.27	-
acetophenone	-	7.63	-	2.94	4.40	-
nonanal	-	1.83	-	1.00	3.73	-
(E)-caryophyllene	3.04	-	-	-	-	-
linalool	-	-	-	-	-	2.88
heptanal	-	-	-	-	-	10.11
4-hydroxybenzaldehyde	-	-	-	-	-	2.04

attractive to stemborer moths (Pickett et al., 1999) but its attraction to parasitoids has not been demonstrated. Infested plants produced a richer blend than the intact plants as also noted in several other studies (Turlings et al., 1991; Geervliet et al., 1997; Ngi-Song et al., 2000). In our study, infested plants produced quite a number of novel compounds mainly sesquiterpenes. Some of these sesquiterpenes are known to be produced in small quantities by mechanically damaged plants but their amounts increase greatly after herbivore damage (Takabayashi et al., 1995; Dicke et al., 1990). The induction of herbivore-induced volatiles is said to be due to elicitors present in the oral secretions of the herbivores (Mattiacci et al., 1994; Alborn et al., 1997; Turlings et al., 2000). These herbivore-induced volatiles are widely reported to be responsible for the recruitment of foraging parasitoids that attack herbivore pests (Alborn, 1997; de Moraes et al., 1998; Dicke & Vet, 1999). This phenomenon could also be in operation in the present study as parasitoids were attracted more to volatiles from infested plants. The following herbivore-induced compounds identified in the present study have been recorded in other studies; (*E*)- β-farnesene, α-humulene, limonene, β-bisabolene and nonanal (Blaakmeer et al., 1994; Dicke, 1999a; Turlings et al., 1998, 2002; Gouinguené, 2001; Hoballah et al., 2002).

The non-host plant molasses grass was attractive to the parasitoid *Cotesia sesamiae*, (Gohole et al., 2003; Chapter 2). This attraction could be attributed to the identified compounds it produced which are similar to those emitted by host plants, particularly (*E*)- β-farnesene, α-humulene and an unidentified sesquiterpene. These compounds were also present in the volatile blend of several other herbivore-damaged cereals (Turlings et al., 1998; Ngi-Song et al., 2000; Gouinguené et al., 2001). Two major homoterpenes that commonly feature in herbivore-induced volatiles blends of several plant species are (*E*)-4,8-dimethy-1,3,7-nonatriene and (*E,E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (Turlings et al., 1991; Takabayashi et al., 1995; Dicke, 1999a). These compounds could not be found in the blends of infested host plants in the present study. (*E*)-4,8- dimethy-1,3,7-nonatriene was also not found in the headspace volatiles of molasses grass used in this study, although previous studies (Khan et al, 1997a; Kimani et al., 2000) had reported its presence in molasses grass volatiles. A number of reasons are possible and not least, the high instability of the two compounds (Turlings & Fritzsche, 1999 - discussion). Studies comparing total production of volatiles by maize plants of different ages revealed that younger plants (1-2 weeks old) released larger amounts of volatiles than older plants (6-8 weeks old) (Turlings et al., 2002). This means that the volatile profile of a plant could be specific to the developmental stage of the plant (Matile & Altenburger, 1988). In most studies in which these two homoterpenes were reported present, seedlings (8-21 days old) were used, while in the present study, 5-week old plants were used. The technique used in collection of the volatiles could also influence the volatile blend produced by plants (Agelopoulos & Pickett, 1998; Agelopoulos et al., 1999). In the present study, volatiles were collected from potted plants which were minimally disturbed while in experiments of Ngi-Song et al. (2000) and Kimani et al. (2000), the plants were uprooted, their roots washed and wrapped in moist cotton wool or placed in water. This disturbance could contribute greatly to a change in the plant's physiology, which would have an effect on volatile production. Furthermore, while handling the plant in preparation for volatile collection, the plants could have been mechanically wounded; this may have contributed to a change in composition of the chemical components.

Based on the reported compounds collected from stemborer host and non-host plants, it can be inferred that the parasitoids could distinguish between plants on the basis of the quality of the volatile blend they produced. In some chemical analyses, differences in blend composition are hardly revealed, yet parasitoids can discriminate among blends (Dicke, 1999b). *Dentichasmias busseolae* distinguished host from non-host plant volatiles. In interactions of plant information and parasitoid responses, there is a continuum of signal-to-noise ratio from a low to a very high level (Vet, 1999). When it is high, discrimination between cues from suitable and unsuitable plants seems not to be a problem to the searching parasitoid. This could be applied to the *D. busseolae* case. This is further evidenced by the fact that *D. busseolae* chose volatiles from a combination of infested host plant and molasses grass over those from molasses grass alone (Chapter 3). This means that the parasitoid still perceives the chemical compounds of the suitable plant even in the presence of the repellent ones from molasses grass. It is hypothesized that its mainly qualitative differences in volatile blends that play a role in plant discrimination by parasitoids (Vet et al., 1998).

In conclusion, the present study did not reveal specific compounds in the volatile blend of molasses grass such as (*E*)-4,8-dimethy-1,3,7-nonatriene that would single out this plant as a promising candidate for enhancing the effectiveness of stemborer parasitoids in cereal pest management. This also explains why molasses grass was not more attractive than the host plants in olfactometric bioassays performed here. However we cannot dismiss the usefulness of molasses grass entirely based on the present study because previous studies had shown it to have compounds that are useful in recruiting parasitoids (Khan et al, 1997a, 2000; Kimani et al., 2000). What still needs to be done is to identify the active components of host plants and molasses grass that elicit sensory and physiological responses in the parasitoids using gas chromatography coupled with electroantennogram recordings (GC-EAD). These sensory-physiological analyses coupled with chemical analysis would shed light on the possible compounds in the blend that the parasitoids use to locate their hosts and explain why molasses grass is attractive to some parasitoids and repellent to others.

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5

Close-range host searching behaviour of the stemborer parasitoids *Cotesia sesamiae* and *Dentichasmias busseolae*: role of a non-host plant *Melinis minutiflora*

Chapter 5

Close-range host searching behaviour of the stemborer parasitoids *Cotesia sesamiae* and *Dentichasmias busseolae*: role of a non-host plant *Melinis minutiflora*.

Abstract

Studies were conducted on the host searching behaviour of the larval parasitoid *Cotesia sesamiae* (Cameron) (Hymenoptera: Braconidae) and the pupal parasitoid *Dentichasmias busseolae* Heinrich (Hymenoptera: Ichneumonidae), both of which attack lepidopteran (Crambidae, Noctuidae) cereal stemborers. The behaviour of individual parasitoids was observed in a semi-field environment of a diversified habitat that consisted of stemborer host plants (maize, *Zea mays* L. and sorghum, *Sorghum bicolour* (L). Moench (Poaceae)) and a non-host plant (molasses grass, *Melinis minutiflora* Beauv. (Poaceae)). In previous olfactometer studies, *C. sesamiae* was attracted to molasses grass volatiles while *D. busseolae* was repelled. The aim of the present study was to investigate how molasses grass influences parasitoid close-range foraging behaviour in a diversified habitat. Both *C. sesamiae* and *D. busseolae* strongly discriminated between host and non-host plants with female wasps spending most time on infested host plants and least time on molasses grass. While on the infested plant, the wasps spent more time foraging on the stem, the site of damage than other areas of the plant. Overall, the results indicate that presence of the non-host plant does not hinder close range foraging activities of either parasitoid species.

Introduction

Foraging behaviour is an important factor that influences the effectiveness of a parasitoid as a biological control agent (Jervis and Kidd, 1996). During foraging, the parasitoid must first locate a host plant, then an infested plant, and finally the host. Parasitoids use chemical, visual, mechanical and acoustic cues, or a combination of two or more of these to locate host habitats and hosts within the habitat (Godfray, 1994, Vet et al., 2002). A sequence of responses to different stimuli brings the parasitoid closer and closer to its potential host. Plants are a major source of information and their role in the host selection process of parasitoids has become increasingly clear (Turlings et al., 1995, Dicke and Vet, 1999, Vet, 1999)

With insect pests becoming tolerant or resistant to conventional chemical pesticides, research is now directed towards other more effective, environmentally friendly and sustainable approaches to combat pests. Habitat management is one such approach that has recently gained attention (Gurr et al., 2000; Landis et al., 2000). In this approach, the habitat is altered to improve the availability of resources required by the natural enemies for optimal performance. Intercropping, a component of habitat management, may improve parasitoid activity because, among other advantages, one of the plants of the intercrop may provide allelochemical attraction to natural enemies (Verkerk et al., 1998). In their studies on habitat management to control cereal stemborers (Lepidoptera: Crambidae, Noctuidae), Khan et al. (1997a,b) devised a system in which molasses grass (*Melinis minutiflora* Beauv. (Poaceae), a non-host plant of stemborers, repelled gravid stemborer moths away from a maize crop. The moths were in turn attracted by a trap crop, reducing infestation and damage on maize. This strategy has been referred to as 'push-pull' or stimulo-deterrent diversionary strategy (Pickett et al, 1997, Agelopoulos et al., 1999). An advantageous phenomenon noted in the same study was the positive effect of the intercropping on the performance of a larval stemborer parasitoid, *Cotesia sesamiae* (Cameron) (Hymenoptera: Braconidae). Higher parasitism rate was found in the intercrop than in the maize monocrop. This observation was attributed to attraction of the parasitoid to the intercrop by chemical volatiles produced by molasses grass.

Recent additional olfactometric Y-tube studies on attraction of parasitoids to plant volatiles confirmed that molasses grass volatiles attracted *C. sesamiae* (Gohole et al., 2003). In contrast, the volatiles were repulsive to another parasitoid species, *D. busseolae* (Chapter 3). Whether attracted or repelled, the question remains, what happens once parasitoids have entered such a diversified habitat? When attracted, do they land and search on the molasses grass that does not harbour hosts thereby wasting valuable foraging time? When repelled, do they recognise the non-host plant at short range and are consequently diverted to the infested host plants? To link results from laboratory olfactometer studies to field results on parasitism rates, it is crucial to understand the short-range interactions involved and how the foraging activities of parasitoids are affected by plant diversity. This is an area that has received limited attention in spite of the numerous theories that have been put forward to explain why some diversified systems harbour more arthropod natural enemies than simple cropping systems.

Cotesia sesamiae is a gregarious endoparasitoid of larvae of several stemborers (Ngi-Song et al., 1995). It employs an 'ingress and sting' foraging strategy, where it locates the entrance hole of the tunnel, contacts the frass, starts antennating and then enters the tunnel by crawling through the frass (Smith et al., 1993). During foraging, *C. sesamiae* uses chemical cues to locate its host, with the most attractive chemical compounds coming from infested host plants and frass produced by the feeding host (Ngi-Song et al., 1996; Ngi-Song and Overholt, 1997; Rutledge and Wiedenmann, 1999). *Dentichasmias busseolae* Heinrich (Hymenoptera: Ichneumonidae) is a solitary pupal endoparasitoid with a narrow host-range that is limited to *Chilo* species (Lepidoptera: Crambidae) (Mohyuddin and Greathead, 1970; Zhou et al., 2003). Most collections of the parasitoid have been from *Chilo partellus* (Swinhoe). *Dentichasmias busseolae* is attracted to the vicinity of its host mainly by chemical cues resulting from the interaction of the host and host plant (Chapter 3). It prefers to oviposit in young pupae (one to two days old) in which development is optimal (Bahana, 1989). Once on the infested plant, the parasitoid is arrested by cues from frass and possibly by mechanosensory and visual cues because it concentrates its antennating at the stem region harbouring the pupa. The parasitoid gains entry into the tunnel by biting off the membranous moth exit window. This window is constructed by the last larval instar of the stemborer prior to pupation to facilitate egress of the adult moth from the stem. Like *C. sesamiae*, *Dentichasmias busseolae* also attacks its pupal host using an 'ingress and sting' tactic (Smith et al., 1993).

The objective of the present work was to examine the foraging behaviour of the stemborer parasitoids *C. sesamiae* and *D. busseolae* in patches with host plants and the non-host plant molasses grass. The investigations will be useful in predicting the influence of the non-host plant on the foraging process of these parasitoids in an intercrop.

Materials and Methods

Plants

The host crop plants sorghum (cv Seredo) and maize (H-511) were planted in plastic pots (ø 20 cm). The non-host plant molasses grass, derived from Thika (37° 5'E, 1° 3'S) situated 35 km from Nairobi, Kenya, was also planted in plastic pots (ø 25 cm) from root splits. Both sorghum and maize were thinned to one plant per pot at 7 days after emergence (DAE). The plants were kept at the International Centre of Insect Physiology and Ecology (ICIPE) at Nairobi, under large cages covered with 400-micron mesh netting to protect them from natural insect attack.

Insects

Chilo partellus larvae were obtained from laboratory colonies reared on artificial diet (Ochieng et al., 1985), and maintained at ICIPE. *Cotesia sesamiae* were also obtained from a laboratory colony maintained at ICIPE (25 ± 2°C, 70-80% RH) according to the method described by Overholt et al. (1994). Females used in the bioassays were 1 to 2-days old and naïve with regard to host plant and ovipositional experience.

A laboratory culture of *D. busseolae* was established with adults reared from *C. partellus* pupae collected from Mbita Point in western Kenya (34° 10'E, 0° 25'S). The parasitoids were reared using a slightly modified version of Mohyuddin's (1972) method. Maize stems of 15 cm length were split longitudinally into two and three depressions, 3 cm apart, excavated in one of the longitudinal sections. Holes (ø 0.4 cm) were bored through the other longitudinal section at locations corresponding to the depressions. The depressions were filled with frass from fifth instar *C. partellus* larvae but ensuring there was space for the parasitoid to manoeuvre its way around the pupae. Two one-day old *C. partellus* pupae were placed in each depression. The longitudinal sections were rejoined and secured in place using rubber bands at each end. The holes were lightly sealed with frass to simulate an exit hole. The stem pieces containing the pupae were offered to the *D. busseolae* females in Perspex cages (30 x 30 x 30 cm) at a ratio of 1 female: 2 pupae. Stems were presented in an upright position and held in place by standing them in plasticine. Pupae were extracted from the stems after 24h and placed on moistened filter paper in Petri dishes. On emergence, both male and female parasitoids were released in a cage and allowed a 48 hours mating period after which the females were separated from the males and placed in separate cages. The parasitoids were provided with a diet of 20%

honey/water solution, supplied in Petri dishes containing cotton wool pads. The pads were renewed every two days to avoid fermentation. The parasitoids also had access to water supplied on wet cotton wool pads. Females used in the experiment were 5 to 7 days old and naïve.

Bioassay with *D. busseolae*

The experiments were conducted in a screen-house at 26 ± 2°C, 45-65% RH. A steel-framed cage, measuring 2m × 2m × 2m and covered with fine white mesh (400 μm) net material, was constructed. The experimental arena consisted of four potted plants placed in a square arrangement on a table centrally positioned in the cage (Figure 1). Two fans were placed at one end of the table to provide an air stream. The wind speed was measured using an anemometer and set at 0.2-0.3 m s^{-1}. The foraging behaviour of *D. busseolae* was observed in the following crop arrangement:

I) Maize monocrop (MM):	two uninfested maize + two infested maize plants
II) Uninfested maize intercrop (UMI):	two uninfested maize + two molasses grass plants
III) Infested maize intercrop (IMI):	two infested maize + two molasses grass plants
IV) Sorghum monocrop (SM):	two uninfested sorghum + two infested sorghum plants
V) Uninfested sorghum intercrop (USI):	two uninfested sorghum + two molasses grass plants
VI) Infested sorghum intercrop (ISI):	two infested sorghum + two molasses grass plants

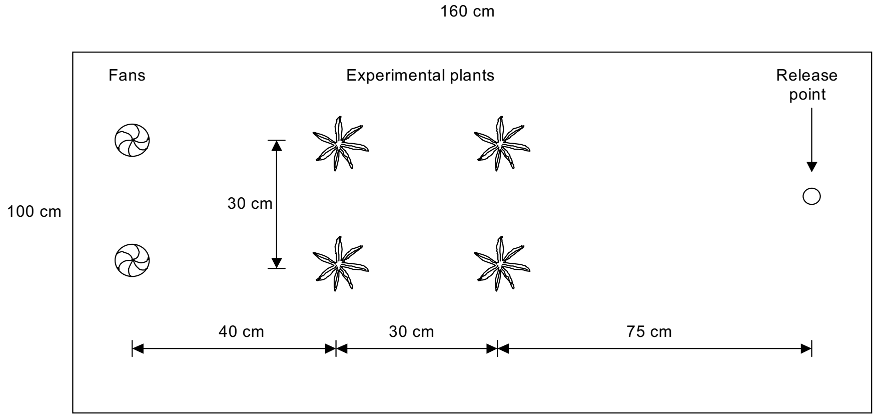

Figure 1. Layout of the semi-field experiment with *D. busseolae*.

Host plants were artificially infested by introducing two fifth instar *C. partellus* larvae into the whorl of each host plant. The larvae were allowed to feed and tunnel into the stem for a period of 5-8 days after which they constructed a moth exit window and pupated in the plant stem. At the start of each observation, a single naïve *D. busseolae* female was released downwind from a vial at the release point. From the moment it flew away from the vial, observations and recording of its behaviour and location were started. Observations were terminated after 30 minutes or when the parasitoid walked or flew away and landed elsewhere (not on plants) and remained there for more than 5 minutes. For each crop arrangement, 15 females were tested and each wasp was used once. Plants were changed after every three individuals, or when the parasitoid bit off the exit hole window. The following behavioural activities were recorded using the computer software package, The Observer (Noldus, 1991):

flying: when the wasp was in flight either within the vicinity of the plants or away from the plants and not in touch with any object.
walking: when the wasp walked constantly on whichever substrate she landed and with antennae swinging but not palpating the substrate.
standing: the wasp remained motionless with very minimal antennal movements.
grooming: the wasp repeatedly drew antennae through forelegs and brushed wings and abdomen with the other legs.
antennating: the wasp walked with irregular speed or stopped, with her antennae bent over her head and palpated the substrate.
window biting: the wasp bit through the stemborer exit window using her mandibles while frequently probing into the tunnel with her antennae.
tunnel entry: the wasp entered the tunnel headfirst or if pupa was close to entrance, she turned and probed at the tunnel entrance with her ovipositor.

Throughout the experiment the location of the wasp was recorded as plant 1 when it was on either of the two plants treated similarly, or plant 2 when on the other two plants in each crop arrangement. All other locations within the cage were recorded as elsewhere.

Bioassay with *C. sesamiae*

Preliminary experiments showed that the bioassay used for *D. busseolae* would not work with *C. sesamiae* due to its small size, which made observations problematic. Thus, the arena was modified and experiments were conducted in a temperature-controlled room at 25 ± 2°C, 60-75% RH. The host plants used were 5 weeks old. Observations were made on single plants. The following plants were used:
I) Infested maize
II) Uninfested maize
III) Infested sorghum
IV) Uninfested sorghum
V) Molasses grass

To obtain infested plants, a hole was bored in the stem of a host plant at 10 cm from soil level. One-third instar *C. partellus* larva was introduced into the hole and allowed 36 hours to bore into the plant stem. At the start of an observation, a single wasp was placed on a host plant's stem 15 cm above soil level. In the case of molasses grass, it was placed 5 cm above soil level on one of the centrally positioned stems. The behaviour and location of the wasp on the plant were recorded using 'The Observer'. A total of 20 female wasps were observed for each treatment and each wasp was used only once. The experimental plants were changed after every three wasps or when the wasp entered the tunnel. The durations (in seconds) of the following parameters were recorded.

a) behaviour of the parasitoid: fly, walk, stand, groom, antennate and tunnel entry (see bioassay with *D. busseolae* for explanations of behaviours).

b) position of the parasitoid: stem, leaves, whorl, tunnel entrance, inside tunnel and elsewhere.

Observations were terminated after 20 minutes or when the parasitoids flew or walked away from the plant and stayed elsewhere for more than 2 minutes.

Data analysis

Since data were not normally distributed and variances were not equal, they were analysed using non-parametric statistics. For each crop arrangement in the *D. busseolae* experiment, total time spent at each position by the wasp and average durations of behaviours were compared using the Friedman's test. When significant, the means were further compared using Wilcoxon's multiple pairwise comparison with Bonferroni correction. Residence time spent on similarly treated plants in the monocrop and intercrop (i.e. residence time on uninfested maize in the monocrop was compared with that on uninfested maize in the intercrop) were compared with the Mann-Whitney-U test. The time it took for the wasp to locate infested host plants in the monocrop and intercrop and frequency of visits to these plants was compared using the Mann-Whitney-U test.

Analyses of differences in time durations between treatments and duration of behavioural parameters in the *C. sesamiae* experiment were performed using the Kruskal-Wallis one-way ANOVA. In cases of analyses being significant, the Mann-Whitney-U test for pairwise comparisons were used with a Bonferroni correction. The Wilcoxon signed rank test was used to compare time spent on the plant to time spent elsewhere (not on the plant), within the same treatment.

Results

Dentichasmias busseolae

In all crop arrangements that had infested host plants (MM, IMI, SM and ISI), *D. busseolae* wasps spent most of their time on the infested plant. There was no difference in time spent on uninfested host plants and time spent elsewhere in the two crop arrangements containing uninfested host plants (UMI and USI). *Dentichasmias busseolae* spent significantly less time on molasses grass than on either infested or uninfested host plants (Figure 2).

Analyses on amount of time spent on uninfested maize and sorghum in the monocrop compared to their counterparts in the intercrop revealed that the wasps spent more time on uninfested plants in the intercrop as compared to the monocrop

(U = 17, p< 0.0001 and U = 20.5, p< 0.0001, for uninfested maize and sorghum respectively). There was no difference in time spent on infested host plants in the monocrop and intercrop (U = 110, p= 0.935 and U = 101, p= 0.653 in infested maize and sorghum respectively).

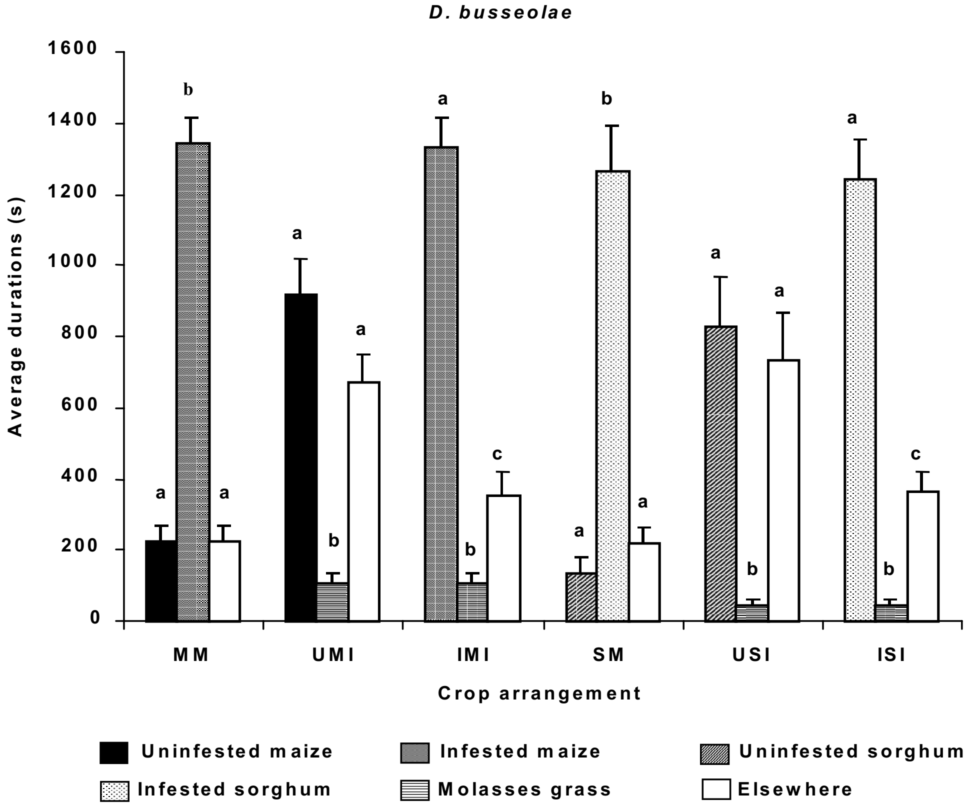

Figure 2. Average residence times (mean ± S.E) of *Dentichasmias busseolae* in a semi-field set up of host and non-host plants. Within each crop arrangement, means sharing the same letter do not differ (Kruskal-Wallis test followed by Mann -Whitney -U test with Bonferroni correction, $\alpha < 0.016$, for multiple pairwise comparison). n = 15. MM Maize monocrop, UMI - uninfested maize intercrop, IMI - infested maize intercrop, SM - sorghum monocrop, USI - uninfested sorghum intercrop, ISI - infested sorghum intercrop.

Durations of behaviours at the three positions in each crop arrangement are shown in Table 1. While on the host plants, the wasps were most involved in walking and standing. On the infested plants, the wasps were also occupied with activities related to close range host finding: antennating, window biting and tunnel entry. On encountering frass, the wasps were arrested and spent time antennating the frass. When the wasp located the portion of the stem harbouring the pupa, it concentrated

Table 1. Average durations (s) (± S.E) of behaviours of *Dentichasmias busseolae* in different cropping arrangements.

Crop arrangement	Position	Behavioural parameters						
		Fly	Walk	Stand	Groom	Antennate	Window biting	Tunnel entry
Maize monocrop	Uninfested maize	13.3±3.2a	77.8±16.2a	103.9±30.3ab	29.6±10.9a	-	-	-
	Infested maize	12.7±2.9a	391.1±41.9b	220.7±33.3b	131.9±14.4b	416.7±64.9	145.9±60.4	24.7±10.0
	Elsewhere	69.2±19.1b	82.2±22.9a	44.5±11.9a	25.8±8.8a	-	-	-
Uninfested maize intercrop	Uninfested maize	22.6±6.6a	260.0±54.5a	508.7±89.4a	123.6±23.4a	0.9±0.8	-	-
	Molasses grass	9.6±2.6a	27.9±6.2b	49.2±17.4b	19.2±8.2b	-	-	-
	Elsewhere	86.7±9.2b	296.9±52.1a	202.3±31.8c	86.0±17.1a	-	-	-
Infested maize intercrop	Infested maize	10.9±2.0a	400.2±52.4a	335.9±57.9a	81.0±10.0a	413.5±52.3	47.5±21.5	42.6±31.7
	Molasses grass	9.5±2.2a	38.6±14.8b	51.5±17.0b	6.0±3.9b	-	-	-
	Elsewhere	42.3±10.9b	148.0±28.8c	103.4±26.1b	57.5±19.4a	-	-	-
Sorghum monocrop	Uninfested sorghum	9.2±5.7a	70.8±31.2a	42.5±17.8a	13.7±7.5a			
	Infested sorghum	11.8±4.2a	384.7±56.1b	143.6±35.9b	169.3±28.2b	370.9±48.4	135.6±53.6	51.6±19.0

	Elsewhere	34.9±7.0b	89.7±21.0a	49.6±14.9ab	44.2±11.9a	-	-	-
Uninfested sorghum intercrop	Uninfested sorghum	17.0±7.7ab	392.0±84.3a	346.1±60.5a	69.7±20.9a	3.8±2.7	-	-
	Molasses grass	5.2±1.4a	9.4±4.1b	27.3±11.7b	4.7±3.1b	-	-	-
	Elsewhere	44.5±9.3b	284.0±68.4a	279.5±66.8a	127.4±31.1a	-	-	-
Infested sorghum intercrop	Infested sorghum	14.3±1.9a	505.1±51.5a	225.2±43.0a	160.9±21.4a	266.0±46.6	56.0±24.7	15.6±7.6
	Molasses grass	5.2±1.6b	6.8±2.9b	26.3±11.6b	6.0±3.1b	-	-	-
	Elsewhere	47.3±10.0c	161.3±32.8c	88.7±21.7ab	67.4±15.4c	-	-	-

Means with same letters in a column within a crop arrangement group are not significantly different (Friedman's test followed by a Wilcoxon test with Bonferroni correction, $\alpha < 0.016$, for multiple pairwise comparisons). N = 15.

its walking around that area, drumming this part of the stem with its antennae until it located the moth exit window. The wasp then bit off part of the window occasionally inserting one of its antennae through the hole in the window and probed the tunnel. When it encountered a host, it continued biting the window until the hole was big enough for the wasp to enter the tunnel. If the pupa was close to the window, the wasp would turn, start probing with its ovipositor until it detected the pupa, and then oviposited. A comparison of duration of various behaviours in uninfested host plants in monocrop and intercrop arrangements revealed that wasps spent more time walking, standing and grooming in the intercrops than monocrops (Mann-Whitney U test, $p < 0.05$). There was no difference in duration of behaviours (fly, walk, stand, groom, antennate, window biting and tunnel entry) on infested host plants in monocrop and intercrop arrangements. The same observation was noted for the non-host plant, molasses grass, when a comparison was made of durations of the different behaviours when the grass was in intercrops with uninfested and infested host plants (Mann-Whitney U test, $p > 0.05$).

The time taken to locate a window and to bite the window was not different between the two crop arrangements for infested maize or sorghum (Table 2). More than 45% of the wasps released in each of the four crop arrangements with infested plants, were able to locate the stemborer exit window. In both the intercrop and monocrop arrangements, 20- 47% of the wasps bit off the window and entered the tunnel. In 63% of the cases where the wasps entered the tunnel (for both maize and sorghum), oviposition took place and adult emergence was recorded. Generally *D. busseolae* spent less time on molasses grass compared to uninfested and infested host plants. Its foraging behaviour while on molasses grass was generally in the following sequence: walk, stand, groom, and then fly to another location. It was never observed antennating any part of the plant.

There was no difference in time taken by *D. busseolae* until its first encounter with a host plant in the monocrop arrangement compared to the first encounter of a similarly treated host plant in the intercrop arrangement. There was also no difference in frequency of visits to infested plants in the two types of crop arrangements, (Table 2). Frequency of visits to uninfested host plants in intercrops was higher than for the uninfested host plants in the monocrop (UM in UMI $= 4.6 \pm 0.5$, UM in MM $= 2.3 \pm 0.46$, Mann-Whitney U test, $U= 41.5$, $p= 0.002$ for uninfested maize and US in USI $= 3.5 \pm 0.7$, US in SM $= 1.0 \pm 0.3$, Mann-Whitney $U= 37$, $p= 0.001$ for uninfested sorghum).

Table 2. Behavioural durations (seconds ± S.E) of *D. busseolae* on infested host plants in different crop arrangements

Parameter	Crop arrangement			
	Infested maize in intercrop	Infested maize in monocrop	Infested sorghum in intercrop	Infested sorghum in monocrop
Mean time to first landing on infested plant	111.7 ± 34.6a	153.6 ± 59.1a	93.8 ± 26.8A	131.7 ± 38.7A
Frequency of visits	3.8 ± 0.5a	3.3 ± 0.6a	3.7 ± 0.5A	2.5 ± 0.3A
Mean latency time to window biting	799.7 ± 152.1a	720.4 ± 162.6a	433.6 ± 58.7A	454 ± 94.4A
Percentage locating window	53% (n = 15)	60% (n = 15)	47% (n = 15)	80% (n = 15)
Percentage entering tunnel	20% (n = 15)	33% (n = 15)	27% (n = 15)	47% (n = 15)
Mean time spent inside tunnel	212.9 ± 130.9a (n = 3)	74.2 ± 11.6a (n = 5)	58.4 ± 13.8A (n = 4)	110.5 ± 26.9A (n = 7)

Means with same case letter in a row are not significantly different from each other (Mann-Whitney-U test, $p < 0.05$).

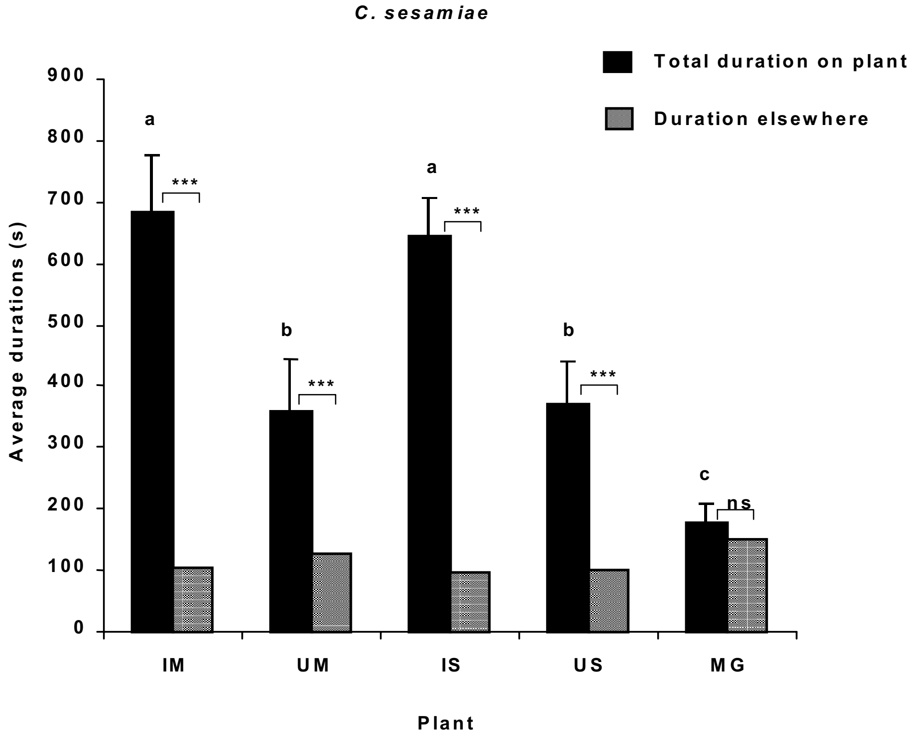

Figure 3. Average residence times (mean ± S.E) of *C. sesamiae* on host plants and a non-host plant and time spent elsewhere. Same letters above black bars (duration on plant) indicate no significant difference between means (Kruskal-Wallis test followed by Mann-Whitney-U test with Bonferroni correction, α < 0.005, for multiple pairwise comparison). Within a treatment, asterisks indicate significant differences between time spent on plant and time spent elsewhere. ** P< 0.0001, ns- not significant (Wilcoxon test). n = 20. IM - infested maize, UM- uninfested maize, IS - infested sorghum, US - uninfested sorghum, MG - molasses grass.

Cotesia sesamiae

Cotesia sesamiae spent more time on the host plants as compared to the non-host plant, molasses grass (Figure 3). None of the parasitoids tested in the molasses grass bioassay stayed in the experimental arena for more than 50% of the whole observation time. On average they spent a total of 329.6 ± 29.9 seconds in this arena before abandoning it completely. Residence times on infested host plants were higher than on uninfested host plants (Figure 3), with 40-55% of the wasps spending more than half of their time in the infested plants compared to 15-25% on uninfested plants. The wasps spent more time on the plant than elsewhere in the case of infested maize, uninfested maize, infested sorghum and uninfested sorghum. Mean times spent on molasses grass and elsewhere did not differ (Figure 3).

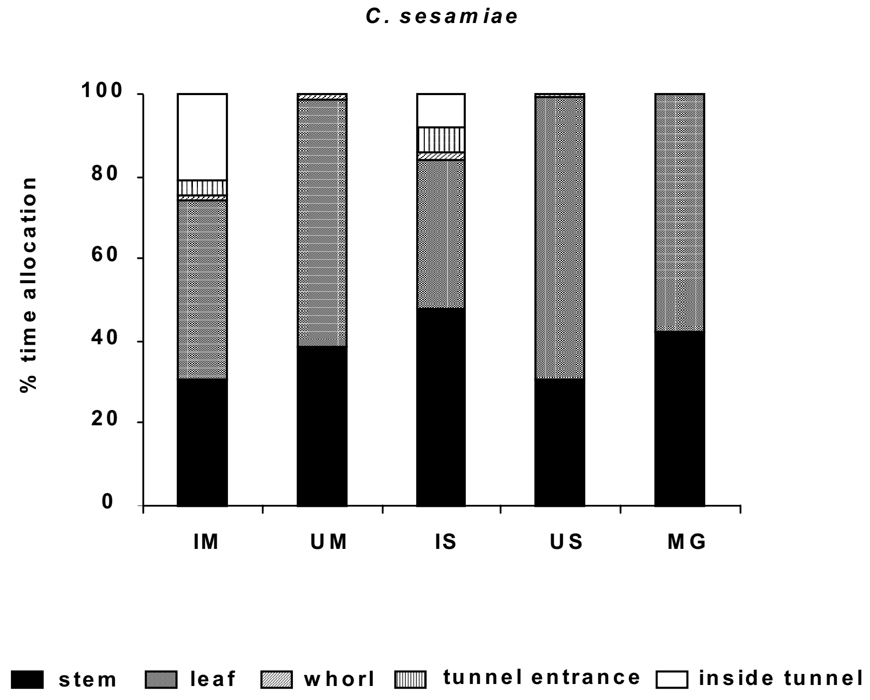

Figure 4. Relative time allocation of *C. sesamiae* at various locations while foraging on host and non-host plants. IM- infested maize, UM- uninfested maize, IS- infested sorghum, US- uninfested sorghum, MG- molasses grass.

While on the infested plants, the wasps spent most of their time on the stem, which also includes time spent at the tunnel entrance (Figure 4). Of the observed wasps, 70% and 85% were able to locate the tunnel entrance on the infested maize and sorghum, respectively. Of the wasps that located the tunnel entrance, 40% entered the infested maize tunnel, with the time spent inside the tunnel varying from 13.8 to 1014.0 seconds. Average time spent in maize tunnel was 356.3 ± 147.9 (SE) (n = 8). In the case of infested sorghum, 25% of the wasps entered the tunnel spending between 5.9 and 1042.9 seconds with average time spent in the tunnel being 421.6 ± 225.2 (SE) (n = 5). Of all the wasps that entered the tunnels, only one managed to crawl out. The movements of the rest were hindered because their bodies and wings adhered to the wet fresh frass.

The average percentages of time of the different foraging behaviours of *C. sesamiae* on different plants are shown in Figure 5. The wasps were most engaged in walking while on host and non-host plants and least in flying. Comparisons were made of the common behavioural elements (fly, walk, stand and groom) on all the plants. The mean duration of flying did not differ among the plants.

Durations of walking, standing and grooming did not differ among host plants but were less on molasses grass (Table 3). There was no difference in the amount of time spent on the behaviours related to host location between the infested plants. *Cotesia sesamiae* spent the same amount of time antennating and in the act of entering the borer tunnel in both infested maize and infested sorghum. Analyses on behaviours within a plant treatment revealed that walking was the dominant activity followed by standing and grooming with flying occupying the least time (Table 3). *Cotesia sesamiae* spent the least time on molasses grass.

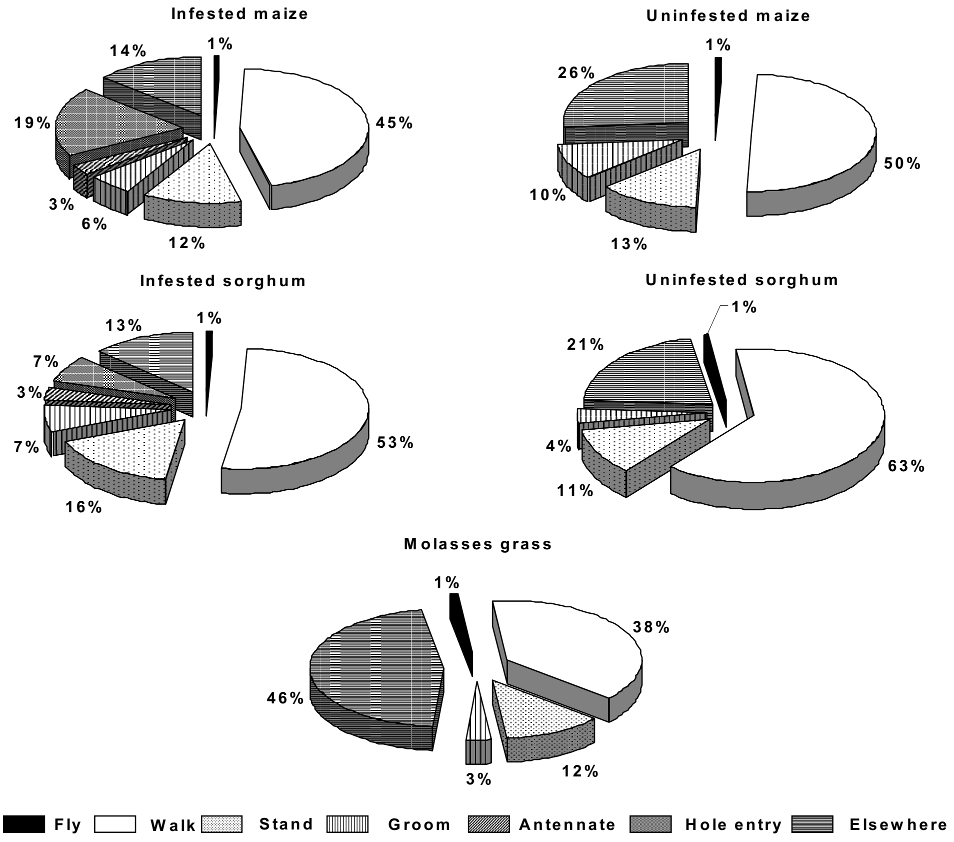

Figure 5. Foraging activities of *C. sesamiae* on host and non-host plants. Numbers next to pie slices indicate % duration of each behaviour while on plant and % duration spent elsewhere.

Discussion

Previous laboratory olfactometer studies have shown that *C. sesamiae* and *D. busseolae* are attracted to the host habitat by volatiles emanating from host plants (Ngi-Song et al., 1996, Rutledge & Wiedenmann, 1999; Chapters 2 & 3). Volatiles from the non-

Table 3. Average durations (s) (± S.E) of behavioural parameters of *Cotesia sesamiae* on uninfested and infested host plants and a non-host plant.

Behavioural parameters	Treatment				
	Infested maize	Uninfested maize	Infested sorghum	Uninfested sorghum	Molasses grass
Fly	3.8 ± 1.5a A	4.7 ± 1.6a A	6.8 ± 3.6a A	2.9 ± 0.7a A	3.8 ± 0.9a A
Walk	350.7 ± 53.4a B	241 ± 41.1ab B	382.5 ± 52.3a B	298.6 ± 56.1a B	125.2 ± 17.0b B
Stand	94.2 ± 27.5ab C	62.3 ± 12.1ab C	121.6 ± 20.3a C	52.6 ± 11.7ab C	39.5 ± 11.5b C
Groom	46.2 ± 11.4a C	49.3 ± 11.4a C	54.7 ± 20.8a AC	17.4 ± 4.7a A	9.8 ± 4.9b A
Antennate	23.8 ± 4.8^x AC	-	23.3 ± 7.2^x A	-	-
Tunnel entry	142.8 ± 69.6^y BC	-	53.8 ± 44.7^y A	-	-
Elsewhere	105.5 ± 17.1ab	127.9 ± 12.5ab	96.9 ± 14.5a	99.5 ± 10.6ab	151.3 ± 8.7b

Mean times in a row followed by the same lowercase letters are not significantly different (Kruskal Wallis test followed by a Mann-Whitney-U test with Bonferroni correction, $\alpha < 0.005$, for multiple pairwise comparisons). n = 20.

[x] Mann-Whitney-U test, p= 0.54

[y] Mann-Whitney-U test, p= 0.13

Means in a column followed by the same uppercase letters are not significantly different (Friedman's test followed by Wilcoxon's test with Bonferroni correction, $\alpha < 0.003$, for multiple pairwise comparisons).

host plant, molasses grass, were shown to be attractive to *C. sesamiae* but repellent to *D. busseolae* (Khan et al., 1997a; Chapters 2 & 3). The present work was designed to determine whether the presence of molasses grass would interfere with close-range foraging of parasitoids once they arrive in the vicinity of the plants. We set out to investigate if *C. sesamiae* was likely to search on the molasses grass thus wasting time on an unrewarding patch, and if *D. busseolae* was repelled from the intercrop. The results show that both parasitoids distinguished between host and non-host plants. *Cotesia sesamiae* was not arrested on molasses grass and the presence of the grass did not interfere with foraging of *D. busseolae* on infested host plants. As part of their search for host plants, insects go through a sequence of predictable behavioural acts, called reaction chains (Atkins, 1980), which eventually lead them to suitable host plants. Part of this sequence involves making a decision on whether to stay on a plant on which it has landed or leave. This has recently been described by Finch and Collier (2000) as the 'appropriate/inappropriate landings' theory. The decision is based on visual, olfactory and tactile cues. Variations in colour, size and shape of plants play an important role in host selection behaviour of insects especially at close range (Prokopy et al., 1983; Schoonhoven et al., 1998). Molasses grass has slender leaves and thin stems. While walking on this grass, the parasitoids may perceive from the size of the stem that the plant could not harbour its hosts. It has been reported that size of leaves was one of the factors influencing residence time of *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae) on different plant species (van Roermund and van Lenteren, 1995; Sütterlin and van Lenteren, 1999).

While on a plant, a parasitoid also evaluates the chemical traits of the plant that could not be perceived at a distance. The insect, using tarsal receptors, assesses whether the chemical stimuli are acceptable or not (Kostal and Finch, 1994). It is most likely that the parasitoid's decision to leave the unsuitable molasses grass in search of a more appropriate patch was based on contact chemicals and visual cues it perceived. Leaving tendency of *C. sesamiae* and *D. busseolae* on molasses grass was high. Of the total time of 1200 seconds allotted for observation, *C. sesamiae* spent 27% of that time in the experimental arena with molasses grass, while 63% of the total foraging time was spent in the infested plants arena. *Dentichasmias busseolae* on average spent 5% of it 1800s allotted time on molasses grass and 72% on infested plants, in intercrop arrangements. Availability of hosts and the correct type of stimuli play a major role on whether a parasitoid should leave a patch or not (Vos et al., 1998; Wang and Keller, 2002). In the present case, molasses grass did not harbour hosts and close-range stimuli perceived must have been unsuitable for further searching by the parasitoids. Searching for infested patches is an energy-consuming venture and it may be more efficient to stay longer in a favourable patch resulting in longer residence time. Therefore, infested host plant patches surrounded by non-host plants should be less abandoned than those surrounded by host plants. This might also explain the longer residence time on infested plants.

More time was spent on the uninfested host plant than on molasses grass, probably because it provided some of the correct stimuli. Several studies have shown the attractiveness of uninfested host plants to parasitoids (Ngi-Song et al., 1996; Rutledge and Wiedenmann, 1999; Ockroy et al., 2001). However, the parasitoids spent more time on infested than uninfested host plants. This finding concurs with several similar studies (Wiskerke and Vet, 1994; van Roermund and Lenteren, 1995; Potting et al., 1999b; Shiojiri et al., 2000; Wang and Keller, 2002). The parasitoids were clearly arrested by the host-damaged plants. Injury to the plant and the presence of frass played a major role in this arrestment. Of the total foraging time of *C. sesamiae*, more than 50% was spent on the stem, including time spent at the tunnel entrance and inside the tunnel. *Cotesia flavipes* was attracted to infested stem pieces, which were more attractive than leaves from an infested plant (Potting et al. 1995). This was attributed to close-range volatiles produced by the injured stem, which guided the parasitoid to the right host microhabitat. Though a large percentage of *C. sesamiae* females located the tunnel entrance, only a small fraction managed to enter the tunnel. This appeared to be due to difficulties in penetrating through the frass, which was fresh and wet. Chinwada and Overholt (2001) speculated that *C. sesamiae* performed poorly in the early part of the maize growing season because stemborer frass was wet and impeded parasitoid movement. There was low success in parasitoids leaving the tunnel, probably due to the wet frass and also the parasitoids being killed by aggressive borers. Aggressive behaviour of borers during stinging by *Cotesia flavipes* has been observed (Potting et al., 1997, 1999a, Takasu and Overholt (1997).

Dentichasmias busseolae dedicated a large percentage of its time antennating frass and drumming parts of the stem that harboured the host. Stemborer frass is attractive to *D. busseolae* and plays a role in host microhabitat location (Mohyuddin, 1972; Bahana, 1989). Frass is an attractive component and is used by searching parasitoids to detect presence of a host (Hailemichael et al., 1994; Potting et al., 1997; Ngi-Song and Overholt, 1997; Tanaka et al., 2001). Arrestment by frass is mediated by both non-volatile contact chemical and volatile chemical cues. Pupal parasitoids often employ mechanosensory cues to locate their hosts. They may detect sounds or vibrations produced by their hosts (Meyhöfer and Casas, 1999). The parasitoids may also produce their own vibrations by drumming the substrate (vibrational sounding) to scan substrate for hidden host pupae (Wäckers et al., 1998; Otten et al., 2001). *Dentichasmias busseolae* was most likely cueing in on mechanosensory stimuli when it was involved in the drumming of the stem with its antennae.

In conclusion, *C. sesamiae* and *D. busseolae* clearly discriminated between host plants, maize and sorghum, and the non-host plant, molasses grass. Foraging behaviour of *D. busseolae* in intercrop arrangements with infested host plants was not different from that of monocrop arrangements. Due to this discrimination of host and non-host plants, we do not expect molasses grass to hinder the foraging behaviour of either parasitoid in an intercrop situation. *Cotesia sesamiae* will not waste time searching on the non-host plant even though the grass is producing volatiles that are attractive to the parasitoid at long-range. However, these predictions need to be confirmed in a more realistic field situation since the present study relates to a semi-field situation that forms a link between olfactometric laboratory bioassays and a field situation. Field study results are reported in chapter 6.

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6

Effects of intercropping sorghum with a non-host plant molasses grass (*Melinis minutiflora*) on cereal stemborers and their parasitoids

Chapter 6

Effects of intercropping sorghum with a non-host plant molasses grass (*Melinis minutiflora*) on cereal stemborers and their parasitoids.

Abstract

Biological control of insect pests can be enhanced by habitat diversification. Field experiments were conducted to determine the effects of intercropping sorghum (*Sorghum bicolor*) with molasses grass (*Melinis minutiflora*) on lepidopteran stemborer population densities and to assess whether intercropping enhances the abundance and impact of stemborer parasitoids. The stemborer complex consisted of four species: *Chilo partellus*, *Busseola fusca*, *Sesamia calamistis* and *Eldana saccharina*, with *C. partellus* being the dominant species. Stemborer density was lower in the sorghum-molasses grass intercrop than in the sorghum monocrop. Five species of hymenopterous parasitoids were reared from stemborer larvae and pupae, the larval parasitoids, *Cotesia sesamiae*, *Cotesia flavipes* and *Stenobracon rufus* and the pupal parasitoids, *Pediobius furvus* and *Dentichasmias busseolae.* A hyperparasitoid *Aphanogmus fijiensis* was also encountered, but only rarely. Parasitism did not differ between the monocrop and intercrop except for pupal parasitism in the long rainy season of 1998, which was higher in the monocrop. Overall larval and pupal parasitism was 2.1 and 11.0 % for the monocrop and 2.0 and 9.8% for the intercrop. The present study indicates that intercropping sorghum and molasses grass reduces stemborer infestation. Although parasitoids are important in regulation of stemborer populations, there is no evidence that intercropping enhances parasitoid activity in the system presently studied.

Introduction

Lepidopteran stemborers are serious cereal pests in eastern Africa. They account for yield losses ranging between 20 and 40 percent (Seshu Reddy, 1998). The most important stemborer species include the spotted borer *Chilo partellus* (Swinhoe) (Crambidae), the African maize borer *Busseola fusca* Fuller, the pink stemborer *Sesamia calamistis* Hampson (Noctuidae) and the sugarcane borer *Eldana saccharina* Walker (Pyralidae). Methods used to manage stemborers include chemical control, biological control, host-plant resistance and cultural control. All have advantages and disadvantages for smallholder farmers in East Africa (reviewed by Minja 1990; Mugo et al., 2001). Current efforts in East Africa focus on developing sustainable and environment friendly strategies that farmers can easily adopt. Habitat management is one such strategy that has received much attention recently, although variable results have been reported (Bugg and Pickett, 1998; Khan et al., 2000). Habitat management is a control approach in which habitats are selectively altered to create environmental conditions that will reduce pest pressure on crops and enhance natural enemy activity (Landis et al., 2000).

Successful attempts in controlling stemborers through habitat management, particularly intercropping, have been reported by Oloo and Ogeda (1990), Ampong-Nyarko et al. (1994) and Skovgård and Päts (1997). Recently, Khan et al. (1997a, b; 2001) introduced the stimulo-deterrent diversionary strategy (SDDS) or 'push-pull' strategy in the battery of approaches against stemborers. It involves the use of both repellent and trap plants cropped with maize. In this approach, stemborers are simultaneously repelled from the crop and attracted to the trap plants, thus lowering the pest densities in the main crop. In addition, in fields with SDDS, parasitism by the stemborer larval parasitoid *Cotesia sesamiae* (Cameron) (Hymenoptera: Braconidae) was increased by a factor of four on the main crop. Attraction of *C. sesamiae* to, and repellence of stemborer moths from the diversified maize agroecosystem was attributed to volatiles produced by molasses grass (*Melinis minutiflora* Beauv. (Poaceae)), a non-host plant of stemborers. One of the volatile compounds produced by molasses grass is the homoterpene (E)-4,8-dimethyl-1,3,7-nonatriene (Khan et al., 1997a). This compound is also emitted by herbivore-injured plants and plays a role in the recruitment of predators and parasitoids (Turlings et al. 1991, Ngi-Song et al. 2000).

Parasitoids have a great potential to control stemborers but the impact of indigenous parasitoids in East Africa is generally low (Oloo and Ogeda, 1990, Overholt et al., 1997, Bonhof et al, 1997). There is a need therefore, to explore strategies that would enhance parasitoid activity in order to increase parasitization rates. Manipulation and exploitation of plant volatiles attractive to parasitoids such as in the SDDS system reported by Khan et al. (1997a,b), offers a promising avenue for enhancing parasitoid activity. An added advantage of interplanting molasses grass is that it is a drought resistant forage grass, which can be fed to livestock especially in semi-arid areas (Fröman & Persson, 1974). The present study was designed to evaluate the role of molasses grass in the control of stemborers in a sorghum-molasses grass intercrop. The study set out to test the hypothesis that there is a reduction in the number of stemborers and increased parasitism in the intercrop as compared to the monocrop.

Materials and methods

Field arrangement

Studies were conducted at ICIPE-Mbita Point Research Station in western Kenya (34° 10' E, 0° 25' S), during the two rainy seasons (March – June and October – December) of the years 1998 and 1999. Two fields were used. The experimental design was a Randomised Complete Block Design (RCBD). One of the fields had four blocks while the second had three blocks. Each block was made up of four plots measuring 9 × 10 m with two replicates of each treatment per block. Treatments were a monocrop consisting of sorghum alone and an intercrop consisting of sorghum (cv. Seredo) and molasses grass. The treatments were assigned randomly to the plots. Rows in the fields were perpendicular to the prevailing winds. Inter-row spacing was 50 cm, while intra-row spacing was 30 cm. In the intercrop, every fourth row of sorghum was substituted by a row of molasses grass. Molasses grass was transplanted from a screen house three weeks prior to planting sorghum to allow it to establish. Distance between plots was two meters and distance between blocks was three meters. The strips between plots and blocks were kept as bare ground (Figure 1). At planting, 100kg/ha of Di-ammonium Phosphate (DAP) fertilizer was applied. Sorghum was thinned to two plants per hill two weeks after plant emergence. Three weeks after plant emergence, the sorghum was top-dressed with 80kg/ha of Calcium Ammonium Nitrate (CAN) fertilizer. Weeding was done manually and there were no pesticide applications. Molasses grass was trimmed to a height of 30cm every six weeks.

During the second year, a third field was introduced with plant rows parallel to the prevailing winds. This was done to investigate whether positioning of the plant rows to the prevailing winds had any effect on borer infestation and parasitism. The third field was set up identically to the first field, with four blocks.

Insect sampling

At weekly intervals starting four weeks after plant emergence and continuing to twelve weeks after plant emergence, insects were sampled. At each sampling time, ten hills of sorghum were randomly selected and sampled from each plot. The two outer rows and the first and last two hills of each row were excluded from sampling. Within each hill, the primary shoot and the tillers were considered as independent sampling units capable of supporting complete borer development. Each tiller was dissected and all stemborer larvae and pupae obtained were brought to the laboratory and reared singly in vials. The larvae were divided into three classes, small (L1 + L2), medium (L3 + L4) and large (L5 + L6), each class corresponding to approximately two developmental larval instars. The larvae were reared in vials and provided with fresh sorghum stems for food every two days until they pupated or died. The pupae were similarly kept in vials lined with moist tissue paper, until emergence of adults or death. The insects were checked on a daily basis for emergence of parasitoids. The parasitoids that emerged were preserved in 70% alcohol and sent to the ICIPE- Biosystematics Unit for identification.

Data analysis

Stemborer infestation

Stemborer density (stemborers/tiller) infestation data, for each plot in each block in fields 1 & 2, were square root-transformed [$\sqrt{(x + 0.5)}$] then subjected to repeated measures analysis of variance using the general linear models (PROC GLM) in SAS version 8 .0 (SAS Institute 1999). In addition, an analysis of variance was carried out to determine the overall effect of year, season and treatment on stemborer density. The Student Newman Keul's (SNK) multiple range-test was used to separate means at $p < 0.05$ level, when ANOVAs were significant. Data obtained from the third field were analysed similarly but separately from those of the first two fields. The overall analysis of variance test was used to determine the effect of season and treatment on stemborer density in the third field.

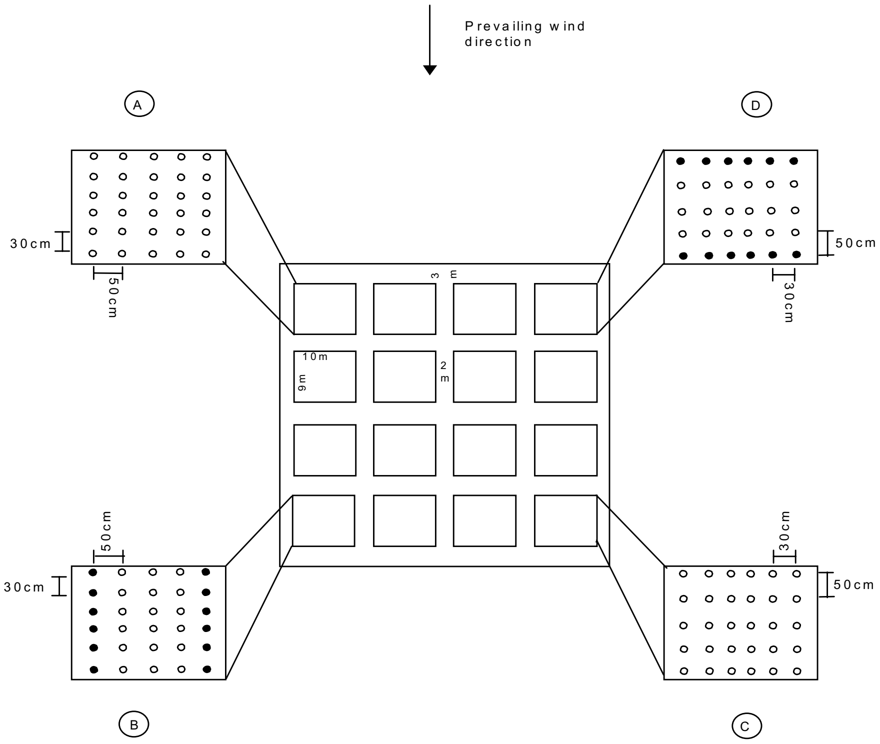


Figure 1. Field layout and row arrangement of the study plots. A - Monocrop plot in fields 1 & 2, B- Intercrop plot in fields 1 & 2, C - Monocrop plot in field 3, D - Intercrop plot in field 3.

Parasitism

The proportion (p) of larvae and pupae parasitized in each treatment in each plot was calculated separately as the total number of hosts parasitized divided by total number of stemborers in the susceptible stage (medium and large classes for larvae) (van Driesche, 1983). The parasitism values were arcsine transformed (arsin $\sqrt{p}$) then subjected to repeated measures analysis of variance. Data for field 3 were analyzed separately from those of fields 1 & 2. ANOVA was performed to test the effect of year, season and treatment.

A correlation coefficient analysis was applied to test the relationship between stemborer density and parasitism for fields 1 and 2 only.

Results

Stemborer species composition and abundance

Four stemborers species were recovered during the sampling period; *Chilo partellus*, *Busseola fusca*, *Sesamia calamistis* and *Eldana saccharina*. *Chilo partellus* was the most abundant stemborer species in all seasons comprising 75.4% of all the recorded stemborers. *Busseola fusca* accounted for 23.2%, while *S. calamistis* and *E. saccharina* together accounted for 1.4% of the totals (Table 1). One *Helicoverpa* spp. larva was collected from sorghum during the long rainy season of 1998.

Main effects of the repeated measures analysis of variance for fields 1 and 2 revealed that in the long and short rainy seasons of the first year (1998) and the short rainy season of the second year (1999), field had a significant effect on stemborer density. There was no treatment effect in both seasons during the two years (Table 2). The interaction term between field and treatment had no effect in all seasons. A general increase in borer density levels was recorded over the weeks in each season. During all seasons, weekly fluctuations of stemborer densities were often lower in the intercrop than in the monocrop though the difference was not significant (Figure 2a). The overall ANOVA on stemborer density for fields 1 and 2 showed a significant effect of year ($F_{(1,52)} = 44.83$, $p = <0.0001$), season ($F_{(1,52)} = 44.83$, $p = <0.0001$) and treatment ($F_{(1,52)} = 8.36$, $p = 0.0056$). There were more borers collected in the first year of sampling (1998) than in the second year (1999). The first season (long rains-LR) of each year posted higher borer densities than the second season (short rains- SR). The highest borer density was recorded in LR-98 season (0.54 ± 0.03/tiller), while the lowest was in SR-99 (0.17 ± 0.01/tiller).

In field 3, treatment had no influence on stemborer density in the long rainy season of 1999 ($F_{(1,14)} = 0.01$, $p = 0.9162$). While there was a significant treatment effect on stemborer density in the short rainy season of the same year ($F_{(1,14)} = 9.34$, $p = 0.0086$). In both seasons, stemborer infestation levels differed between the weeks ($F_{(7,98)} = 4.94$, $p = < 0.0001$ and $F_{(7,48)} = 8.42$, $p = <0.0001$) for the long and short rains seasons respectively (Figure 2b). The interaction between treatment and time (weeks) significantly influenced borer density in both seasons. Overall ANOVA on data from the third field revealed significant season effect on borer infestation levels ($F_{(1,52)} = 58.59$, $p = < 0.0001$), but no treatment effect ($F_{(1,12)} = 1.52$, $p = 0.241$). Borer density was higher in the long rainy season than the short rainy season.

Table 1. Species abundance (percentage) of stemborers collected in sorghum monocrops and sorghum-molasses grass intercrop plots during the long and short rains seasons of 1998 and 1999 expressed as total counts with percentages of seasonal totals in parenthsis. LR- longs rains, SR- short rains, F1- field 1, F2- field 2 and F3- field 3.

Season	Number of plants	*Chilo partellus*	*Busseola fusca*	*Sesamia calamistis*	*Eldana saccharina*	Total borers collected
LR-98 F1						
Monocrop	1417	494 (53.8)	417 (45.5)	0 (0.0)	6 (0.7)	917
Intercrop	1424	346 (46.3)	399 (53.3)	0 (0.0)	3 (.04)	748
LR-98 F2						
Monocrop	1331	408 (63.0)	239 (37.0)	0 (0.0)	0 (0.0)	647
Intercrop	1338	286 (55.2)	232 (44.8)	0 (0.0)	0 (0.0)	518
SR-98 F1						
Monocrop	1320	490 (96.6)	15 (3.0)	0 (0.0)	2 (0.4)	507
Intercrop	1360	322 (93.0)	24 (7.0)	0 (0.0)	0 (0.0)	346
SR-98 F2						
Monocrop	1058	500 (95.8)	22 (4.2)	0 (0.0)	0 (0.0)	522
Intercrop	978	427 (97.3)	12 (2.7)	0 (0.0)	0 (0.0)	439
LR-99 F1						
Monocrop	1321	486 (89.5)	27 (5.0)	0 (0.0)	30 (5.5)	543
Intercrop	1327	473 (93.4)	17 (3.4)	0 (0.0)	16 (3.2)	506
LR-99 F2						
Monocrop	1073	453 (97.8)	10 (3.4)	0 (0.0)	0 (0.0)	463
Intercrop	996	295 (96.7)	10 (3.3)	0 (0.0)	0 (0.0)	305
LR-99 F3						
Monocrop	1267	220 (46.5)	251 (53.1)	0 (0.0)	2 (0.4)	473
Intercrop	1257	225 (47.1)	253 (52.9)	0(0.0)	0 (0.0)	478
LR-99 F1						
Monocrop	1174	190 (92.2)	8 (3.9)	3 (1.5)	5 (2.4)	206
Intercrop	1056	115 (91.2)	5 (5.0)	6 (4.8)	0 (0.0)	126
LR-99 F2						
Monocrop	927	162 (88.0)	5 (2.7)	17 (9.3)	0 (0.0)	184
Intercrop	859	156 (94.5)	6 (3.7)	3 (1.8)	0 (0.0)	165
LR-99 F3						
Monocrop	1237	198 (92.1)	3 (1.4)	8 (3.7)	6 (2.8)	215
Intercrop	1132	105 (97.2)	0 (0.0)	0 (0.0)	3 (2.8)	108
Total						
Monocrop	12,125	3,601(77.0)	997 (21.3)	28 (0.6)	51 (1.1)	4,677 (55.6)
Intercrop	11,727	2,750 (73.5)	958 (25.6)	9 (0.3)	22 (0.6)	3,739 (44.4)

Table 2. Repeated measures analysis of variance on stemborer density in sorghum monocrop and sorghum-molasses grass intercrop plots in Fields 1 and 2 in different seasons.

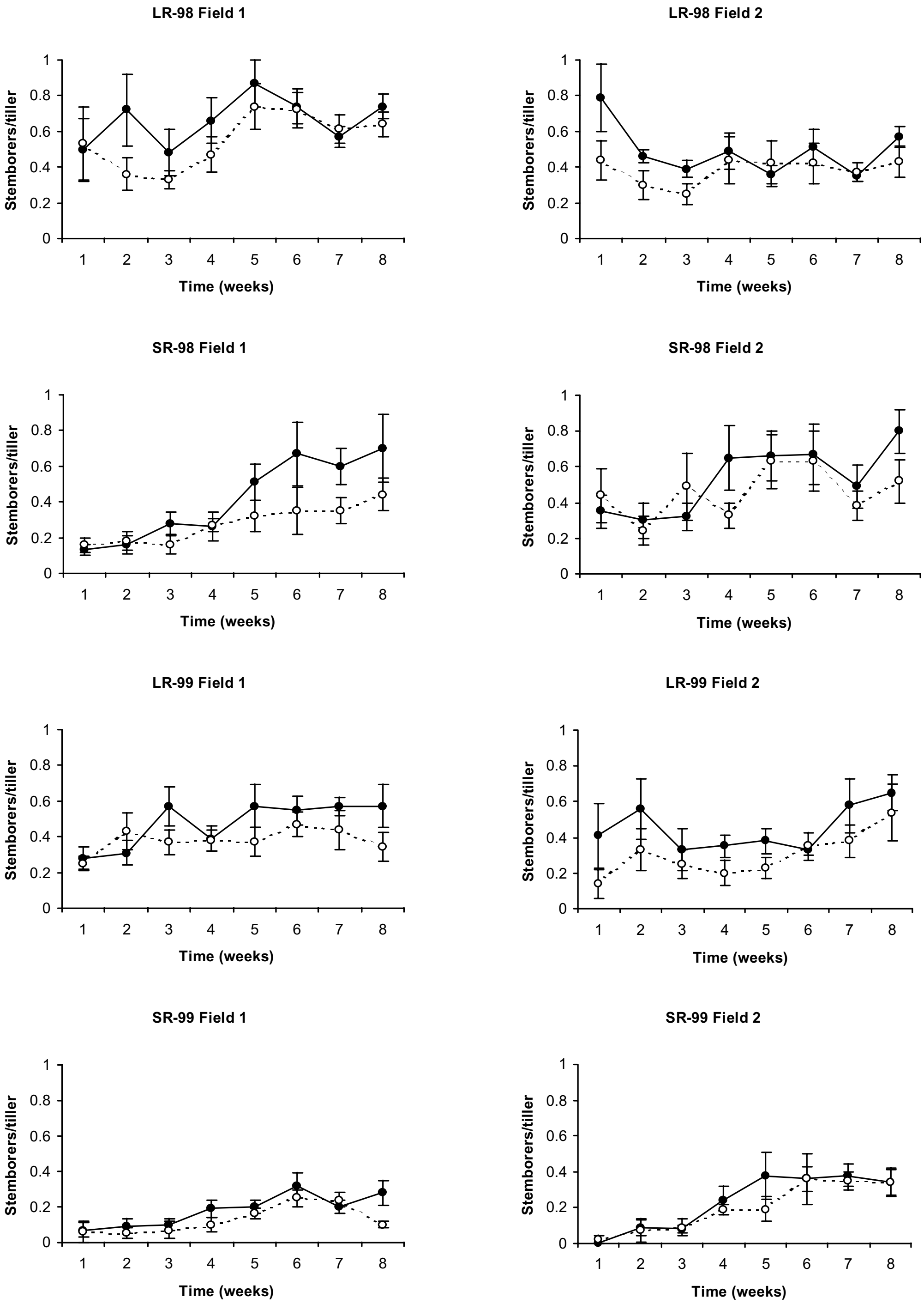
Season	Source	df	MS	F	P
LR- 98	Field	1	0.253	7.71	0.0148
	Treatment	1	0.110	3.36	0.0792
	Field*treatment	1	0.003	0.09	0.7552
	Error	24	0.033	-	-
SR- 98	Field	1	0.328	7.15	0.0133
	Treatment	1	0.131	2.85	0.1040
	Field*treatment	1	0.014	0.31	0.5818
	Error	24	0.046	-	-
LR- 99	Field	1	0.007	0.19	0.6637
	Treatment	1	0.095	2.77	0.1093
	Field*treatment	1	0.043	1.26	0.2719
	Error	24	0.820	-	-
SR- 99	Field	1	0.039	4.28	0.0495
	Treatment	1	0.016	1.82	0.1896
	Field*treatment	1	0.010	1.15	0.2936
	Error	24	0.009	-	-

Chilo partellus was the only stemborer species contributing to any noted differences in stemborer density between the treatments in all seasons. The densities of the other borer species were not different between the two treatments in any of the seasons. *Busseola fusca* densities were generally higher in the first season than the second season of each year (Table 3).

Table 3. Mean number of stemborers per tiller of each species in the different treatments in the cropping seasons of 1998 and 1999.

	Statistical parameters	*Chilo partellus*	*Busseola fusca*	*Sesamia calamistis*	*Eldana saccharina*
		Fields 1 & 2	combined		
LR-98					
Monocrop		0.33 ± 0.035a	0.25 ± 0.032a	0.0	0.003 ± 0.002a
Intercrop		0.23 ± 0.020b	0.23 ± 0.038a	0.0	0.001 ± 0.001a
	F	5.95	0.03	.	0.37
	df	1, 12	1, 12	.	1, 12
	P	0.0312	0.8736	.	0.5517
SR-98					
Monocrop		0.42 ± 0.041a	0.02 ± 0.003a	0.0	0.0
Intercrop		0.33 ± 0.049a	0.02 ± 0.005a	0.0	0.0
	F	2.05	0.07	.	.
	df	1, 12	1, 12	.	.
	P	0.1776	0.7962	.	.
LR-99					
Monocrop		0.39 ± 0.037a	0.02 ± 0.006a	0.0	0.01 ± 0. 01a
Intercrop		0.33 ± 0.03a	0.01 ± 0.007a	0.0	0.008 ± 0.004a
	F	1.56	0.42	.	0.28
	df	1, 12	1, 12	.	1, 12
	P	0.2361	0.5308	.	0.6045
SR-99					
Monocrop		0.17 ± 0.015a	0.006 ± 0.002a	0.01 ± 0.004a	0.001 ± 0.001a
Intercrop		0.04 ± 0.019a	0.005 ± 0.003a	0.004 ± 0.003a	0.0a
	F	1.26	0.00	1.17	1.00
	df	1, 12	1, 12	1, 12	1, 12
	P	0.2833	0.9970	0.3006	0.3370
		Field	3		
LR-99					
Monocrop		0.2 ± 0.019a	0.2 ± 0.024a	0.0	0.0
Intercrop		0.2 ± 0.014a	0.02 ± 0.029a	0.0	0.0
	F	0.01	0.02	-	-
	df	1, 6	1, 6	-	-
	P	0.9145	0.9055	-	-
SR-99					
Monocrop		0.16 ± 0.02a	0.003 ± 0003a	0.005 ± 0.003a	0.003 ± 0.003a
Intercrop		0.09 ± 0.015b	0.0a	0.0a	0.003 ± 0.003a
	F	6.59	1.00	3.00	0.00
	df	1, 6	1, 6	1, 6	1, 6
	P	0.0425	0.3359	0.1340	1.00

Treatment means followed by the same letter within a season under each stemborer species are not significantly different from each other (F-test).



LR-98 Field 1

LR-98 Field 2

SR-98 Field 1

SR-98 Field 2

LR-99 Field 1

LR-99 Field 2

SR-99 Field 1

SR-99 Field 2

Figure 2a. Mean number (± S.E) of stemborers per tiller during the long rains (LR) and short rains (SR) seasons of 1998 and 1999 at ICIPE-Mbita Point in sorghum monocrop (-●-) and sorghum molasses grass intercrop (-○-) for the Fields 1 & 2. The first sampling (week 1) followed at four weeks after plant emergence.

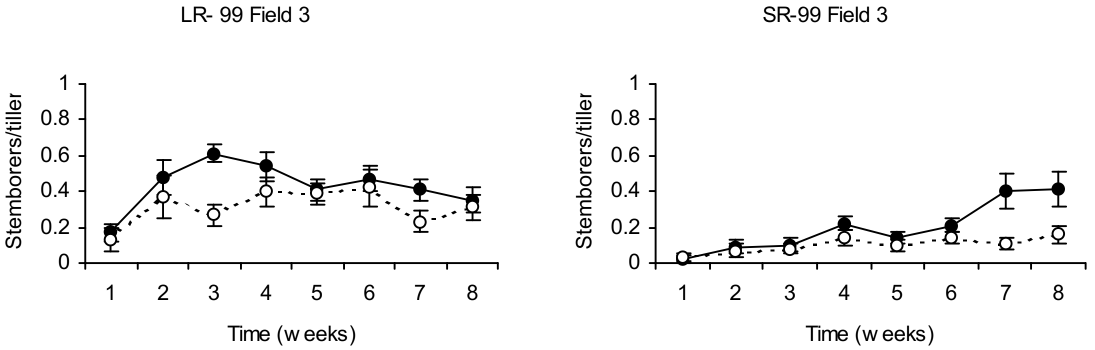


Figure 2b. Mean number (± S.E) of stemborers per tiller during the long rains (LR) and short rains (SR) seasons of 1998 and 1999 at ICIPE-Mbita Point in sorghum monocrop (-●-) and sorghum molasses grass intercrop (-○-) for the Field 3. The first sampling (week 1) followed at four weeks after plant emergence.

Parasitoid species and parasitism

The parasitoid species reared from stemborers collected during the sampling period were *Cotesia sesamiae, Cotesia flavipes, Pediobius furvus,* and *Dentichasmias busseolae.* The parasitoids were recovered only from *C. partellus*, and *B. fusca.* No parasitoids were reared from *S. calamistis* or *E. saccharina. Stenobracon rufus*, an ectoparasitoid of *C. partellus* and *Aphanogmus fijiensis* a hyperparasitoid of *C. sesamiae*, were infrequently encountered.

The repeated measures analysis for fields 1 & 2 showed there was no field or treatment effect on both larval and pupal parasitism. In field 3, treatment had no effect on larval and pupal parasitism (Table 4). For fields 1 & 2, larval parasitism levels differed between weeks only in the first season of the first year ($F_{(7,168)} = 4.53$, $p = 0.0011$). In the rest of the seasons, larval parasitism did not differ with time. Pupal parasitism differed between weeks in the first season of 1998 and 1999 ($F_{(7,168)} = 6.25$, $p = 0.0003$ and $F_{(7,168)} = 2.51$, $p = 0.0206$ respectively) , and second season of 1998 ($F_{(7,168)} = 3.58$, $p = 0.0029$). There was no pupal parasitism in the first weeks and parasitism gradually increased with time. In field 3, there was no difference in larval and pupal parasitism with time. Overall ANOVA for larval parasitism in fields 1 and 2 show an effect of year ($F_{(1,51)} = 97.40$, $p = <0.0001$), season ($F_{(1,51)} = 109.08$, $p = <0.0001$) and the interaction of year and season ($F_{(1,51)} = 94.91$, $p = <0.0001$). Higher larval parasitism levels were noted in the 1998 than 1999. More borer larvae were parasitized in the long rainy seasons than the short rainy seasons. Treatment had no effect on larval parasitism ($F_{(1,51)} = 0.11$, $p = 0.7374$) . There was no difference in pupal parasitism between the two years ($F_{(1,51)} = 2.05$, $p = 0.1588$). Within a year, pupal parasitism was different between the two seasons ($F_{(1,51)} = 7.55$, $p = 0.0083$), with higher parasitism levels noted in the long rainy than the short rainy season.

Table 4. Repeated measures analysis of variance on larval and pupal parasitism in sorghum monocrop and sorghum-molasses grass intercrop plots in different seasons.

			Larval parasitism			Pupal parasitism		
Season	Source	df	MS	F	P	MS	F	P
				Fields		**1 & 2**		
LR- 98	Field	1	570.88	2.16	0.1551	1304.09	2.42	0.1330
	Treatment	1	229.64	0.87	0.3611	2393.03	4.44	0.0458
	Field*treatment	1	29.78	0.11	0.7403	403.67	0.75	0.3954
	Error	24	264.91	-	-	539.13	-	-
SR- 98	Field	1	26.05	2.64	0.1172	694.32	1.72	0.2022
	Treatment	1	1.01	0.10	0.7517	76.50	0.19	0.6673
	Field*treatment	1	1.01	0.10	0.7517	101.26	0.25	0.6211
	Error	24	9.87	-	-	403.84	-	-
LR- 99	Field	1	126.83	3.91	0.0595	29.44	0.08	0.7855
	Treatment	1	126.83	3.91	0.0595	1401.10	3.61	0.0697
	Field*treatment	1	126.83	3.91	0.0595	2.42	0.01	0.9377
	Error	24	32.40	-	-	388.55	-	-
SR- 99	Field	1	7.85	1.39	0.2499	108.48	1.47	0.2372
	Treatment	1	0.45	0.08	0.7807	0.0	0.00	1.000
	Field*treatment	1	0.45	0.08	0.7807	0.0	0.00	1.000
	Error	24	5.65	-	-	73.83	-	-
				Field		**3**		
LR- 99	Treatment	1	63.28	1.00	0.3343	19.12	0.09	0.7712
	Error	14	63.28	-	-	217.44	-	-
SR- 99	Treatment	1	5.51	1.00	0.3343	0.00	0.00	1.00
	Error	14	5.51	-	-	126.56	-	-

There was no treatment effect on pupal parasitism ($F_{(1,51)} = 3.20$, $p = 0.0794$). The interaction of year and season had an effect on pupal parasitism ($F_{(1,51)} = 9.23$, $p = 0.0038$). In field three between the two seasons, there was no difference in larval parasitism ($F_{(1,12)} = 0.46$, $p = 0.5116$) or pupal parasitoids ($F_{(1,12)} = 0.80$, $p = 0.3898$). Treatment had no influence on either larval or pupal parasitism ($F_{(1,12)} = 1.54$, $p = 0.2380$, $F_{(1,12)} = 0.05$, $p = 0.8201$ respectively).

Correlation coefficients of larval (monocrop, $r = 0.221$, $p = 0.049$; intercrop, $r = 0.322$, $p = 0.004$) and pupal parasitism (monocrop, $r = 0.245$, $p = 0.029$; intercrop, $r = 0.149$, $p = 0.197$), with host density, though positive and significant, were not high suggesting that the relationship between parasitism and stemborer density was weakly density-dependent.

Table 5. Percent parasitism (± standard error) of pooled data of stemborers collected in monocrop and intercrop in different seasons.

		Parasitoid				
		Cotesia sesamiae	*Cotesia flavipes*	*Stenobracon rufus*	*Pediobius furvus*	*Dentichasmias busseolae*
	Host	CP, BF	CP	CP	CP, BF	CP
	Host stage	L3-L6	L3-L6	L3-L6	P	P
			Fields	**1 & 2**		
Season						
LR-98	Mono	8.6 ± 1.53a	1.5 ± 0.67a	0.3 ± 0.19a	3.1 ± 1.8a	18.3 ± 4.88a
	Inter	8.8 ± 1.46a	0.4 ± 0.39a	0.0a	3.1 ± 1.5a	6.6 ± 3.77b
SR-98	Mono	0.7 ± 0.49a	0.0	0.1 ± 0.09a	0.8 ± 0.62a	13.0 ± 4.29a
	Inter	0.0a	0.0	0.1 ± 0.09a	2.8 ± 2.15a	10.9 ± 3.62a
LR-99	Mono	0.4 ± 0.25a	0.0	0.0	0.9 ± 0.89a	22.9 ± 6.15a
	Inter	0.0a	0.0	0.0	0.0a	16.7 ± 7.46a
SR-99	Mono	0.2 ± 0.22a	0.0	0.0	0.0	2.5 ± 2.50a
	Inter	0.8 ± 0.6a	0.0	0.0	0.0	4.2 ± 4.17a
Overall parasitism	**Mono**	**2.5 ± 0.60a**	**0.4 ± 0.18a**	**0.1 ± 0.06a**	**1.2 ± 0.53a**	**14.2 ± 2.4a**
	Inter	**2.4 ± 0.60a**	**0.1 ± 0.09a**	**0.02 ± 0.02a**	**1.5 ± 0.67a**	**9.6 ± 2.51a**
			Field	**3**		
LR-99	Mono	0.0a	0.0	0.0	0.0	7.7 ± 5.09a
	Inter	0.2 ± 0.20a	0.0	0.0	0.0	18.8 ± 1.32a
SR-99	Mono	0.0	0.0	0.0	0.0	0.0
	Inter	0.0	0.0	0.0	0.0	0.0

Mono- monocrop, Inter- intercrop; L3- L6- larval instars, P- pupa; BF- *Busseola fusca*, CP- *Chilo partellus*

Treatment means followed by the same letter within a season under each parasitoid species are not significantly different from each other (F-test).

Total stemborer parasitism across the seasons was generally low, with 5.1% in the monocrop and 4.4% in the intercrop. *Cotesia sesamiae* and *D. busseolae* were the most abundant parasitoids recorded during the study (Table 5). *Cotesia sesamiae* accounted for 90.4% of all the parasitized larvae, while *D. busseolae* accounted for 88.5% of the parasitized pupae.

Discussion

Diversification of the agroecosystem may lead to a decrease in the incidence of insect pest outbreaks observed in some monocultures (Risch et al., 1983, Andow, 1991). Two hypotheses proposed by Root (1973) attempt to explain the lower pest incidence in such agroecosystems. The 'resource concentration hypothesis' suggests that herbivores will easily locate and build up populations in monocultures because host plants are concentrated in time and space. The lower concentration of resources in diversified agroecosystems would interfere with the herbivore's ability to find a host plant, particularly when specialist species are involved. The 'natural enemies hypothesis' posits high herbivore mortality caused by a more abundant population of natural enemies in diversified agroecosystems due to provision of alternative prey, food, refugia and additional allelochemical attractants. In their theory on 'appropriate and inappropriate landings' by host finding insects Finch & Collier (2000), state that the presence of another plant in the cropping system interferes with the sequence of landing on host plants and receiving adequate stimuli to oviposit. The insect spends more time searching and oviposits less or leaves that habitat in search of a better one.

The present study demonstrated that intercropping sorghum and molasses grass reduced the abundance of *C. partellus* in sorghum. There are three likely explanations for this observation, which may support the 'resource concentration hypothesis'. First, molasses grass volatiles may have repelled gravid stemborer moths from the intercrop plots leading to deposition of fewer eggs. In a laboratory oviposition bioassay, gravid female stemborers were repelled by volatiles of molasses grass extracted by hydro-distillation (Khan et al. (1997a). Fewer eggs were laid on filter paper discs with molasses grass extract than on untreated discs. Second, in intercrop plots, the probability of female moths encountering host plants might have been lower due to the presence of molasses grass resulting in the deposition of fewer eggs. A third possibility could be that the molasses grass acted as a mechanical barrier or interference to dispersing first instar larvae. A large proportion of *C. partellus* neonate larvae are known to disperse by ballooning on silken threads from the plant of oviposition (Berger, 1989). This mechanism of companion crops interfering with larval dispersal was also reported to have contributed to the reduced maize borer abundance in several maize intercrops (Litsinger et al., 1991). As the present study did not measure density of stemborer eggs, it is difficult to separate and identify the mechanisms responsible for the lower stemborer abundance. Interestingly, molasses grass seemed to have only repelled *C. partellus* and not *B. fusca* another stemborer that was in pest densities during the long rainy seasons (Table 1). The two insects could be having different behaviours of finding host plants in which *C. partellus* is affected by presence of molasses grass.

Stemborer densities varied with seasons with the highest densities recorded during the long rainy season of 1998 and the lowest densities in the short rainy season of 1999 (Table 3). A likely explanation for the higher stemborer densities in the LR-98 could be the uncharacteristically high and extended rainfall experienced in the short rainy season of the previous year due to the El Niño weather phenomenon. With an abundance of vegetation, there was build-up of stemborers populations that attacked the crop in the subsequent season. Stemborer densities slightly rose after the fourth week of sampling in each season (Figure 2a). This observation could possibly be due to the appearance of a second generation of *Chilo partellus,* which is known to have two to three generations in a cropping season (Overholt et al., 1994). *Busseola fusca* emerge from diapause much later then *C. partellus* (Kfir et al., 2002). Their late infestation also contributed to the higher stemborer densities recorded later in each of the cropping seasons. *Busseola fusca* numbers were lower in the short rainy seasons than the long rainy seasons. According to Okuda (1988), *B. fusca* diapause termination requires sustained exposure to wet conditions, which do not usually take place during the short rainy season.

Several mechanisms have been postulated as contributing to the effects of habitat diversification on parasitism of herbivore pests. For example, Murphy et al. (1998) reported higher egg parasitism rates in grape vineyards located downwind of prune trees than in vineyards not associated with prune trees. The prune trees provided refuge to the egg parasitoids especially as overwintering sites. The presence of wild brassicas in the vicinity of cultivated brassicas contributed to higher parasitism of the diamond back moth because of provision of extra nectar to it larval parasitoid *Diadegma insulare* (Cresson) (Hymenoptera: Ichneumonidae) (Idris and Grafius, 1996). The microclimate of a diversified agroecosystem can also be altered to favour activity of parasitoids leading to more herbivores being attacked (Coll and Bottrell, 1996). Similar to the mechanism investigated in the present study, is the attraction of parasitoids to a diversified habitat by volatiles of the companion crop. Collards planted near beet, enhanced biological control of beet pests because parasitoids were attracted to that habitat by collards (Read et al., 1970).

There is no evidence from the present study that intercropping sorghum and molasses grass increased parasitoid abundance or their effectiveness. Parasitism rates of stemborers did not differ among the treatments in all seasons. It is clear that molasses grass did not attract more parasitoids to this system. A previous field study by Khan (1997a) reported increased stemborer parasitism by *C. sesamiae* in a maize-molasses grass intercrop, a phenomenon attributed to attraction of more wasps by molasses grass volatiles (Khan, et al., 1997a). However, in Khan's study, a different host plant species was involved i.e. maize. Sorghum, and all the parasitoids encountered in the present study, with the exception of *C. flavipes*, are indigenous to Africa, while maize is a new introduction. The native parasitoids have evolved with sorghum and probably respond strongly to the sorghum volatile blend that additional volatiles from molasses grass do not increase attraction to the host habitat as was hypothesized for the maize situation. Alternatively, volatiles produced by sorghum are in higher amounts or can be more easily perceived by the parasitoids than those released by maize. It seems that the attractive ability of molasses grass in some locations may not be permanent. Another factor may be variation in attractiveness of molasses grass. Khan et al. (2000) observed that with continued cultivation of molasses grass at Mbita Point, there was a drop in parasitism of stemborers to non-significant levels in the maize intercrop. The molasses grass cultivated at Mbita Point originated from a cooler highland area of western Kenya. Molasses grass used in the present studies was from a stock earlier propagated by Khan's group at Mbita. Additional olfactometer studies by Gohole et al. (2003) confirmed that molasses grown in Mbita was no longer attractive to *C. sesamiae*. This change is ascribed to growing conditions in Mbita, which most likely contributed to alterations in the volatile blend. Findings of the present field study fully agree with previous olfactometer results, where volatiles from sorghum were more attractive to *C. sesamiae* than volatiles from a combination of sorghum and molasses grass (Gohole et al., 2003). Olfactometric investigations on response of *D. busseolae* to molasses grass volatiles showed that the wasp was repelled by the volatiles (L.S. Gohole, unpublished data). It was noted that pupal parasitism by *D. busseolae* was lower in the intercrop than in the monocrop in the long rainy season of 1998. This could possibly be because of the repelling effect of the molasses grass. However, in the subsequent seasons, no difference in pupal parasitism by the wasp was noted between monocrop and intercrop. Khan (2000) pointed out, with continued multiplication of the grass, its attractiveness to *C. sesamiae* diminished, probably the same could be applied for the *D. busseolae* case in that the grass was lost its repellency over time.

It should be noted that not all diversification programmes enhance parasitism. Some result in no change and some in even lower parasitism (Russell, 1989; Williams et al., 1995; Ogol et al., 1998 and Coll, 1998 for review). Some of the reasons put forward as contributing to low parasitism rates in intercrops include the masking of visual and olfactory cues used by foraging parasitoids, interference of parasitoid movement by non-host plants and provision of alternative hosts that distract the parasitoid away from the target pests. It is not yet possible to generalise about the effects of habitat diversification on pests and their natural enemies owing to the great diversity of systems and competing processes, which vary under different conditions (Letourneau, 1987; Päts et al., 1997). Therefore, studies in a specific crop-based system are a prerequisite for the selection of the most appropriate intercropping regime.

It is interesting that no parasitoids were recovered from *S. calamistis*, yet suitability studies showed it to be the preferred host of *C. sesamiae* (Ngi-Song et al., 1995; Mochiah et al., 2001). The occurrence of this borer species was very low and was only noted in the fourth sampling season (Table 1). None of the small larvae collected were parasitised. This is a confirmation that small larvae (first and second instar) are not attacked by the parasitoid species encountered in the present study (Smith et al., 1993, Ngi-Song et al., 1995). When calculating parasitism, it is important to know the susceptible stages of the host because including other non-susceptible stages in parasitism calculations, leads to underestimation of parasitism (van Driesche, 1993). However this does not mean that the small larvae are not subject to natural enemies. Earwigs and cockroaches were observed to prey on stemborer eggs and small larvae (Bonhof, 2000, Gohole personal observations).

In conclusion, results of the present study did not support the natural enemies hypothesis that lower pest densities in diversified cropping systems are the result of increased activity of natural enemies (Root, 1973). The intercropping regime had no effect on levels of parasitism. Generally, parasitism rates recorded were low and not significantly different from previous reports (Oloo and Ogeda, 1990). However, the resource concentration theory was given credence in that herbivore densities were lower in the intercrop. Based on the present study, the reduction of stemborers in the sorghum-molasses grass intercrop cannot be attributed to the action of parasitoids. This study shows that although habitat management holds great potential in the war against stemborers as evidenced by the 'push-pull' studies with maize and molasses grass by Khan et al., (1997a,b), there is great need to identify the exact mechanisms that underlie this strategy. The situation for sorghum is clearly different from the maize one. The key elements of diversity that interact with natural enemies need to be elucidated so that systems can be developed to better exploit these mechanisms. Finally, investigations into other methods that can complement habitat management, such as host plant resistance and various cultural practices, would be an added advantage.

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7

Summarizing discussion

Chapter 7

Summarizing discussion

As part of continuing efforts to manage cereal stemborers in eastern Africa, studies have been underway at the International Centre of Insect Physiology and Ecology (ICIPE) in which an intercropping and trap crop system has been developed. This system, using a 'push-pull' strategy or stimulo-detterent diversionary strategy (SDDS) attempts to control stemborers in smallholder farming systems. This strategy involves the use of both trap and repellent plants, enabling stemborers to be simultaneously repelled from the main cereal crop and attracted to trap plants (Khan et al., 1997a, b, 2000, 2001). Napier grass and Sudan grass have shown potential for use as trap plants, while molasses grass and two leguminous desmodium species, repel ovipositing stemborers. Molasses grass not only reduced infestation of maize by stemborers, it also contributed to an increase in stemborer parasitism by a parasitoid, *Cotesia sesamiae*. This result was attributed to the emission of volatile chemical compounds by molasses grass that repelled gravid stemborer moths but attracted *C. sesamiae* to the system. However, the exact mechanisms responsible for the observed results are not well elaborated and understood. We were interested in one component of Khan et al.'s hypothesis that allelochemical activity was responsible for the higher parasitism levels recorded in the maize-molasses grass intercrop. We went on further to investigate whether their findings could also be translated to systems containing another cereal crop different from maize, and the influence of the system on behaviour of other stemborer parasitoid species. This was deemed important since plans of expanding the approach to other cereal growing areas are in the pipeline and preliminary knowledge on how wide the approach can operate is necessary for the expansion process. The main aim of the present study was to examine the multitrophic interactions involved in a diversified cereal agroecosystem with particular emphasis on the foraging behaviour of stemborer parasitoids and how this behaviour might be influenced by diversification of the system. The system under study consisted of the host plants maize and sorghum, the non-host plant, molasses grass, stemborers, the larval parasitoid, *Cotesia sesamiae* and the pupal parasitoid, *Dentichasmias busseolae*.

Chemical ecology of *C. sesamiae* and *D. busseolae* in a diversified system

The study of chemical interactions between organisms and their environment is generally termed chemical ecology. This was investigated for a cereal-based system diversified by the addition of a non-host plant, molasses grass in laboratory-based bioassays. The findings of these olfactometer studies are reported in chapters 2 & 3. Parasitoids make extensive use of volatile chemical stimuli from their host's natural habitat during host location (Vet & Dicke, 1992). Plants, having a larger biomass than the herbivorous hosts, produce large quantities of volatiles that aid the parasitoids in locating their victims. A direct location of herbivores is hampered since herbivores are under strong selection to be inconspicuous. Hence, the information they provide

Summary

for their natural enemies will be as limited as possible. In the present study, the role of host plant volatiles in the foraging behaviour of *C. sesamiae* and *D. busseolae* was elucidated. Both parasitoids were strongly attracted to volatiles emanating from uninfested host plants (maize and sorghum). Volatiles from uninfested host plants have been demonstrated to be attractants for parasitoids in a number of studies (Lewis et al., 1990; Ngi-Song et al., 1996, Rutledge & Wiedenmann, 1999). We found that volatiles from infested host plants were the most attractive. These results indicate that in our system host plant volatiles also guide parasitoids to a habitat likely to be harbouring their hosts. The uninfested host plants provide detectable cues but these cues are far less reliable for host location. This dilemma is solved by parasitoids narrowing their search by responding to volatiles released by host plants after damage by herbivores (Vet et al., 1991; Dicke & Vet, 1999).

In a diversified cropping system, natural enemies are confronted with non-host plant species in their foraging arena. These non-host plants also provide olfactory and visual cues that may influence the foraging behaviour of the natural enemies. Khan et al. (1997a) noted higher stemborer parasitism rates in an intercrop of maize and molasses grass than in the monocrop by the parasitoid *C. sesamiae*. This was said to be due to molasses grass producing volatile compounds similar to those produced by herbivore-infested plants that increased foraging activity of this parasitoid. Molasses grass was thus recommended as a candidate for intercropping with maize to enhance parasitization by stemborer parasitoids. In chapter 2 we showed that volatiles from the non-host plant molasses grass were indeed attractive to *C. sesamiae*. However, molasses grass was not more attractive than uninfested host plants. The implication here is that molasses grass may play a role in the long-range attraction of *C. sesamiae* to the diversified habitat. Molasses grass was not attractive to *D. busseolae*, the pupal parasitoid we studied. It was clearly demonstrated that *D. busseolae* was repelled by molasses grass volatiles when molasses grass was presented alone in a Y-tube olfactometer (chapter 3). This parasitoid has a very narrow host range (Zwart, 1998) and is more of a specialist than *C. sesamiae*. According to Sheehan (1986), plant diversity often decreases the effectiveness of specialist parasitoids. Specialists appear to use more specific chemical cues while orienting to plants than generalists (Vinson, 1976) and the presence of non-host plants may disrupt orientation to the patch. However, our results show that *D. busseolae* was not repelled by volatiles emanating from a combination of molasses grass and infested host plants. Despite the presence of the repellent molasses grass volatiles, infested host plant cues seem to be sufficiently specific for the parasitoid to discriminate them from those of the non-host plant.

Our results show an intriguing environmental effect on plant volatile production. The same cultivar of molasses grass grown in two different locations emitted different volatile blends (chapter 4) that elicited divergent responses from the parasitoid *C. sesamiae* (chapter 2). This is a pointer to the fact that a successful outcome of a habitat strategy in one region does not necessarily mean that it can be generalized and extrapolated to other zones that grow the same crops. The careful screening and selection of agroecological zones in which a habitat management strategy is to be established should also be taken into consideration.

Close-range behaviour of *C. sesamiae* and *D. busseolae*

The gap between laboratory studies such as olfactometer experiments and what actually happens in the filed can be a large one. Hence, it is important to try to extrapolate volatile attraction laboratory studies to more realistic situations in which the foraging behaviour of parasitoids on and between plants is investigated. Therefore, semi-field studies were conducted to observe and compare close-range behaviour of these two stemborer parasitoids in arenas consisting of either intercrops of host plants with the non-host plant molasses grass or host plant monocrops (chapter 5). The diminutive size of many parasitoids makes observation of their foraging behaviour in the field often difficult, if not impossible. Use of simulative semi-field arenas is an attempt to solve this problem, but still has shortcomings, as in some cases the environment may still not be representative of a real field situation. The case of *C. sesamiae*, measuring approximately 2-mm body length, is a good example of this. Even after modifying the arena to include only four plants, it was still difficult to follow the small insect's movements on the plant foliage. Thus, observations could only be made on a single plant. Though a single plant is a poor representation of a diversified system, predictions on how the parasitoid might behave while in the field can still be made from the results obtained. Both *C. sesamiae* and *D. busseolae* recognized host plants and spent more time on the host plants than the non-host plant. Molasses grass therefore would not be an impediment to patch exploitation by these parasitoids in an intercrop.

Specificity of chemical stimuli of the studied system

To facilitate interpretation of behavioural observations of the parasitoids *C. sesamiae* and *D. busseolae* in olfactometer bioassays, chemical analyses of headspace volatiles of the plants used were conducted. The analyses revealed qualitative differences in the volatile blend of the plants under investigation. Infested host plants released a richer volatile blend than the uninfested host plants, with many of the compounds identified reported to be commonly released by plants after herbivore damage (Turlings et al, 1991; Ngi-Song et al., 2000; Gouinguené et al., 2001). These compounds most likely played the role of making these plants more attractive in bioassays (chapters 2 & 3). Thika molasses grass was attractive to *C. sesamiae*, a parasitoid considered to be a generalist (Ngi-Song et al., 1995; Mochiah et al., 2001). The volatile blend from Thika molasses grass had some compounds produced by herbivore-damaged plants and this may account for its attractiveness. However, *D. busseolae* was not attracted to Thika molasses grass. Being a monophagous parasitoid, it probably used very specific compounds to locate host plants, a specificity that was not provided by the Thika molasses grass blend. Mbita molasses grass released the least number of identifiable compounds. This volatile blend did not have any compounds noted in the infested host plants and this could account for its unattractiveness. As noted in chapter 2, the environmental conditions influenced the volatiles released by Mbita molasses grass. From the chemical data collected, the infested host plants sorghum and maize emitted compounds noted to be produced by herbivore-damaged plants, some of which were noted in Thika molasses grass. This possibly explains why volatiles from infested host plants were generally more attractive to parasitoids than those from a combination of uninfested host plants and molasses grass. Addition of molasses grass volatiles did not elicit an enhanced response from the parasitoids as hypothesized. These chemical analyses provide more information on why parasitoids responded in the manner they did in olfactometer bioassays. They also give insight into the results obtained from the field (chapter 6), whereby there was no enhanced stemborer parasitism in intercrop plots.

Impact of habitat diversification on stemborer populations and their parasitoids

To investigate the effect of molasses grass on composition and abundance of stemborers and their parasitoids in a diversified habitat with sorghum, field experiments were conducted (chapter 6). Stemborer density was generally lower in the intercrop than in the monocrop plots. There was no evidence that intercropping sorghum with molasses grass enhanced parasitoid effectiveness as postulated. Apparently none of the probable mechanisms responsible for enhancing parasitoid activity, such as provision of food resources, alternative hosts, or refuges, operated in this system. The success of the system seems to be due to mechanisms acting directly on the stemborers that were however not investigated in this study. Khan et al. (1997a) reported a four-fold increase in parasitism by *C. sesamiae* in maize-molasses grass plots as compared to monocrop plots. In the same system, stemborer densities were lower in the intercrop. They hypothesized that these observations were due to production of chemical compounds by molasses grass that are similar to those produced by damaged maize plants. They particularly singled out the compound (3E)-4,8-dimethyl-1,3,7-nonatriene. These compounds were thought to play a dual role of first repelling gravid stemborer females, leading to lower deposition of eggs on host plants in the intercrop and second the recruitment of parasitoids. Indeed repulsion of gravid moths by herbivore-induced volatiles seems a valid possibility. De Moraes et al. (2001) and Keßler & Baldwin (2001) investigated this phenomenon in different plant-herbivore systems and confirmed this form of direct defence strategy by plants. However, there is limited direct proof that the molasses grass actually recruits extra parasitoids to intercrop plots. An equally parsimonious explanation for the reported higher parasitism in maize intercrop plots in Khan et al.'s (1997a, b) study, would be a decrease in densities of stemborers due to the repellent plants, with no additional recruitment of parasitoids, resulting in a higher parasitoid to host ratio and thus higher parasitism.

The broader perspective of habitat management

Implementing habitat management to enhance natural enemies

For a habitat management strategy to be successful, careful identification and selection of the key organisms to be incorporated in the diversified system are a crucial first step (Gurr et al., 1998). This is a difficult but necessary task since it avoids the hit-and-miss nature of providing diversity. The first challenge is the selection of alternative or companion plants that will be added to the cropping system to bring about diversity that will lower pest densities and at the same time enhance natural enemy effectiveness. This process involves surveys of candidate plants having a potential of providing resources needed by natural enemies but missing in the main agroecosystem of interest (Baggen & Gurr, 1998; Khan et al., 2000). It is important to note that the plants selected in diversifying the habitat must in themselves be of value for the communities involved (Pickett et al., 1997). For example, molasses grass used in the present study has the main advantage of being a forage grass for livestock (Khan et al., 2000). This is an advantage especially in semi-arid regions where pasture is scarce. It is in these same regions that sorghum is grown.

A more enlightened knowledge of the ecology of the organisms involved in the multitrophic system, and the circumstances under which the natural enemies are most likely to be effective is required for the success of any habitat management approach. Understanding the host searching behaviours of natural enemies in a diversified ecosystem is one such requirement. This is because it is of utmost importance for the natural enemies to locate their hosts and to attack them successfully; otherwise the exercise of manipulating the habitat to enhance natural enemy activity would be jeopardised. The present study concentrated on the aspect of influence of volatiles from the companion plant on stemborer parasitoid host location. Although molasses grass did not enhance parasitoid effectiveness as hypothesized, it did not obscure volatiles directly involved in recruitment of the parasitoids either. Therefore, overall we think molasses grass has a neutral effect in this diversified system. The chemical ecology of diversified systems is an area that is poorly exploited. Yet it could serve as one of the major explanations to mechanisms underlying increased natural enemy activity in habitat management systems.

Once agents of a habitat management strategy have been identified and preliminary bioecology studies conducted on them, the last stage is implementation and evaluation of the biological control efficacy (Naranjo, 2001). The evaluation methods are varied and could include comparison of parasitism rates; recording natural enemy numbers to correlate pest and natural enemy densities or in the case of predators, analysis of the gut contents to obtain predation rates. It should be noted that some methods engaged in during the study stage could still be employed in the evaluation stage such as the determination of factors that disrupt or enhance biological control. A number of reviews have addressed the evaluation of biological control approaches (Luck et al., 1988; Sunderland, 1988; Kidd & Jervis, 1996; Barbosa, 1998; Pickett & Bugg, 1998). The results are varied and seem to be case specific. It is not possible to show universal trends owing to the great diversity of the systems and competing processes, which vary under different conditions. Figure 1 summaries the important steps followed in establishing a habitat management strategy to enhance biological control.

Shortcomings of habitat management on natural enemy effectiveness

Habitat management as a pest control strategy is not without its pitfalls of which proponents of this strategy should be aware. Once a habitat is modified, little can be done to control the myriad of organisms that might exploit it (Stephens et al., 1998).

Vegetational diversity can provide a barrier to the parasitoid searching ability, including olfaction barriers whereby the volatiles from companion plants mask the odours of host plants and confuse the parasitoid (Costello & Altieri, 1995). Presence of a companion crop can interfere with the movement of natural enemies and lead to emigration from the diverse habitat (Coll & Bottrell, 1996). The companion crop could also decrease the apparency of the target plant to the natural enemy, although this decreased apparency could be advantageous from a pest management point of view (Finch & Collier, 2000). Some natural enemies may orient to the non-host plants before entering the searching mode to locate potential insect hosts, thus wasting time (Lewis et al., 1994; Bugg et al., 1987). This negative impact may be especially acute for short-lived parasitoids. Baggen & Gurr (1998) reported exploitation of nectar and pollen resources, meant for parasitoids, by their herbivorous hosts as well. The presence of other crops in the agroecosystem could provide enemy-free space to a host herbivore when these additional crops are inaccessible to some of its parasitoids. For these reasons, researchers must remain cognisant of the potential conflicts in the development of a habitat management strategy meant to enhance parasitoid activity.

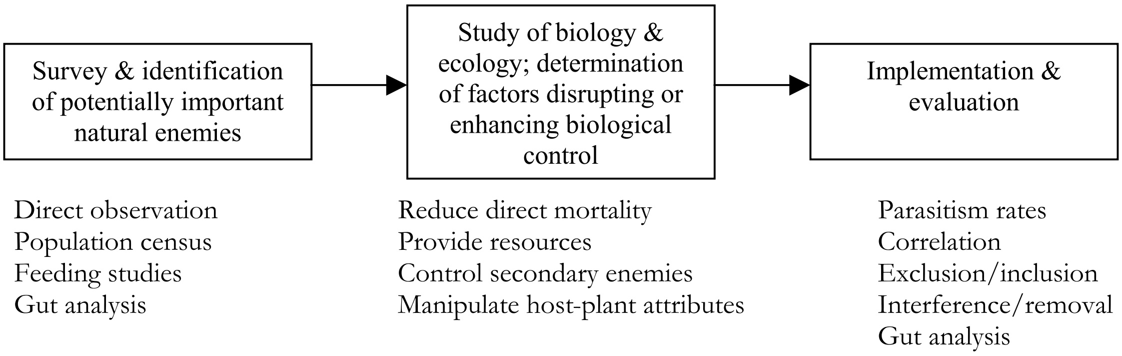

Figure 1. Components and approaches of conservation biological control (Adapted from Naranjo, 2001).

Conclusion

Most studies on the effects of habitat diversity on natural enemies do not attempt to explain why there are differences in population densities between simple and diversified systems. Without better mechanistic understanding of the influence of plant diversity on natural enemies it is difficult to make useful predictions on the multitrophic interactions and their outcome in diversified systems. Such predictions are essential if agroecosystems are to be designed to enhance biological control of herbivorous insect by natural enemies. The work presented in this thesis investigated the chemical ecology and behavioural aspects of the stemborer parasitoids *C. sesamiae* and *D. busseolae*. Particular emphasis was on how these parasitoids would operate in a cereal agroecosystem diversified by addition of a non-host plant molasses grass that was purported to aid in recruitment of parasitoids to the diverse system. Our findings suggest that molasses grass will probably not play an important role in enhancing the activity of the two parasitoids. This revelation is important because it narrows the candidate list of plants that hold potential of being used in the habitat management strategy particularly aimed at enhancing parasitoid foraging behaviour. Our results do not support the hypothesis by Khan et al (1997a) that increased attraction of a stemborer parasitoid by molasses grass is playing a significant role in suppressing stemborer populations in an intercropping system. To really understand why there is enhanced control of stemborers in the diversified crop system, we may have to focus more on the stemborers themselves. In field experiments (chapter 6), we noted lower borer densities in sorghum-molasses grass intercrops than in monocrop plots. We believe that there is a clear need to further investigate the role of the non-host plant molasses grass on the host plant location, host plant acceptance and oviposition behaviour of the stemborer moths and the host plant quality for its development. Therefore, our major recommendation on use of molasses grass in stemborer management efforts is to investigate and expound on the interrelations between molasses grass, stemborers and the host plants.

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Summary

Maize and sorghum are important staple food sources for the majority of human populations living in tropical Africa. Full production of these crops is not realised due to various constraining factors including attack by herbivorous insects. Habitat management is a pest control approach that aims at reducing pest pressure on crops and enhance effectiveness of natural enemies. A clear understanding of the effect of vegetational diversity on natural enemy activity is vital for the success of the strategy. This thesis focused on the influence of habitat diversification on the behaviour of stemborer parasitoids. The general objective was to investigate the functioning of multitrophic interactions involved in a cereal-based agroecosystem diversified with the addition of a non-host plant molasses grass (*Melinis minutiflora*). The results provide insight as to whether habitat diversification can be used to enhance the effectiveness of the parasitoids against stemborers. In **chapter 1**, background information on the economic importance of cereal stemborers, and the need to search for environmentally sound and sustainable control measures is presented. The need to understand multitrophic interactions in a diverse agroecosytem is also discussed.

In **chapter 2**, olfactory responses of the larval stemborer parasitoid, *Cotesia sesamiae* to volatiles emitted by host plants, maize and sorghum and the non-host plant, molasses grass, were studied in a Y-tube olfactometer. The hypothesis was that volatiles from host plants and the non-host plant in combination would be more attractive than the host plants alone. Volatiles from the host and non-host plants were attractive to *C. sesamiae.* Infested plants were the most attractive, while molasses grass was as attractive as the uninfested host plants. Combining molasses grass with maize or sorghum did not increase attraction as compared to host plants alone. Wasps were more attracted to volatiles from infested sorghum plants than volatiles from a combination of infested sorghum and molasses grass. Sorghum was a preferred host plant over maize. Local environmental conditions had an influence on the blend of volatiles released by the same cultivar of molasses grass grown in two different locations, Thika and Mbita. Volatiles from the two grass populations elicited divergent olfactory responses from *C. sesamiae*, with the Thika population being attractive while the Mbita one was not.

The role of volatile plant stimuli on the foraging behaviour of a stemborer pupal parasitoid *Dentichasmias busseolae* was investigated in **chapter 3**. Volatiles from sorghum and maize plants infested with stemborers and uninfested plants were attractive to *D. busseolae*, with volatiles from the infested plants being more attractive to the parasitoid. Further tests to explore the possibility of enhancing the foraging activity of this pupal parasitoid by diversifying the agroecosystem with molasses grass showed that the grass was repellent to the parasitoid. Combinations of molasses grass and host plants were less attractive than sole host plants. *Dentichasmias busseolae* could not discriminate between volatiles emitted by maize plants infested with two stemborer species, *Chilo partellus* and *Busseola fusca*, but it preferred volatiles from sorghum to those from maize. In **chapter 4**,

a report on analyses of headspace volatiles of the plants used in the bioassays in **chapters 2** and **3** is provided. Qualitative differences in compound composition of the emitted volatiles were noted between plant species and between infested and uninfested host plants. Infested host plants produced a richer blend than uninfested plants. The Thika molasses grass blend had some compounds common to infested host plants, while Mbita molasses grass produced few identifiable compounds, which were common to all plants under investigation.

Results reported in **chapter 5** were a link between laboratory and field studies. Investigations were conducted on how the presence of molasses grass influences close-range foraging behaviour of *C. sesamiae* and *D. busseolae* in a semi-field situation. A comparison of foraging behaviour of the parasitoids was made between host plant monocrop and host and non-host plant intercrop patches. Both parasitoids distinguished between host and non-host plants. *Cotesia sesamiae* was not arrested on the molasses grass, while the presence of the grass did not interfere with the foraging of *D. busseolae* on stemborer-infested host plants. Foraging behaviour of *D. busseolae* in intercrops was not significantly different from that in monocrops. The parasitoids also spent more time on infested host plants than on uninfested ones, implying there were stimuli on the infested plants that arrested them.

The effects of intercropping a non-host plant, molasses grass, with a host plant, sorghum, on stemborer abundance and parasitism levels by stemborer parasitoids were tested in field experiments (**chapter 6**). Four stemborer species were encountered; *Chilo partellus, Busseola fusca, Sesamia calamistis* and *Eldana saccharina* with *C. partellus* being the dominant species. The parasitoid species reared from stemborers were: *Cotesia sesamiae, Cotesia flavipes* and *Stenobracon rufus* from larvae and *Dentichasmias busseolae* and *Pediobius furvus* from pupae. *Aphanogmus fijiensis*, a hyperparasitoid of *Cotesia* spp. was also infrequently encountered. Stemborer density was generally lower in the intercrop than the monocrop. There were differences in stemborer abundance among the years and seasons, with higher densities in 1998 than in 1999; and higher densities in the long rainy season than the short rainy season. Intercropping sorghum and molasses grass did not enhance stemborer parasitism in the intercrop. Overall larval and pupal parasitism during the rainy seasons of 1998 and 1999 was 2.1 and 11.0% in the monocrop and 2.0 and 9.8% in the intercrop.

In **chapter 7** a synthesis of the aspects studied in this thesis is made and discussed in relation to implementation of habitat management to enhance natural enemies. The conclusions drawn from the results of this thesis are as follows:

- Plant volatiles play an important role in the host searching behaviour of the stemborer parasitoids *Cotesia sesamiae* and *Dentichasmias busseolae.*
- Combining molasses grass with host plants was not more attractive to *C. sesamiae* than host plants alone.

- Molasses grass volatiles were repellent to *D. busseolae*, but the presence of molasses grass did not impede patch exploitation by this parasitoid.
- The environmental conditions in which molasses grass was grown had an effect on the blend of volatiles emitted by the grass, which elicited divergent responses from *C. sesamiae*.
- Variations in the volatile blends of infested and uninfested host plants and molasses grass resulted in differences in responses elicited by parasitoids to these plants.
- Intercropping molasses grass with cereals had the effect of lowering stemborer densities in the intercrop but did not enhance parasitism of the stemborers.

Future perspectives

The results presented in this thesis are not an end in themselves but lay a foundation for further research. With that, I suggest the following future investigations:

- Investigations on the role of molasses grass on host plant location, host plant acceptance and oviposition behaviour of the stemborer moths, and the effect of molasses grass on the dispersal of early instar stemborer larvae.
- Identification of other plants to be used in habitat diversification that provide refuge, alternative prey and food to natural enemies.
- Gas chromatography coupled with electroantennogram recording (GC-EAD) bioassays to identify active components in host plant and molasses grass volatiles that elicit sensory and physiological responses in parasitoids.
- Investigations into other control methods that compliment habitat management such as host plant resistance, classical biological control, and other cultural practices to increase pest suppression and enhance natural enemy activity.

Samenvatting

Maïs en sorghum zijn belangrijke voedselbronnen voor de meerderheid van de bevolking van tropisch Afrika. Volledige productie van deze gewassen wordt niet gerealiseerd door verschillende beperkende factoren waaronder schade verzaakt door herbivore (plantenetende) insecten. Habitat management is een plaagbestrijdingsmethode die tracht de druk van de plaag op de gewassen te reduceren en de efficiëntie van natuurlijke vijanden te vergroten. Het begrijpen van het effect van gewas diversiteit op de activiteit van natuurlijke vijanden is van vitaal belang voor het succes van deze strategie. Dit proefschrift richt zich op de invloed van habitat diversiteit op het gedrag van stengelboorder parasieten (sluipwespen). Het algemene doel was het onderzoeken van het functioneren van multitrofe interacties in een op graan gebaseerd agro-ecosysteem waarin de diversiteit is verhoogd door toevoeging van een niet-gastheerplant, het molasse gras (*Melinis minutiflora*). De resultaten bieden inzicht in de vraag of habitat diversiteit gebruikt kan worden om de effectiviteit van de parasieten van stengelboorders te vergoten. In **hoofdstuk 1** wordt achtergrond informatie over het economisch belang van graan stengelboorders gegeven en de noodzaak van het zoeken van milieuvriendelijke en duurzame bestrijdingsmethoden gepresenteerd. Daarnaast wordt het belang van het begrijpen van multitrofe interacties in een divers agro-ecosysteem bediscussieerd.

In **hoofdstuk 2**, geur respons van de larvale stengelboorder parasiet, *Cotesia sesamiae* naar vluchtige stoffen geproduceerd door gastheerplanten, mais en sorghum, en de niet-gastheerplant, het molasse gras, werden onderzocht in een Y-buis olfactometer. De hypothese was dat een combinatie van vluchtige stoffen van gastheerplanten en de niet-gastheerplant aantrekkelijker is dan vluchtige stoffen van gastheerplanten apart. Vluchtige stoffen van de gastheer en niet-gastheer planten waren aantrekkelijk voor *C. sesamiae.* Geïnfecteerde planten waren het meest aantrekkelijk, terwijl het molasse gras net zo aantrekkelijk was als niet-geïnfecteerde gastheerplanten. Een combinatie van molasse gras met maïs of sorghum vergrootte de aantrekkelijkheid niet vergeleken met alleen gastheerplanten. Sluipwespen werden meer aangetrokken tot vluchtige stoffen van geïnfecteerde sorghumplanten dan tot vluchtige stoffen van een combinatie van geïnfecteerde sorghum en molasse gras. Sorghum werd geprefereerd boven maïs als gastheerplant. Locale omgevingsfactoren hadden een invloed op het geurpatroon geproduceerd door dezelfde mollasse gras cultivar gekweekt op twee verschillende locaties, Thika en Mbita. Vluchtige stoffen van twee gras populaties veroorzaakten verschillende responzen van *C. sesamiae*, waarbij de Thika populatie aantrekkelijk was en de Mbita populatie niet.

De rol van vluchtige plant stimuli op het foerageer gedrag van de parasiet *Dentichasmias busseolae* van het popstadium van de stengelboorder werd onderzocht in **hoofdstuk 3**. Vluchtige stoffen van sorghum en maïs planten geïnfecteerd met stengelboorders en ongeïnfecteerde planten waren aantrekkelijk voor *D. busseolae*, waarbij vluchtige stoffen van de geïnfecteerde planten aantrekkelijker waren voor de parasiet. Andere toetsen om de foerageer activiteit van de popparasiet te vergroten d.m.v. het diversificeren van het agro-ecosysteem met molasse gras lieten zien dat het gras afstotend was voor de parasiet. Combinaties van molasse gras en gastheerplanten waren minder aantrekkelijk dan gastheerplanten apart. *Dentichasmias busseolae* kon geen onderscheid maken tussen vluchtige stoffen geproduceerd door maïs planten geïnfecteerd met twee stengelboorder soorten, *Chilo partellus* of *Busseola fusca*, maar gaf de voorkeur aan vluchtige stoffen van sorghum boven maïs. In **hoofdstuk 4** wordt een analyse van de headspace van geurpatronen van de gebruikte planten in de biotoetsen van **hoofdstuk 2** en **3** gepresenteerd. Kwalitatieve verschillen waren te zien tussen de geurpatronen van de verschillende plantensoorten en tussen de geurpatronen van geïnfecteerde en niet-geïnfecteerde planten. Geïnfecteerde gastheerplanten produceerden een rijker geurpatroon dan ongeïnfecteerde planten. Het Thika molasse gras patroon bevatte stoffen die algemeen waren voor geïnfecteerde gastheerplanten, terwijl Mbita molasse gras weinig identificeerbare stoffen produceerde die algemeen waren voor alle onderzochte planten.

Het onderzoek gepresenteerd in **hoofdstuk 5** was een link tussen laboratorium- en veld studie. In dit onderzoek werd bepaald hoe de aanwezigheid van molasse gras het foerageer gedrag van *C. sesamiae* en *D. busseolae* op korte afstand beïnvloedt in een semi-veldsituatie. Een vergelijking werd gemaakt tussen het foerageer gedrag van de parasieten in een gastheerplant monocultuur en een gastheer en niet-gastheerplant mengteelt. Beide parasieten maakten onderscheid tussen gastheer en niet-gastheerplanten. *Cotesia sesamiae* bleef niet op molasse gras zitten, terwijl de aanwezigheid van het gras niet interfereerde met het foerageren van *D. busseolae* op stengelboorder-geïnfecteerde gastheerplanten. Foerageer gedrag van *D. busseolae* in de mengteelt was niet significant verschillend van dat in de monocultuur. De parasieten spendeerden ook meer tijd op geïnfecteerde gastheerplanten dan op niet geïnfecteerde gastheerplanten wat duidt op de aanwezigheid van zgn. "arrestatie stimuli" op de geïnfecteerde planten.

De effecten van een mengteelt van een niet-gastheerplant, molasse gras, met een gastheerplant, sorghum, op de hoeveelheid aanwezig stengelboorders en parasiteringsgraden door stengelboorderparasieten werden getest in veldexperimenten (**hoofdstuk 6**). Vier stengelboorersoorten werden gevonden; *Chilo partellus, Busseola fusca, Sesamia calamistis* en *Eldana saccharina* waarbij *C. partellus* de dominante soort was. De parasieten gekweekt op stengelboorders waren: *Cotesia sesamiae, Cotesia flavipes* en *Stenobracon rufus* op larven en *Dentichasmias busseolae* en *Pediobius furvus* op poppen. *Aphanogmus fijiensis*, een hyperparasiet van *Cotesia* spp. werd ook in kleine hoeveelheden gevonden. De dichtheid aan stengelboorders was over het algemeen lager in de mengteelt dan in de monocultuur. Er waren verschillen in de aantallen stengelboorders tussen de jaren en seizoenen, met daarbij hogere dichtheden in 1998 dan in 1999; daarnaast waren er hogere dichtheden in het lange regenseizoen dan in het korte regenseizoen. Een mengteelt van sorghum en molasse gras vergrootte de parasiteringsgraad van de stengelboorders niet. De totale parasitering van de stengelboorderlarven en poppen gedurende de regenseizoenen van 1998 en 1999 was 2.1 en 11.0% in de monocultuur en 2.0 en 9.8% in de mengteelt.

In **hoofdstuk 7** wordt een synthese gemaakt van de bestudeerde aspecten in dit proefschrift. Dit bestudeerde aspecten worden bediscussieerd in relatie met het toepassen van habitat management om de effectiviteit van natuurlijke vijanden te vergroten. De conclusies van de resultaten zijn:

- Vluchtige plantenstoffen spelen een belangrijke rol in het gastheer zoekgedrag van de stengelboorderparasieten *Cotesia sesamiae* en *Dentichasmias busseolae*.
- Het combineren van molasse gras met gastheerplanten was niet aantrekkelijker voor *C. sesamiae* dan gastheerplanten apart.
- Vluchtige molasse gras stoffen waren afstotend voor *D. busseolae*, maar de aanwezigheid van molasse gras beïnvloedde de "patch" exploitatie door de parasiet niet negatief.
- De omgevingsfactoren waaronder molasse gras werd gekweekt hadden een effect op het geurenpatroon dat geproduceerd werd door het gras, wat verschillende responzen van *C. sesamiae* tot gevolg had.
- Variaties in geurpatronen van geïnfecteerde en niet-geïnfecteerde gastheerplanten en molasse gras resulteerden in verschillende responzen van de parasieten op de planten.
- Een mengteelt van molasse gras met graan verlaagde de dichtheid aan stengelboorders maar vergrootte de parasiteringsgraad van de stengelboorders niet.

Toekomstperspectieven

De resultaten gepresenteerd in dit proefschrift betekenen zeker niet een einde van het onderzoek maar zijn een fundering voor toekomstig onderzoek. Ik stel hierbij het volgende voor:

- Onderzoeken naar de rol van molasse gras op gastheerplant locatie, gastheerplant acceptatie en eileg gedrag van de stengelboorder, en het effect van molasse gras op de dispersie van vroege larvale stadia van de stengelboorder.
- Identificatie van andere planten die gebruikt kunnen worden bij habitat diversificatie die schuilplaatsen, alternatieve prooien en voedsel voor natuurlijke vijanden bieden.
- Gas chromatografie gekoppeld met electroantennogram opnames (GC-EAD) en biotoetsen met actieve componenten in gastheerplant en molasse gras geurpatronen die een sensorische en fysiologische respons van de parasiet teweeg brengen.
- Onderzoeken naar andere methoden van bestrijding die iets kunnen toevoegen aan habitat management zoals bijvoorbeeld gastheerplant resistentie, klassieke biologische bestrijding, en andere culturele praktijken om de plaag te onderdrukken en de effectiviteit van de natuurlijke vijand te vergroten.

Curriculum vitae

Linnet Serenge Gohole was born on 19th December 1968 in Kakamega district, Kenya. She attended Kaimosi Junior School and Mumias Girls Boarding School for her primary education between 1975 and 1981. She proceeded to Loreto High School - Limuru where she completed her secondary school education in 1987. She joined Kenyatta University in 1988 to pursue a Bachelor of Education-Science (Botany and Zoology) degree course, which she completed in 1991. She was posted by the Teachers' Service Commission (TSC), Kenya to Mbale Boys High School where she taught Biology and Geography. However, her teaching stint at this school lasted only 4 months for in September of the same year she was awarded a scholarship by Kenyatta University to study Agricultural Entomology at Masters level. She obtained her MSc degree in 1994. On completion, she was re-deployed to St. Teresa's Girls Secondary School in Nairobi. In November 1996, she was employed as an assistant lecturer by Moi University at the department of Crop Production and Seed Technology (CPST), Faculty of Agriculture. Her area of specialization is crop protection. In September 1997, Linnet joined Wageningen University to pursue PhD studies with funding from MHO/NUFFIC under a collaborative project between Wageningen and Moi University. Her research focused on enhancing biological control of stemborers. The study, which was a sandwich program, was partially undertaken in her home country, Kenya, where she was based at the International Centre of Insect Physiology and Ecology (ICIPE) as a Dissertation Research Internship Program (DRIP) scholar. Linnet will be based at the CPST department, Moi University where she will be involved in teaching, research, student supervision, and conducting other administrative duties.

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