Visual and olfactory cues used in host location by the blister beetle *Hycleus apicicornis* (Coleoptera: Meloidae), a pest of *Desmodium* (Fabaceae) species

Lefulesele Nteletsana Lebesa

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Visual and olfactory cues used in host location by the blister beetle *Hycleus apicicornis* (Coleoptera: Meloidae), a pest of *Desmodium* (Fabaceae) species

by

Lefulesele Nteletsana Lebesa

Submitted in partial fulfilment of the requirements for the degree

***Philosophiae Doctor* (Entomology)**

In the Faculty of Natural and Agricultural Science

Department of Zoology & Entomology

University of Pretoria

December 2012

For my family

My lovely daughters, Khabiso, Boitumelo and Boikhabo, this piece of work is dedicated to you. I thank you because you waited for me to come back home and be a mother after a long absence. I thank God, Almighty for sustaining you throughout the waiting period. I thank my two mothers, my late mom, 'Maitumeleng Masakale and my mother-in law, 'Makatleho Lebesa. My mom single-handedly raised me and it was with her trust in the Lord, her determination and encouragement that I knew any heights could be reached. Mom-in-law, 'thank you for being the pillar of support and portraying the true qualities of a parent.' The rest of the family, I thank you for your continued support and for standing as a united team behind me.

Men love to wonder, and that is the seed of science. (Ralph Waldo Emerson)

May we keep on planting the seed.

Visual and olfactory cues used in host location by the blister beetle *Hycleus apicicornis* (Coleoptera: Meloidae), a pest of *Desmodium* (Fabaceae) species

Student: Lefulesele Nteletsana Lebesa

Supervisors: Prof. Kerstin Krüger (University of Pretoria)

Co-supervisor: Dr Zeyaur R. Khan (ICIPE)

Department: Department of Zoology & Entomology, University of Pretoria

Degree: Philosophiae Doctor

Abstract

Desmodium spp. are leguminous plants mainly used as livestock fodder. In Kenya and neighbouring countries they are also used in a 'push-pull' strategy by smallholder farmers to protect maize (*Zea mays*) against two major groups of pests, stemborers (Lepidoptera) and witchweed, *Striga* spp., by repelling the former away from the cereal crop and suppressing growth of the latter. However, smallholder seed production of *Desmodium* spp. is compromised by blister beetles *Hycleus* spp. (Coleoptera: Meloidae) which feed on the flower petals and adversely affect seed setting. The objective of this study was to determine the behavioural responses of *Hycleus apicicornis* (Guér.) (Coleoptera: Meloidae) towards olfactory and visual cues from host plants, and the impact of these cues on resource-finding by this beetle. The present findings should be of future use in developing a trapping system to manage this pest.

A farmer questionnaire and a field survey were used to determine the pest status and host range of blister beetles. *Desmodium* spp. were identified as the most preferred followed by beans (*Phaseolus vulgaris*) and sweetpotato (*Ipomoea batatas*). Fewer farmers mentioned cowpea (*Vigna unguiculata*) and maize as additional host plants. Based on the questionnaire, the field survey and a literature review on potential host plants for blister beetles, *D. uncinatum*, *I. batatas*, *P. vulgaris*, *V. unguiculata*, *Abelmoschus esculentus*, and *Ipomoea hildebrandtii* (a local wild host) were chosen for subsequent experiments.

Behavioural studies were undertaken to determine i) the impact of visual cues on their host locating and landing behaviours, ii) responses of beetles towards volatiles released by individual host plants, iii) responses of beetles towards extracts of flowers of host plants and two synthetic compounds and iv) orientation and settling preferences of *Hycleus apicicornis* towards different host plants in the laboratory.

Beetles were significantly attracted to sky blue (with maximum spectral reflectance of 73% around 450 nm), which was preferred over other colours tested (black, red, yellow, white and green) and other shades of blue (turquoise and dark blue). Beetles also showed a high preference for odours of cut flowers of *D. uncinatum*, *I. batatas* and *P. vulgaris,* and 2-phenylethanol and phenylacetaldehyde but not for intact flowers in olfactometer bioassays. In the orientation and settling experiment, cut flowers of *A. esculentus* were attractive in addition to *P. vulgaris*, *D. uncinatum* and *I. batatas*. Volatiles were collected from *A. esculentus, D. uncinatum*, *P vulgaris* and *V. unguiculata through air-entrainment techniques and analysed* with gas chromatography coupled with mass spectrometry (GC-MS) to identify volatile compounds that may be inducing behavioural responses of adult beetles. Qualitative and quantitative differences were observed between and within the chemical profiles of the four host plant species.

This study provides baseline information required for the development of a trapping system for the management of blister beetles on *Desmodium* spp. or other potential host crops by making use of visual and olfactory cues. A stronger response was obtained with the visual cues compared to olfactory cues suggesting that *H. apicicornis* may be using visual cues for long range while the olfactory is used at close range during host location.

Acknowledgements

First and foremost I am grateful to my supervisors, Dr Khan and Dr Krüger for tirelessly providing guidance throughout this study and who did not only give academic support but also provided or facilitated provision for my personal needs both in Kenya and in South Africa. I am also thankful to the rest of the supervisory team, Prof. A. Hassanali, Prof. J.A. Pickett and Dr Toby Bruce who guided me and transferred their skills to me. Prof. Hassanali and Dr Bruce, I am forever grateful for your presence because there was a time I did not want to continue but you believed in me, you were very patient with me and inspired me to move on.

My sincere gratitude to the Habitat Management Project team: i) Dr D. Amudavi, Mr I. Mbeche, Dr C. Midega, Dr A. Murage, Dr E. Njuguna, Mr J. Pittchar and Dr A. Tameru for their expertise in the design of my experiments, reading and providing valuable comments and inputs and ii) Mr Paul Diela, Mr George Genga, Mr Salim Juma, Mr Elisha Kongere, Mr Samuel Mukaya, Mr Aloice Ndiege, Mr Dickens Nyagol, Mr Phillip Akelo, Mr Charles Oyugi, Mr Silas Ouko, Mr Joseph Ondiju, Mr Harrison Odhiambo and Mr Jacob Odhiambo for the support in carrying out the experimental work and many more that I came across in my practical work.

I am indebted to Dr. B. Torto who allowed me to use his laboratory and resources, offered suggestions and advices in the analyses of plant volatiles for my samples as well as providing his team (Mr Onesmo Wanyama, Mr Xavier Cheseto, Ms Peris Amwayi, and Mr Vincent) for helping out and providing an enabling environment for learning. My gratitude goes to the following people (Dr M. Liphoto, Dr M. Phoofolo but most importantly Mr S. Ratšiu) who critically went through my work and helped me put it in order.

I am also grateful for the financial support for the living and travel expenses provided by DAAD through the *icipe* Capacity Building & Instititional Development (CB&ID) Department. Some financial and logistical support provided by the University of Pretoria and the Lesotho Government when working in South Africa is also appreciated. I thank the whole CB&ID team, Lilian Igweta, Lisa Omondi & Margaret Nyagol for their dedication in facilitating and ensuring that my stay in Kenya and my exit from Kenya were a smooth process. The *icipe* Mbita family, friends and colleagues who saw me through this challenging period are also thanked.

I would like to thank my family for being there for me and taking me tirelessly through the whole process. My mother-in-law, ‘me ‘Makatleho Lebesa, you were the pillar of strength. You stood by me during the darkest hours and encouraged me to move on. I thank all my sisters and brothers who stood in for me to help with the kids. Mr Katleho Lebesa, I thank you for being both parents to our daughters when I was away. I cannot leave out the family support I got from my church, Shofar, Pretoria. I thank you, pastor H. Titus for being my spiritual father and giving me the support of a father I never had.

My friends (E.M. Njuguna, L. Serutla, A. Murage, N. Makio, K. Morobe, M. Lebete, T. Molomo, M. Mohloboli, T. Au, P. Nkhabutlane, K. Lehloenya, M. Semuli, L. Ntsane, M. Likhang, P. Mojela, L. Mashile, M. Matete and N. Tsiboho – to name a few), you displayed the true meaning of friendship, you cried with me when it was tough, laughed with me when things seemed better but most importantly you prayed with me and for me, held my hand and pushed me on.

I am grateful to the Department of Agricultural Research (DAR) for providing me an opportunity in their training and capacity building program to further my studies for the

betterment of the institution. The management team (especially DAR director, Dr M. Ranthamane) is being thanked for providing an enabling atmosphere (time and resources) for completion of this work.

Overall I thank God Almighty, the provider, the guide and the father who surrounded me with his tender mercies and grace and gave me the ability to go through this process to the end.

Declaration

I, Lefulesele Nteletsana Lebesa, declare that the thesis titled

Visual and olfactory cues used in host location by the blister beetle *Hycleus apicicornis* (Coleoptera: Meloidae), a pest of *Desmodium* (Fabaceae) species

which I hereby submit for the degree *Philosophiae Doctor* (Entomology) at the University of Pretoria is my own work and has not previously been submitted by me or anyone else for a degree at this or any other tertiary institution. All sources used in this study have been indicated and acknowledged by means of references.

Signature……………………………………………….

Date…………………………………………………….

Thesis organisation

The aim of this study was to evaluate how the blister beetle *H. apicicornis* uses visual and olfactory cues to locate its host plants. The knowledge gained can be used in future studies for the development of efficient control strategies to reduce the impact of this species on desmodium seed production. *Hycleus* spp. only recently emerged as pests of desmodium (*Desmodium* spp.), thus knowledge of their dynamics and management as a pest is critical for the East African smallholder farming community.

The first chapter provides an introduction to the study, giving background information, the objectives and expected outcomes, as well as a review of previous work related to this study. Chapter 2 highlights the importance of *Hycleus* species as emerging pests of desmodium in East Africa and how these beetles are perceived by small-scale farmers in western Kenya as well as observations made in the field. This chapter is followed by Chapter 3, which evaluates how *H. apicicornis* uses colour as one of the visual cues for locating its host plants.

Chapter 4 seeks to determine whether this pest species also makes use of olfactory cues to locate its host plants. This work involved laboratory behavioural bioassays, where responses of *H. apicicornis* to odours of six different host plants, *viz.* beans (*Phaseolus vulgaris*), cowpea (*Vigna unguiculata*), desmodium (*Desmodium uncinatum*), morning glory (*Ipomoea batatas*), okra (*Abelmoschus esculentus*) and sweetpotato (*Ipomoea hildebrandtii*) and two synthetic compounds, phenylacetaldehyde and 2-phenylethanol were investigated through the use of olfactometers.

Subsequently, in Chapter 5 air-entrainment of these plants was employed to identify the volatile components of the four host plants (*A. esculentus, D. uncinatum, P. vulgaris and V. unguiculata*) through gas-chromatography and mass spectrometry (GC-MS). Qualitative and quantitative analyses were then undertaken to compare these compounds and identify possible compounds responsible for attraction to the preferred host plants according to the results of the olfactometer tests. The final chapter (Chapter 6) summarises and discusses the findings from which conclusions and recommendations for future work were made.

Contents

CHAPTER 1

General introduction

1.1 Interactions of insects with other organisms

Plants and insects have co-evolved over millions of years (Metcalf & Metcalf 1992; Bruce *et al*. 2005). Their relationship goes far beyond plants merely providing food resources to insects (Schoonhoven *et al*. 2005; Schoonhoven 1999); it can involve complex processes that sustain their survival, for example, in pollination or in tri-trophic interactions among plants, plant-herbivores and natural enemies. Insects also interact with other living organisms such as other insects (their own species, natural enemies, predators etc.), micro-organisms and higher animals (Schoonhoven *et al*. 2005). Amongst all living organisms there is some form of communication that sustains all these complex interactions. These communication systems involve chemicals used to send messages to other organisms that may have originally evolved to benefit the emitter, although such messages can also be to the detriment of the emitter (Schoonhoven *et al*. 2005; Jönsson 2005).

The chemicals emitted by organisms to effect various interactions between them are known as semiochemicals (Isaacs 1994; Ansebo 2004; Bruce *et al*. 2005). The senses involved in detecting chemical odours are particularly sensitive in insects and have a great impact on their behavioural processes (Schoonhoven *et al*. 2005). The male moth of *Bombyx mori* Author (Lepidoptera; Bombycidae) is a well-known example (Plimmer *et al*. 1982; Steinbrecht 1987).

While chemicals play a central role in shaping behaviour of insects towards plants and other organisms it is also important to note that plants or other organisms may have other properties with which they communicate with insects (Prokopy & Owens 1983; Raguso 2004). Such properties are usually perceived by insects through their senses of vision, smell and touch and may include colour, shape and texture of these organisms (Jönsson 2005). It is therefore crucial to evaluate the role played by different cues (e.g. olfactory, visual, mechanical, gustatory) to explain the different behaviours of insects or interactions with their environment. As the mobile party, compared with plants, an insect has to use different tactics to find suitable plants for its survival (Bell 1990).

1.2 Host-plant selection

In general, plants are suitable for insects if they provide all necessary requirements, such as food and oviposition sites, for the development and survival of the latter (Bernays & Chapman 1994). Depending on the needs of insects, they may specialize not only on certain plants but also on parts of plants. For example, different herbivorous insect species can co-exist on a plant mainly because its different parts are able to satisfy different dietary requirements (Price 1997; Schoonhoven *et al.* 2005). To find the correct host plant, insect herbivores are faced with the challenge of detecting and locating their potential host plants and finally confirming their appropriateness (Bernays & Chapman 1994).

Host location

The process of finding a host or mate can be complex, due to the cues provided by the emitted semiochemicals from hosts/mates. It follows a series of steps undertaken before eventually accepting or rejecting the potential source of emission (Dethier 1983). Therefore, characterizing both host and mate location processes separately can be important.

The common assumption that the ability of insects to select food plants has been optimised by natural selection may suggest that, regardless of how insects end up on or amongst plants, they should be able to select the plants suitable for their growth and survival (Mayhew 1997). However, in practice this is not as simple as stated by this theoretical model of natural selection (Mayhew 1997; Bernays 2001). Many factors such as quality of host plants or presence or absence of natural enemies play a role when an insect chooses a host plant (Cunningham *et al*. 2001; Harris *et al*. 2001). As a result of taking into account various factors, such as extra energy wasted on searching for the most suitable host or threats by natural enemies present on the most suitable host plant (Videla *et al.* 2006), an adult insect may eventually select a food source that is not suitable or of lesser quality for the fitness of its offspring (Cunningham *et al.* 2001; Mayhew 2001).

Host location involves receiving the appropriate stimuli, which leads to search and assessment behaviour by an insect (Dethier 1983; Schoonhoven *et al.* 2005). The ability of an insect correctly to identify its hosts is dependent upon the visual and olfactory sensory systems that receive the stimuli from the environment. These systems have the ability to detect and code the stimuli (Dethier 1982, 1983; Murlis *et al.* 1992; Bernays & Chapman 1994). Olfactory and visual stimuli often play an interactive role in the first stage of search, with the former often playing a major role (Ansebo 2004). The relative importance of the two, however, differs between species (Schoonhoven *et al.* 2005). The final stage involves either or both the mechanical and gustatory sensory cues of an insect to accept the chosen plants as appropriate for food or oviposition (Bernays & Chapman 1994).

Search Behaviour

The ability of an insect to locate or find its host, food or other resources is influenced by a number of factors which are associated with the genetic make-up of or experience of an insect (Dicke 1999; Heard 2000). These characteristics often have an influence on the response of insects towards different stimuli in host searching. Many phytophagous insects have evolved to exploit the crucial characteristics associated with acquiring resources such as food, oviposition sites, mating sites etc. (Weiss & Papaj 2003; Cunningham *et al.* 1998a, 1998b; Prokopy *et al.* 1982).

Learning and Experience

Most insects employ the learned or adaptive strategy, which is an integration of information from different cues resulting in directed searching as this produces a higher success ratio per unit of time and energy invested in searching behaviour (Prokopy *et al.* 1982; Schoonhoven *et al.* 2005). It is important to note that the most abundant host plant may eventually be the most preferred even though it is not the most suitable. This can happen as a result of learning or experience due to previous encounters of such a plant (Cunningham *et al.* 2001). Mayhew (2001) also argues that when the best larval food differs from the best adult food an adult may choose the foods that best sustain its fitness so they can increase the quantity of offspring produced. The quantity may then balance the problem of offspring quality.

Many reports of how insects use learning of cues and relating them to finding their hosts are on Hymenoptera, e.g. bumblebees, (Dyer & Chittka 2004a, 2004b), honeybees (Giurfa *et al.* 1999; Giurfa 2004), wasps (Desouhant *et al.* 2010) and Lepidoptera (butterflies (Weiss 1997; McNeely & Singer 2001) and moths (Balkenius & Kelber 2006; Goyret & Raguso 2006). It is, however, possible that learning is also present in other taxa as has been shown in some Diptera (Prokopy *et al.* 1982; Prokopy *et al.* 1989) and Orthoptera (Bernays & Wrubel 1985).

If an insect is able to learn from past experiences it can then modify its choices to be able to recognize accurately or forage selectively for the hosts with maximum rewards (Greggers & Menzel 1993; Heard 2000; Cunningham *et al.* 2001). There are two types of learning, *associative learning*, where stimuli from previously encountered plants make an insect associate such stimuli with either rewards or avoidance (Prokopy *et al.* 1982; Schoonhoven *et al.* 2005; Dejean *et al.* 2003) and *non-associative/aversion learning,* where the response of an insect to certain stimuli is reduced due to repeated non-rewarding encounters (Dethier 1982; Dejean *et al.* 2003).

Foraging

The optimal foraging strategy states that when an insect forages for food it has to deal with both maximization of food or energy and minimization of risk to increase its fitness (Pyke *et al*. 1977; Hassell & Southwood 1978). Foraging strategies may differ between species and even within species, e.g. between males and females due to the different purposes of foraging, e.g. foraging for oviposition sites, foraging for food or searching for mates (Ne'eman *et al*. 2006). Other factors that affect the foraging strategies and efficiency in insects are the way resources are distributed temporally and spatially (Schoonhoven *et al*. 2005; Scheirs *et al*. 2002). Availability of resources may differ according to seasons or even different times of the day or differ according to the level of distribution in a habitat, and even further into a patch (Bell 1990). The insect should therefore be capable of using both innate and external sensory cues efficiently to locate the necessary resources (Riffell *et al*. 2008; Ômura & Honda 2005).

A search for a plant host by an insect may start as a random event in the absence of stimuli that elicits foraging and direction to a source of stimuli (Becher & Guerin 2009; Schoonhoven *et al*. 2005). An insect in this case is motivated to start searching for food by the hunger levels triggering a random search. An insect will use the internally stored memory or the information from experience to locate resources and start moving in an undirected movement. In this case an insect may perform scanning movements to increase chances of detecting hosts (Schoonhoven *et al*. 2005). In the absence of a stimulus an insect will make circular movements, which change with the receipt and increasing intensity of a stimulus until they become straight or directed (Visser 1988; Schoonhoven *et al*. 2005).

Orientation of insects towards host stimuli

Detection of appropriate stimuli by insects from a distance causes them to move from their current position (Bernays & Chapman 1994) and to start orientating towards such stimuli. These search or orientation mechanisms towards host stimuli are described as undirected (kinesis) and directed (taxes) reactions, which depend on locomotion patterns displayed by insects in response to the stimuli received (Kennedy 1986; Visser 1988; Schoonhoven *et al*. 2005).

These locomotion patterns differ between flying and walking insects. Flying insects mainly use anemotaxis, the orientation with respect to wind to reach the source of chemical stimuli (Cardé 1984; Kennedy 1986; Murlis *et al*. 1992; Bernays & Chapman 1994). Insects need to detect the wind direction, and to do so flying insects rely mainly on visual stimuli when they are in the air although on the ground they will depend on the mechano-chemical stimuli such as deterrents or stimulants (Bernays & Chapman 1994; Schoonhoven *et al*. 2005). Various types of orientation mechanisms are involved in insect orientation towards host cues (Murlis *et al*. 1992) and these can be commonly summed up together as the kinesis-taxes, which still maybe under some criticism of not fully incorporating the impact of the internal memory (Visser 1988).

Kinesis and Taxes

Kinesis involves non-directional movements, whereby insects may change the movement frequency (*orthokinesis*) or may change the rate of frequency of turning (*klinokinesis*). Movements are not directed by the stimuli but are effected by the insect itself once near the source of a stimulus (Kennedy 1986; Visser 1988; Schoonhoven *et al*. 2005).

Taxes are the directed movements due to stimuli (one or in combination with another) enabling the receiver to sense the direction to or away from the source of emission. In this situation there is temporal comparison of information from olfactory receptors (*klinotaxis*) or instantaneous comparison of intensities from bilateral pairs of olfactory receptors (*tropotaxis*) (Visser 1986; Schoonhoven *et al*. 2005). There is also *menotaxis* (including *anemotaxis* and *phototaxis*) whereby an insect maintains a constant angle to stimuli. Anemotaxis and phototaxis are orientation responses in respect to wind and light cues respectively (Schoonhoven *et al*. 2005).

Host-plant acceptance

Once an insect has reached a potential host and has established contact it undergoes an evaluation or assessment processes that may lead to acceptance or rejection (Miller & Strickler 1984; Schoonhoven *et al*. 2005). During this contact phase insects use both mechanosensory (tactile) and contact chemosensory (taste or gustatory) stimuli offered by the plant to inspect the potential host (Harris *et al*. 1993; Schoonhoven *et al*. 2005). The two behavioural responses observed in this phase are scanning and nibbling (Miller & Strickler 1984). Upon arrival on the host, insects scan the host plant by examining it first before attempting feeding behaviour. Thereafter, insects start the nibbling process whereby small amounts of food from the potential host food are ingested. In the case of oviposition an insect inserts the ovipositor without depositing an egg (Miller & Strickler 1984). If after the nibbling an insect continues feeding or it ends up ovipositing then it is considered to have accepted the host plant. It should be noted that it is at this stage that stimulants, arrestants and deterrents play a significant role for host plant recognition (Nielsen *et al*. 1977; Mitchell 1988; Schoonhoven *et al*. 2005; Pelletier & Dutheil 2006). The physical characteristics of the potential host plant also have a major role to play during a contact phase because they can deter an insect away from feeding or oviposition. For example the feeding behaviour in the final stage of host location of *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae) is interrupted by the presence of both trichomes and the phagodeterrent on the leaves of *Solanum tarijense* Hawkes (Solanaceae) once the insect comes in contact with the plant (Pelletier & Dutheil 2006).

Mate selection by insects

Mate selection is almost the same process as host selection in that both follow detection and recognition phases. It has been observed that when an insect perceives a sex pheromone, the pheromone stimulates a sequence of behaviours that eventually lead it to the partner for mating purposes. As in host location it goes through an activation stage whereupon behaviours such as antennal elevation, restlessness and wing vibration are observed (Plimmer *et al*. 1982). Having been aroused by the chemical stimuli an insect will then orientate towards the source (a calling insect) by flying upwind (positive anemotaxis) (Plimmer *et al*. 1982).

The insect has to recognize a mate before it can approach. It will therefore, use one or both of the stimuli (olfactory and visual) given off by the calling mate (Fukaya *et al*. 2005, 2006). For example, Wee & Tan (2005) observed that the searching behaviour of the female fruit fly, *Bactrocera carambolae* Drew & Hancock (Diptera: Tephritidae), is influenced by the male-produced pheromone odour. When detecting the odour females were observed to be flying anemotactically in a non-stop zig-zag pattern towards the male source of emission. Employing the two cues together for mate location may be a strategy that insects use to increase the search efficiency to save on the energy that may be wasted if only one stimulus is depended on. Odour alone may not always be very reliable to get them directly to the source since it is airborne and the wind may change the direction and speed (Fukaya *et al.* 2006). In some insects other pheromones e.g. aggregation pheromone are used to enhance mate location. Common examples of insects with aggregation pheromones are various species of beetles (Aldrich *et al*. 2003; Rao *et al*. 2003; Aukema & Raffa 2004; Lacey *et al*. 2004, 2008).

1.3 Sensory cues in host selection

Insects are able to use different senses for selection of their hosts and these are senses of smell, vision, touch and taste (Bernays & Chapman 1994). However the most crucial are senses of smell and vision for locating and finding their hosts while others are employed in the final stages for acceptance or rejection of potential hosts (Schoonhoven *et al*. 2005).

Olfactory cues and their effects on the behaviour of insects

Natural vegetation comprises various plant species both host and non-hosts, presenting insects with an array of chemicals from which to choose the ones that are attractive to them. Often chemicals used for host recognition are common to a wide range of plants including non-host plants thus insects have to have specific mechanisms for distinguishing between host and non-host plants. In a few cases there may be taxon-specific common chemicals among the host plant for some insects (Bernays & Chapman 1994; Bruce *et al*. 2005). Alternatively the detection of specific blends or ratios of chemical components that are generally found in different plant species can result in the recognition of host plants from non-host plants (Dethier 1983; Bernays & Chapman 1994; Bruce *et al*. 2005).

Semiochemicals

Classification of semiochemicals is based on whether the interaction they mediate is within (intra-specific) or between (inter-specific) species (Tumlinson & Teal 1987; Isaacs 1994; Blum 1996) (Fig. 1.3 below). The two major classes involved in these interactions are pheromones (intra-) and allelochemicals (inter-) (Isaacs 1994). There is however a considerable overlap between chemicals of different classes thus a chemical can serve more than one function, depending on the receiver and the type of behaviour displayed by the receiver, or even the organism that is producing it (Tumlinson & Teal 1987; Isaacs 1994, Ansebo 2004).

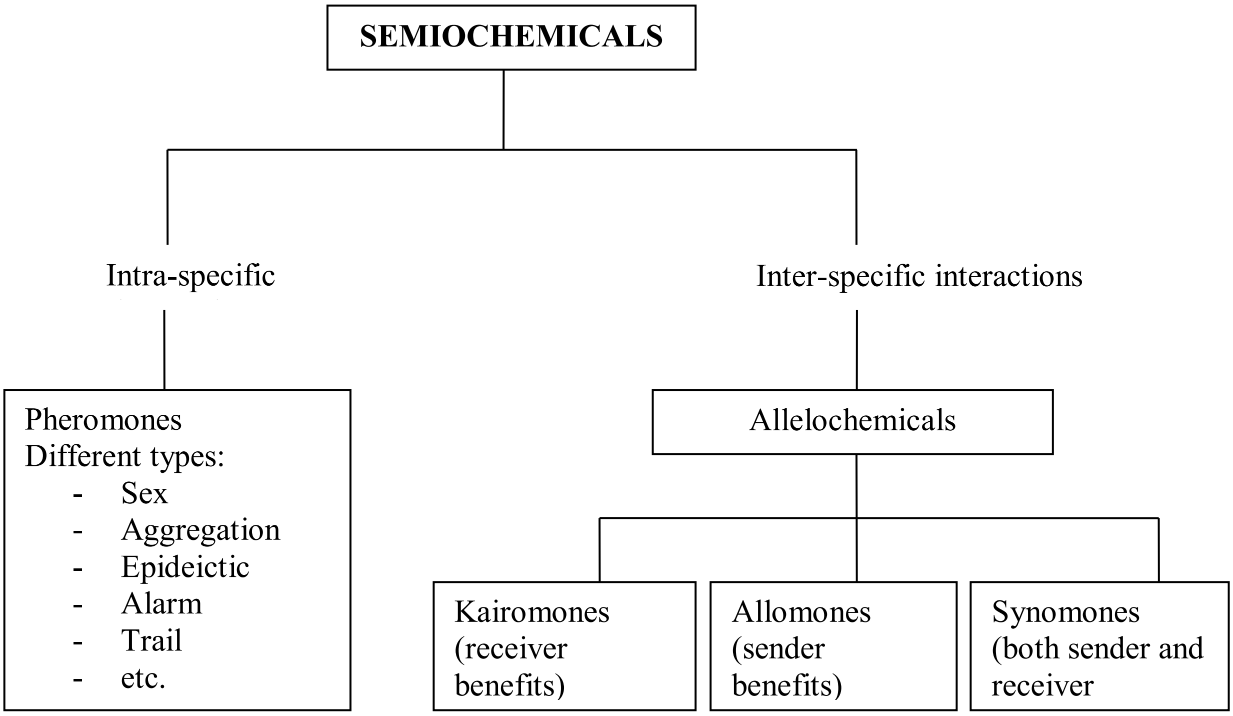


Figure 1. Diagrammatic presentation of the classification of semiochemicals and the interactions they mediate in organisms

Pheromones

These are chemical stimuli that may elicit some behavioural or physiological responses from the individuals of the same species (Regnier & Law 1968; Leoncini *et al.* 2004). There are two main classes of pheromones under which different types of pheromones fall, the releaser and primer pheromones. The former elicit an immediate behavioural response on the receiver while the latter cause long-term physiological changes and may lead to certain behaviour in the future (Regnier & Law 1968; Wyatt 2003; Leoncini *et al.* 2004). Most of the pheromone types fall into the releaser pheromones although it is important to note that in some insects one compound can serve as a releaser or a primer depending on the purpose it is produced for (Regnier & Law 1968).

Sex pheromones are the most commonly studied pheromones in insects. Sex pheromones are often released by one sex of a species to attract the opposite sex for mating purposes (Plimmer *et al*. 1982). In most cases the emitter is the female.

Alarm pheromones are produced by insects when they are attacked by natural enemies. Their release results in the dispersal or avoidance behaviour by conspecifics receiving such stimulus (Blum 1969; Sleeper *et al*. 1980; Blum 1996). Aphid species are among the well-known examples of insects producing an alarm pheromone and most of them produce (E)-β-farnesene (Pickett & Griffiths 1980; Pickett *et al*. 1992; Shah *et al*. 1999; Byers 2005).

Aggregation pheromones; when these pheromones are released both sexes of the same species are attracted towards the source of emission, resulting in the formation of aggregations on the host plant (Wertheim *et al*. 2005). Aggregation pheromones often have a synergistic interaction with some volatiles released by host plants if the emitting sex is feeding (Rao *et al*. 2003).

Epideictic pheromone: These are pheromones that may be produced by insects to avoid intraspecific or interspecific competition (Byers *et al*. 1980, 1984; Jutsum & Gordon 1989; Ruther & Thiemann 1997). They may be used to deter their conspecifics away from the host plants that are already occupied by the emitters.

Trail pheromones are much more common in the social insects such as ants. Release of trail pheromones as the name suggests leaves evidence for the conspecifics either to follow or to stay away depending on the message it is supposed to send to the receiver (Blum 1977).

Allelochemicals

These semiochemicals generally mediate interactions between species and are allotted to sub-groups depending on who benefits in the information exchange (Isaacs 1994). Unlike pheromones, they can be produced by both plants and insects.

Kairomones benefit the receiver without necessarily benefiting the sender. For example, Ruther *et al*. (2002) and Reinecke *et al*. (2005) have shown that the cockchafer beetles of *Melolontha genus, M. hippocastani* and *M. melolontha* L. (Coleoptera: Scarabaeidae) use green leaf volatiles released by host plants during feeding to locate females for mating purposes.

Allomones: these are semiochemicals that benefit the emitter rather than the receiver (Plimmer *et al.* 1982). For example, some plants while either in intact or in damaged state may release volatiles that deter feeding or oviposition by insects (Pichersky & Gershenzon 2002).

Synonomes: Release of these semiochemicals brings benefits to both the sender and the receiver. These chemicals are commonly encountered within tritrophic interactions where plants as a result of insect feeding release chemicals that in turn attract parasitoids that control the herbivorous insects (Powell 1998; Fatouros *et al*. 2005) thus both the emitter (plants) and the receiver (parasitoids) benefit from the synomone emission.

Behavioural responses to semiochemicals

Different behavioural responses in insects are elicited by semiochemicals. Due to the different behavioural responses they evoke, semiochemicals have been classed into five major categories (Isaacs 1994; Schoonhoven *et al*. 2005), (table 1). The first two have a long-distance effect, making an insect move toward or away from the source of emission while the remaining three act over a very short distance only, such as when insects are near the host plant or are already in contact with it (Bernays & Chapman 1994).

Table 1: A list of types of semiochemicals, their description and behavioural response they evoke on insects

Types of semiochemicals	Description
Attractants	transmit messages that induce insects to orientate towards resources (food, mates, oviposition sites etc) (Plimmer *et al*. 1982).
Repellents	these are volatile chemicals that result in negative behaviour or orientation of the insect away from the source of emission. Examples are volatiles emitted by plants to signal their unsuitability as host plants (Chapman *et al*. 1981; Byers *et al*. 1998; Mauchline *et al.* 2005).
Stimulants	usually arouse feeding or oviposition behaviour in an insect (Bernays & Chapman 1994; Schoonhoven *et al*. 2005).
Deterrents	If these semiochemicals are present in a host, they inhibit insects' feeding or ovipositioning (Schoonhoven *et al*. 2005).
Arrestants	the presence of these chemicals in a host slows down an insect's host-finding behaviour.

Visual cues and their effects on the behaviour of insects

Like olfaction, vision is also crucial to insects for location of resources such as hosts, mates and oviposition sites (Allan 1987; Van der Entl & Visser 1991; Kühnle & Müller 2010). Unlike the human eye the insect's eye is more sensitive to the shorter wavelengths (300-650 nm) than to the longer ones (> 650 nm) (Kevan & Baker 1983; Prokopy & Owens 1983; Jönsson 2005). There are three properties of host plants or other resources that have an influence on the vision of insects. These are spectral quality (colour), size and shape (Prokopy & Owens 1983; Kevan & Baker 1983). The way insects perceive colours of their host or non-host plants differs from the human vision (Arnold *et al*. 2008; Kevan *et* *al*. 1996; Chittka *et al*. 1994; Kelber 2001). For example in Arnold *et al*. (2008) a flower of opium poppy, *Papaver somniferum* that looks red to the human eye appears ultra-violet to the bee.

Although vision has not received much attention in some insects some studies directly or indirectly highlight the important role played by vision in insects' behaviour or survival (Foster & Harris 1997). These include trapping studies whereby colours are used singly or in conjunction with chemicals to give positive (Hall 1984; Smart *et al*. 1997) or negative orientation (Strom *et al*. 1999, 2001) towards such colours in pest management. Colour plays a critical role in the visual attraction of insects irrespective of whether true colour vision or wavelength-specific behavior is involved (Menzel 1979).

Colour vision requires that an insect must have at least two types of photoreceptors that differ in spectral sensitivity and have an opponent interaction (Kelber 1999, Briscoe & Chittka 2001; Kelber *et al*. 2003; Skorupski & Chittka 2009). Insects with true colour vision are able to discriminate between colours according to wavelength composition irrespective of light intensity (Menzel 1979; Kelber & Pfaff 1999; Kelber *et al*. 2003) and should be able to recognize colour irrespective of shape (Skorupski & Chittka 2009). In the case of wavelength-specific behaviour an insect is colour blind but may have different behaviours at different wavelengths due to either one receptor signal or additive interactions of different receptors (Lunau & Maier 1995; Kelber 1999).

Apart from colour, shape and pattern may also be employed together with colour in complementing each other for correct recognition of potential hosts (Kevan & Baker 1983; Wäckers & Lewis 1999; Dafni & Potts 2004). For example large flowers tend to have more insect visitors than smaller ones although in some situations insects may view small flowers as corporal image (Ne'eman & Kevan 2001).

Interaction between visual and olfactory cues

The two cues, olfactory and visual are often employed together during host location by an insect. The use of the two cues may differ between taxa but depending on species one may work at longer range for orientation towards the resource, while the other is employed at short range for landing or identifying the resources (Raguso 2001; Weiss 2001; Ômura & Honda 2005; Fukaya *et al*. 2006). They have also been employed in pest management where an enhanced behavioural response is observed when they are together (Schmera *et al*. 2004; Blackmer & Cañas 2005; Campbell & Borden 2006). Trap catches may be either additive (the total is equal to the sum of individual catches due to each stimulus) or synergistic (in which the total exceeds the sum of the catches made from traps of individual cues) (Björklund *et al*. 2005; Fukaya *et al*. 2005; Campbell & Borden 2006).

1.4 Host plants of blister beetles

Blister beetles (Coleoptera: Meloidae) are polyphagous; feeding on the floral parts of plants belonging to different families. The main host plant considered in this study is silverleaf desmodium (*Desmodium uncinatum,* Fabaceae). In addition to the main host plant *D. uncinatum*, five alternate host plants on which blister beetles feed in Kenya were used in the study. These are okra (*Abelmoschus esculentus,* Malvaceae), cowpea (*Vigna unguiculata,* Leguminoseae) (Jackai & Daoust 1986), sweetpotato (*Ipomoea batatas,* Convolvulaceae), common bean (*Phaseolus vulgaris,* Fabaceae) (Abate & Ampofo 1996) and morning glory (*Ipomoea hildebrandtii*, Convolvulaceae). Detailed description of the host plants used in this study and others mentioned elsewhere are shown in Table 2.

Table 2. List of host plants for *Hycleus* spp. including those used in the study for maintenance and various experiments.

Common name	Species	Family	Referenced by
Common/french beans	*Phaseolus vulgaris* (L.)	Fabaceae	*Abate & Ampofo 1996
Cowpea	*Vigna unguiculata* (L.) Walp	Fabaceae	*Jackai & Daoust 1986, Gahukar 1991
Greenleaf desmodium	*Desmodium intortum* (Mill.) Urb.	Fabaceae	*
Maize	*Zea mays*, L.		*
Morning glory	*Ipomoea hildebrandtii* Vatke	Convolvulaceae	*
Okra	*Abelmoschus esculentus* (L.) Moench	Malvaceae	*Robinson 2005
Pearl millet	*Pennisetum glaucum* (L.) R. Br.		Lale & Sastawa 2000, Tanzubil & Yakubu 1997
Pigeon pea	*Cajanus cajan* (L.) Mills	Fabaceae	Hillocks *et al*. 2000
Roselle and kenaf	*Hibiscus cannabinus* L.		Robinson 2005
Silverleaf desmodium	*Desmodium uncinatum* (Jacq.) DC.	Fabaceae	*
Sorghum	*Sorghum bicolor* Moench	Poaceae	Gahukar 1991
Sweetpotato	*Ipomoea batatas* (L.) Lam.	Convolvulaceae	*

* observed feeding on flowers of the mentioned host plants in Kenya

Desmodium species

Desmodium species is a leguminous plant that is mainly used as fodder for livestock throughout the world (Dzowela 1986; Barnes 1988; Rumbaugh 1990; Kfir *et al*. 2002; Roder *et al*. 2002; Lopez *et al*. 2004; Cook *et al*. 2005). Different species of *Desmodium* are documented to be natives of different regions of the world, for example, *D. uncinatum* and *D. intortum* are said to be natives of Brazil (Agnew & Agnew 1994; Cook *et al*. 2005) while approximately 15 other species are recorded to be natives of Sudan (Andrews 1952; Trout 1997). Trout (1997) also lists 12 *Desmodium* spp, nine of which were recorded by Andrews (1952). Trout (1997) indicates that all of these are native to Sudan, and the majority of these species have a wider distribution in Africa than any other region in the world.

According to Agnew & Agnew (1994) the only *Desmodium* species recorded in Kenya is *Desmodium uncinatum* whose origin is stated as South America. This species was recorded in Kenya as far back as 1969. Cook *et al*. (2005) also mentioned only two species *D. intortum* and *D. uncinatum,* and as being introduced into Kenya from Australia. These validate the records of Andrews (1952), which did not include *D.intortum* and *D. uncinatum* among the *Desmodium* species that are believed to be native to Africa, Sudan in particular.However, Trout (1997) lists seven *Desmodium* species in addition to *D. intortum* and *D. uncinatum* as occuring in Kenya. Because these species are native to Sudan (Andrews 1952) it is possible that they are native to Kenya also or may have been introduced or expanded their ranges to Kenya from Sudan.

As a forage legume *Desmodium* spp. generally provide foliage of higher nutritional quality (Lopez *et al*. 2004) than grasses resulting in an increase in yield in terms of milk and beef production when integrated into the livestock feeding patterns (Imrie *et al*. 1983; Dzowela 1986; Muyekho F.N. and Odongo J.A., unpublished data). Production of seed of *Desmodium* spp. and other leguminous forages allows their subsequent cultivation which enhances both livestock (its use as animal feed) and crop production (e.g. *Desmodium* spp., for use in crop protection in the push-pull system).

Production constraints for Desmodium spp.

Like other crops, *Desmodium* spp. face both biotic and abiotic challenges. *Desmodium* spp. are generally tropical forage legumes and show an optimal yield under tropical conditions, although tolerance to different extreme weather conditions may differ between species. Compared to other leguminous plants *Desmodium* spp. are less tolerant of frost conditions with *D. uncinatum,* being more vulnerable than *D. intortum* (Imrie *et al*. 1983). However, in this study the abiotic constraints do not play a major role since east Africa has tropical conditions. The main emphasis in this study is on biotic constraints due to attack by insects especially the blister beetles.

In Australia *Amenmus quadrituberculatus* Boheman and A. *superciliaris* Pascoe and *Graphognathus leucoloma* Boheman (Coleoptera: Curculionidae) were recorded as pests of *Desmodium* spp. They attack leaves and roots with adults feeding on leaves while larvae chew on the roots (Davis 1966; Elder *et al*. 1979; Jones 1989; Cook *et al*. 2005). Jones (1989) recorded the nematodes, *Meloidogyne javanica*, Treub (Nematoda: Heteroderida) and *Radopholus similis* Cobb (Nematoda: Pratylenchidae), as root pests of *Desmodium* spp., but regarded them as insignificant because plant growth was not affected. Roder *et al*. (2002) who evaluated both *D. intortum* and *D. uncinatum* for seed production in Bhutan, mentioned that an attack by insect pests feeding on the aerial parts after flowering leads to significant yield loss in seed production. Most insect pests are regarded as insignificant pests where *Desmodium* spp. are grown for fodder. However, it is important to note that the presence of pests, such as blister beetles feeding on flowers, is likely to pose a serious challenge for desmodium grown for seed production especially in plots of small holders’ as is the practice in east Africa.

1.5 Study insects

The object of this study is the blister beetle *Hycleus apicicornis* (Guérin-Méneville) (Coleoptera: Meloidae), also referred to in the literature as *Coryna apicicornis* (e.g. Bologna *et al*. 2008). *Hycleus* spp. are of particular interest because they have a negative impact on seed production of *Desmodium* spp. in Kenya by feeding on their flowers. In this study a complex of *Hycleus* spp. were observed to be pests of *Desmodium* spp. in different parts of Kenya where *Desmodium* spp. are grown (Fig. 1). The available literature on meloids in Kenya, however, documents the taxa recorded on other leguminous crops as belonging to *Coryna* and *Mylabris* (Abate & Ampofo 1996; Hillocks *et al*. 2000). *Mylabris* spp. and *Coryna* spp. have been recorded as often co-occurring on one plant crop in various sub-Saharan countries. For example, Abate & Mpofo (1996) noted their widespread occurrence on beans throughout sub-Saharan African countries. In Ghana and Nigeria they are considered to be a serious problem on pearl millet (Tanzubil & Yakubu 1997; Lale & Sastawa 2000).

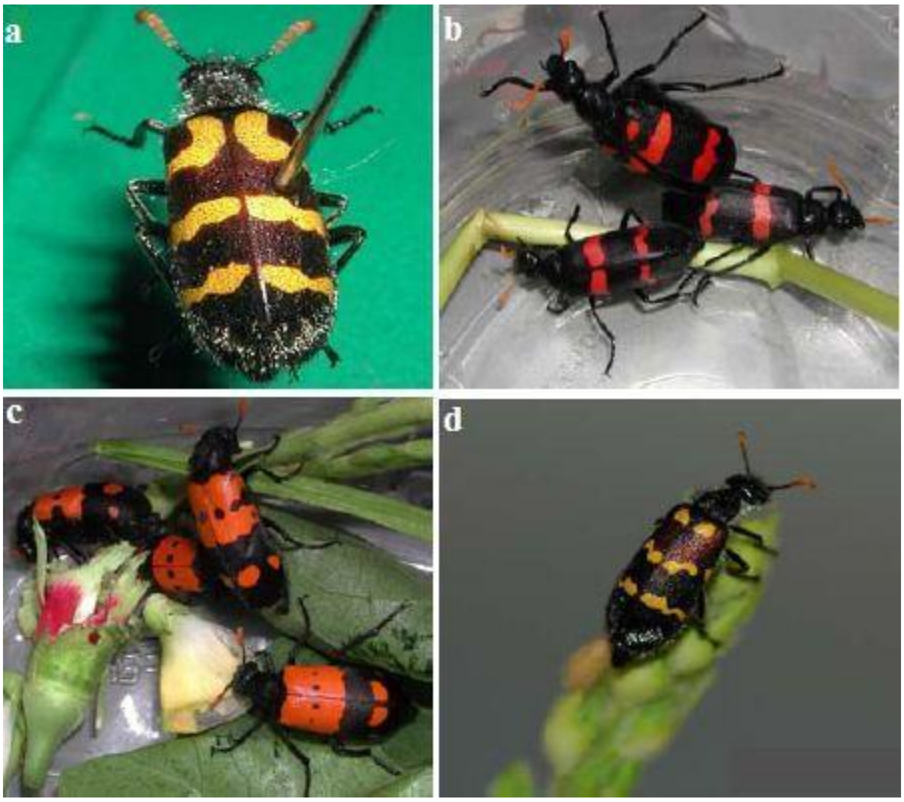


Figure 1. Various *Hycleus* species: a) *H. vestitus*, b) *H. sjoestedti* (top) and *H. dicinctus* (bottom) c) *H. dubiosus* feeding on okra and cowpea flowers and d) *H. apicicornis* feeding on developing seeds of *D. uncinatum.*

General description and biology of blister beetles

Blister beetles belong to the family Meloidae, which consists of approximately 2500 species worldwide (Bologna & Pinto 2001; Selander & Fasulo 2003). *Hycleus* spp. are placed in the tribe Mylabrini of the subfamily Meloinae (Bologna & Pinto 2001). Mylabrini constitute the most species-rich tribe in this subfamily, with *Hycleus* and *Mylabris* as the largest genera (Bologna & Pinto 2002; Bologna *et al*. 2005). *Hycleus* spp. are often considered as or confused with *Mylabris* spp. (Bologna & Pinto 2002; Bologna *et al*. 2005).

Adult blister beetles may range from 5 - 40 mm long depending on species (Scholtz & Holm 1986). They are elongate, soft bodied with long and slender legs, a deflexed head with the neck narrower than either the head or the elytra (Özbek & Szaloki 1998; Nikbakhtzadeh & Tirgari 2002). Most mylabriine meloids are characterized by aposematic yellow, orange or red colour bands or spots on their elytra (Hill 1975; Abate & Ampofo 1996) and the antennae are often also colourful, being red, orange or yellow (Abate & Ampofo 1996) (Fig. 1). In addition, adult blister beetles secrete an irritant called cantharidin ($C_{10}H_{12}O_4$) (Fig. 2), which is used for defence against predators (Scholtz & Holm 1986; Bologna *et al.* 2005). According to Bologna *et al.* (2005) and McCormick & Carrel (1987), cantharidin may be an aggregation pheromone. This substance causes blistering if it comes in contact with human skin, hence the name blister beetles (Carrel & Eisner 1974; McCormick & Carrel 1987).

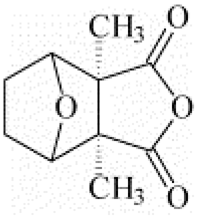


Figure 2. Structure of cantharidin

The adult female lays cylindrical eggs (Fig. 3a) in batches in the soil. These eggs hatch into larvae called triungulins, the most active larval stage (Fig. 3b). Triungulins crawl over soil surfaces entering into cracks to look for grasshopper egg pods, which are usually laid about 2.5 cm beneath the soil surface. After finding the grasshopper eggmass, blister beetle larvae will feed on them (Hill 1975; Selander 1986; Abate & Ampofo 1996; Özbek & Szaloki 1998; Bologna & Pinto 2001; Zhu *et al.* 2005) and develop into various larval stages (see Fig. 4) inside the eggpod (Nikbakhtzadeh 2004). As a result of this blister beetles may be considered as beneficial to man (Özbek & Szaloki 1998). Some blister beetles feed on provisions and larvae of Hymenoptera (Selander 1981; Bologna & Pinto 2001).

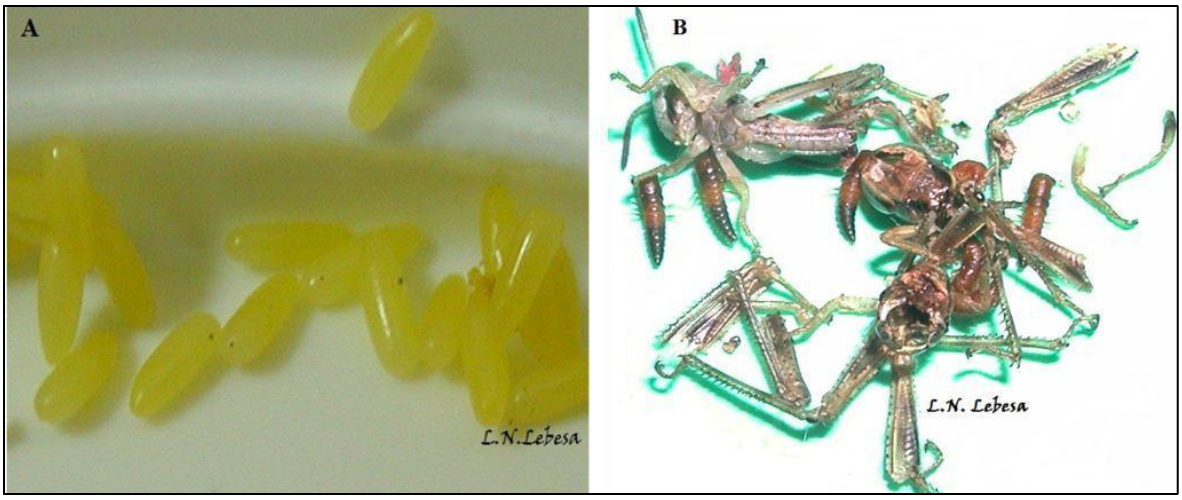


Figure 3. Some immature stages of *H. apicicornis* (a) eggs and (b) triungulins (1st instar) feeding on grasshopper nymphs.

The larvae of blister beetles differ morphologically in different life stages (Abate & Ampofo 1996). The number of instars may differ both between species and according to environmental conditions. The first instar may be followed by two to five instars, although in some instances some instars can be skipped (Selander 1986; Nikbakhtzadeh 2004). The larval instars after the triungulin consist of the first grub stage, which may comprise of one or two feeding larval instars that look like grubs, followed by a coarctate instar. The coarctate instar may diapause if conditions are unfavourable until favourable conditions return, whereupon it will undergo ecdysis and then become a grub-like form again and start feeding (this is the second grub phase). For example, the larval cycle of *H. dubiosus* includes the coarctate phase, which is not the case with *H. apicicornis*. Pupation takes place shortly after ecdysis and is followed by adult emergence. Development from egg to adult takes several months, with most of the time spent in the larval stage (Fig. 4).

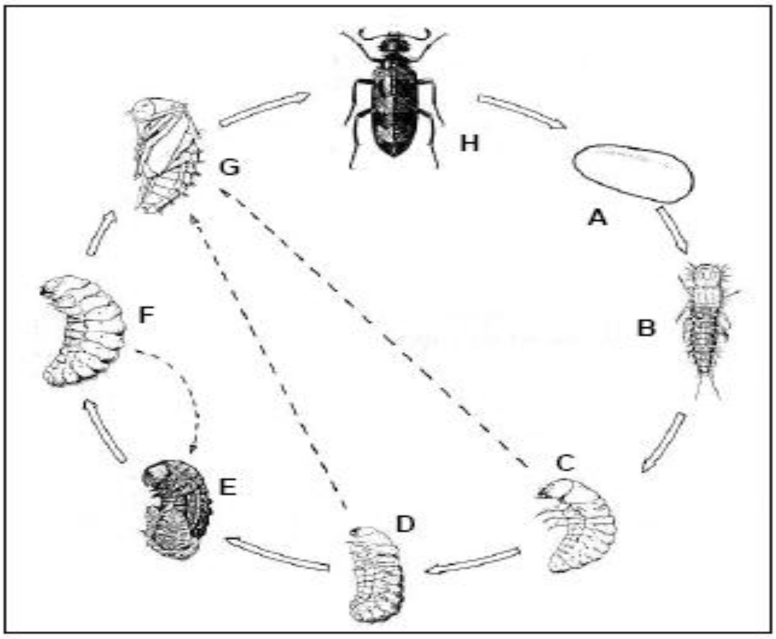


Fig 4: Life-cycle of *Mylaris variabilis*. (a) A= egg, B= triungulin, C&D= first grub phase instars, E: coarctate larva (CL), F= second grub phase (SG), G: pupa, H: adult. Arrows indicate normal life-cycle which can be shorter (as indicated by dotted arrows) in some species e.g. by jumping from FG1 or FG2 to pupa without passing SG and CL. They can also change from SG to CL in a reverse trend. (*Redrawn from Nikbakhtzadeh 2004*).

Distribution

The tribe Mylabrini is restricted to the Old World, with *Mylabris* spp. and *Coryna* spp. occurring throughout the whole of Africa and elsewhere (Hill 1975). Several *Mylabris* species commonly occurr in East and West Africa (Singh & Van Emden 1979). It should, however, be noted that *Hycleus* spp. have been incorrectly documented as either *Mylabris* or *Coryna* (Bologna *et al*. 2005). Importance of blister beetles in Africa and in most other parts of the world, e.g. Asia, Turkey etc., has been mostly associated with crop damage (Singh & Van Emden 1979; Özbek & Szaloki 1998; Hillocks *et al*. 2000). In other parts such as North America, the most important genus is *Epicauta* due to its importance in the livestock industry. Blister beetles in this case pose a serious threat of cantharidin poisoning if animals feeding on hay or alfalfa ingest them (Capinera *et al*. 1985; Blodgett *et al*. 1991; Ward 1997). Cantharidin can also be poisonous to humans if ingested, whether by mistake (Tagwireyi *et al*. 2000) or as a potent aphrodisiac (Polettini *et al*. 1992; Karras *et al*. 1996). When absorbed, cantharidin causes serious damage to the internal system and depending on the amount consumed the level of damage may even lead to the death (Tagwireyi *et al*. 2000; Bailey *et al*. 2005).

Damage

Only adult beetles inflict damage on host plants. Larvae are beneficial to man because they feed on grasshopper eggs (Hill 1975; Selander 1986; Özbek & Szaloki 1998). Blister beetles are polyphagous pests, feeding on flowers and foliage of several plants such as cucurbits, legumes, millets, sorghum, maize, weeds, fruit trees and ornamentals. Most blister beetles feed on floral parts, such as flowers, pods, buds, though some feed on foliage or pollen (Singh & Van Emden 1979). In addition to causing damage on flowers, *H. apicicornis* also feeds on the pollen of some of its host plants, e.g. maize, okra (L.N. Lebesa pers. obs.) and pearl millet (Gahukar 1984; Lale & Sastawa 2000), and as such it is also referred to as pollen beetle (Hill 1975). Although blister beetles are not considered a major pest (Hill 1975; Zhu *et al*. 2005), they can cause considerable damage due to their gregarious nature (Hall 1984, Selander & Fasulo 2003; Nikbakhtzadeh 2004). In such cases they often completely defoliate or chew all floral parts and young pods, depending on the type of species, thus adversely affecting fruit/seed setting (Evans 1990; Zhu *et al*. 2005).

Blister beetles can cause serious yield reduction when invading plots of smallholder farmers and so are becoming important pests in Kenya. Production of *Desmodium* spp. seed is generally undertaken on smallholdings. The usual cultural control practice for this pest is to manually collect adults and destroy them. While the practice may suffice when infestations are low, this is not always practical because it is labour-intensive when infestations are high. Furthermore, with the presence of cantharidin in their body, collecting adults needs exercising a lot of caution to avoid skin blistering. It is further unlikely that resource-poor farmers can use chemicals for the control of blister beetles, because of the cost involved. Thus, an affordable pest management strategy is required.

1.6 Use of semiochemicals and other cues in pest management

Exploitation of semiochemicals and visual cues for pest management is likely to offer an alternative that may reduce or even replace pesticide application in some cases (Cook *et al.* 2007). Chemical pesticides have safeguarded farmers against losses due to various major pests but they have several drawbacks necessitating the use of alternative control strategies. These include increased resistance to pesticides (Pickett 1998; Isman 2002; Cook & Denholm 2008) and being the less economically viable option, especially for resource-poor farmers who are either unable afford them or do not have easy access to them (Khan & Pickett 2004; Mendesil *et al.* 2007). The exclusive application of chemical pesticides has been associated with increasing harmful effects to the environment (Smart *et al.* 1994). Pesticides will, however, continue to play a considerable role in pest management (Pickett 1998). Thus integrating or alternating chemical control with other strategies such as semiochemicals is likely to be a better approach to address some of the challenges mentioned and develop effective pest control strategies (Smart *et al.* 1994; Pickett 1998).

Studying the effects of semiochemicals on the behaviour of insects is useful for using them to manipulate the behaviour of insects to obtain desired pest control results (Ruther & Mayer 2005). For example, normally an attractant plant volatile will attract specific insect pest(s) towards the emittant plant. The same volatile compound/s may be used in pest management as bait/lures in a trapping system to attract such pest(s) to a trap for monitoring or control purposes (Pickett *et al*. 1997; Jones 1998; Pickett 1998).

Semiochemicals have been deployed in many instances in trap system where they can be used alone as stimuli signalling messages to pests or in conjunction with visual stimuli to send those messages (Hesler & Sutter 1993; Schmera *et al*. 2004). The effectiveness of traps to attract pests is dependent upon many factors that may influence the behaviour of an insect (Millar *et al*. 2002). Those factors include but are not limited to the following; environmental conditions (Reinecke *et al.* 2005), concentration of the semiochemical(s) (Athanassiou *et al*. 2002), trap design, colour of traps and location of the trap (Hesler & Sutter 1993; Athanassiou *et al*. 2002; Rull & Prokopy 2005; Bacca *et al*. 2006) among others. This shows that in order to develop an efficient trap numerous factors have to be taken into account. Other examples of applications of attractant semiochemicals include employment of the pheromones as sex attractants for attraction of female/males whereby mating processes maybe disrupted and thus reproduction capacity decreased (Schmidt-Tiedemann *et al*. 1999).

Semiochemicals may on the other hand be used as repellents driving pests away from the host crop, so as to prevent pests from colonizing it. Examples of these in pest management include the use of alarm pheromones, as in the successful application against aphids, where application of synthetic (E)-β-farnesene, a naturally occurring alarm pheromone in several aphid species to the crop, resulted in deterring aphids from colonizing such a crop (Pickett *et al.* 1992; Shah *et al.* 1999; Jing-Gong 2002). Some plants release volatile compounds that are repellent to some insects and thus drive the pests away from such host plants (Khan *et al*. 2010; Mithöfer & Boland 2012).

The use of the two approaches of 'attract and repel' system combined together is commonly known as "push-pull" which is alternately called a stimulo-deterrent diversionary strategy (Miller and Cowles 1990; Isman 2002; Pickett *et al*. 2006) is used to provide a sustainable pest control strategy. In this strategy insect pests are pushed away from the host by tactics that deter the pest, e.g. plants/crops that release repellent volatiles, oviposition deterrent pheromones, alarm pheromones or antifeedants. This is coupled with a trap crop that provides the attractant semiochemicals or strong visual cues that attract the pest to the trap crop and pull it away from the main crop (Pickett *et al*. 2006).

With this strategy, ICIPE (International Centre of Insect Physiology and Ecology) and its partners (Rothamsted Research and Kenya Agricultural Research Institute) developed a system for East Africa whereby maize (main crop) is intercropped with non-host fodder crops, silverleaf desmodium, *Desmodium uncinatum* (Jacq). DC. (Fabaceae), and molasses grass, *Melinis minutiflora* (P. Beauv.) (Cyperales: Poaceae) to provide the push effect. The pull effect is then provided by planting the host fodder crops such as Napier grass, *Pennisetum purpureum* Schumach (Poaceae) as perimeter/trap crops around the maize plots (Khan *et al*. 2002). In addition to their ability to control stemborers in the push-pull strategy *Desmodium uncinatum* also provides effective control for *Striga* spp. through a variety of mechanisms ranging from its nitrogen fixation to smothering of the emerging weeds and allelopathy (Khan *et al*. 2002; Khan *et al*. 2008; Khan *et al*. 2010). These three mechanisms leading to suppression of Striga spp. have been investigated and the latter seem to play a major role (Khan *et al* 2002).

This strategy proved to be a major success for subsistence farmers in eastern Africa by controlling stemborers in maize and soghurm (Khan *et al*. 1997; Khan & Pickett 2004; Cook *et al* 2007). The strategy employs various semiochemicals in conjunction with habitat management (Khan *et al.* 2000, 2001; Mauchline *et al.* 2005; Midega *et al* 2006). The use of this strategy under different settings, such as subsistence and commercial farming and the benefits or disadvantages associated with it have been extensively reviewed by Cook *et al.* (2007). Semiochemicals are also useful in some situations by enhancing the efficiency of natural enemies thus increasing protection of crops (Khan *et al.* 1997; Cook *et al.* 2007). For example, an application of cis-jasmone on beans renders them repellent to herbivores while leaving them attractive to parasitoids (Matthes *et al.* 2003; Birkett *et al.* 2000).

Studying the behaviour of insects towards different semiochemicals and visual cues needs further research in different insects of economic importance in order to offer alternative protection strategies. As has been outlined with the examples above on the successes of application of semiochemicals, studying the effect of different semiochemicals and visual cues on the behaviour of blister beetles for future use in designing trapping system holds the promise of resulting in a sustainable pest control strategy for these beetles.

1.7 Relevance of this study

Maize is the third most important cereal crop under production, and most of it is grown in developing countries (Doswell 1996) where it forms a staple food, particularly in Africa (Pingali & Pandey 2000; Kfir *et al.* 2002). Production of this crop throughout Africa and in sub-Saharan Africa in particular is adversely affected by various lepidopteran stemborers (Seshu Reddy 1998; Khan *et al.* 2000; Kfir *et al.* 2002). Yield loss due to stemborers varies widely according to region and species, but may be as high as 100% (Bosque- Pérez & Mareck 1991; Seshu Reddy 1998). Additional biotic constraints to cereal production, particularly maize are parasitic witchweeds, *Striga* spp., which may cause substantial yield losses of more than 50% if no effective control is employed (Khan *et al*. 2000; Labrada 2000).

To combat stemborers and *Striga* spp., development of the push-pull strategy by ICIPE and its partners has produced excellent control results (Khan *et al*. 2001) and was readily adopted in East Africa, particularly in Kenya. The shortage of *Desmodium* seeds is regarded as the main factor limiting uptake by farmers. To resolve this, ICIPE in collaboration with a local seed company, established community based seed production units. However, production of *Desmodium* spp. seed by community-based producers is now facing a challenge from blister beetles, (*Hycleus* spp.).

Blister beetles are polyphagous, feeding on floral parts of several crops, weeds and ornamentals and are sometimes considered a minor pest (Singh & van Emden 1979). However, due to their gregarious nature (Selander 1964; Pinto & Bologna 1999), they can cause severe damage to flowers resulting in significant yield reductions, if they attack crops that flower early or those that are cultivated on smallholdings. For example, yield reductions of approximately 51% have been reported on pearl millet due to *Coryna* spp. (Tanzubil & Yakubu 1997). Seed production of *Desmodium* spp. in East Africa is undertaken by smallholder farmers and feeding on flowers by blister beetles affects seed setting which eventually results in reduced seed production.

As *Desmodium* spp. seed production is undertaken by smallholder farmers who may not afford the costs of pesticides to control this pest. Failure to acquire pesticides may leave farmers with only cultural control options. The commonly employed cultural practice for their control is that of collecting and destroying them, and it is also not suitable, for two reasons; it may pose danger of blistering by these beetles if care is not taken and it is also very labour-intensive. As a result, there is an urgent need to develop alternative control methods that combine effectiveness and safety while being simple and affordable to use. Various studies have been carried out before in pest management of whereby visual stimuli and chemical stimuli are used either singly or in combination in strategies (e.g. pest monitoring, mass trapping etc.) to control damage brought about by pests. These behavioural studies have in some cases employed with varying successes the pheromones of the insects to disrupt the usual resource (food, mating sites, oviposition sites, etc.) finding patterns. There are also a number of successful situations whereby colours and or shapes of host plants are used either for pest monitoring or for mass trapping. Therefore, an understanding of the role played by visual (colour) and olfactory (semiochemicals) cues may help in devising the strategies that may be applied for management of this pest on *Desmodium spp*.

Objectives

The main objective of this study was to understand the different roles played by chemical and visual cues in host-plant finding by blister beetles. The study also involved undertaking brief surveys to determine the host range of the pest to establish those most preferred besides *Desmodium* spp. This information should prove useful for developing future strategies that may reduce the impact of *H. apicicornis* on seed multiplication plots of *Desmodium* spp. The specific objectives were:

1. to determine perceptions and knowledge of farmers on the pest status of blister beetles on *Desmodium* spp., as well as alternative hosts and the incidence of this pest on different host plants,
2. to identify the most suitable colour/s involved in eliciting behavioural responses associated with host finding, if any,

3. to determine the feeding preferences of blister beetles on different host plants,
4. to study the behaviour of adult beetles towards semiochemicals released from different host plants, and
5. to identify volatile compounds that play a major role in host location of H. apicicornis.

References

Abate, T. & Ampofo, J.K.O. (1996) Insect pests of beans in Africa: their ecology and management. *Annual Review of Entomology* **41**: 45-73.

Agnew, A.D.Q. & Agnew, S. (1994) *Upland Kenya wild flowers – a flora of the ferns and herbaceous flowering plants of Upland Kenya*. 2nd edition. East Africa Natural History Society, Nairobi, Kenya.

Aldrich, J.R., Bartelt, R.J., Dickens, J.C., Knight, A.L., Light, D.M. & Tumlinson, J.H. (2003) Insect chemical ecology research in the United States Department of Agriculture – Agricultural Research Service. *Pest Management Science* **59**: 777-787.

Allan, S.A., Day, J.F. & Edman, J.D. (1987) Visual ecology of biting flies. *Annual Review of Entomology* **32**: 297-316.

Andrews, F.W. (1952) *The flowering plants of the Anglo-Egyptian Sudan. Vol. II Sterculiaceae–Dipsaceae*. 1st edition. T. Buncle & Co. Ltd, Scotland.

Ansebo, L. (2004) Odour Perception in the Codling Moth *Cydia pomonella* L. – from Brain to Behaviour. *Doctoral thesis.* Swedish University of Agricultural Sciences, Alnarp, Sweden**.**

Arnold, S.E.J., Savolainen, V. & Chittka, L. (2008) FReD: The floral reflectance spectra database. *Nature Precedings* doi:10.1038/**npre.2008.1846**

Athanassiou, C.G., Kavallieratos, N.G., Gravanis, F.T. Koukounitsas, N.A. & Roussou, D.E. (2002) Influence of trap type, pheromone quantity and trapping location on capture of the pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae). *Applied Entomology and Zoology* **37**: 385-391.

Aukema, B.H. & Raffa, K.F. (2004) Does aggregation benefit bark beetles by diluting predation? Links between a group-colonisation strategy and the absence of emergent multiple predator effects. *Ecological Entomology* **29**: 129-138.

Bacca, T, Lima, E.R., Picanço, M.C., Guedes, R.N.C. & Viana, J.H.M. (2006) Optimum spacing of pheromone traps for monitoring the coffee leaf miner *Leucoptera coffeella. Entomologia Experimentalis et Applicata* **119**: 39-45

Bailey, W.C., Enns, W.R. & Loch, W. (2005) Blister beetle management in alfalfa. *University of Missouri Extension Papers,* USA.

Balkenius, A. & Kelber, A. (2006) Colour preferences influences odour learning in the hawkmoth, *Macroglossum stellatarum*. *Naturwissenschaften* **93:** 255-258.

Barnes, G.R. (1988) Desmodium: a Low-Cost Pasture for the Eastern Cape Coastal Region. *Journal of the Grassland Society of Southern Africa* **5**: 138-142.

Becher, P.G. & Guerin, P.M. (2009) Oriented responses of grapevine moth larvae *Lobesia botrana* to volatiles from host plants and an artificial diet on a locomotion compensator. *Journal of Insect Physiology* **55**: 384-393.

Bell, W.J. (1990) Searching behaviour patterns in insects. *Annual Review of Entomology* **35**: 447-67.

Bernays, E.A. & Chapman, R.F. (1994) *Host-plant Selection by Phytophagous Insects. Contemporary Topics in Entomology 2* Chapman & Hall, London.

Bernays, E.A. & Wrubel, R.P. (1985) Learning in grasshoppers: association of colour and light intensity with food. *Physiological Entomology* **10**: 359-369.

Birkett, M.A., Campbell, C.A.M., Chamberlain, K., Guerrieri, E., Hick, A.J., Martin, J.L., Matthes, M., Napier, J.A., Pettersson, J., Pickett, J.A., Poppy, G.M., Pow, E.M., Pye, B.J., Smart, L.E., Wadhams, G.H., Wadhams, L.J. & Woodcock, C.M. (2000) New roles for cis-jasmone as an insect semiochemicals and in plant defense *Proceedings of the National Academy of Science* **97**: 9329-9334.

Björklund, N. Nordlander, G. & Bylund, H. (2005) Olfactory and visual stimuli used in orientation to conifer seedlings by the pine weevil, *Hylobius abietis. Physiological Entomology* **30**: 225-231.

Blackmer, J.L. & Cañas, L.A. (2005) Visual cues enhance the response of *Lygus hesperus* (Heteroptera: Miridae) to volatiles from host plants. *Environmental Entomology* **34**: 1524-1533.

Blodgett, S.L., Carrell, J.E. & Higgins, R.A. (1991) Cantharidin content of blister beetles (Coleoptera: Meloidae) collected from Kansas alfalfa and implications for inducing cantharidiasis. *Environmental Entomology* **20**: 770-780.

Blum, M.S. (1969) Alarm pheromones. *Annual Review of Entomology* **14**: 57-80.

Blum, M.S. (1977) Behavioural responses of Hymenoptera to pheromones and allomones. In: Shorey, H.H. & McKelvey, Jr. J.J. (Eds.), *Chemical Control of Insect Behaviour – Theory to application*. 149-167. John Wiley & sons, New York.

Blum, M.S. (1996) Semiochemical parsimony in the arthropoda. *Annual Review of Entomology* **41**: 353-74.

Bologna, M.A. & Pinto, J.D. (2001) Phylogenetic studies of Meloidae (Coleoptera), with emphasis on the evolution of phoresy. *Systematic Entomology* **26**: 33-72.

Bologna, M.A. & Pinto, J.D. (2002) The Old World genera of Meloidae (Coleoptera): a key and synopsis. *Journal of Natural History* **36**: 2013–2102.

Bologna, M.A., Di Giulio, A. & Pitzalis, M. (2008) Systematics and biogeography of the genus *Actenodia* (Coleoptera: Meloidae: Mylabrini). *Systematic Entomology* **33**: 319-360.

Bologna, M.A., D'Inzillo B., Cervelli, M., Oliverio, M. & Mariottini, P. (2005) Molecular phylogenetic studies of the Mylabrini blister beetles (Coleoptera, Meloidae). *Molecular Phylogenetics and Evolution* **37**: 306-311.

Bosque-Pérez, N.A. & Mareck, J.H. (1991) Effect of the stem borer *Eldana saccharina* (Lepidoptera: Pyralidae) on the yield of maize. *Bulletin of Entomological Research* **81**: 243-47.

Briscoe, A.D. & Chittka, L. (2001) The evolution of color vision in insects. *Annual Review of Entomology* **46**: 471-510.

Bruce, T.J.A., Lester, L.J. & Woodcock, C.M. (2005) Insect host location: a volatile situation. *Trends in Plant Science* **10**: 26-274.

Byers, J.A. (2005) A cost of alarm pheromone production in cotton aphids, *Aphis gossypii*. *Naturwissenschaften* **92**: 69-72.

Byers, J.A. & Wood, D.L. (1980) Interspecific inhibition of the response of the bark beetles, *Dendroctonus brevicomis* and *Ips paraconfusus*, to their pheromones in the field. *Journal of Chemical Ecology* **6**: 149-164.

Byers, J.A., Wood, D.L., Craig, J. & Hendry, L.B. (1984) Attractive and inhibitory pheromones produced in the bark beetle, *Dendroctonus brevicomis*, during host colonization: Regulation of inter- and intraspecific competition. *Journal of Chemical Ecology* **10**: 861-877.

Byers, J.A., Zhang, Q-H., Schlyter, F. & Birgersson, G. (1998) Volatiles from nonhost birch trees inhibit pheromone response in Spruce bark beetles. *Naturwissenschaften* **85**: 557-561.

Campbell, S.A. & Borden, J. (2006) Close-range, in-flight integration of olfactory and visual information by a host-seeking bark beetle. *Entomologia Experimentalis et Applicata* **120**: 91-98.

Capinera, J.L., Gardner, D.R., Stermitz, F.R. (1985) Cantharidin levels in blister beetles (Coleoptera: Meloidae) associated with Alfalfa in Colorado. *Journal of Economic Entomology* **78**: 1052-1055.

Cardé, R.T. (1984) Chemo-orientation in flying insects. In: Bell W.J. & Cardé R.T. (Eds.), *Chemical Ecology of Insects*. 111-124. Chapman & Hall, London.

Carrel, J.E. & Eisner, T. (1974) Cantharidin: potent feeding deterrent to insects. *Science* **183**: 755-757.

Chapman, R.F., Bernays, E.A. & Simpson, S.J. (1981) Attraction and repulsion of the aphid, *Cavariella aegopodii* by plant odours. *Journal of Chemical Ecology* **7**: 881-888.

Chittka, L., Shmida, A., Troje, N. & Menzel, R. (1994) Ultraviolet as a component of flower reflections, and the colour perception of Hymenoptera. *Vision Research* **34**: 1489-1508.

Cook, S.M. &. Denholm, I. (2008) Ecological approaches to the control of pollen beetles in oilseed rape. *OEPP/EPPO Bulletin* **38**: 110-113.

Cook, B.G., Pengelly, B.C., Brown, S.D., Donnelly, J.L., Eagles, D.A., Franco, M.A., Hanson, J., Mullen, B.F., Partridge, I.J., Peters, M. & Schultze-Kraft, R. (2005) *Tropical Forages: an interactive selection tool, [CD-ROM]*, CSIRO, DPI&F (Qld), CIAT and ILRI, Brisbane, Australia.

Cook, S.M., Khan, Z.R. & Pickett, J.A. (2007) The use of push-pull strategies in integrated pest management. *Annual Review of Entomology* **52**: 375-400.

Cunningham J.P., Jallow, M.F.A., Wright, D.J. & Zalucki, M.P. (1998a) Learning in oviposition behaviour in *Helicoverpa armigera* (Lepidoptera: Noctuidae) *Animal behaviour* **55**: 227-234.

Cunningham J.P., West S.A., Wright D.J. (1998b) Learning in the nectar foraging behaviour of *Helicoverpa armigera*. *Ecological Entomology* **23**: 363-369.

Cunningham, J.P., West, S.A. & Zalucki, M.P. (2001) Host selection in phytophagous insects: a new explanation for learning in adults. *Oikos* **95**: 537-542.

Dafni, A. & Potts, S.G. (2004) The role of flower inclination, depth, and height in the preferences of a pollinating beetle (Coleoptera: Glaphyridae). *Journal of Insect Behavior* **17**: 823-834.

Davis, J.J. (1966) Records of *Amnemus quadrituberculatus* (Boh.), *A. superciliaris* (Pasc.), *Pantomorus cervinus* (Boh.) and *Graphognathus leucoloma* (Boh.) (Coleoptera:

Curculionidae) from Queensland. *Australian Journal of Entomology* **5**: 69-70.

Dejean, A. Gibernau, M., Lauga, J. & Orivel, J. (2003) Coccinellid learning during capture of alternative prey. *Journal of Insect Behavior* **16**: 859-864.

Dethier, V.G. (1982) Mechanism of host-plant recognition. *Entomologia Experimentalis et Applicata* **31**: 49–56.

Dethier, V.G. (1983) Introduction. In: Ahmad, S. (Ed.), *Herbivorous Insects – host-seeking behaviour and mechanisms*. Academic Press, London.

Desouhant, E., Navel, S., Foubert, E., Fischbein, D., Théry, M. & Bernstein, C. (2010) What matters in the associative learning of visual cues in foraging parasitoid wasps: colour or brightness? *Animal Cognitive* **13**: 535-543.

Dicke, M. (1999) Are herbivore-induced plant volatiles reliable indicators of herbivore identity to foraging carnivorous arthropods? *Entomologia Experimentalis et Applicata* **91**: 131-142.

Doswell, C.R., Paliwal, R.L. & Cantrell, R.P. (1996) *Maize in the Third World*. Westview Press, USA.

Dyer, A.G. & Chittka, L. (2004a) Biological significance of distinguishing between similar colours in spectrally variable illumination: bumblebees (*Bombus terrestris*) as a case study. *Journal of Comparative Physiology A* **190**: 105-114.

Dyer, A.G. & Chittka, L. (2004b) Bumblebees (*Bombus terrestris*) sacrifice foraging speed to solve difficult colour discrimination tasks. *Journal of Comparative Physiology A* **190**: 759-763.

Dzowela, B.H. (1986) Value of a forage legume component in bummer beef fattening systems in Malawi. In: Haque, I., Jutzi, S. & Neate, P.J.H. (Eds.), *Potentials of forage legumes in farming systems of sub-Saharan Africa.* Proceedings of a workshop held at ILCA, Addis Ababa, Ethiopia, 16-19 September 1985. ILCA, Addis Ababa.

Elder RJ, Brown, JD, Wicks R. (1979) The biology and laboratory rearing of a new Leptopiine weevil, a pest of legumes in Queensland. *Journal of Australian Entomological Society* **18**: 81-89.

Evans, E.W. (1990) Dynamics of an aggregation of blister beetles (Coleoptera: Meloidae) attacking a prairie legume. *Journal of the Kansas Entomological Society* **63**: 616-625.

Fatouros, N.E., Bukovinszkine'Kiss, G., Kalkers, L.A., Gamborena, R.S., Dicke, M. & Hilker, M. (2005) Oviposition-induced plant cues: do they arrest *Trichogramma* wasps during host location? *Entomologia Experimentalis et Applicata* **115**: 207-215.

Foster, S.P. & Harris, M.O. (1997) Behavioral manipulation methods for insect pest-management. *Annual Review of Entomology* **42**: 123-146.

Fukaya, M., Wakamura, S., Arakaki, N., Yasui, H., Yasuda, T. & Akino, T. (2006) Visual 'pinpoint' location associated with pheromonal cue in males of the black chafer *Holotrichia loochooana loochooana* (Coleoptera: Scarabaeidae). *Applied Entomology and Zoology* **41**: 99-104.

Fukaya, M., Yasui, H., Yasuda, T., Akino, T & Wakamura, S. (2005) Female orientation to the male in the white-spotted longicorn beetle, *Anoplophora malasiaca* (Thomson) (Coleoptera: Cerambycidae) by visual and olfactory cues. *Applied Entomology & Zoology* **40**: 63-68.

Gahukar, R.T. (1984) Insect pests of millet in West Africa: a review. *Tropical Pest Management* **30**: 142-147.

Gahukar, R.T. (1991) Pest status and control of blister beetle in West Africa. *Tropical Pest Management* **37**: 415-420.

Giurfa, M. (2004) Conditioning procedure and color discrimination in the honeybee *Apis mellifera*. *Naturwissenschaften* **91**: 228-231.

Goyret, J. & Raguso, R.A. (2006) The role of mechanosesnory input in flower handling efficiency and learning by *Manduca sexta*. *The Journal of Experimental Biology* **209**: 1585-1593.

Greggers, U, & Menzel, R. (1993) Memory dynamics and foraging strategies of honeybees. *Behavioural Ecology and Sociobiology* **32**: 17-29.

Hall, M.J.R. (1984) Trap-oriented behaviour of red-banded blister beetles, *Mylabris designata* var. *hacolyssa rochebrune* (Coleoptera: Meloidae), in the Sudan. *Bulletin of Entomological Research* **74**: 103-112.

Hassell, M.P. & Southwood, T.R.E. (1978) Foraging Strategies of Insects. *Annual Review of Ecology and Systematics* **9**: 75-98.

Harris, M.O., Rose, S. & Malsch, P. (1993) The role of vision in the host plant-finding behaviour of the Hessian fly. *Physiological Entomology* **18**: 31-42.

Harris, M.O., Sandanayaka, M. & Griffin, W. (2001) Oviposition preferences of the Hessian fly and their consequences for the survival and reproductive potential of offspring. *Ecological Entomology* **26**: 473-486.

Heard T.A. (2000) Concepts in insect host-plant selection behavior and their application to host specificity testing. In: Van Driesche, R., Heard, T.A. McClay, A.S. & Reardon, R. (Eds.), *Proceedings of Session: Host Specificity Testing of Exotic Arthropod Biological Control Agents - The Biological Basis for Improvement in Safety - August 2000*. 1-10. USDA Forest Service, Publication no. FHTET-99-1.

Hesler, L.S. & Sutter, G.R. (1993) Effect of trap color, volatile attractants, and type of toxic bait dispenser on captures of adult corn rootworm beetles (Coleoptera: Chrysomelidae). *Environmental Entomology* **22**: 743-750.

Hill, D. (1975) *Agricultural insect pests of the tropics and their control*. 1st edition. Cambridge University Press, London.

Hillocks, R.J., Minja, E., Mwaga, A., Nahdy, M.S. & Subrahmanyam, P. (2000) Diseases and pests of pigeonpea in eastern Africa: a review. *International Journal of Pest Management* **46:** 7-18.

Imrie, B.C. Jones, R.M. & Kerridge, P.C. (1983) Desmodium. In: Burt, R.L., Rotar, P.P., Walker, J.L. & Silvey, M. (Eds), *The role of* Centrosema*,* Desmodium *and* Stylosanthes *in improving tropical pastures.* 97-140. Westview Press, USA.

Isaacs, R. (1994) Studies on the chemical ecology of the black bean aphid, *Aphis fabae*. *PhD Thesis*. University of London, U.K.

Isman, M. (2002) Insect Antifeedants. *Pesticide Outlook* **8**: 32–38.

Jackai, L.E.N. & Daoust, R.A. (1986) Insect pests of cowpeas. *Annual Review of Entomoogy* **31**: 95-119

Jing-Gong, X., Feng, Z., Yu-Ling, F., Wei, K., Guang-Xue, Z. & Zhong-Ning, Z. (2002) Behavioural response of aphids to the alarm pheromone component (E)-β-farnesene in the field. *Physiological Entomology* **27**: 307-311.

Jones, O.T. (1998) The Commercial Exploitation of Pheromones and other Semiochemicals. *Pesticide Science* **54**: 293-296.

Jones, R.M. (1989) Productivity and population dynamics of silverleaf desmodium (*Desmodium uncinatum*), greenleaf desmodium (*D. intortum*) and two *D. intortum* x *D. sandwicense* hybrids at two stocking rates in coastal south-east Queensland. *Tropical Grasslands* **23**: 43-55.

Jönsson, M. (2005) Responses to oilseed rape and cotton volatiles in insect herbivores and parasitoids. *Doctoral thesis*. Swedish University of Agricultural Sciences, Alnarp, Sweeden.

Jutsum, A.R. & Gordon, R.F.S. (1989) Introduction. Pheromones: importance to insects and role in pest management. In: Jutsum, A.R. & Gordon, R.F.S (Eds.), *Insect*

Pheromones in Plant Protection. 1-13. John Wiley & Sons Limited, Chichester, UK.

Karras, D.J., Farrell, S.E., Harrigan, R.A., Henretig, F.M. & Gealt, L. (1996) Poisoning from 'spanish fly' (cantharidin). *American Journal of Emergency Medicine* **14**: 478-483.

Kelber, A. (1999) Ovipositing butterflies use a red receptor to see green. *Journal of Experimental Biology* **202**: 2619-2630.

Kelber, A. (2001) Receptor based models for spontaneous colour choices in flies and butterflies. *Entomologia Experimentalis et Applicata* **99**: 231-244.

Kelber, A. & Pfaff, M. (1999) True colour vision in the orchard butterfly, *Papilio aegeus*. *Naturwissenschaften* **86**: 221-224.

Kelber, A., Vorobyev, M. & Osorio, D. (2003) Animal colour vision – behavioural tests and physiological concepts. *Biological Reviews* **78**: 81-118.

Kennedy, J.S. (1986) Some current issues in orientation to odour sources. In: Payne, T.L., Birch, M.C. & Kennedy, C.E.J. (Eds.), *Mechanisms in Insect Olfaction* Oxford, Clarendon.

Kevan, P.G. & Baker, H.G. (1983) Insects as flower visitors and pollinators. *Annual Review of Entomology* **28**: 407-53.

Kevan, P., Giurfa, M. & Chittka, L. (1996) Why are there so many and so few white flowers? *Trends in Plant Science* **1**: 280-284.

Kfir, R., Overholt, W.A., Khan, Z.R. & Polaszek, A. (2002) Biology and management of economically important lepidopteran cereal stemborers in Africa. *Annual Review of Entomology* **47**: 701-731.

Khan, Z.R., Ampong-Nyarko, K., Chiliswa, P., Hassanali, A., Kimani, S., Lwande, W., Overholt, W.A., Pickett, J.A., Smart, L.E., Wadhams, L.J. & Woodcock, C.M. (1997) Intercropping increases parasitism of pests. *Nature* **388**: 631-632.

Khan, Z.R., Pickett, J.A., Van denBerg, J., Wadhams, L.J. & Woodcock, C.M. (2000) Exploiting chemical ecology and species diversity: stem borer and striga control for maize and sorghum in Africa. *Pest Management Science* **56**: 957-962.

Khan, Z.R., Pickett, J.A., Wadhams, L.J. & Muyekho, F. (2001) Habitat management strategies for the control of cereal stemborers and striga in maize in Kenya. *Insect Science and Its Application* **21**: 375-380.

Khan, Z.R., Hassanali, A., Overholt, W., Khamis, T.M., Hooper, A.M., Pickett, J.A., Wadhams, L.J. & Woodcock, C.M. (2002) Control of witchweed *Striga hermonthica* by intercropping with *Desmodium* spp. and the mechanism defined as allelopathic. *Journal of Chemical Ecology* **28**: 1871-1885.

Khan, Z.R. & Pickett, J.A. (2004) The ‘push-pull’ strategy for stemborer management: a case study in exploiting biodiversity and chemical ecology. In: Gurr, G.M., Wrattem, S.D. & Altieri, M.A. (Eds), *Ecological Engineering for Pest Management: Advances in Habitat Manipulation for Arthropods*. 155-164. CABI, Wallington, UK.

Khan, Z.R., Midega, C.A.O., Amudavi, D.M., Hassanali, A. & Pickett, J.A. (2008) On-farm evaluation of the ‘push-pull’ technology for the control of stemborers and striga weed on maize in Western Kenya. *Field Crops Research* **106**: 224-233.

Khan, Z.R., Midega, C.A.O., Bruce, T.J.A., Hooper, A.M. & Pickett, J.A. (2010) Exploiting phytochemicals for developing a ‘push–pull’ crop protection strategy for cereal farmers in Africa. *Journal of Experimental Botany* **61**: 4185-4196.

Kühnle, A & Müller, C. (2010) Relevance of visual and olfactory cues for host location in the mustard leaf beetle *Phaedon cochleariae*. *Physiological Entomology* **36**: 68-76.

Labrada, R. (2000) An overview of parasitic weed control. *Proceedings of the 3rd International Weed Science Society. Foz do Iguassu, Brazil, 6-11 June 2000. (Abstract)*

Lacey, E.S., Ginzel,M.D., Jocelyn G., Millar, J.G. & Hanks. L.M. (2004) Male-produced aggregation pheromone of the Cerambycid beetle *Neoclytus acuminatus acuminatus*. *Journal of Chemical Ecology* **30**: 1493-1507.

Lacey, E.S., Moreira, J.A., Millar, J.G. & Hanks, L.M. (2008) A male-produced aggregation pheromone blend consisting of alkanediols, terpenoids, and an aromatic alcohol from the Cerambycid beetle *Megacyllene caryae*. *Journal of Chemical Ecology* **34**: 408-417.

Lale, N.E.S. & Sastawa, B.M. (2000) Evaluation of host plant resistance, sowing date modification and intercropping as methods for the control of *Mylabris* and *Coryna* species (Coleoptera: Meloidae) infesting pearl millet in the Nigerian Sudan savanna. *Journal of Arid Environments* **46**: 263-280.

Leoncini, I., Le Conte, Y., Constagliola, G., Plettner, E., Toth, A.L., Wang, M., Huang, Z., Bécard, J-M., Crauser, D., Slessor, K.N. & Robinson, G.E. (2004) Regulation of behavioural maturation by a primer pheromone produced by adult worker honey bees. *Proceedings of the National Academy of Social Sciences of the United States of America* **101**: 17559-17564.

Lopez, J., Tejada, I., Vasquez, C., de Dios Garza, J. & Shimada, A. (2004) Condensed tannins in humid tropical fodder crops and their *in vitro* biological activity: Part 1. *Journal of the Science of Food and Agriculture* **84**: 291-294.

Lunau, K., & Maier, E.J. (1995) Innate colour preferences of flower visitors. *Journal of Comparative Physiology A* **177**: 1-19.

Matthes, M., Napier, J.A., Pickett, J.A. & Woodcock, C.M. (2003) New chemical signals in plant protection against herbivores and weeds. In: *Proceedings of BCPC International Congress – Crop Science & Technology 10-12 November 2003, SECC Glasgow,9C-4*. 1227-1236.

Mauchline, A.L., Osborne, J.L., Martin, A.P., Poppy, G.M & Powell, W. (2005) The effects of non-host plant essential oil volatiles on the behaviour of the pollen beetle *Meligethes aeneus*. *Entomologia Experimentalis et Applicata* **114**: 181-188.

Mayhew, P.J. (2001) Herbivore host choice and optimal bad motherhood. *Trends in Ecology & Evolution* **16**: 165-167.

McCormick, J.P. & Carrel, J.E. (1987) Cantharidin biosynthesis and function in meloid beetles. In: Prestwitch, G.D. & Blomquist, G.J. (Eds.) *Pheromone Biochemistry*. 307-350. Academic Press, Orlando.

McNeely, C. & Singer, M.C. (2001) Contrasting the roles of learning in butterflies foraging for nectar and oviposition sites. *Animal Behaviour* **61**: 847-852.

Mendesil, E., Abdeta, C., Tesfaye, A., Shumeta, Z. & Jifar, H. (2007) Farmers' perceptions and management practices of insect pests on stored sorghum in southwestern Ethiopia. *Crop Protection* **26**: 1817-1825.

Menzel, R. (1979) Spectral sensitivity and color vision in invertebrates. In: Autrum, H. (Ed.), *Handbook of Sensory Physiology*, vol VII/6A. *Vision in Invertebrates* 503-580. Springer-Verlag, Berlin.

Metcalf, R.L. & Metcalf, E.R. (1992) *Plant Kairomones in Insect Ecology and Control* Chapman & Hall, London.

Midega, C.A.O., Khan, Z.R., van den Berg, J., Ogol, C.K.P.O, Pickett, J.A. & Wadhams, L.J. (2006) Maize stemborer predator activity under 'push-pull' system and Bt-maize: A potential component in managing Bt resistance. *International Journal of Pest Management* **52**: 1-10.

Millar, J.G., McBrien, H.L., Ho, H.-Y., Rice, R.E., Cullen, E., Zalom, F.G. & Uokl, A. (2002) Pentatomid bug pheromones in IPM: possible applications and limitations. *International Organic and Biological Control Bulletin* **25**: 241-250.

Miller, J.R. & Cowles, R.S. (1990) Stimulo-deterrent diversion: A concept and its possible application to onion maggot control. *Journal of Chemical Ecology* **16**: 3197-3212.

Miller, J.R. & Strickler, K.L. (1984) Finding and accepting host plants. In: Bell, W.J. & Cardé, R.T. (Eds.), *Chemical Ecology of Insects*. 127-157. Chapman & Hall, London.

Mitchell, B.K. (1988) Adult leaf beetles as models for exploring the chemical basis of host-plant recognition. *Journal of Insect Physiology* **34**: 213-225.

Mithöfer, A. & Boland, W. (2012) Plant defense against herbivores: chemical aspects. *Annual Review of Plant Biology* **63**: 431-50.

Murlis, J., Elkinton, J.S. & Cardé, R.T. (1992) Odor plumes and how insects use them. *Annual Review of Entomology* **37**: 505-532.

Ne'eman, G. & Kevan, P.G. (2001) The effect of shape parameters on maximal detection distance of model targets by honeybee workers. *Journal of Comparative Physiology A* **187**: 653-660.

Ne'eman G., Shavit, O., Shaltiel, L. & Shmida, A. (2006) Foraging by male and female solitary bees with implications for pollination. *Journal of Insect Behavior* **19**: 383-401.

Nielsen, J.K., Larsen, L.M. & Sørenson, H. (1977) Cucurbitacin E and I in *Iberis amara*: feeding inhibitors for *Phyllotreta nemorum*. *Phytochemistry* **16**: 1519-1522.

Nikbakhtzadeh, M.R. & Tirgari, S. (2002) Blister beetles (Coleoptera: Meloidae) in Nahavand county (Hamedan Province, Iran) and their ecological relationship to other Coleopteran families. *Iranian Journal of Public Health* **31**: 55-62.

Nikbakhtzadeh, M.R. (2004) Transfer and distribution of cantharidin within selected members of blister beetles (Coleoptera: Meloidae) and its probable importance in sexual behaviour. *PhD Dissertation*. Universität Bayreuth. Bayreuth, Germany

Ômura, H. & Honda, K. (2005) Priority of color over scent during flower visitation by adult *Vanessa indica* butterflies. *Oecologia* **142**: 588-596.

Özbek, H. & Szaloki, D. (1998) A contribution to the knowledge of the Meloidae (Coleoptera) fauna of Turkey along with new records. *Tropical Journal of Zoology* **22**: 23-40.

Pelletier, Y. & J. Dutheil, J. (2006) Behavioural responses of the Colorado potato beetle to trichomes and leaf surface chemicals of *Solanum tarijense*. *Entomologia Experimentalis et Applicata* **120**: 125-130.

Pichersky, E. & Gershenzon, J. (2002) The formation and function of plant volatiles: perfumes for pollinator attraction and defense. *Current Opinion in Plant Biology* **5**: 237-243.

Pickett, J.A. (1998) Pest semiochemicals in arable crop protection. *Pesticide Science* **54**: 290-299.

Pickett, J.A. & Griffith, D.C. (1980) Composition of aphid alarm pheromones. *Journal of Chemical Ecology* **6**: 349-360.

Pickett, J.A., Wadhams, L.J., Woodcock, C. M. & Hardie, J. (1992) The chemical ecology of aphids. *Annual Review of Entomology* **37***:* 67-90.

Pickett, J. A., Wadhams, L. J., & Woodcock, C.M. (1997) Developing sustainable pest control from chemical ecology. *Agriculture, Ecosystems and the Environment* **64**: 149-156.

Pickett, J.A., Bruce, T.J.A., Chamberlain, K., Hassanali, A., Khan, Z.R., Matthes, M.C., Napier, J.A., Smart, L.E., Wadhams, L.J. & Woodcock, C.M. (2006) Plant volatiles yielding new ways to exploit plant defence. In: Dicke, M. & Takken, W. (Eds.), *Chemical Ecology: From Gene to Ecosystem*. 161-173. Springer, Netherlands.

Pingali, P.L. & Pandey, S. (2001) Meeting world maize needs: Technological opportunities and priorities for the public sector. In: Pingali, P.L. (Ed.), *CIMMYT 1999-2000*

World Maize Facts and Trends. Meeting world maize needs: Technological opportunities and priorities for the public sector. CIMMYT, Mexico, D.F.

Pinto, J.D. & Bologna, M.A. (1999) The new world genera of Meloidae (Coleoptera): a key and synopsis. *Journal of Natural History* **33**: 569-620.

Plimmer, J.R., Inscoe, M.N. & McGovern, T.P. (1982) Insect attractants. *Annual Review of Pharmacology and Toxicology* **22**: 297-320.

Polettini, A., Crippa, O., Ravagli, A. & Saragoni, A. (1992) A fatal case of poisoning with cantharidin. *Forensic Science International* **56**: 37-43.

Powell, W. (1998) Semiochemicals to increase parasitism in aphid pest control. *Pesticide Science* **54**: 291-293.

Price, P.W. (1997) *Insect Ecology*. Third edition. Chapter 1. John Wiley & Sons, Inc. New York.

Prokopy, R.J. & Owens, E.D. (1983) Visual detection of plants by herbivorous insects. *Annual Review of Entomology* **28**: 337-364.

Prokopy, R.J., Averill, A.L., Cooley, S.S. & Roitberg, C.A. (1982) Associative learning in egglaying site selection by apple maggot flies. *Science* **218**: 76-77

Prokopy, R.J., Green, T.A. & Wong, T.T.Y. (1989) Learning to find fruit in *Ceratitis capitata* flies. *Entomologia Experimentalis et Applicata* **53**: 65-72.

Pyke, G.H., Pulliam, H.R. & Charnov, E.L. (1977) Optimal foraging: a selective review of theory and tests. *Quarterly Review of Biology* **52**: 137-154.

Raguso, R.A. (2004) Why do flowers smell? The chemical ecology of fragrance-driven pollination. In: Cardé, R.T. & Millar, J.G. (Eds.), *Advances in Insect Chemical Ecology*. 151-178. University Press, Cambridge.

Raguso, R.A. (2001) Floral scent, olfaction, and scent-driven foraging behavior. In: L. Chittka, L. & Thomson, J. (Eds.), *Cognitive Ecology of Pollination* 83-105. Cambridge University Press, Cambridge.

Rao, S., Cossé, A.A., Zilkowski, B.W. & Bartelt, R.J. (2003) Aggregation pheromone of the cereal leaf beetle: field evaluation and emission from males in the laboratory. *Journal of Chemical Ecology* **29**: 2165-2175.

Regnier, F.E. & Law, J.H. (1968) Insect pheromones. *Journal of Lipid Research* **9**: 541-551.

Reinecke, A., Ruther, J. & Hilker, M. (2005) Electrophysiological and behavioural responses of *Melolontha melolontha* to saturated and unsaturated aliphatic alcohols. *Entomologia Experimentalis et Applicata* **115**: 33-40.

Riffell, J.A., Alarcón, R., Abrell, L., Davidowitz, G., Bronstein, J.L. &. Hildebrand, J.G. (2008) Behavioral consequences of innate preferences and olfactory learning in hawkmoth–flower interactions. *Proceedings of the National Academy of Science* **105**: 3404-3409.

Robinson, J. (2005) Pests and integrated pest management in western Equatoria, southern Sudan. *International Journal of Tropical Insect Science* **25**: 224-235.

Roder, W., Tshering, S., Dorji, J., Samdup, C. & Wangchuk, P. (2002) Experiences with Greenleaf desmodium (*Desmodium intortum*) seed production in Bhutan. *Tropical Grasslands* **36**: 159-164.

Rull, J. & Prokopy, R.J. (2005) Interaction between natural and synthetic fruit odor influences response of apple maggot flies to visual traps. *Entomologia Experimentalis et Applicata* **114**: 79-86.

Rumbaugh, M.D. (1990) Special purpose forage legumes. In: Janick, J. & Simon, J.E. (Eds.), *Advances in New Crops*. 183-190. Timber Press, Portland.

Ruther, J. & Mayer, C.J. (2005) Response of garden chafer, *Phyllopertha horticola*, to plant volatiles: from screening to application. *Entomologia Experimentalis et Applicata* **115**: 51-59.

Ruther, J. & Thiemann, K. (1997) Response of the pollen beetle *Meligethes aeneus* to volatiles emitted by intact plants and conspecifics. *Entomologia Experimentalis et Applicata* **84**: 183-188.

Ruther J, Reinecke, A. & Hilker, M. (2002) Plant volatiles in the sexual communication of *Melolontha hippocastani*: response towards time dependent bouquets and novel function of (*Z*)-3-hexen-1-ol as a sexual kairomone. *Ecological Entomology* **27**: 76-83.

Scheirs, J. De Bruyn, L. & Verhagen, R. (2002) Seasonal changes in leaf nutritional quality influence grass miner performance. *Ecological Entomology* **27**: 84-93.

Schmera, D., Tóth, M., Subchev, M., Sredkov, I. Szarukán, I. Jermy, T. & Szentesi, Á. (2004) Importance of visual and chemical cues in the development of an attractant trap for *Epicometis* (*Tropinota*) *hirta* Poda (Coleoptera: Scarabaeidae). *Crop Protection* **23**: 939‑944.

Schmidt-Tiedemann, A., Louis, F., Fukumoto, T., Zebitz, C.P.W. & Arn, H. (1999) Mating disruption tests to control *Sparganothis pilleriana* (Schiff.) (Lepidoptera: Tortricidae). In: Witzgall, P. & El-Sayed, A. (Eds.), *Scents in Orchards - Plant and Insect Semiochemicals from Orchard Environments*. In *Proceedings of IOBC wprs Symposium, Dachau 1998*. *IOBC/wprs Bulletin* **22**.

Scholtz, C.H. & Holm, E. (1986) *Insects of Southern Africa*. Butterworth Publishers (Pty) Ltd Mayville, Durban.

Schoonhoven, L.M. (1999) Insects and plants: two worlds come together**.** *Entomologia Experimentalis et Applicata* **91**: 1–6.

Schoonhoven, L.M., Jermy, T. & van Loon, J.J.A. (2005) *Insect-Plant Biology*. 2nd edition. Chapman & Hall, London.

Selander, R.B. (1964) Sexual behavior in blister beetles (Coleoptera: Meloidae) I. The genus *Pyrota*, *The Canadian Entomologist* **96**: 1037-1081.

Selander, R.B. (1981) Evidence for a third type of larval prey in blister beetles (Coleoptera: Meloidae). *Journal of the Kansas Entomological Society* **54**: 757-783.

Selander, R.B. (1986) Rearing blister beetles. *Insecta Mundi*. **1**: 209-220.

Selander, R.B. & Fasulo, T.R. (2003) Blister beetles (Insecta: Coleoptera: Meloidae). *EENY-166 originally published as DPI Entomology Circular 268).* Entomology and Nematology Department, Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida.

Seshu Reddy, K.V. (1998) Maize and Sorghum: East Africa. In: Polaszek, A. (Ed.), *African Cereal Stem Borers: Economic Importance*, *Taxonomy*, *Natural Enemies and Control*. 25-27. CAB International, Wallingford, UK.

Shah, P.A., Pickett, J.A. & Vandenburg, J.D. (1999) Responses of Russian wheat aphid (Homoptera: Aphididae) to aphid alarm pheromone. *Environmental Entomology* **28**: 983-985.

Singh, S.R. & van Emden, H.F. (1979) Insect pests of grain legumes. *Annual Review of Entomology* **24**: 255-278

Skorupski, P. & Chittka, L. (2009) Is colour cognitive? *Optics & Laser Technology* **43**: 251-260.

Sleeper, H.L., Paul, V.J. & Fenical, W. (1980) Alarm pheromones from the marine opisthobranch *Navanax inermis*. *Journal of Chemical Ecology* **6**1: 57-70.

Smart, L.E., Blight, M.M. & Hick, A.J. (1997) Effect of visual cues and a mixture of Isothiocyanates on trap capture of cabbage seed weevil, *Ceutorhynchus assimilis. Journal of Chemical Ecology* **23**: 889-902.

Smart, L.E., Blight, M.M., Pickett, J.A. & Pye, B.J. (1994) Development of field strategies incorporating semiochemicals for the control of the pea and bean weevil, *Sitona lineatus* L. *Crop Protection* **13**: 127-135.

Steinbrecht, R.A. (1987) Functional morphology of pheromone-sensitive sensilla. In Prestwich, G.D. & Blomquist, G.J. (Eds.), *Pheromone Biochemistry*. 353-379. Academic Press, Inc. Orlando.

Strom, B.L., Roton, L.M., Goyer, R.A. & Meeker, J.R. (1999) Visual and semiochemical disruption of host finding in the southern pine beetle. *Ecological Applications* **9**: 1028-1038.

Strom, B.L., Goyer, R.A. & Shea, P.J. (2001) Visual and olfactory disruption of orientation by the western pine beetle to attractant-baited traps. *Entomologia Experimentalis et Applicata* **100**: 63-67.

Tagwireyi, D. Balla, D.E. Logab, P.J. & Moyo, S. (2000) Cantharidin poisoning due to ``Blister beetle" ingestion. *Toxicon* **38**: 1865-1869.

Tanzubil, P.B. & Yakubu, E.A. (1997) Insect pests of millet in Northern Ghana. 1. Farmers' perceptions and damage potential *International Journal of Pest Management* **43**: 133-136.

Trout, K. (1997) Trout's notes on the genus D*esmodium* (chemistry, ethnomedicine, pharmacology, synonyms and miscellany). Mydriatic Productions.

Tumlinson, J.H. & Teal, P.E.A. (1987) Relationship of structure and function to Biochemistry in insect pheromone systems. In: Prestwitch, G.D & Blomquist, G.J. (Eds.), *Pheromone Biochemistry* 3-26. Academic Press, INC. Orlando.

Van der Entl, L.J. & Visser, J.H. (1991) The visual world of the Colorado potato beetle. *Proceedings of the Experimental & Applied Entomology* **2**: 80-85.

Videla, M., Valldares, G. & Salvo, A. (2006) A tritrophic analysis of host preference and perfomance in a polyphagous leafminer. *Entomologia Experimentalis et Applicata* **121**: 105-114.

Visser, J.H. (1986) Host odor perception in phytophagous insects. *Annual Review of Entomology* **31**: 121-144.

Visser, J.H. (1988) Host-plant finding by insects: Orientation, sensory input and search patterns. J*ournal of Insect Physiology* **34**: 259-268.

Wäckers, F.L. & Lewis, W.J. (1999) A comparison of color-, shape- and pattern-learning by the hymenopteran parasitoid *Microplitis croceipes*. *Journal of Comparative Physiology A* **184**: 387-393.

Ward, C.R. (1997) Blister beetles in alfalfa. *Cooperative Extension Service, Circular no.536*. College of Agriculture and Home Economics, New Mexico State University, Las Cruces, New Mexico.

Wee, S. & Tan, K. (2005) Female sexual response to male rectal volatile constituents in the fruit fly, *Bactrocera carambolae* (Diptera: Tephritidae). *Applied Entomology and Zoology* **40**: 365-372.

Weiss, M.R. (1997) Innate colour preferences and flexible colour learning in the pipevine swallowtail. *Animal Behaviour* **53**: 1043-1052.

Weiss, M.R. (2001) Vision and learning in some neglected pollinators: beetles, flies, moths and butterflies. In: L. Chittka, L. & Thomson, J. (Eds.), *Cognitive Ecology of Pollination* 171-189. Cambridge University Press, Cambridge.

Weiss, M.R. & Papaj, D.R. (2003) Colour learning in two behavioural contexts: how much can a butterfly keep in mind? *Animal Behaviour* **65**: 425-434.

Wertheim, B., van Baalen, E.A., Dicke, M. & Vet, L.E.M. (2005) Pheromone-mediated aggregation in nonsocial arthropods: an evolutionary ecological perspective. *Annual Review of Entomology* **50**: 321-46.

Wyatt, T.D. (2003) *Pheromones and Animal Behaviour – Communication by Smell and Taste*. Cambridge University Press, UK.

Zhu, F., Lei, C.L. & Xue, F. (2005) The morphology and temperature-dependent development of *Mylabris phalerata* Pallas (Coleoptera: Meloidae). *The Coleopterist's Bulletin* **59**: 521-527.

CHAPTER 2

Farmers' knowledge and perceptions of blister beetles, Hycleus spp. (Coleoptera: Meloidae), as pest herbivores of Desmodium legumes in western Kenya

Paper published as: Lebesa, L.N., Khan, Z.R., Krüger K., Bruce, T.J., Hassanali, A. & Pickett, J.A. (2012) Farmers' knowledge and perceptions of blister beetles, *Hycleus* spp. (Coleoptera: Meloidae), as pest herbivores of *Desmodium* legumes in western Kenya. *International Journal of Pest Management* **58**: 165-174.

Abstract

A survey was undertaken to determine the pest status of blister beetles, *Hycleus* spp., in western Kenya where they attack crops such as *Desmodium* spp. and sweetpotato. *Desmodium* spp. are important intercrops in the 'push-pull' strategy adopted for *Striga* and stemborer control in maize and sorghum. Production of desmodium seed is adversely affected by blister beetles, which feed on the flowers and negatively affect seed setting. To assess farmers' knowledge and perceptions of *Hycleus* species as pests, a questionnaire survey was conducted in three sites in Bungoma district, western Kenya, in 2007. The survey was followed by field sampling of *Desmodium* spp. and sweetpotato to compare the results with the responses received from farmers. *Hycleus* spp. were mentioned by 75% of the respondents as major pests of *Desmodium* spp. During field sampling *Hycleus* spp. comprised 70% of the insect pests collected. According to farmers, blister beetles were more abundant on *Desmodium* spp. than on sweetpotato. However, field sampling revealed that differences in beetle abundance on the two crops were not consistent across different sites, suggesting that these crops may function as alternative hosts. The study provides baseline information for the development of a management strategy for blister beetles.

Keywords: *Desmodium* spp.; questionnaire; field survey; pest status; sweetpotato

2.1 Introduction

Desmodium species (Fabaceae), such as *Desmodium uncinatum* Jacq. DC., play an important role as intercrops for maize and sorghum in 'push-pull' crop protection strategies due to their repellent ('push') properties against maize stemborers (Lepidoptera), and their ability to cause suicidal germination of dormant seeds of witchweed, *Striga* spp. (Scrophulariaceae) and so suppress population levels of that parasitic weed (Khan *et al*. 2000, 2002, 2006a, 2006b, 2008a). The 'push-pull' strategy was developed for small scale farmers by ICIPE (International Centre of Insect Physiology and Ecology) and its two partners, KARI (Kenya Agricultural Research Institute, Kenya) and Rothamsted Research, UK (Pickett 1998; Cook *et al*. 2007; Khan *et al*. 2008a). Apart from its role as an intercrop in the 'push-pull' technology, *Desmodium* spp. (hereafter referred to as desmodium) are also nutritious fodder crops for livestock (PANESA/ARNAB 1990; Paterson *et al*. 1998; Khan *et al*. 2006a). Thus, desmodium is valuable for small-scale farmers. The increased demand for desmodium seed associated with the widespread adoption of the 'push-pull' technology prompted ICIPE and its partners to establish community-based desmodium seed production units in East Africa (Khan *et al*. 2008b).

The main constraint upon the production of desmodium seed is infestation by blister beetles, *Hycleus* spp. (Coleoptera: Meloidae; formerly *Coryna*) (Pickett *et al*. 2010; Lebesa *et al*. 2011), which feed on flowers causing damage and preventing the development of seed. Apart from *Desmodium* spp., they have been observed feeding on floral parts of other crops such as sweetpotato, cowpea and okra in several parts of western Kenya (L.N. Lebesa unpublished observations). The available literature on meloids in Kenya, however, mainly documents *Coryna* and *Mylabris* as being associated with other leguminous crops (Abate & Ampofo 1996; Hillocks *et al*. 2000). *Mylabris* spp. and *Coryna* spp. are reported to co-occur frequently on the same plant crop in various sub-Saharan countries (Abate & Ampofo 1996; Tanzubil & Yakubu 1997; Lale & Sastawa 2000). In

West Africa, several species of blister beetles, e.g. *Psalydolytta* spp., *Mylabris* spp. and *Hycleus* spp. (formerly *Coryna*); (Bologna & Pinto 2002) have been observed feeding on pollen or flowers of various grain crops such as sorghum, pearl millet and cowpea, and to reduce grain yields (Gahukar 1991; Lale & Sastawa 2000).

Adult blister beetles may range from 5 – 40mm in body length, depending on the species (Scholtz & Holm 1986). The adult female lays cylindrical eggs in batches in the soil. Upon hatching into active larvae called triungulins, the progeny search for grasshopper eggs for feeding (Hill 1975; Selander 1986; Abate & Ampofo 1996; Özbek & Szaloki 1998). The larvae develop into higher larval stages inside the grasshopper egg pod (Nikbakhtzadeh 2004). Damage to plants is inflicted by adult beetles only; these feed on both floral parts as well as on the developing seeds thereby negatively affecting seed setting and, consequently, yields. Meloids are largely polyphagous, feeding on a wide range of host plants within families such as Fabaceae, Malvaceae, Convolvulaceae and Solanaceae (Selander 1986; Bologna & Pinto 2002; Zhu *et al*. 2005; Lebesa *et al*. 2011).

Although the presence of blister beetles in different crops is usually not considered to be a serious constraint (Hill 1975; Zhu *et al*. 2005), infestations of crops grown in small-holder plots may cause considerable damage because of the gregarious nature of adult blister beetles (Hall 1984; Nikbakhtzadeh 2004). Evans *et al*. (1989) reported that more than 80% of flowers and developing pods of a prairie legume, *Baptisia australis* (L.) R. Br. (Fabaceae) were damaged by the blister beetle *Epicauta fabricii* LeConte, thereby adversely affecting seed production. Blister beetles are similarly becoming a very important pest in East Africa because of increasing demand for *Desmodium* spp. seeds by small-holder farmers adopting the push-pull strategy for controlling stemboreres and striga. As an introduced crop in eastern Africa (Agnew & Agnew 1994; Cook *et al*. 2005), there is limited information on its pests, especially blister beetles, which represent a significant challenge to desmodium seed production and the push-pull farming system.

We undertook a survey to provide baseline information required for the development of management strategies for blister beetles on desmodium. A questionnaire was provided to small-holder farmers to: (i) assess their knowledge and perception of the pest status of blister beetles, *Hycleus* species, (ii) document current control methods and their effectiveness, and (iii) record the farmers' perceptions of the host range of the blister beetles. In addition, a field survey to assess the abundance of blister beetles on desmodium and alternative host plants was undertaken to compare the results with the responses of farmers.

2.2 Material & Methods

Household survey

A household survey was undertaken in the Bungoma district (0°34´ to 0°39´S, 34°30´ to 34°32´E) in the Western Province in Kenya in October and November 2007 to identify potential crops infested by blister beetles; this was followed by a field survey. A questionnaire was developed and enumerators with knowledge of the local language were trained on how to interview farmers. Thereafter, enumerators undertook face-to-face interviews in 92 farm households. Participating farmers were selected randomly within the three categories of farmers from three sub-locations in the Bungoma district. Of these, 44 were from Marakaru and Tutii and involved in producing desmodium seed, 33 farmers from the same areas were practising the 'push-pull' technology, and 15 farmers from Kapchai were not involved in either the 'push-pull' technology or desmodium seed production. The latter group of farmers was, however, planting sweetpotato (*Ipomoea batatas* (L.) Lam., Convolvulaceae), an alternative host of *Hycleus* spp. (Table 1). Care was taken to balance gender, where possible, to avoid potential sex bias.

Table 1. Study sites, geographical zones and farmers' sample distribution.

Sub-location	Elevation (m a.s.l.)	Coordinates	No. of farmers per farm type			Total no. of farmers
			'push-pull' technology	Seed production	No desmodium	
Marakaru	1430 - 1530	0°38´ - 0°40´N, 34°31´ - 34°32´E	13	18	-	31
Tutii	1230 - 1540	0°35´ - 0°39´N, 34°30´ - 34°33´E	20	26	-	46
Kapchai	1390 - 1490	0°35´ - 0°36´N, 34°31´ - 34°32´E	-	-	15	15

The questionnaire was prepared to obtain information on: (i) demographic information on farmers, which included gender and the highest education level attained; (ii) farming practices, including farm sizes, type of crops planted, and mode of farming (mono- or mixed-cropping); and (iii) farmers' perception of pest problems on desmodium and other crops (pest status, extent of damage caused by blister beetles, control methods used, if any) to compare this with previously received reports from desmodium seed producers.

To improve accuracy of information gathering, the questionnaire included pictures of the different blister beetle species (Figure 1). If farmers were able to identify one or more species, they were asked to provide further information on the blister beetles. This included: (i) their abundance during different times of the day and seasons, (ii) the species that were dominant, (iii) the parts of the plant that were observed to be damaged, and (iv) estimated loss due to blister beetle attack compared to years where damage by beetles were minimized through pesticide application. Farmers' responses were categorised either in 'yes' or 'no' categories, e.g. recognition of blister beetles, or in three- to five-point codes representing a response to each question e.g. pest recognised and mentioned by a farmer had five-point codes.

H. apicicornis

H. dubiosus

H. vestitus

H. sjoestedti

H. vestitus

H. dicinctus

Figure 1. *Hycleus* spp. recorded on *Desmodium* spp. and sweetpotato in different parts of Kenya between 2006 and 2009. These sample pictures were shown to farmers in Bungoma to identify which of the beetles attacked their crops (identification of beetles by Marco Bologna, Roma TRE University).

Field sampling

Although farmers mentioned desmodium, sweetpotato and beans (*Phaseolus vulgaris* (L.), Fabaceae) as the main host crops of *Hycleus* species in Bungoma, only desmodium and sweetpotato had reached flowering stages and therefore were available for sampling. Sampling for the presence of blister beetles and other pests was undertaken in the sub-locations of Marakaru, Tutii and East Bukusu (another desmodium-producing area) in the Bungoma district, western Kenya. East Bukusu replaced Kapchai, because the latter had neither sweetpotato nor desmodium plants at the time of the survey.

Sampling was done twice in the space of two weeks (week 43 in October and 46 in November 2007) to capitalize on availability of flowers. During the first sampling, three fields of each crop were sampled except for Marakaru where only two sweetpotato fields were available. Desmodium, on the other hand, was sampled only from Tutii and Marakaru because in East Bukusu desmodium plants had been slashed off and only sweetpotato could be sampled. *Hycleus* spp. have two peak activity periods which occur in the morning between 09h00 and 11h00 and in the afternoon between 15h00 and 17h00 (L. Lebesa, unpublished data). Accordingly, sampling of blister beetles on the two crops was undertaken during these peak activity periods. Separate records were kept for the morning and afternoon periods. During the second sampling period, two fields per crop (desmodium and sweetpotato) were sampled.

During each survey, sampling was carried out in a standard area of 100 m^2 within each field because of variations in field size. Because desmodium and sweetpotato plants are creepers, it was difficult to distinguish between individual plants. Therefore, twenty 1 x 1 m sub-plots within a field were chosen at a spacing of 2 m and sampling followed a W-pattern across the field. Leaves and flowers present within the 1 x 1 m sub-plots were visually inspected for the presence of beetles and other pests.

Since blister beetles tend to drop to the ground and feign death when disturbed, aerial parts of plants were lifted to search the ground underneath for the beetles. Beetles were collected and kept in aerated containers that were labelled with the field number and location, host plant, date and time of sampling for inspection in the laboratory to separate species and sex.

Statistical analysis

Calculation of the percentage of farmers giving the same response to a question was based on the total number of farmers that responded to the question. If a farmer gave more than one answer to a question, each was included in the appropriate group of responses to the question. As a result, percentages for some questions exceeded 100% (Ebenebe *et al*. 2001; Tefera 2004; Obopile *et al*. 2008). Chi-squared analyses of the relevant frequencies were analysed as 2 x 2 contingency tables to determine if the beetle abundances were related to the two practices of growing desmodium ('push-pull' or seed production) and to the number of years a farmer had been involved with desmodium production. Gamma correlation was further used to determine correlations between years of experience of growing desmodium and the pest status of blister beetles (Healey 1990).

Data from the field sampling were not normally distributed even after transformation, therefore non-parametric tests were employed for analyses. Kruskal-Wallis ANOVA was used to determine the significance of differences in the abundances of beetles between different fields, and within and between the host plants sampled in each location. Paired tests were used to test for any significant differences between the abundances of beetles on the two hosts at two sub-locations (Mann-Whitney U-test) and their abundances in the morning compared to the afternoon (Wilcoxon Matched Pairs test). Data were analysed with Statistica (Version 7.0, StatSoft, Inc, 1984-2004).

2.3 Results

Household surveys

Demographic characteristics and production practices

The overall male:female ratio for all interviewed farmers was approximately 1:1, with slightly more females (53%) than males (47%). The overall proportion of females and males interviewed was similar for desmodium farmers (49% males and 51% females), although at Kapchai (a non-desmodium growing location) more female (67%) than male (33%) farmers were interviewed. The majority of the interviewed farmers had formal education, 42% having attended primary and 50% having attended secondary school. Only 8% had no formal education (Table 2).

Table 2. Summary of socio-economic characteristics and production practices of farmers interviewed (numbers in brackets denote %).

Variable	Marakaru	Tutii	Kapchai	Total responses
	n (%)	*n* (%)	*n* (%)	*n* (%)
Gender				
Female	16 (52)	22 (48)	10 (67)	48 (53)
Male	15 (48)	24 (52)	5 (33)	44 (47)
Education				
No formal education	5 (16)	1 (2)	1 (6)	7 (8)
Primary (Std1-8)	7 (23)	25 (54)	7 (47)	39 (42)
Secondary (Form1-4)	19 (61)	20 (44)	7 (47)	46 (50)
No. of crops per field				
4-5	6 (19)	9 (19)	2 (13)	17 (18)
6-7	16 (52)	27 (59)	11 (74)	54 (59)
>7	9 (29)	10 (22)	2 (13)	21 (23)
Desmodium farm size: (ha)				
*NA	- -	- -	15 (16)	15 (16)
<0.40	17 (55)	32 (70)	- -	49 (53)
0.40 – 0.80 (1-2)	11 (35)	13 (28)	- -	25 (27)
0.81 – 2.02 (2-5)	3 (10)	1 (2)	- -	4 (4)
Years of experience with desmodium				
1	2 (7)	3 (7)	- -	5 (7)
2	12 (39)	12 (26)	- -	24 (31)
3	9 (29)	14 (29)	- -	23 (30)
>3	8 (26)	17 (37)	- -	25 (32)

* refers to those farmers that were not growing desmodium.

Farms were characterized by small-holdings, ranging from less than 0.4 hectares to approximately two hectares per farm. On average, farmers' plots were 0.4 – 0.8 hectares per crop, including desmodium. The majority of the farmers (80%) grew five or more crops, most of which were intercropped (Table 2). The only two crops that were almost exclusively mono-cropped were sweetpotato (99%) and cowpea (92%). Maize and beans were the most important crops, grown by 100% and 98% farmers, respectively. Desmodium was grown by 84% of farmers who either practised the 'push-pull' technology (39%) or were involved in seed multiplication (52%), while 9% were involved in both practices. Other crops grown included cassava (*Manihot esculenta* Crantz, Euphorbiaceae), bananas (*Musa acuminata* Colla, Musaceae), coffee (*Coffea arabica* L., Rubiaceae), groundnuts (*Apios americana* Medik., Fabaceae) and Napier grass (*Pennisetum purpureum* Schumach, Poaceae).

The majority of farmers (62%) growing desmodium had been exposed to the crop for three years or more. Farmers with three of more years of experience tended to be seed bulking farmers (39%). Other than for 'push-pull' and seed bulking, a small percentage of farmers (3%) grew desmodium for fodder production. Two desmodium species, silverleaf (*D. uncinatum*) and greenleaf (*D. intortum*), were almost equally planted by farmers. Forty-seven per cent and 51% of farmers mentioned *D. uncinatum* and *D. intortum,* respectively, while 2% were growing both.

Farmers' perception of pest incidence on different crops

The pests mentioned by farmers for crops other than desmodium were stemborers (Lepidoptera), *H. armigera* (Lepidoptera), aphids (Hemiptera: Aphididae), blister beetles, *Hycleus* spp., sweetpotato weevils (Coleoptera: Curculionidae) and mole rats (Bathyergidae), depending on the crop. For desmodium only two pests, *Hycleus* spp. and *H. armigera*, were mentioned. *H. armigera* was not considered a serious pest of desmodium and was mentioned by less than 10% of farmers who grew desmodium (Figure 2).

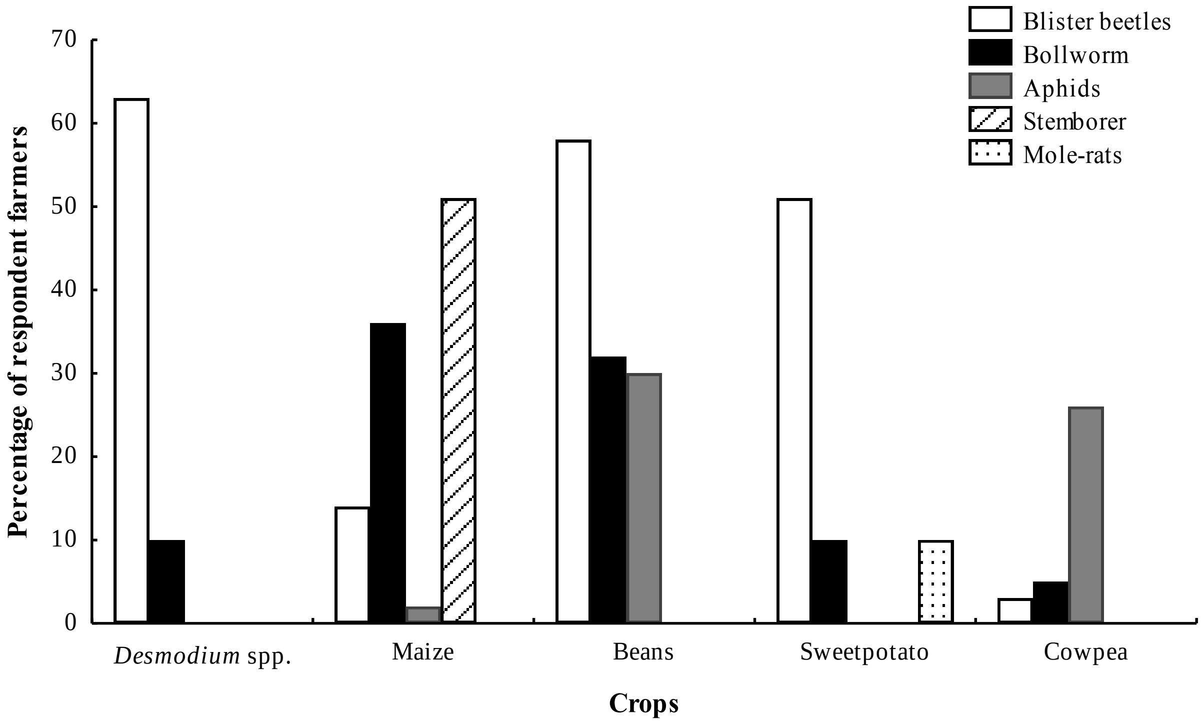


Figure 2. Percentage of farmers that mentioned various pests that attack different crops in Bungoma.

Knowledge of blister beetles, their host plants and damage caused

Of the 92 interviewed farmers, the highest number of farmers reported blister beetles as pests of desmodium (63%) and beans (58%), followed by sweet-potato (51%). Only a few farmers mentioned blister beetles as pests of maize (14%) and cowpea (3%) (Fig. 2). Seventeen per cent of the farmers believed that *Desmodium* spp. were free of pests. Farmers reported damage by blister beetles on flowers, seeds, leaves, and stems of crops attacked. For all crops, farmers reported the highest damage on flowers of beans (73%), desmodium (60%), sweetpotato (92%) and cowpea (100%), as well as pollen feeding on maize tassels (53%). For desmodium, damage to seed pods was considered most common after damage to flowers and was mentioned by 45% of desmodium-growing farmers. Damage to leaves and stems was mentioned by 14% and 3% of the desmodium farmers, respectively.

When the pictures of different species of blister beetles were presented to farmers, 86 of them (93%) were able to identify the insects they were familiar with. The three species that were recognised were *Hycleus apicicornis* (72%), *H. dubiosus* Marseul (43%) and *H. sjostedti* Borchmann (40%). Farmers mentioned that all three species were observed feeding on desmodium, maize, sweetpotato and beans, while on cowpea farmers noted only *H. apicicornis* (Figure 3).

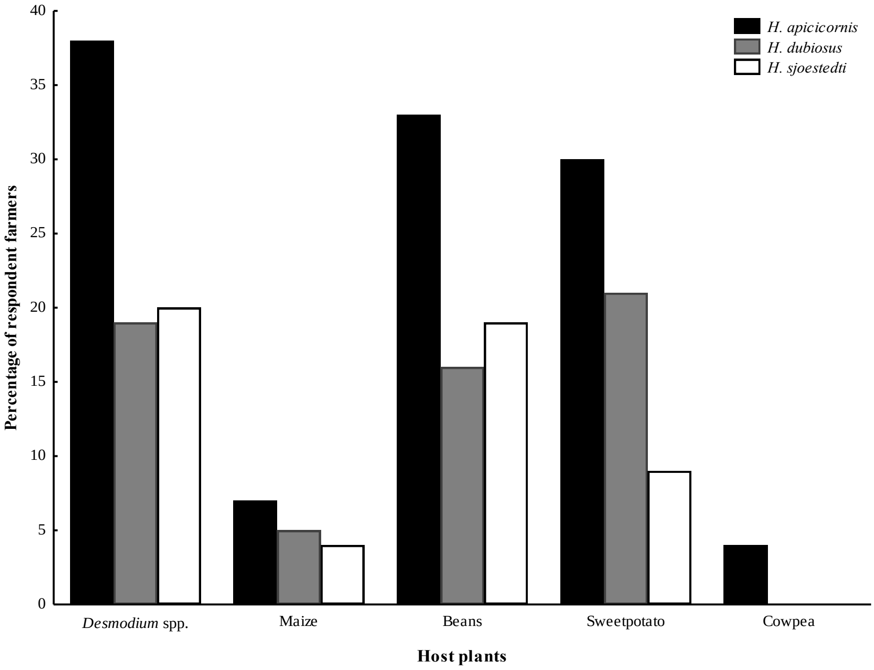


Figure 3. Percentage of respondent farmers who identified with the aid of pictures, three *Hycleus* spp. attacking various crops.

Farmers were able to specify the time of peak activity of the blister beetles during the day and different seasons of the year. Seventy-two per cent of farmers mentioned the long rainy season (May - July) as the period when blister beetles were most abundant. Sixty-seven per cent and 79% farmers of the farmers mentioned 06h00-08h00 and 17h00-19h00, respectively, as the hours at which activity of beetles peaked during the day.

The incidence of blister beetles reported by farmers at the two locations was compared. The percentage of farmers who mentioned the presence of blister beetles on *Desmodium* spp. was higher in Tutii (82%) than in Marakaru (62%); however, differences in the number of famers reporting blister beetles on *Desmodium* spp. in the two sub-locations were not significant (χ^2 = 3.42, df = 1, P = 0.064). There was a significant positive correlation between the experience farmers had in growing desmodium and the reported pest status of blister beetles on desmodium (γ = -0.35, n = 77, P = 0.015).

Current control methods used against blister beetles

Although blister beetles were recorded as a pest by the majority of farmers on the three major crops (beans, desmodium, and sweetpotato), only a few farmers were using control measures against the beetles. For desmodium, 33% of farmers were using some control measures, while for all other crops this was below 10%. The three control measures mentioned were chemical pesticides, physical methods (hand- picking of beetles and crushing) and application of ash. Of these, application of chemical pesticides was the most common, mentioned by 75% of farmers who were applying some form of control. Reasons given by farmers who did not employ any form of control measures against blister beetles included lack of knowledge on what to use (72%), unaffordable cost of pesticides (21%) and low pest damage (5%).

Although the majority of farmers with blister beetle problems (82%) were not using any form of control, they rated it as a serious pest on both desmodium and beans. Severity of blister beetle damage on beans was said to be higher in mixed cropping systems (81%) than in bean mono-crops (15%). However, for desmodium the opposite was considered to be true with more blister beetle damage reported by farmers producing desmodium seed (62%) than by those practising the 'push-pull' technology (29%); significantly more seed-producing than 'push-pull' farmers reported blister beetle damage ($\chi^2 = 6.24$, df = 1, $P = 0.012$). A small percentage of both groups (9%) reported no difference.

Of the 58 farmers growing desmodium and who mentioned blister beetles as pests, 49 were able to estimate the amount of seed considered to be lost as a result of attack by these beetles. Damage estimates ranged from less than 2.5 kg to more than 11 kg per hectare which is approximately 2% – 8% of their seed yield (130kg/ha). The majority of farmers (47%) estimated the loss at less than 2.5 kg per hectare. Nineteen farmers (32%) estimated yield losses ranging between 2.5 kg and 11 kg per hectare, while only three farmers gave estimated losses of more than 11 kg per hectare.

Field sampling

Observed pests

Two blister beetle species were recorded during the field sampling: *H. apicicornis* and *H. dubiosus*. The former was the more abundant species (Table 3). Another pest recorded in this study was the African bollworm *H. armigera.* It was found on both desmodium and sweetpotato, although it was present in very low numbers (<10 larvae in each sample site) (Table 3). Other insects collected included the common stink bug, *Nezara viridula* L. (Heteroptera: Pentatomidae) and other coleopteran pests such as *Cylas* spp. (Coleoptera: Curculionidae) on sweetpotato and the black maize beetle *Heteronychus arator* Fabricius (Coleoptera: Scarabaeidae) on desmodium. Due to the low abundance of *H. dubiosus,* only *H. apicicornis* was selected for comparison among hosts and sampling locations. Abundance of *Hycleus apicicornis* did not differ between morning and afternoon hours

(Marakaru: Wilcoxon Matched Pairs test; Z = 1.33, df = 55, *P* = 0.18; East Bukusu: Wilcoxon Matched Pairs test; Z = 0.55, df = 37, *P* = 0.58), except in the Tutii sub-location where significantly more beetles were recorded in the morning (Wilcoxon Matched Pairs test; Z = 3.93, df = 85, *P* < 0.0001).

No sampling of desmodium was undertaken in East Bukusu because all plots had been slashed off at the time when farmers were visited. There were no significant differences (Kruskal-Wallis test; $H_{2,24}$ = 0.775, *P* = 0.679) in the abundances of beetles on sweetpotato across all sampled sites. A comparison of the abundances of beetles between the two crops was made for the Marakaru and Tutii sub-locations. In Marakaru, significantly more *H. apicicornis* adults were recorded on desmodium than on sweetpotato (Mann-Whitney U test; $Z_{adj.}$ = 3.095, df = 178, *P* = 0.002). However, in Tutii, there were significantly more beetles on sweetpotato than on desmodium (Mann-Whitney U test; $Z_{adj.}$ = -2.080, df = 198, P = 0.038).

Table 3. List of observed insect pests on *Desmodium* spp. and sweetpotato fields in each of the three sub-locations in Bungoma. N = total number of units sampled throughout the whole sampling period.

Sub-locations	Host plants Insect pests	Time of occurrence		Mean total catches
		09h00 - 11h00	15h00 - 17h00	
Marakaru	*Desmodium* spp.			
	Hycleus apicicornis	0.51 ± 0.078	0.36 ± 0.069	0.87 ± 0.109
	Hycleus dubiosus	0.00 ± 0.000	0.01 ± 0.010	0.01 ± 0.010
	Helicoverpa armigera	0.01 ± 0.010	0.01 ± 0.010	0.02 ± 0.014
	Nezara viridula	0.08 ± 0.027	0.15 ± 0.048	0.23 ± 0.055
	Sweetpotato			
	Hycleus apicicornis	0.22 ± 0.067	0.21 ± 0.055	0.44 ± 0.096
	Hycleus dubiosus	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000

Sub-locations	Host plants Insect pests	Time of occurrence		Mean total catches
		09h00 - 11h00	15h00 - 17h00	
	Helicoverpa armigera	0.01 ± 0.013	0.04 ± 0.021	0.05 ± 0.025
	Nezara viridula	0.08 ± 0.043	0.00 ± 0.000	0.08 ± 0.043
Tutii	*Desmodium* spp.			
	Hycleus apicicornis	0.28 ± 0.060	0.27 ± 0.053	0.55 ± 0.077
	Hycleus dubiosus	0.00 ± 0.000	0.10 ± 0.041	0.10 ± 0.041
	Helicoverpa armigera	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000
	Nezara viridula	0.55 ± 0.007	0.38 ± 0.087	0.93 ± 0.151
	Sweetpotato			
	Hycleus apicicornis	0.71 ± 0.091	0.12 ± 0.046	0.83 ± 0.095
	Hycleus dubiosus	0.12 ± 0.046	0.00 ± 0.000	0.12 ± 0.046
	Helicoverpa armigera	0.01 ± 0.010	0.00 ± 0.000	0.01 ± 0.010
	Nezara viridula	0.05 ± 0.022	0.20 ± 0.064	0.25 ± 0.069
East Bukusu	Sweetpotato			
	Hycleus apicicornis	0.39 ± 0.071	0.44 ± 0.070	0.83 ± 0.106
	Hycleus dubiosus	0.02 ± 0.014	0.02 ± 0.014	0.04 ± 0.020
	Helicoverpa armigera	0.00 ± 0.000	0.02 ± 0.014	0.02 ± 0.014
	Nezara viridula	0.04 ± 0.020	0.01 ± 0.010	0.05 ± 0.022

2.4 Discussion

Information from the farmers was unlikely to be biased by gender because this was balanced. Likewise, the majority of farmers had attended school; therefore, opinions regarding knowledge of different pests were not likely to be influenced by different levels of education between different locations. Although farmers were producing all their crops on very small areas of less than one hectare, most farmers were growing six or more crops, most of which were intercropped. This practice was useful for assessing the occurrence of blister beetle, because farmers' responses to the questionnaire were not based only on their desmodium crop.

Responses to the questionnaire revealed that farmers were generally aware of the blister beetles even before pictures were shown to them. This was the case for both desmodium and non-desmodium growing farmers and was confirmed by correct identification by the farmers of the plant parts that are damaged by blister beetles on different host plants. Flowers were mentioned as the most severely damaged plant organs, followed by seed. Blister beetle damage of developing seeds has been observed on desmodium in this study (L.N. Lebesa, unpublished observations). Damage on seeds by blister beetles has also been documented on other crops such as pearl millet (Zethner & Laurense 1988; Lale & Sastawa 2000) and a prairie legume (Evans *et al*. 1989).

In addition to desmodium, blister beetles were also mentioned by the majority of farmers growing beans and sweetpotato but by fewer farmers growing maize and cowpea. Cowpea was cultivated by fewer farmers making it less available as a host. However, a multiple choice feeding study in the laboratory confirmed that cowpea was the least preferred crop compared with desmodium, sweetpotato, okra, and beans (L.N. Lebesa, unpublished observations). On maize, *H. apicicornis* has been observed to feed on pollen only (L.N. Lebesa unpublished observations), hence could not have had a serious impact on maize yields and may account for the small number of farmers who noticed its presence on plants. However, in some crops, feeding on pollen by blister beetles may affect the number of grains and thereby reduce yields as was observed with pearl millet in West Africa (Gahukar 1984; Lale & Sastawa 2000). Other than blister beetles, the African bollworm, *H. armigera* was also mentioned by a few farmers as occurring on desmodium. This observation was supported by field sampling where the pest was found in very low numbers (<10 larvae at each site), although *H. armigera* can be a problem for desmodium seed production (Boonman 1993).

Although blister beetles were mentioned as pests by most respondents, few farmers were using any control measures. This could be attributed to a number of factors, some of which were highlighted in the interviews. These included lack of knowledge of effective control methods and non-availabilty and/or high cost of chemical pesticides, which have been been also mentioned previously (Wightman & Wightman 1994; Mendesil *et al*. 2007). The practice of collecting beetles and crushing them, mentioned by a few farmers, may be harmful since farmers handle the beetles with bare hands. Blister beetles produce cantharidin (McComick & Carrel 1987; Nikbakhtzadeh & Tirgari 2002), a toxic terpenoid responsible for blistering if it comes into contact with skin (Carrel & Eisner 1974; McComick & Carrel 1987). Levels of cantharidin in *Hycleus* spp. have been found to vary from negligible concentrations to high levels (Mebs *et al*. 2009). Although in this particular study, farmers did not complain about blistering, suggesting that levels may be low, there may be need for caution in this regard in the longer term.

Farmers rated blister beetle damage as more serious on the seed bulking plots (desmodium monocrops) than on the 'push-pull' stands (desmodium intercropped with maize). This is most likely due to less efficient location of desmodium (the preferred host) by the pest in a 'push-pull' system. It is also possible that the impact on desmodium is lower in the mixed stands because beetles alternate between the two hosts to obtain different food resources to balance nutritional requirements (Cook *et al*. 2004; Bernays & Minkenberg 1997; Miura & Ohsaki 2004; Marques *et al*. 2000).

Although the field survey to determine the abundance of blister beetles on different crop hosts was carried out during the short rainy season only, which is characterized by low populations of blister beetles, the outcome largely supported the perceptions of farmers. According to the farmers, the abundance of blister beetles on desmodium is slightly higher than on sweetpotato. However, this was not the case in both sites sampled. On the other hand, follow-up collections of *H. apicicornis* on both crops at full flowering stage were almost the same on the two crops (L. Lebesa, unpublished observations). However, the blister beetle, *H. dubiosus* and the bollworm *H. armigera* were more abundant on sweetpotato than on desmodium. Moreover, although the abundance of blister beetles on desmodium was higher at the Marakaru than the Tutii sub-location, where blister beetles were more abundant on sweetpotato than on desmodium, an overall comparison shows that occurrence of beetles was similar on the two crops. Thus, sweetpotato seems to be a good alternative host for this pest, and it would be interesting to explore the effect of intercropping sweetpotato with desmodium in seed production systems as a tactic in the management of blister beetles.

In conclusion, the results of this survey show that the famers in the study area are cognisant of the blister beetles, especially *H. apicicornis*, as a pest of desmodium. Their perceptions have been confirmed by field surveys. If not controlled, blister beetles have the potential to reduce the yield of desmodium seed. This in turn may negatively affect the success of the 'push-pull' system of crop protection that requires desmodium as a companion plant to suppress *Striga* (Cook *et al*. 2007).

Acknowledgements

Research funds for this study were provided by Gatsby Charitable Foundation (UK) and the Kilimo Trust (East Africa). DAAD, the German Academic Exchange Service provided a grant to cover living expenses in Kenya for the first author. The University of Pretoria and the Lesotho Government are also acknowledged for providing both financial and logistical support in South Africa. We would like to express our gratitude to the following persons Prof. Marco Bologna, Roma TRE University who identified the blister beetles free of charge; Mr J. Pitchaar and Dr A. Amudavi for commenting on the design of the questionnaires; Dr E.M. Njuguna, and anonymous referees for critical evaluation of the manuscript; Dr C. Midega for his comments on an earlier version of this work; the icipe field staff based in Bungoma and Kitale, specifically Aloice Ndiege and Phillip Akelo, who provided logistical support.

References

Agnew, A.D.Q. & Agnew, S. (1994) *Upland Kenya wild flowers – a flora of the ferns and herbaceous flowering plants of upland Kenya.* 2nd edition. East Africa Natural History Society, Nairobi, Kenya.

Abate, T. & Ampofo, J.K.O. (1996) Insect pests of beans in Africa: their ecology and management. *Annual Review of Entomology* **41**: 45-73.

Bernays, E.A. & Minkenberg, O.J.M. (1997) Insect herbivores: different reasons for being a generalist. *Ecology* **78**: 1157-1169.

Bologna, M.A. & Pinto, J.D. (2002) The Old World genera of Meloidae (Coleoptera): a key and synopsis. *Journal of Natural History* **36**: 2013-2102.

Boonman, J.G. (1993) East Africa's grasses and fodders: their ecology and husbandry. London: Kluwer Academic Publishers. Chapter 10, Legumes in sown and grazed pastures; p.190-206.

Carrel, J.E. & Eisner, T. (1974) Cantharidin: potent feeding deterrent to insects. *Science* **183**: 755-757.

Cook, S.M., Murray, D.A. & Williams, I.H. (2004) Do pollen beetles need pollen? The effect of pollen on oviposition, survival, and development of a flower-feeding herbivore. *Ecological Entomology* **29**: 164–173.

Cook, B.G., Pengelly, B.C., Brown, S.D., Donnelly, J.L., Eagles, D.A., Franco, M.A., Hanson, J., Mullen, B.F., Partridge, I.J., Peters, M. & Schultze-Kraft, R. (2005) *Tropical Forages: an interactive selection tool [CD-ROM].* Brisbane, Australia, CSIRO, DPI&F (Qld), CIAT and ILRI.

Cook, S.M., Khan, Z.R. & Pickett, J.A. (2007) The use of push-pull strategies in integrated pest management. *Annual Review of Entomology* **52**: 375-400.

Ebenebe, A.A., Van den Berg, J. & Van der Linde, T.C. (2001) Farm management practices and farmers' perceptions of stalk-borers of maize and sorghum in Lesotho. *International Journal of Pest Management* **47**: 41-48.

Evans, E.W., Smith, C.C. & Gendron, R.P. (1989) Timing of reproduction in a prairie legume: seasonal impacts of insects consuming flowers and seeds. *Oecologia* **78**: 220-230.

Gahukar, R.T. (1984) Insect pests of pearl millet in West Africa: a review. *Tropical Pest Management* **30**: 142-147.

Gahukar, R.T. (1991) Pest status and control of blister beetles in West Africa. *Tropical Pest Management* **37**: 415-420.

Hall, M.J.R. (1984) Trap-oriented behaviour of red-banded blister beetles, *Mylabris designata* var. *hacolyssa* Rochebrune (Coleoptera: Meloidae), in the Sudan. *Bulletin of Entomological Research* **74**: 103-112.

Healey, J.F. (1990) Statistics: A tool for social research. 2nd edition. Belmont, California. Wadsworth Publishing Co.

Hill, D.S. (1975) Agricultural insect pests of the tropics and their control. 1st edition. London. Cambridge University Press.

Hillocks, R.J., Minja, E., Mwaga, A., Nahdy, M.S. & Subrahmanyam, P. (2000) Diseases and pests of pigeonpea in eastern Africa: a review. *International Journal of Pest Management* **46**: 7-18.

Khan, Z.R., Pickett, J.A., Van den Berg, J., Wadhams, L.J. & Woodcock, C.M. (2000) Exploiting chemical ecology and species diversity: stem borer and striga control for maize and sorghum in Africa. *Pest Management Science* **56**: 957-962.

Khan, Z.R., Hassanali, A., Overholt, W., Khamis, T.M., Hooper, A.M., Pickett, J.A., Wadhams, L.J. & Woodcock, C.M. (2002) Control of witchweed *Striga* *hermonthica* by intercropping with *Desmodium* spp., and the mechanism defined as allelopathic. *Journal of Chemical Ecology* **28**: 1871-1885.

Khan, Z.R., Pickett, J.A., Wadhams, L.J., Hassanali, A. & Midega, C.A.O. (2006a) Combined control of *Striga hermonthica* and stemborers by maize–*Desmodium* spp. intercrops. *Crop Protection* **25**: 989–995.

Khan, Z.R., Midega, C.A.O., Hassanali, A., Pickett, J.A., Wadhams, L.J. & Wanjoya, A. (2006b) Management of witchweed, *Striga hermonthica*, and stemborers in sorghum, *Sorghum bicolor*, through intercropping with greenleaf desmodium, *Desmodium intortum*. *International Journal of Pest Management* **52**: 297–302.

Khan, Z.R., Midega, C.A.O., Amudavi, D.M., Hassanali, A. & Pickett, J.A. (2008a) On-farm evaluation of the 'push-pull' technology for the control of stemborers and striga weed on maize in Western Kenya. *Field Crops Research* **106**: 224–233.

Khan, Z.R., Amudavi, D.M., Midega, C.A.O., Wanyama, J.M. & Pickett J.A. (2008b) Farmers' perceptions of a 'push-pull' technology for control of cereal stemborers and *Striga* weed in western Kenya. *Crop Protection* **27**: 976-987.

Kyneb, A. & Toft, S. (2006) Effects of maternal diet quality on offspring performance in the rove beetle *Tachyporus hypnorum*. *Ecological Entomology* **31**: 322-330.

Lale, N.E.S. & Sastawa, B.M. (2000) Evaluation of host plant resistance, sowing date modification and intercropping as methods for the control of *Mylabris* and *Coryna* species (Coleoptera: Meloidae) infesting pearl millet in the Nigerian Sudan savanna. *Journal of Arid Environments* **46**: 263–280.

Lebesa, L.N., Khan, Z.R., Hassanali, A., Pickett, J.A., Bruce, T.J.A., Skellern, M. & Krüger, K. (2011) Responses of the blister beetle *Hycleus apicicornis* to visual stimuli. *Physiological Entomology* **36**: 220-229.

Marques, E.S.A., Price, P.W. & Cobb, N.S. (2000) Resource abundance and insect herbivore diversity on woody Fabaceous desert plants. *Environmental Entomology* **29**: 696-703.

Mebs, D., Pogoda, W., Schneider, M. & Kauert, G. (2009) Cantharidin and demethylcantharidin (palasonin) content of blister beetles (Coleoptera: Meloidae) from southern Africa. *Toxicon* **53**: 466-468.

Mendesil, E., Abdeta, C., Tesfaye, A., Shumeta, Z. & Jifar, H. (2007) Farmers' perceptions and management practices of insect pests on stored sorghum in southwestern Ethiopia. *Crop Protection* **26**: 1817-1825.

Miura, K. & Ohsaki, N. (2004) Diet mixing and its effect on polyphagous grasshopper nymphs. *Ecological Research* **19**: 269-274.

Nikbakhtzadeh, M.R. (2004) Transfer and distribution of cantharidin within selected members of blister beetles (Coleoptera: Meloidae) and its probable importance in sexual behaviour. *PhD Dissertation*. Universität Bayreuth. Bayreuth, Germany

Nikbakhtzadeh, M.R. & Tirgari, S. (2002) Cantharidin component of Iranian blister beetles (Col: Meloidae) and their differences between Iranian and exotic species. *Iranian Journal of Public Health* **31**: 113-117.

Obopile, M., Munthali, D.C. & Matilo, B. (2008) Farmers' knowledge, perceptions and management of vegetable pests and diseases in Botswana. *Crop Protection* **27**: 1220-1224.

Özbek, H. & Szaloki, D. (1998) A contribution to the knowledge of the Meloidae (Coleoptera) fauna of Turkey along with new records. *Trends in Journal of Zoology* **22**: 23-40.

[PANESA/ARNAB] Pastures Network for Eastern and Southern Africa/African Research Network for Agricultural By-Products. (1990) Utilization of research results on forage and agricultural by-product materials as animal feed resources in Africa.

Proceedings of the first joint workshop held in Lilongwe, Malawi; 1988 Dec 5-9; Addis Ababa, Ethiopia. PANESA/ARNAB.

Paterson, R.T., Karanja, G.M., Roothaert, R.L., Nyaata, O.Z. & Kariuki, I.W. (1998) A review of tree fodder production and utilization within smallholder agroforestry systems in Kenya. *Agroforestry Systems* **41**: 181–199.

Pickett, J.A. (1998) Extended summaries, SCI Pesticides Group Meeting Semiochemicals in Integrated Crop Management: Commercial prospects. *Pesticide Science* **54**: 290-299.

Pickett, J.A., Hamilton, M.L., Hooper, A.M., Khan, Z.R. & Midega, C.A.O. (2010) Companion cropping to manage parasitic plants. *Annual Review of Phytopathology* 48: 161-177.

Scholtz, C.H. & Holm, E. (1986) Insects of Southern Africa. Butterworth Publishers (Pty) Ltd: Mayville, Durban.

Selander, R.B. (1986) Rearing blister beetles (Coleoptera, Meloidae). *Insecta Mundi* **1**: 209-220.

Tanzubil, P.B. & Yakubu, E.A. (1997) Insect pests of millet in Northern Ghana. 1. Farmers' perceptions and damage potential *International Journal of Pest Management* **43**: 133-136.

Tefera, T. (2004) Farmers' perceptions of sorghum stem-borer and farm management practices in eastern Ethiopia. *International Journal of Pest Management* **50**: 35–40.

Wightman, J.A. & Wightman, A.S. (1994) An insect, agronomic and sociological survey of groundnut fields in southern Africa. *Agriculture, Ecosystems & Environment* **51**: 311-331.

Zethner, O. & Laurense, A.A. (1988) The economic importance and control of the adult blister beetle *Psalydolytta fusca* Olivier (Coleoptera: Meloidae). *Tropical Pest Management* **34**: 407-412.

Zhu, F., Lei, C-L. & Xue, F. (2005) The morphology and temperature-dependent development of *Mylabris phalerata* Pallas (Coleoptera: Meloidae). *Coleoptrist Bullettin* **59**: 521–527.

CHAPTER 3

Responses of the blister beetle *Hycleus apicicornis* to visual stimuli

Paper published as: Lebesa, L.N., Khan, Z.R., Hassanali, A., Pickett, J.A., Bruce, T.J., Skellern, M. & Krüger, K. (2011) Responses of the blister beetle *Hycleus apicicornis* to visual stimuli. *Physiological Entomology* **36**: 220-229.

Abstract

Insect attraction to host plants may be partly mediated by visual stimuli. In this study, the responses of adult *Hycleus apicicornis* (Guér.) (Coleoptera: Meloidae) to plant models of different colours, different combinations of two colours, or three hues of blue of different shapes were compared. Single-colour models comprised the colours sky blue, bright green, yellow, red, white and black. Sky blue (reflecting light in the 440 nm – 500 nm region) was the most attractive followed by white, which reflected light in a broader range (400-700 nm). On landing on sky blue targets, beetles exhibited feeding behaviour immediately. When different hues of blue (of different shapes) were compared, sky blue was preferred over turquoise followed by dark blue, indicating that *H. apicicornis* is more attracted to lighter hues of blue than to darker ones. No significant differences were found between the three shapes (circle, square and triangle) tested, suggesting that reflectance associated with colour could be a more important visual cue than shape for host location by *H. apicicornis*. The preference of *H. apicicornis* for sky blue can be exploited in the future for designing an attractive trap for its management.

Key words: Blister beetles; colour targets; shape; spectral reflectance; visual cues.

3.1 Introduction

Insects make use of stimuli from their external environment, including visual and olfactory cues, to detect resources appropriate for their different needs, including their preferred hosts and mates (Bernays & Chapman 1994; Rojas & Wyatt 1999; Raguso & Willis 2002; Schmera *et al*. 2004; Campbell & Borden 2006a). Colour plays a major role in the visual ecology of insects for locating their mates (Allan *et al*. 1987; Brunton & Majerus 1995; Jiggins *et al*. 2001) and for locating and identifying food resources (Menzel 1979; Harris & Miller 1983; Kelber *et al*. 2003b; Zaccardi *et al*. 2006). It is especially important for flower-visiting insects to discriminate between different flowers (Bernays & Chapman 1994; Balkenius & Kelber 2004; Hirota & Kato 2004; Goyret *et al*. 2007). Depending on species, certain cues (visual or olfactory) work at longer range for orientation towards the resource, while others are employed at short range for landing or identifying the resources (Raguso 2001; Ômura & Honda 2005; Balkenius *et al*. 2006; Campbell & Borden 2006b; Fukaya *et al*. 2006; Jönsson *et al*. 2007; Kulahci *et al*. 2008). The size, shape and colour of a potential host plant are often the three key components allowing an insect to differentiate visually between host and non-host plants (Prokopy & Owens 1983).

Colour can play a critical role in the visual attraction of insects irrespective of whether true colour vision or wavelength-specific behaviour is involved (Menzel 1979). True colour vision has been confirmed in a few orders such as Hymenoptera (Chittka & Menzel 1992; Giurfa *et al*. 1997; Kelber *et al*. 2003b; Giurfa 2004; Dyer & Chittka 2004a,b) and Lepidoptera (Weiss 1997; Kelber *et al*. 2003a,b; Goyret & Raguso 2006). However, in other groups such as Coleoptera the presence or absence of true colour vision has not been established, although in some studies beetles show behaviours associated with colour detection (Hausmann *et al*. 2004; Döring & Skorupski 2007).

The ability of insects to perceive and detect colours enables them to make use of these as indicators of plant identity (Prokopy *et al*. 1983a; Lunau 1993) and quality (Döring & Skorupski 2007). In such cases, colours either mediate long-range orientation of an insect towards its host or landing on its host (Prokopy *et al*. 1983a,b). If the colour that stimulates an insect for host searching is known it can be employed singly or in combination with other stimuli to manipulate its behaviour in pest management. Traps of certain colours and shapes attractive to specific insect taxa have been used for monitoring pest populations (Epsky & Heath 1998; Blight & Smart 1999) or mass trapping of pests (Hesler & Sutter 1993).

Hycleus apicicornis (Guér.) (Coleoptera: Meloidae) is one of approximately five *Hycleus* Latreille species in western Kenya that have been observed feeding on floral parts of crops such as *Desmodium* spp., sweetpotato, cowpea and okra (L.N. Lebesa, unpublished observations). *Desmodium* spp. intercropped with maize or sorghum are an important component of ‘push-pull’ technology in the control of stemborers (Lepidoptera) and *Striga* spp. weeds (Khan *et al*. 2000, 2002, 2008). Production of *Desmodium* spp. seed is affected adversely by adult *Hycleus* spp. that feed on flowers. Consumption of flowers by blister beetles affects seed setting negatively (Evans *et al*. 1989; Gahukar 1991; Lale & Sastawa 2000). The flowers of different host plants of *H. apicicornis* differ in shape, size and colour, although the majority of them are hues of violet colours, as perceived by the human eye. By contrast to adults, larvae are carnivorous. Females lay their eggs in the soil. Upon hatching larvae search for and feed on grasshopper eggs (Nikbakhtzadeh 2004).

H. apicicornis may be making use of visual cues associated with flowers of host plants together with olfactory cues to locate their food resources. In this study, the behavioural responses of *H. apicicornis* to different colours (and associated reflectance spectra) and shapes were evaluated to determine the role of visual cues in the location of floral food resources by the beetle, and as part of the development of an efficient trapping system for this beetle.

3.2 Materials and Methods

Insects

Because *H. apicicornis* larvae are cannibalistic and go into diapause for extended periods, rearing of blister beetles proved difficult (Selander 1986). Therefore, field-collected adults were used in the experiments. To ensure representation of beetles that had learned to search for flowers of different host plants in different locations, adults were collected from different host species in three districts: on-station plots of silverleaf desmodium (*Desmodium uncinatum* at ICIPE, Thomas Odhiambo Campus, Mbita (0°25’S, 34°12’E, 1200 m a.s.l.), sweetpotato and beans fields in Bungoma (0°34´S, 34°30´E, app. 1,700 m a.s.l.), and from desmodium and sweetpotato fields in Kitale (1°28'S, 37°16' E, app. 1890 m a.s.l). In addition, adults were also collected on morning glory, *Ipomoea hildebrandtii*, a wild alternate host widely found at Mbita and neighbouring places.

The insects were kept in cages in the laboratory and maintained on a mixed diet of differently coloured fresh flowers of different host plant species (silverleaf desmodium; morning glory; sweetpotato (*Ipomoea batatas*, Convolvulaceae); okra (*Abelmoschus esculentus*, Malvaceae); cowpea (*Vigna unguiculata*, Fabaceae) and bean (*Phaseolus vulgaris*, Fabaceae)). A stock of beetles was maintained by regular collection in the field throughout the duration of the experiments. Field-collected insects used in the experiments were maintained in the laboratory for a minimum of one week for acclimatization and were not kept longer than three months.

Choice Experiments of Response to Visual Stimuli

Three different sets of experiments were conducted using three different types of paper models as colour targets for *H. apicicornis*. A preliminary trial showed that when beetles were exposed to non-shiny cotton fabric targets and to printed papers they landed more frequently on the latter indicating that they preferred the reflective paper surfaces. Therefore, colours for the targets were generated using the RGB (red:green:blue) colour coordinates system and reproduced on 80 GSM printing paper (P.T. Pindo Deli Pulp & Paper Mills, Karawang, Indonesia) using an HP Laserjet 2600 printer. All three experiments were undertaken in a screenhouse (12 m length, x 7 m width x 8 m height), screen-walled (black screen mesh on the side walls and plexi-glass for the back and front walls and roof top) (Seyoum *et al.* 2002). Plant models were placed 4 m from the rear side of the screenhouse, and *c.* 7 m from the front side. Targets were rearranged after observing every 10 individuals and papers were changed daily.

Adult beetles were separated by sex and starved for 25-26 h prior to use. Beetles were released at the middle point on the ground 3 m from colour targets. The ground was selected as the release point because beetles were observed to take off more readily after orientating themselves on the ground. Numbers of beetles landing on targets were quantified as in a previous study by Harris *et al.* (1993). Beetles were recorded as having made a choice when they took off from the ground, landed on a target and exhibited feeding behaviour. To avoid any interference or influence of conspecifics in their choice, one beetle was released at a time and was removed after making a choice. Each beetle was given 5 min to make a choice. If a beetle did not take off from the ground within 3 min it was excluded from the experiment.

To reduce variation in behaviour, observations for all experiments were made on cloud-free days between 10h00 and 13h30, the time at which *H. apicicornis* is most active. Experiments were run for six to seven consecutive days using new sets of beetles every day. Depending on the availability of beetles, 30 to 70 beetles were used per day. Light intensity (daylight) averages throughout the study period ranged from 55983 to 67700 lux. Means for temperature and relative humidity were 35 °C and 66 % RH, respectively. Daily measurements for light, temperature and relative humidity were recorded with a digital light meter (RS 180-7133, RS Components, Corby, United Kingdom) and a dual Tinytag data logger for temperature and RH, TGP 4500 (Gemini data Loggers (UK) Ltd), respectively.

Spectral reflectance measurements of coloured inks printed on white paper

The reflectance spectra of the coloured inks on white paper and of the white paper on which they had been printed, were measured using a Stellarnet EPP2000C spectroradiometer (Stellarnet Inc., Oldsmar, Florida, USA; calibrated to an operating range of 300 – 850 nm). The instrument was calibrated against a high-reflectivity white standard (SRS-99-010, Labsphere Inc., North Sutton, New Hampshire, USA). Measurements were taken at a distance of 35 cm below an overhead light source, which emitted daylight wavelengths. The sensor of the spectroradiometer was positioned 5 cm away from the printed samples or reflectance standard, and at an angle of 45 degrees to prevent shading. The spectra were recorded in 0.5 nm steps, and the reflectance of the sample expressed as a percentage of that reflected by the white standard at each wavelength.

Experiment 1: models with single colour and different heights

To determine the attractiveness to beetles of the different colours as perceived by the human eye, six individual colours using the RGB colour system were used as paper targets in plant models (sky blue (0:204:255), bright green (0:255:0), yellow (255:255:0), red (255:0:0), white (255:255:255) and black (0:0:0)).

Blister beetles feed on flowers of different heights. Therefore, two different heights were evaluated to determine the preferred height for the subsequent experiments in this study, and later, for the field trapping experiments. The models were constructed from five 4 x 4 cm^2 printed papers affixed on a wooden stick. Short plants were simulated by placing the models at 0.5 m and tall plants at 1 m above ground. Adults of *H. apicicornis* feed on flowers of different shapes and sizes, making it difficult to have one flower model representing all. Therefore, paper pieces were arranged to mimic the appearance of flowers of the target host plant, *D. uncinatum,* which look like small leaflets (Fig. 1, model A).

Twenty-four plant models (12 short, 12 tall) were placed in a straight line at a spacing of 0.3 m. Heights and colours were arranged randomly with the restriction that same heights and/or colours were not placed next to one another.

Experiment 2: models with different colours combined and different heights

To determine if colour contrasts enhance landing preference, two colours in combination were used in one plant model. Sky blue was the most attractive colour in Experiment 1. Therefore, sky blue and one of the other colours (except black, because it did not attract any beetles) were combined and used together in plant models as is explained in Experiment 1. The model was similar to that in the first experiment except blue paper squares were alternated with another colour, thus six squares instead of five were used. The blue and the other colour were assigned randomly to either side of the model (Fig. 1, model B). As in Experiment 1, models were evaluated at two different heights, 0.5 m and 1 m above ground. A sky blue, leaf-like model used in Experiment 1 was included for comparison. Twenty plant models (10 short, 10 tall) were placed in a straight line at a spacing of 0.3 m. Heights and colours were arranged randomly with the restriction that same heights and/or colours were not placed next to one another.

Experiment 3: models of shapes and hues of blue

Because *H. apicicornis* adults feed on flowers of different shapes, the response of adult beetles to shapes and hues of blue (which was the most preferred colour) was evaluated. Three hues, sky blue (0:204:255), turquoise (0:255:255) and dark blue (0:0:128), and three shapes, square (8 x 8 cm), circle (9 cm diameter) and triangle (14 cm, 9 cm, base and height respectively), were used in combination (Fig. 1, model C). Printed papers with different combinations of hues of blue and shape were affixed on wooden sticks to serve as plant models. A sky blue, leaf-like model used in Experiment 1 was included as control. For this experiment, only short plant models (0.5 m above ground) were used. Twenty models were placed in a straight line at a spacing of 0.3 m. Each of the models was represented twice in the line. Shapes and different hues of blue were arranged randomly with the restriction that similar hues were not placed next to each other.

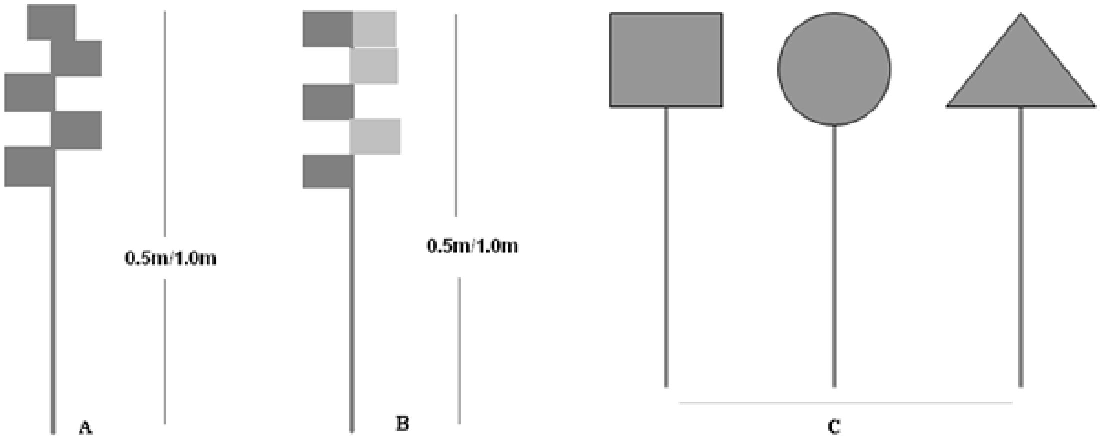


Figure 1. A schematic drawing illustrating the plant models used as colour targets for adult blister beetles. Model A was used to determine the most attractive colour(s). Each plant model consisted of five (4 x 4cm) square pieces of the same colour (Experiment 1). Model B consisted of the most attractive colour (blue), determined in Experiment 1, in combinations with one of the following colours: green, yellow, red, or white. The lighter shade of grey in the figure represents any of the other colours while the dark grey indicates blue. Each plant model consisted of six (4 x 4 cm) square pieces: three blue and three of one of the other colours (Experiment 2). To identify the most attractive hues of blue (sky, turquoise and dark blue) and shape (triangle, square and circle) plant models C were used (Experiment 3). The height of models tested was 0.5 and 1.0 m each for models A and B to simulate short and tall plants, and 0.5 m for model C.

Statistical Analyses

Heterogeneity tests were carried out to determine whether data for different replicates could be pooled for subsequent analyses (Zar 1984; Sokal & Rohlf 1969). When data were homogeneous chi-square tests for testing the goodness-of-fit were carried out on pooled data. Where landing responses were found to differ significantly, paired chi-square tests corrected with the Bonferroni adjustment (Norman & Streiner 1994) were used. Data were analysed with GenStat® (Payne *et al*. 2007) and Statistica (Version 7.0, StatSoft, Inc, 1984-2004).

3.3 Results

General Observations

Prior to taking off from the point of release, generally beetles would tilt their head upwards and fan their wings just before flying. Sometimes, a beetle would spin around in one position as if surveying the whole background before take-off. Some beetles did not fly directly towards the targets but flew straight to the roof of the screenhouse, especially towards the position of the sunlight and then would wander around randomly on the roof before flying down to the targets. Upon reaching a chosen target, a beetle appeared to explore the target and start to nibble as if feeding. Sometimes a target was approached by a beetle, that circled once or several times (while still in flight) before moving away and finally landing and settling on another target. In such a situation the circled target was not considered to be a choice.

Experiment 1: models with a single colour and different heights

Of the 310 (males and females in equal numbers), beetles released in the presence of blue, green, yellow, red, white, and black plant models, 74 (24%) did not make a choice. These were excluded from the subsequent analyses. Of the other 236, 169 (71.6%) beetles landed on sky blue plant models, followed by white (12.3%), yellow (8.9%), red (4.7%) and green (2.5%). Black did not attract any beetles and models of this colour were, therefore, excluded from subsequent experiments.

There were no significant differences between the numbers of male and female beetles attracted to the targets ($\chi^2 = 1.373$, df = 1, $P = 0.241$) (Fig. 2a), so subsequent analyses were based on data pooled for both sexes. With the exception of green plant models, which attracted six beetles only, short plant models were preferred as landing targets by 67 % of beetles that made a choice (Yates corrected $\chi^2 = 25.779$, df = 1, $P <$ 0.001); within each colour. 55 to 76 % of beetles landed on short plant models (Fig. 2b). Therefore, models of different heights were analysed separately.

Numbers of beetles landing on the variously-coloured models differed significantly for both heights (short plant models: $\chi^2 = 259.401$, df = 4, $P < 0.0001$; tall plant models: χ^2 = 134.987, df = 4, $P < 0.0001$) (Figs 2c,d). A comparison of the numbers attracted to the short blue, green, yellow, red, and white plant models showed that the sky blue ones differed from all others and were preferred by 71% of the beetles that landed. White models attracted 14% of beetles, yellow ones 9%, red ones 4% and green ones 2%. The number of beetles landing on white models was not significantly different from yellow but differed from red and green (Fig. 2c). Similarly, for tall plant models, 71% of beetles preferred those that were sky blue (Fig. 2d). However, unlike the models of short plants, there was no significant difference in the number of beetles landing on white (9 %), yellow (10 %), red (6 %) or green (4 %) taller models (Fig. 2d).

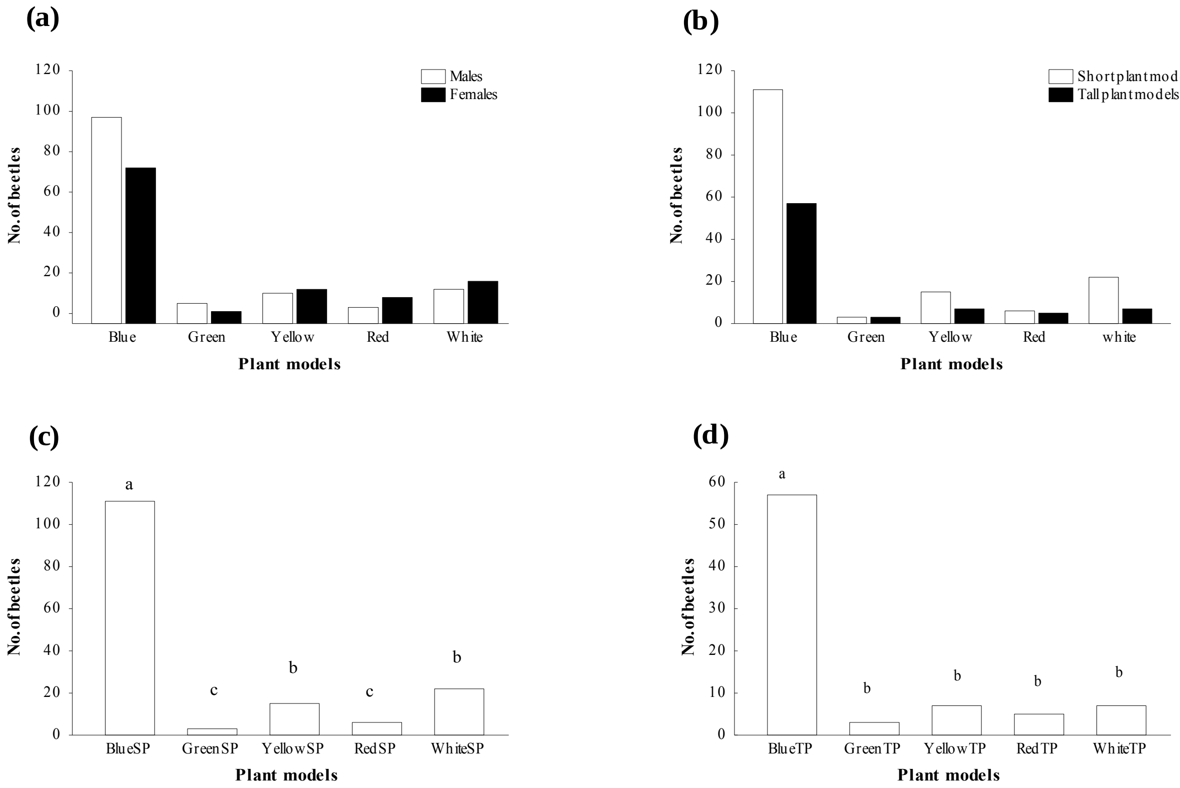


Figure 2. Response of adult blister beetles to the coloured plant models: (a) males and females (b) short and tall plant models (0.5 m and 1.0 m), (c) short plant models (SP), and (d) tall plant models (TP); blue refers to sky blue and green to bright green. Different letters above bars indicate significant differences (*P* < 0.05) (paired chi-square tests corrected with Bonferroni adjustment).

Experiment 2: models with different colours combined and different heights

A total of 290 (males and females in equal numbers) beetles were tested. Sixty one beetles (38 females and 23 males) did not make a choice and were excluded from the analyses. Of the 229 that made a choice for a specific plant model, more beetles (31%) landed on non-mixed sky blue plant models, followed by sky blue-white (26%), sky blue-red (17%), sky blue-green (14%), and sky blue-yellow (12%) models. There were no significant differences between the numbers of male and female beetles making a choice across all colour combinations ($\chi^2 = 0.983$, df = 1, $P = 0.322$) (Fig. 3a), so data for both sexes were pooled. As was observed in Experiment 1, short plant models were significantly more attractive than tall ones ($\chi^2 = 46.328$, df = 1, $P < 0.001$) (Fig. 3b) and these were analysed separately. Landing rates on different colours differed significantly for the short plant models ($\chi^2 = 42.680$, df = 4, $P < 0.0001$), while differences were not significant for the tall models ($\chi^2 = 8.349$, df = 4, $P = 0.079$) (Figs 3c,d). The non-mixed short sky blue plant model, was more attractive than short models in colour combinations (blue-green, blue-yellow, blue-red), except for the blue-white combination. Numbers of beetles landing on all colour combinations did not differ significantly (Fig. 3c).

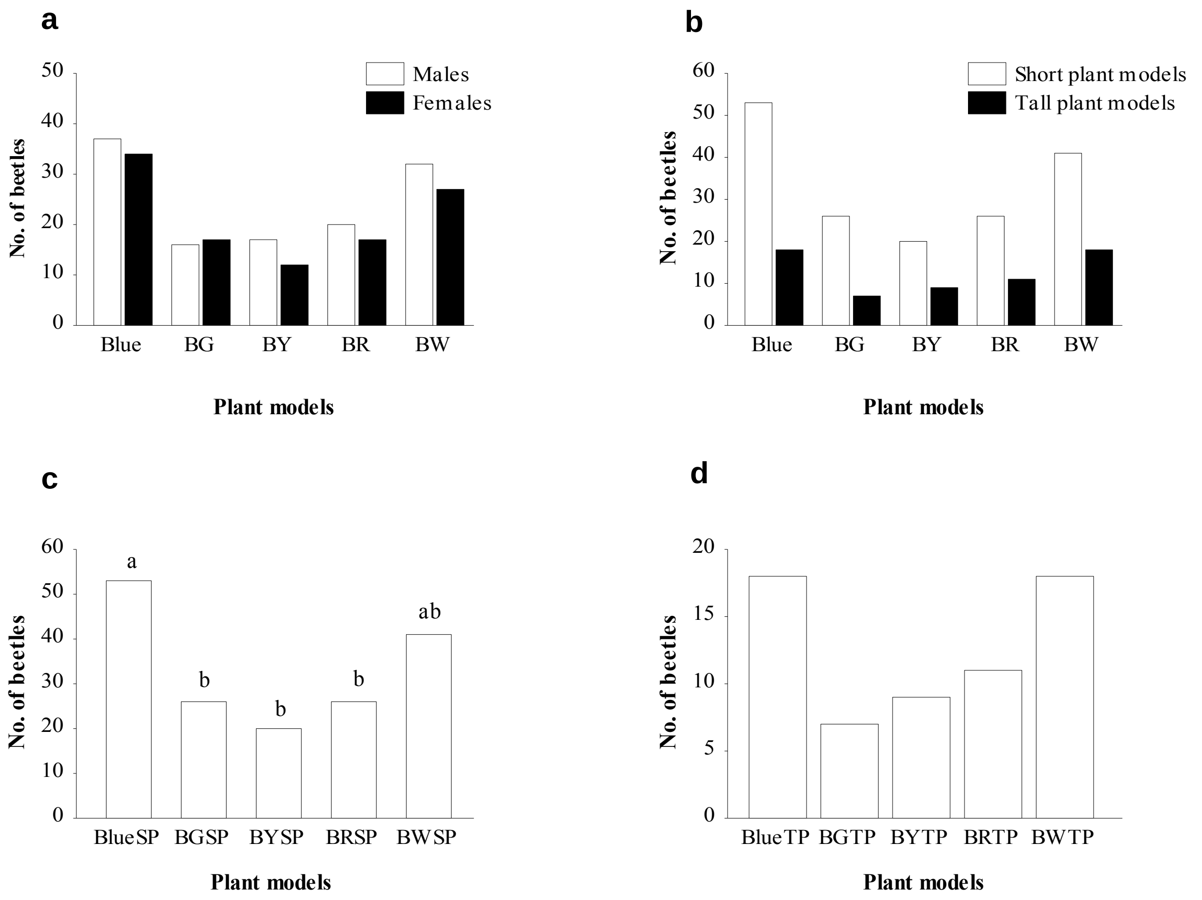


Figure 3. Responses of blister beetles to the sky blue plant models (single colour (blue) and in combination with bright green (BG), red (BR), yellow (BY), and white (BW): (a) males and females (b) short and tall plant models (0.5 and 1.0 m), and (c) short plant models (SP), and (d) tall plant models (TP). Different letters above bars indicate significant differences ($P < 0.05$) (paired chi-square tests corrected with Bonferroni adjustment).

Experiment 3: models with shapes and hues of blue

Of the 266 (males and females in equal numbers) beetles released 74% landed on one of the targets of different shapes (circle, square and triangle) or hues of blue (sky, turquoise and dark blue) combined. As was observed in the previous two experiments, there was no significant difference between the numbers of males and females landing on these plant models ($\chi^2 = 0.505$, df = 1, $P = 0.477$) (Fig. 4a) so data for both sexes were pooled.

The preference of beetles for different plant models differed significantly ($\chi^2 = 99.475$, df = 9, $P < 0.0001$). The round sky blue and square sky blue models attracted 23% of beetles each, and were the most preferred targets while least preference was observed for the square dark blue models which attracted 2% of the beetles that made a choice (Fig. 4b). The round sky blue and square sky blue models were more attractive to the beetles than the leaf-like sky blue model which attracted only 10 % of beetles.

The response of beetles towards the models was dependent on the two characteristics, shape and colour ($\chi^2 = 20.349$, df = 6, $P = 0.02$). Therefore, each type of model was analysed separately. Landing preference of beetles on plant models differed significantly between the three hues of blue ($\chi^2 = 164.419$, df = 2, $P < 0.0001$). Sky blue was preferred by 65% of beetles followed by turquoise blue (23%), and dark blue (12%) was the least preferred (Fig. 4c). When shapes were compared, choice of beetles differed significantly ($\chi^2 = 30.121$, df = 3, $P < 0.0001$). The highest number of beetles was recorded on the round (34%), square (31%) and triangular (25%) plant models. Significantly fewer beetles (9%) landed on the leaf-like shape (Fig. 4d).

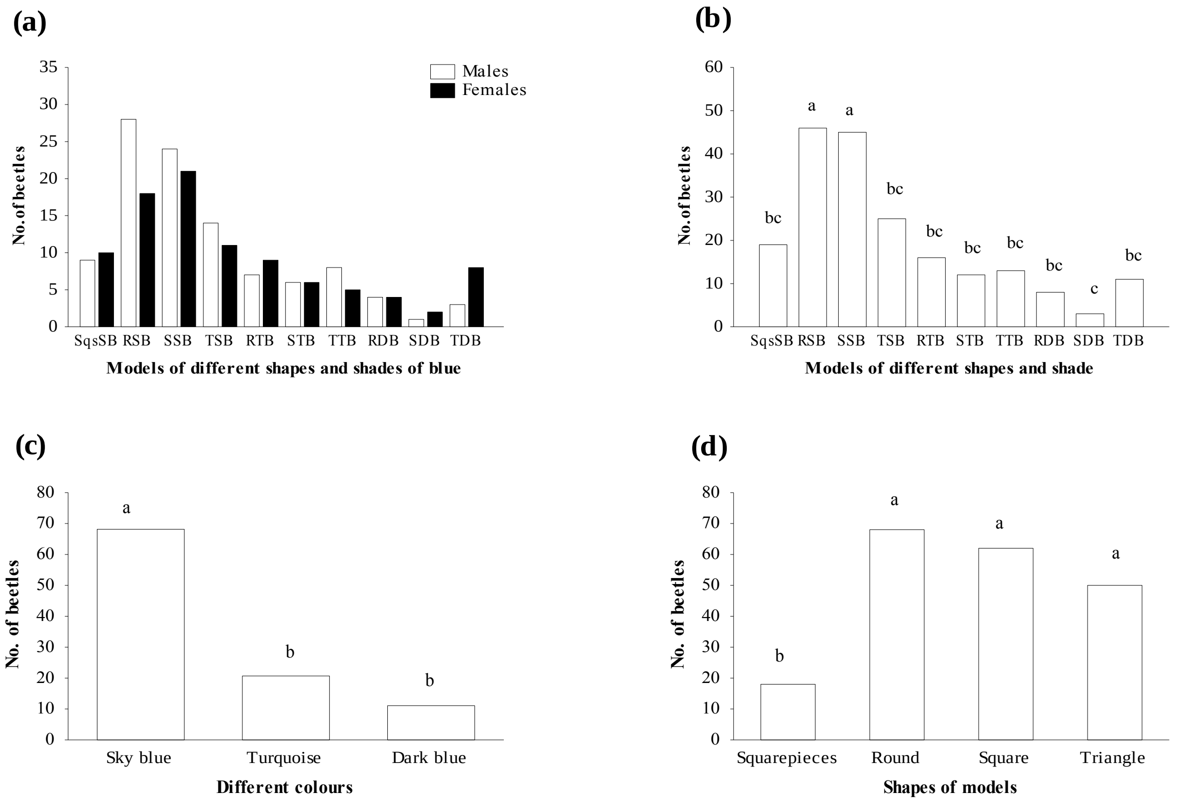
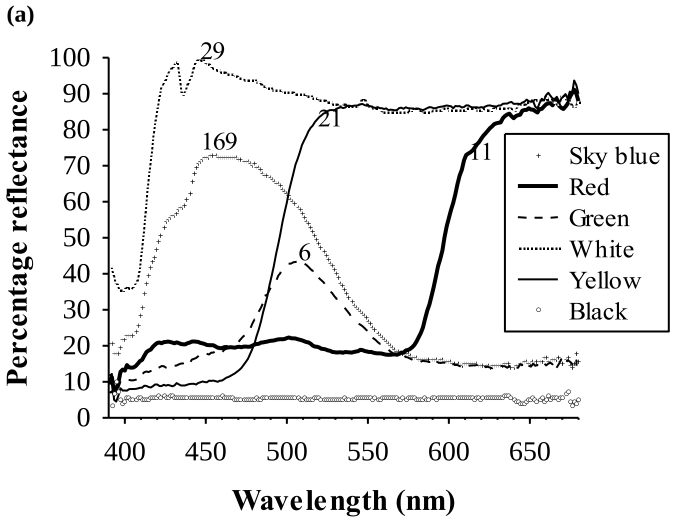


Figure 4. Responses of blister beetles to plant models of different shapes and hues of blue: (a) males and females, (b) shapes and hues combined, (c) hues of blue (SB = sky blue, TB = turquoise, DB = dark blue), and (d) shapes. LLSB = leaflike, sky blue models; RSB = round, sky blue models; SSB = square sky blue models; TSB = triangular, sky blue models; RTB = round, turquoise blue models; STB = square, turquoise blue models; TTB = triangular, turquoise blue models; RDB = round, dark blue models; SDB = square, dark blue models and TDB = triangular dark blue. Different letters above bars indicate

significant differences ($P < 0.05$) (paired chi-square tests corrected with Bonferroni adjustment).

Reflectance spectra of the coloured paper samples

The reflectance of the coloured papers used in experiments ranged from 400 – 650 nm. The highest percentage reflectance was observed with white paper whose reflectance ranged from 41 to 98% with double-peak wavelengths at 430 and 446 nm. Yellow and red had the next highest percentage reflectance (both above 80%) peaking at 530 and 630 nm, respectively. Black had the lowest percentage reflectance at around 5% (Fig. 5a). Sky blue on the other hand had the highest percentage reflectance (72%) among the hues of blue and this occurred at the peak wavelength of 450 nm while reflectance of dark blue (39%) at the dominant wavelength of 450 nm was the lowest (Fig. 5b). The data on total number of beetles responding to the different colours (including different hues of blue) described above suggest that beetles could have a high preference for colours with reflectance in the region between 440 - 500 nm (Figs 5a,b).



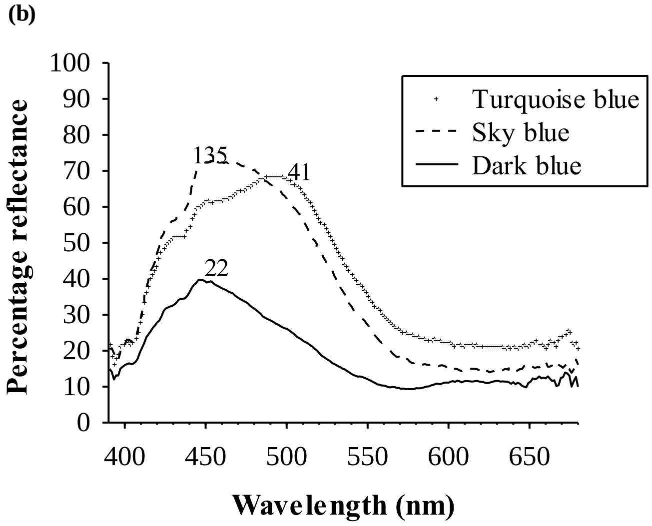


Figure 5. Spectral reflectance of printed coloured papers used as visual targets for *Hycleus apicicornis*: (a) reflectance of papers used in plant models to test attractiveness of different colours and (b) three hues of blue. The numbers close to lines show the number of beetles that landed on a colour represented by that line.

3.4 Discussion

In this study, *H. apicicornis* exhibited a strong preference for blue. This finding is similar to a previous study by Hall (1984) who caught more of another of blister beetle (*Mylabris designata* var. *hacolyssa*) in blue traps. The current study shows that white, yellow, red, and green attract relatively few beetles. However, white is the next most attractive colour after blue. The preference for sky blue followed by white is demonstrated further when evaluating plant models of two colours combined (sky blue plus any other colour except black). Whereas white paper in a blue-white combination does not elicit a significantly different response from *H. apicicornis* to plant models with blue paper alone, the presence of yellow, red or green resulted in fewer beetles landing on those models.

Black reflects minimum level of light (*c*. 5% throughout the whole reflectance spectra) and failure to attract any beetles in this study shows that *H. apicicornis* needs reflective materials to be attracted to a target. This was also observed in a preliminary experiment (L.N. Lebesa, unpublished observations) where differently coloured non-shiny cotton fabrics failed to attract *H. apicicornis* in significant numbers. Previously, Blight & Smart (1999) observed that a black coloured trap failed to attract pollen beetles, *Meligethes aeneus* (Coleoptera: Nitidulidae), presumably because of poor reflectance. Green is preferred by many insects because it falls within one of their common sensitive areas (Prokopy & Owens 1983) and presumably is associated with foliage. However, in the current study, only a few beetles landed on green plant models suggesting that even if *H. apicicornis* adults can detect green they are not attracted to it. This makes sense as adults feed on flowers not foliage. Although green and yellow have very close maximum wavelengths (510 nm and 530, respectively), they differ in their percentage reflectance (43% and 85%, respectively). Yellow attracted more beetles, indicating that reflectance intensity plays a role in attracting *H. apicicornis*.

White, which has a high reflectance (around 99%), is the next preferred colour after sky blue among short plant models and is more attractive to *H. apicicornis* than red and green. In addition to having a high percentage reflectance, white has a broader wavelength reflectance range (peaking from 430 to 650 nm) that overlaps with blue (whose maximum reflectance falls between 440-480 nm), which has the highest attraction for *H. apicicornis* Therefore, the broad wavelength reflectance covering the sky blue maximum reflectance region coupled with the high intensity could explain why white models were the next attractive models to *H. apicicornis*. This may, therefore, argue against intensity being the main factor behind the choice, because the red and yellow colours used here have intensities that are higher than that of sky blue. The high spectral reflectance (80% and above) and a broad wavelength range for white is well known from previous studies (e.g. Hardie *et al*. 1996; Strom *et al*. 1999; Yaku *et al*. 2007) despite the differing reflectance of various white papers or materials. Because the white models reflected a wider range of wavelengths , which is generally the case with white flowers as perceived by the human eye (Chittka *et al*. 1994; White *et al*. 1994; Kevan *et al*. 1996), they may also be seen as violet (as perceived by humans) flowers by *H. apicicornis*.

According to Chittka *et al*. (1994) violet, pink, purple and blue flowers (in accordance with human perception) are known as blue (bee UV-blue) flowers. Although the colour space for *H. apicicornis* is not known it is possible that majority of flowers of its host plants fall into this category. To the human eye, flowers of most host plants of *H. apicicornis* (e.g. silverleaf desmodium, sweetpotato, cowpea, morning glory and beans) have a light purplish (violet) appearance. Sky blue models attract more beetles than turquoise or dark blue targets, irrespective of shape. Of the reflectance spectra, sky blue had the highest percentage reflectance. Although percentage reflectance of turquoise was close to that of sky blue, its maximum reflectance range was slightly outside the blue region (440 nm). This indicates that *H. apicicornis* has the ability to distinguish flowers of host plants from those of similarly coloured non-host plants. This ability may be important if *H. apicicornis* uses primarily colour for locating its host plants, as is the case with the butterfly *Vanessa indica* (Lepidoptera: Nymphalidae) (Ômura & Honda 2005). It enables an insect to avoid wasting energy on visiting flowers that would not be rewarding (Chittka & Menzel 1992).

Other important traits in the visual system of insects for correct host recognition are shape and/or patterns and size of flowers (Wäckers & Lewis 1999; Ne'eman & Kevan 2001; Dyer & Chittka 2004a). Shape may be a complementary factor that ensures that insects do not make mistakes when confronted with a choice of similarly coloured host and non-host plants (Dyer & Chittka 2004a). However, in this study, shape does not seem to be of importance for *H. apicicornis*, possibly because this species feeds on several host plant species that are often unrelated and that differ in their morphological appearance. Ne'eman & Kevan (2001) suggest that floral area is important and that while larger flowers may seem more attractive, simultaneously blooming small flowers may be equally attractive because insects view them as a combined image. However, visual cues such as shape, pattern or size may not necessarily complement colour during host search and may have to be coupled with olfactory cues for an insect to make correct choices (Rojas & Wyatt 1999; Kulahci *et al*. 2008).

In the present study, there was no significant difference between the landing preferences of males and females, suggesting that the distribution of the photoreceptors in the eyes of the two sexes is similar. It is possible that *H. apicicornis* does not use visual cues for mate location or for locating oviposition sites. In insects that do, there are differences in the arrangement of receptors to allow for mate recognition (Bernard & Remington 1991; Brunton & Majerus 1995) and recognition of host plants for oviposition (Prokopy *et al*. 1983b; Kelber 1999; Briscoe & Chittka 2001).

Although spectral sensitivity of *H. apicicornis* is not known and was not determined in this study the results of the study show that adult *H. apicicornis* is attracted mainly to targets that reflect in the blue wavelength region (440-500 nm), suggesting the possibility of sensitivity of the main receptor in this region. However, follow up physiological and behavioural studies, including experiments on any relationship between colour targets and rewards (Giurfa *et al*. 1995; Kelber & Hénique 1999; Kelber *et al*. 2003a; Balkenius & Kelber 2006; Goyret & Raguso 2006), would have to be undertaken to determine whether the observed feeding behaviour in *H. apicicornis* is due to true colour vision or just wavelength dependent.

The present findings should be of use in designing traps that can be used for the management of *H. apicicornis* on *Desmodium* spp. on which they have recently become a pest in East Africa. No studies have been undertaken on possible mediation of attractive plant semiochemicals for *H. apicicornis* or indeed any other blister beetle. If such semiochemicals also play a role in attracting *H. apicicornis* they could be used together with sky blue coloured materials to design an effective trap for the management of this beetle.

Acknowledgements

Research funds for this study were provided by Gatsby Charitable Foundation (UK) and the Kilimo Trust (East Africa). DAAD, German Academic Exchange Service (Germany) provided grant for the living expenses for the first author. For providing help the authors are very grateful to Dr Sam Cook, Professor Marco Bologna, Marie Smith, ARC-Biometry Unit and technical staff of the Habitat Management section at ICIPE.

References

Allan, S.A., Day, J.F. & Edman, J.D. (1987) Visual ecology of biting flies. *Annual Review of Entomology* **32**: 297-316.

Balkenius, A. & Kelber, A. (2004) Colour constancy in diurnal and nocturnal hawkmoths. *Journal of Experimetal Biology* **207**: 3307-3316.

Balkenius, A. & Kelber, A. (2006) Colour preferences influences odour learning in the hawkmoth, Macroglossum stellatarum. Naturwissenschaften **93**: 255-258.

Balkenius, A., Rosén, W. & Kelber, A. (2006) The relative importance of olfaction and vision in a diurnal and a nocturnal hawkmoth. *Journal of Comparative Physiology A* **192**: 431-437.

Bernard, G.D. & Remington, C.L. (1991) Color vision in *Lycaena* butterflies: Spectral tuning of receptor arrays in relation to behavioral ecology. *Proceedings of the National Academy of Science* **88**: 2783-2787.

Bernays, E.A. & Chapman, R.F. (1994) *Host-plant Selection by Phytophagous Insects. Contemporary Topics in Entomology 2.* Chapman & Hall, London.

Blight, M.M., & Smart, L.E. (1999) Influence of visual cues and isothiocyanate lures on capture of the pollen beetle, *Meligethes aeneus* in field traps. *Journal of Chemical Ecology* **25**: 1501-1516.

Briscoe, A.D. & Chittka, L. (2001) The evolution of color vision in insects. *Annual Review of Entomology* **46**: 471-510.

Brunton, C.F.A. & Majerus, M.E.N. (1995) Ultraviolet colours in butterflies: intra- or inter-specific communication? *Biological Sciences* **260**: 199-204.

Campbell, S.A. & Borden, J. (2006a) Integration of visual and olfactory cues of hosts and non-hosts by three bark beetles (Coleoptera: Scolytidae). *Ecological Entomology* **31**: 437-449.

Campbell, S.A. & Borden, J. (2006b) Close-range, in-flight integration of olfactory and visual information by a host-seeking bark beetle. *Entomologia Experimentalis et Applicata* **120**: 91-98.

Chittka, L. & Menzel, R. (1992) The evolutionary adaptation of flower colours and the insect pollinators' colour vision. *Journal of Comparative Physiology A* **171**: 171-181.

Chittka, L., Shmida, A., Troje, N. & Menzel, R. (1994) Ultraviolet as a component of flower reflections, and the colour perception of Hymenoptera. *Vision Research* **34**: 1489-1508.

Döring, T.F. & Skorupski, P. (2007) Host and non-host leaves in the colour space of the Colorado potato beetle (Coleoptera: Chrysomelidae). *Entomologia Generalis* **29**: 81–95.

Dyer, A.G. & Chittka, L. (2004a) Biological significance of distinguishing between similar colours in spectrally variable illumination: bumblebees (*Bombus terrestris*) as a case study. *Journal of Comparative Physiology A* **190**: 105-114.

Dyer, A.G. & Chittka, L. (2004b) Bumblebees (*Bombus terrestris*) sacrifice foraging speed to solve difficult colour discrimination tasks. *Journal of Comparative Physiology A* **190**: 759–763.

Epsky, D.E. & Heath, R.R. (1998) Exploiting the interactions of chemical and visual cues in behavioral control measures for pest tephritid fruit flies Behavioral Ecology Symposium ’97. *Florida Entomologist* **81**: 273-282.

Evans, E.W., Smith, C.C. & Gendron, R.P. (1989) Timing of reproduction in a prairie legume: seasonal impacts of insects consuming flowers and seeds. *Oecologia* **78**: 220-230.

Fukaya, M., Wakamura, S., Arakaki, N., Yasui, H., Yasuda, T. & Akino, T. (2006) Visual ‘pinpoint’ location associated with pheromonal cue in males of the black chafer

Holotrichia loochooana loochooana (Coleoptera: Scarabaeidae). *Applied Entomology and Zoology* **41**: 99-104.

Gahukar, R.T. (1991) Pest status and control of blister beetle in West Africa. *Tropical Pest Management* **37**: 415-420.

Giurfa, M. (2004) Conditioning procedure and color discrimination in the honeybee *Apis mellifera*. *Naturwissenschaften* **91**: 228-231.

Giurfa, M., Backhaus, W. & Menzel, R. (1995) Color and angular orientation in the discriminiation of bilateral symmetric patterns in the honeybee. *Naturwissenschaften* **82**: 198-201.

Giurfa, M., Vorobyev, M., Brandt, R., Posner, B. & Menzel, R. (1997) Discrimination of coloured stimuli by honeybees: alternative use of achromatic and chromatic signals. *Journal of Comparative Physiology A* **180**: 235-244.

Goyret, J. & Raguso, R.A. (2006) The role of mechanosensory input in flower handling efficiency and learning by *Manduca sexta*. *The Journal of Experimental Biology* **209**: 1585-1593.

Goyret, J., Markwell, P.M. & Raguso, R.A. (2007) The effect of decoupling olfactory and visual stimuli on the foraging behavior of *Manduca sexta*. *The Journal of Experimental Biology* **210**: 1398-1405.

Hall, M.J.R. (1984) Trap-oriented behaviour of red-banded blister beetles, My*labris designata var. hacolyssa* Rochebrune (Coleoptera: Meloidae), in the Sudan. *Bulletin of Entomological Research* **74**: 103-112.

Hardie, J., Storer, R.J., Cook, F.J., Campbell, C.A.M., Wadhams, L.J., Lilley, R. & Peace, L. (1996) Sex pheromone and visual trap interactions in mate location strategies and aggregation by host-alternating aphids in the field. *Physiological Entomology* **21**: 97-106.

Harris, M.O. & Miller, J.R. (1983) Color stimuli and oviposition behavior of the onion fly, *Delia antique* (Meigen) (Diptera: Anthomyiidae). *Annals of the Entomological Society of America* **76**: 766-771.

Harris, M.O., Rose S. & Malsch, P. (1993) The role of vision in the host plant-finding behaviour of the Hessian fly. *Physiological Entomology* **18**: 31-42.

Hausmann, C., Samietz, J. & Dorn, S. (2004) Visual orientation of overwintered *Anthonomus pomorum* (Coleoptera: Curculionidae). *Environmental Entomology* **33**: 1410-1415.

Hesler, L.S. & Sutter, G.R. (1993) Effect of trap color, volatile attractants, and type of toxic bait dispenser on captures of adult corn rootworm beetles (Coleoptera: Chrysomelidae). *Environmental Entomology* **22**: 743-750.

Hirota, T. & Kato, Y. (2004) Color discrimination on orientation of female Eurema hecabe (Lepidoptera: Pieridae). *Applied Entomology and Zoology* **39**: 229-233.

Jiggins, C.D., Naisbit, R.E., Coe, R.L. & Mallet, J. (2001) Reproductive isolation caused by colour pattern mimicry. *Nature* **411**: 302-305.

Jönsson, M., Rosdahl, K. & Anderson, P. (2007) Responses to olfactory and visual cues by over-wintered and summer generations of the pollen beetle, *Meligethes aeneus. Physiological Entomology* **32**: 188-193.

Kelber, A. (1999) Ovipositing butterflies use a red receptor to see green. *Journal of Experimental Biology* **202**: 2619-2630.

Kelber, A. & Hénique, U. (1999) Trichromatic colour vision in the hummingbird hawkmoth, *Macroglossum stellatarum* L. *Journal of Comparative Physiology A* **184**: 535-541.

Kelber, A., Balkenius, A. & Warrant, E.J. (2003a) Colour vision in diurnal and nocturnal hawkmoths. *Integrative and Comparative Biology* **43**: 571-579.

Kelber, A., Vorobyev, M. & Osorio, D. (2003b) Animal colour vision – behavioural tests and physiological concepts. *Biological Reviews* **78**: 81-118.

Kevan, P., Giurfa, M. & Chittka L. (1996) Why are there so many and so few white flowers? *Trends in Plant Science* **1**: 280-284.

Khan, Z.R., Pickett, J.A., Van den Berg, J., Wadhams, L.J. & Woodcock, C.M. (2000) Exploiting chemical ecology and species diversity: stem borer and striga control for maize and sorghum in Africa. *Pest Management Science* **56**: 957-962.

Khan, Z.R., Hassanali, A., Overholt, W., Khamis, T.M., Hooper, A.M., Pickett, J.A., Wadhams, L.J. & Woodcock, C.M. (2002) Control of witchweed *Striga hermonthica* by intercropping with *Desmodium* spp. and the mechanism defined as allelopathic. *Journal of Chemical Ecology* **28**: 1871-1885.

Khan, Z.R., Midega, C.A.O., Amudavi, D.M., Hassanali, A. & Pickett, J.A. (2008) On-farm evaluation of the push-pull technology for the control of stemborers and striga weed on maize in western Kenya. *Field Crops Research* **106**: 224-233.

Kulahci, I.G., Dornhaus, A. & Papaj, D.R. (2008) Multimodal signals enhance decision making in foraging bumble-bees. *Proceedings of the Royal Society B* **275**: 797-802.

Lale, N.E.S. & Sastawa, B.M. (2000) Evaluation of host plant resistance, sowing date modification and intercropping as methods for the control of *Mylabris* and *Coryna* species (Coleoptera: Meloidae) infesting pearl millet in the Nigerian Sudan savanna. *Journal of Arid Environments* **46**: 263-280.

Lunau, K. (1993) Interspecific diversity and uniformity of flower colour patterns as cues for learned discrimination and innate detection of flowers. *Experientia* **49**: 1002-1010.

Menzel, R. (1979) Spectral sensitivity and color vision in invertebrates. In: Autrum, H. (Ed.), *Handbook of Sensory Physiology*, vol VII/6A. *Vision in Invertebrates* 503-580. Springer-Verlag, Berlin.

Ne'eman, G. & Kevan, P.G. (2001) The effect of shape parameters on maximal detection distance of model targets by honeybee workers. *Journal of Comparative Physiology A* **187**: 653-660.

Nikbakhtzadeh, M.R. (2004) Transfer and distribution of cantharidin within selected members of blister beetles (Coleoptera: Meloidae) and its probable importance in sexual behaviour. *PhD Dissertation*. Universität Bayreuth. Bayreuth, Germany.

Norman, G.R. & Streiner, D.L. (1994) *Biostatistics, the Bare Essentials*, Mosby-Year Book, Inc., St Louis, Baltimore.

Ômura, H. & Honda, K. (2005) Priority of color over scent during flower visitation by adult *Vanessa indica* butterflies. *Oecologia* **142**: 588–596.

Payne, R.W., Harding, S.A. Murray, D.A., Murray, D.A., Soutar, D.M., Baird, D.B., Welham, S.J., Kane, A.F., Gilmour, A.R., Thompson, R., Webster, R. & Wilson T.G. (2007) *GenStat for Windows® Introduction*. 10th edition. VSN International, Hempstead.

Prokopy, R.J. & Owens, E.D. (1983) Visual detection of plants by herbivorous insects. *Annual Review of Entomology* **28**: 337-364.

Prokopy, R.J., Collier, R.H. & Finch, S. (1983a) Leaf color used by cabbage root flies to distinguish among host plants. *Science* **221**: 190-192.

Prokopy, R.J., Collier, R.H. & Finch, S. (1983b) Visual detection of plants by cabbage root flies. *Entomologia Experimentalis et Applicata* **34**: 85-89.

Raguso, R.A. (2001) Floral scent, olfaction, and scent-driven foraging behavior. In: L. Chittka, L. & Thomson, J. (Eds.), *Cognitive Ecology of Pollination* 83-105. Cambridge University Press, Cambridge.

Raguso, R.A. & Willis, M.A. (2002) Synergy between visual and olfactory cues in nectar feeding by naïve hawkmoths, *Manduca sexta*. *Animal Behavior* **64**: 685-695.

Rojas, J.C. & Wyatt, T.D. (1999) Role of visual cues and interactions with host odour during the host-finding behaviour of the cabbage moth. *Entomologia Experimentalis et Applicata* **91**: 59-65.

Schmera, D., Tóth, M., Subchev, M. Sredkov, I. Szarukán, I. Jermy, T. & Szentesi, Á. (2004) Importance of visual and chemical cues in the development of an attractant trap for Epicometis (Tropinota) hirta Poda (Coleoptera: Scarabaeidae). *Crop Protection* **23**: 939-944.

Selander, R.B. (1986) Rearing blister beetles. *Insecta Mundi* **1**: 209-220.

Seyoum, A., Pålsson, K., Kung'a, S., Kabiru, E.W., Lwande, W., Killeen, G.F., Hassanali, A., & Knols, B.G. J. (2002) Traditional use of mosquito-repellent plants in western Kenya and their evaluation in semi-field experimental huts against *Anopheles gambiae*: ethnobotanical studies and application by thermal expulsion and direct burning. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **96**: 225-23.

Sokal, R.R. & Rohlf, F.J. (1969) *Biometry – the Principle and Practice of Statistics in Biological Research*. 2nd edition. W.H. Freeman and Company, New York.

Strom, B.L., Roton, L.M., Goyer, R.A. & Meeker, J.R. (1999) Visual and semiochemical disruption of host finding in the southern pine beetle. *Ecological Applications* **9**: 1028-1038.

Yaku, A., Walter, G.H. & Najar-Rodriguez, A.J. (2007) Thrips see red – flower colour and the host relationships of a polyphagous anthophilic thrips. *Ecological Entomology* **32**: 527-535.

Wäckers, F.L. & Lewis, W.L. (1999) A comparison of color-, shape- and pattern-learning by the hymenopteran parasitoid *Microplitis croceipes*. *Journal of Comparative Physiology A* **184**: 387-393.

Weiss, M.R. (1997) Innate colour preferences and flexible colour learning in the pipevine swallowtail. *Animal Behaviour* **53**: 1043-1052.

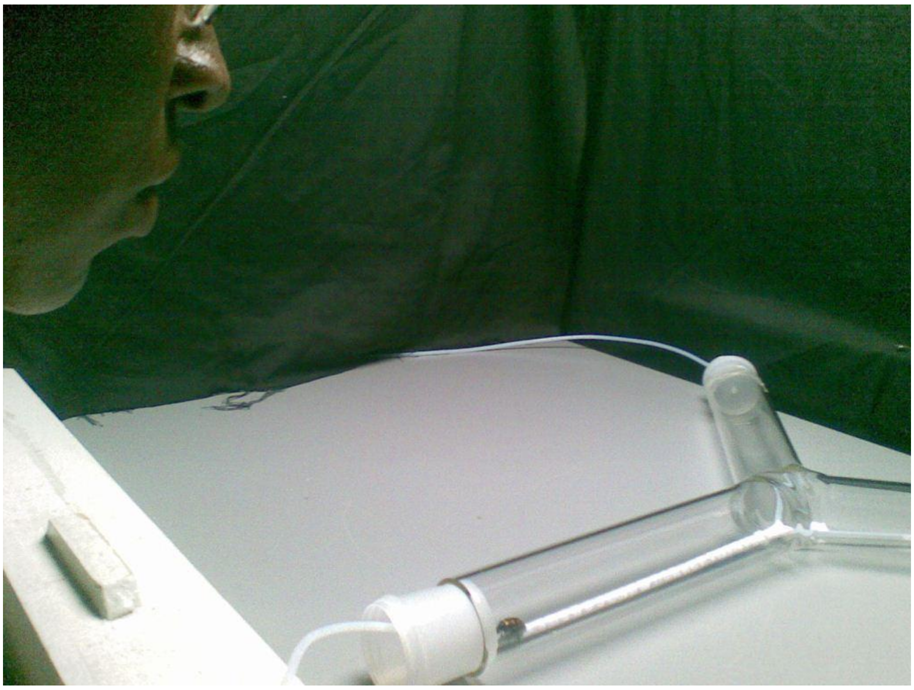
White, R.H., Stevenson, R.D, Bennett, R.R., Cutler, D.E. & Harber, W.A. (1994) Wavelength discrimination and the role of Ultraviolet vision in the feeding behavior of hawkmoths. *Biotropica* **26**: 427-435.

Zaccardi, G., Kelber, A., Sison-Mangus, M.P. & Briscoe, A.D. (2006) Color discrimination in the red range with only one long-wavelength sensitive opsin. *Journal of Experimental Biology* **209**: 1944-1955.

Zar, H.J. (1984) *Biostatistical Analysis*. 2nd edition. Prentice Hall International, London.

CHAPTER 4

Behavioural responses of the blister beetle *Hycleus apicicornis* to odours of host plants in the laboratory



Abstract

The role played by olfactory cues in host plant finding by the blister beetle *Hycleus apicicornis* was investigated. Responses of individual adults to odours of flowers of *Abelmoschus esculentus, Desmodium uncinatum, Ipomoea batatas, Ipomoea hildebrandtii, Phaseolus vulgaris* and *Vigna unguiculata,* as well as two synthetic volatiles, 2-phenyl ethanol and phenylacetaldehyde were tested in dual choice experiments using a Y-tube olfactometer. Adults of *H. apicicornis* were highly attracted to odours of cut flowers of *P. vulgaris*, *D. uncinatum*, *I. batatas*, and the two synthetic compounds. Odours from flowers of intact plants were not attractive to the beetles. 2-phenylethanol was more attractive than phenylacetaldehyde. Results for settling and orientation laboratory experiments with adult *H. apicicornis* differed slightly from the olfactometer tests. Cut flowers of *A. esculentus* were preferred in addition to *P. vulgaris*, *D. uncinatum* and *I. batatas*. Feeding responses did not differ significantly between all plants except for *V. unguiculata*, which was the least visited in both choice and no-choice experiments. *H. apicicornis* was attracted to cut flowers of *P. vulgaris*, *D. uncinatum* and *I. batatas* in olfactometer tests and these three plants also emerged amongst most preferred in the orientation and settling preference study.

Keywords: *Desmodium uncinatum;* olfaction; olfactometer; orientation and settling preference; plant odours.

4.1 Introduction

Plants emit numerous volatile organic compounds to cope with various adversities affecting their survival (Paré & Tumlinson 1996). Plants may emit phytochemicals that assist them in coping with heat when experiencing high temperatures (Paré & Tumlinson 1996). They are also released as indirect defence against herbivorous insects (Chapman *et al*. 1981, Raguso & Pellmyr 1998; Dicke *et al*. 1999; Pichersky & Gershenzon 2002), and in attracting pollinators (Pichersky & Gershenzon 2002; Röse & Tumlinson 2004; Kaiser 2006; Whitney & Glover 2007). In addition, some herbivorous insects are attracted by volatile compounds and use them as cues to locate mating partners, oviposition sites, food needed for survival (Plimmer *et al.* 1982; Al Abassi *et al*. 1998; Agelopoulos *et al*. 1999a; Anderson & Alborn 1999; Stökl *et al*. 2005; Bengtsson *et al*. 2006; Kost & Heil 2006). Once herbivores are attracted to plants and start feeding on them, there is further release of herbivore induced volatiles (Bolter *et al*. 1997; Dicke & van Loon 2000; De Moraes *et al*. 2001; Watkins *et al*. 2006).

Volatile compounds released by a plant may differ qualitatively and or quantitatively depending on the type of damage, whether mechanical or due to herbivory (Turlings *et al*. 1995; Paré & Tumlinson 1999; Dicke & van Loon 2000; De Moraes *et al*. 2001; Arimura *et al*. 2009). The type of damage may also differ between different herbivores resulting in emission of different chemical profiles (Paré & Tumlinson 1999; De Moraes *et al*. 2001; Leitner *et al*. 2005; Kost & Heil 2006; Arimura *et al*. 2009).

Blister beetles that are pests are polyphagous. They feed on floral parts of plants belonging to different families including several garden crops, weeds, ornamentals and fruit trees (Singh & Van Emden 1979). *Hycleus apicicornis* (Guér.) (Coleoptera: Meloidae) is one of about five *Hycleus* species forming a species complex in western Kenya. *Hycleus apicicornis* was observed feeding on floral parts of *Desmodium* spp. (Fabaceae), sweetpotato (*Ipomoea batatas* (L.) Lam., Convolvulaceae), morning glory

(*Ipomoea hildebrantii* Vatke, Convolvulaceae), cowpea (*Vigna unguiculata* (L.) Walp, Leguminoseae), beans (*Phaseolus vulgaris* (L.) (Fabaceae) and okra (*Abelmoschus esculentus* (L.) Moench, Malvaceae) (Lebesa *et al*. 2012). Olfactory cues from floral parts may play an important role in host plant location by *H. apicicornis*. As a pest that feeds on flowers of different morphologies olfactory cues may be more important than visual cues therefore determining key volatiles compounds or blend(s) responsible for the attraction of *H. apicicornis* to its resources is critical for the development of a control strategy for the pest.

In large scale agriculture blister beetles are generally not considered serious pests but if they invade a crop on small holdings they can cause considerable damage due to their gregarious nature (Selander 1964; Evans 1990; Pinto & Bologna 1999). The aggregation behaviour of blister beetles is not well understood but may be due to an aggregation pheromone produced by one or both sexes (Selander 1964; Nikbakhtzadeh 2004). Often insect species that feed gregariously possess an aggregation pheromone which one or both sexes release upon feeding on host plants and that attracts both sexes to the point of release (Smyth & Hoffman 2003; Wertheim *et al*. 2005; Lacey *et al*. 2008). An aggregation pheromone is often synergised by the kairomones from the host plants to signal their availability to conspecifics (Aldrich *et al*. 2003; Rao *et al*. 2003; Wertheim *et al*. 2005).

To identify the most attractive host plant species, the behavioural responses of *H. apicicornis* to intact or mechanically damaged flowers of host plant species were determined in the laboratory through olfactometer tests and no-choice and multiple-choice assays. This study provides the basis for comparing the chemical profiles of different plant species to identify key volatile compounds that are responsible for the attraction of *H. apicicornis*.

4.2 Material & Methods

Insects

Collection and maintenance of beetles

Adult *H. apicicornis* were collected from silverleaf desmodium, *Desmodium uncinatum* Jacq. DC and greenleaf desmodium, *Desmodium intortum* Urb. (Fabaceae), plots at *icipe*, Thomas Odhiambo Campus, Mbita (ITOC) (0°25'S, 34°12'E, app. 1 200 m a.s.l.). To ensure representivity, adults were also collected on morning glory, *I. hildebrandtii*, an abundant wild alternative host at Mbita and neighbouring places. Adults were kept in cages in the laboratory and maintained on a mixed diet of fresh flowers of different host plants, *Desmodium* spp., *P. vulgaris, I. hildebrandtii, I. batatas, A. esculentus* and *V. unguiculata.* A stock of beetles was maintained by regular collection in the field throughout the duration of experiments. Field-collected insects used in experiments were maintained for a minimum of one week for acclimatization and were not kept longer than three months. Beetles were kept at ambient room temperatures ranging from 28 °C to 38 °C, 50 % - 70 % RH and lighting regimes of 14L:10D. Field-collected insects were used because *H. apicicornis* larvae are cannibalistic and go into diapause for extended periods and the rearing of blister beetles has proven difficult (Selander 1986). Insects were removed from their maintenance cages and starved for 18 to 24 h prior to use.

Host plants

The main host plant used in this study was *D. uncinatum*. In addition, five alternative host plant species of *H. apicicornis* in Kenya were selected for comparison. The alternative host plant species were *A. esculentus*, *I. batatas*, *I. hildebrandtii, P. vulgaris,* and *V. unguiculata*. All plants were planted in field plots and in pots in a screenhouse at ITOC, Mbita. Potted plants in the screenhouse were kept under natural light and thus received similar amounts of light as plants in the field. Planting and fertilization followed the normal planting and cropping recommendations for these crops.

Plant selection criteria

The choice of plants was based on one or a combination of the following: i) host status especially the floral parts being fed upon in sub-Saharan Africa (de Pury 1968; Singh & Van Emden 1979; Scholtz & Holm 1986; Jackai & Daoust 1986; Abate & Ampofo 1996) ii) presence of plants, either as cultivated or wild plants in Kenya, and iii) personal observations made to confirm that adult *H. apicicornis* were indeed feeding on the floral parts of documented host plants.

Behavioural response to plant volatiles

Prior to behavioural experiments in the laboratory, tests were done to determine the most suitable olfactometers for behavioural studies. Three potential candidates were evaluated: the four-arm olfactometer (Pettersson 1970), the linear track olfactometer (LTO) (Sakuma & Fukami 1985) and Y-tube olfactometer (Ngi-Song *et al.* 1996). The four-arm olfactometer was found unsuitable due to the narrow end of its arms that did not allow beetles to turn. However, the linear track and the Y-tube olfactometer allowed beetles to turn easily despite their large body size. Good response was observed with the Y-tube olfactometer while results with LTO were inconclusive because some beetles did not respond well and remained in the holding port. Therefore, evaluations were made with the Y-tube olfactometer.

Y-tube olfactometer

The experimental arena consisted of a glass box covered inside with black cloth to avoid interference of colours from the surrounding area. Light was provided by a fluorescent tube (TLD 36W, 0.34A, 220v; Philips Electronics, Holland) placed on top of the glass box to provide illumination from above. Experiments were done at 27.5 ± 1.0 °C and 57 ± 5 % RH. Dual-choice bioassays were carried out using a modified Y-tube (length: 20 cm base, 15 cm arms; 3.5 cm internal diameter). The Y-tube was modified by inserting a thin wire (covered in Teflon tube) made into a Y extending from the central arm to the side arms for the insects to walk on because adult beetles were not able to walk on the smooth walls of the glass (Fig.1). Teflon tubing (5.5 mm internal diameter) connected the arms of the Y-tube, the flow-meters and the odour source chambers.

Air purified by passing through an activated charcoal filter was pumped into the chambers (one enclosing host plant part/parts and the other blank air) via controlled flow meters and drawn into the arms of the y-tube at 0.5 l/min. Adult beetles were released individually into the central arm. Each beetle was given 5 min to choose one of the arms. If a beetle failed to respond within that time it was removed from the experiment and another beetle of the same sex was used to repeat that trial. A choice of the right or left arm was recorded when the insect had passed the decision line, i.e. the junction, by 2 cm and remained there for more than one minute.

To avoid any directional bias the position of the odour source was changed after a set of 8 to 10 beetles with an equal number of each sex. The odour source itself was changed after testing the next set of beetles. During each observation the behaviour of individuals in response to the stimulus and the number of individuals reaching each arm was recorded. To verify there was no equipment bias a control trial of purified air versus purified air was undertaken. Before use, between changing position of the odour source, and after changing the odour source, all glassware and other equipment used was cleaned thoroughly with a detergent and water. Glassware and metal were rinsed with acetone and distilled water and subsequently baked at 180 ˚C for at least 2 hours before use. Perspex and plastic items were rinsed with absolute alcohol and distilled water and left to dry completely before use.

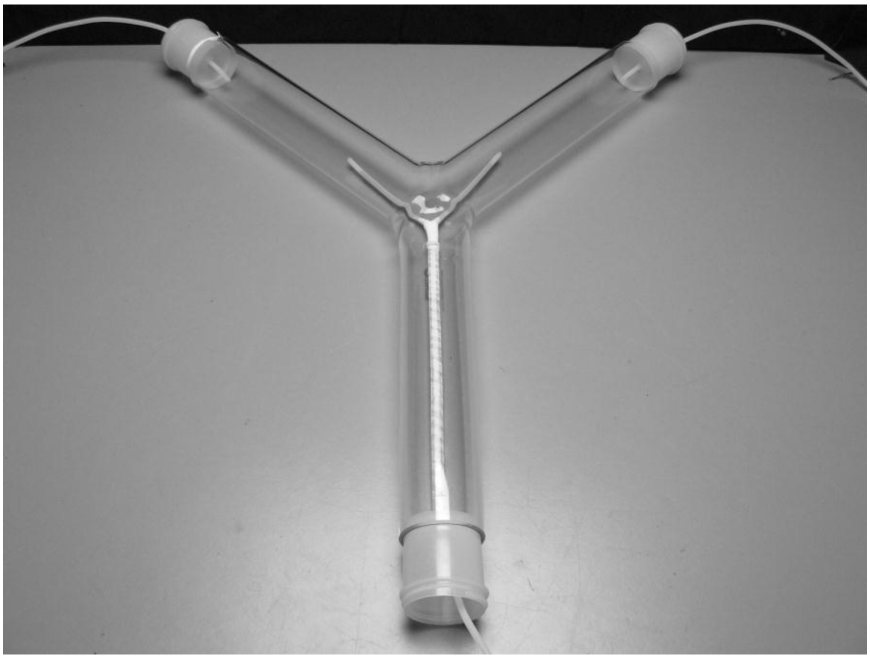


Figure 1. Y-tube olfactometer for measuring behavioural responses of insects to olfactory cues.

Odour sources

Volatiles from cut, intact and extracts of flowers of *D. uncinatum*, *A. esculentus*, *I. batatas*, *I. hildebrandtii*, *P. vulgaris* and *V. unguiculata* were tested in the Y-tube olfactometer. Flowers were cut at the pedicel to exclude leaves and stems, except for flowers of *D. uncinatum* which were cut at the stem.

Plant extracts from cut and intact flowers were obtained from volatiles collected through air entrainments (Birkett *et al*. 2004; Chamberlain *et al*. 2006, Chapter 5) eluted with dichloromethane (GC/GC-MS–grade dichloromethane (Burdick and Jackson, Muskegon, Michigan, USA)). Only extracts from flowers of *D. uncinatum*, *I. batatas* and *P. vulgaris* were used because only they elicited positive responses from beetles. The volatile extracts were used as chemical stimuli while the solvent (dichloromethane) was used as control. Extracts of intact flowers were evaluated separately against the control and against extracts of cut flowers (Table 1).

For cut flowers or flower extract, 5 mg, cut flowers and 20 μl extracts placed on 1 cm^2 pieces of paper were placed in the odour chambers (1 l glass containers) while intact flowers were enclosed in cooking bags (Pick 'n Pay multi-purpose oven bags, South Africa). Air from the pump was drawn through the odour chambers into the olfactometer arms at 0.5 l/min. Intact flowers of *I. batatas* could not be used because potted plants did not produce flowers.

Sample sizes differed among experiments but the total number tested ranged between 30 and 238 beetles per dual bioassay experiment. Six different sets of dual choice bioassays were undertaken: i) cut flowers versus control (purified air); ii) intact flowers versus control, iii) cut flowers versus intact flowers, iv) extracts of cut flowers versus control, v) extracts of intact flowers versus control, and vi) extracts of cut flowers versus intact flowers. Details of experiments are given in Table 1.

Authentic Compounds

The two compounds, phenylacetaldehyde and 2-phenylethanol found in the preliminary GC-MS analysis of *I. batatas* and *D. uncinatum* respectively were used as odour source in Y-tube dual choice experiments. 2-phenylethanol was chosen because in a preliminary trapping study conducted in Bungoma in 2004 a trap baited with the compound had higher catches of *Hycleus* spp. compared to traps baited with with linalool, 4-acetophenone and a mixture of 4-acetophenone and 2-phenylethanol (L. Wadhams, unpublished data). Responses of beetles to 2-phenylethanol and phenylacetaldehyde were evaluated against the control (purefied air) and with each other.

Table 1. Sources of odours tested against adults of *H. apicicornis* in the dual-choice olfactometer bioassays

Experiment type	Source of odour	Number of beetles tested[a]
i) cut flowers versus control (purified air)	*A. esculentus*	100
	D. uncinatum	238
	I. batatas	212
	I. hildebrandtii	98
	P. vulgaris	100
	V. unguiculata	100
ii) intact flowers versus control (purified air)	*A. esculentus*	140
	D. uncinatum	100
	I. hildebrandtii	100
	P. vulgaris	60
	V. unguiculata	100
iii) cut flowers versus intact flowers	*D. uncinatum*	80
	P. vulgaris	100
iv) extracts of intact flowers versus control (dichloromethane 99%)	*D. uncinatum*	30
	I. batatas	30
	P. vulgari	30
v) extracts of cut flowers versus extracts of intact flowers	*D. uncinatum*	40
	I. batatas	50
	P. vulgaris	40
vi) synthetic compounds versus control (purified air)	2-phenylethanol	152
	Phenylacetaldehyde	222
vii) synthetic compounds with each other	2-phenylethanol Phenylacetaldehyd	160
viii) purified air in arm 1 and arm 2	Olfactometer 1	120
	Olfactometer 2	110

[a] Equal numbers of males and females.

Orientation and Settling preference

Cut flowers of the host plants tested in the olfactomerter trial were used in a cage trial to assess settling preference of the beetles towards host plants. The experiment followed a randomized complete block design (Sokal & Rohlf 1969, Gomez & Gomez 1984). Six host plants, *D. uncinatum, P. vulgaris, I. hildebrandtii, I. batatas, A. esculentus* and *V. unguiculata* served as treatments, while cages and days served as replicates and blocks respectively. Two types of experiments, multiple and no-choice experiments, were carried out. For both experiments, four screen cages of wire mesh and wooden frames (60 cm x 60 cm x 60 cm) were placed on tables and spaced at 0.5 m apart. Flowers of predetermined weight (1.6 g and 1.9 g) were placed loosely in 7 cm diameter Petri dishes. Petri dishes were arranged in a circle, equidistant from each other. Beetles were released in the centre of the circle. To offset the difference in weight loss due to insects feeding control Petri dishes were covered with nylon mesh. Beetles were starved for 15 to 16 h prior to use. To determine differences in weight loss due to insects feeding, control Petri dishes were covered with nylon mesh. Beetles were starved for 15 to 16 h prior to use.

Multiple-choice experiments

In the multiple-choice experiments, Petri dishes with cut flowers of all six plant species were placed in a single cage. Two types of Petri dishes per plant species were used; open Petri dishes with cut flowers of predetermined weight and nylon mesh covered Petri dishes containing cut flowers of predetermined weight as controls. For each treatment and control each of four Petri dishes contained flowers of predetermined weight of 1.6 g for all host plants except for *A. esculentus* which weighed 1.9 g. Twelve Petri dishes were randomly arranged in a circle with the restriction that Petri dishes containing the same plant species were not placed next to each other. Forty beetles (20 males and 20 females) were released in a Petri dish placed in the centre of the circle. Behaviour and response of beetles to the flowers of the different host plant species were observed for 15 min. Experiments were carried out between 08h00 and 12h00 to coincide with the morning peak activity for the beetles (Lebesa *et al*. 2012) as well as avoiding wilting when flowers were kept for more than four hours. The number of beetles on each of the host plant species in each Petri dish was then recorded at four time intervals; after 30 min for orientation, and at one-hour intervals thereafter for settling preference. Upon termination, the weights of the host plants in both control and experimental Petri dishes were determined. The experiment was repeated for four consecutive days, each day with new beetles and fresh flowers.

No-choice experiments

In the no-choice experiments cut flowers of all six host plant species were individually evaluated in cages that contained eight Petri dishes with cut flowers form a single plant species comprising four open and, as controls, four nylon mesh-covered Petri dishes. The Petri dishes were arranged in a circle, equidistant from each other and alternating open and closed Petri dishes. Twenty beetles, 10 males and 10 females, were released from a Petri dish placed in the centre of a circle. Behaviour and responses were observed for 15 min. The number of beetles on each of the host plants was recorded at three intervals, initially after 30 min, and at 2 and 3 h thereafter. Upon termination, the weight of the flowers in the open and closed Petri-dishes were recorded. The experiment was repeated for four consecutive days, each day with new beetles and fresh flowers.

Statistical Analysis

For the olfactometric assays data were analyzed with two-tailed binomial tests to assess whether responses of beetles to two sources of stimuli, plant volatiles or synthetic compounds versus control (purefied air), and comparison of two odours, e.g. intact versus cut flowers, differed from a 50:50 distribution using Statistica (Version 9.0, StatSoft, Inc, 1984-2009). The number of beetles settling on cut flowers of six plant species in multiple choice and no-choice experiments was analysed with linear mixed models using residual maximum likelihood (REML) in GenStat (Payne et al. 2011). For the multiple choice experiment Time, Species and the Time x Species interaction were included as fixed effects and the Cage x Time interaction as a random effect with a power covariance model fitted over time. The effect of Day was not significant and excluded from the analysis. For the no-choice experiment Day, Species, Time and the Species x Time interaction were included as fixed effects and the Cage x Time interaction as a random effect with a power covariance model fitted over time. Bonferroni tests were used to separate means. Although beetles were observed feeding on flowers in the multiple- and no-choice orientation and settling experiments, the weight loss of flowers (wet weight) could not be used as an indirect measure of feeding because of uneven weight loss among plant species. The level of significance was set at $P < 0.05$ for all analyses.

4.3 Results

Behavioural bioassays

No bias was found between the two arms of the Y-tube olfactometer (two-tailed binomial test, Tube A: $z = 0.274$, $n = 120$; Tube B: $z = 0.095$, $n = 110$; $P > 0.05$). There were no significant differences between sexes for any of the dual bioassay tests, with the exception of odours of intact flowers of *I. hildebrandtii,* where responses of males and females differed significantly ($\chi^2 = 6.78$, $df = 1$, $P < 0.01$). Consequently, data for both males and females were pooled for all analyses except for the comparison of odours from flowers of intact plants of *I. hildebrandtii* with purified air.

Of the six plant species tested, *H. apicicornis* showed a significant preference for odours of cut flowers of *D. uncinatum* (z = 2.917, $P < 0.01$), *I. batatas* (z = 2.679, $P < 0.01$) and *P. vulgaris* (z = 2.3, p = 0.021), but not for those of *V. unguiculata*, *I. hildebrandtii* and *A. esculentus* (Fig. 2a). There were no significant differences when beetles were given a choice between odours of flowers of intact plants and purified air for all plant species tested except for *I. hildebrandtii* (Fig. 2b). Females preferred purified air to odours of intact flowers of this plant species (two-tailed binomial test, z = 2.121, $P < 0.05$), whereas males did not show a significant preference for either (Fig. 2b). When comparing odours from cut flowers with flowers of intact plants of *D. uncinatum* and *P. vulgaris*, respectively, beetles did not show a preference (Fig. 2c). Cut flowers and flowers of intact plants of *I. batatas* could not be compared because potted plants failed to produce flowers.

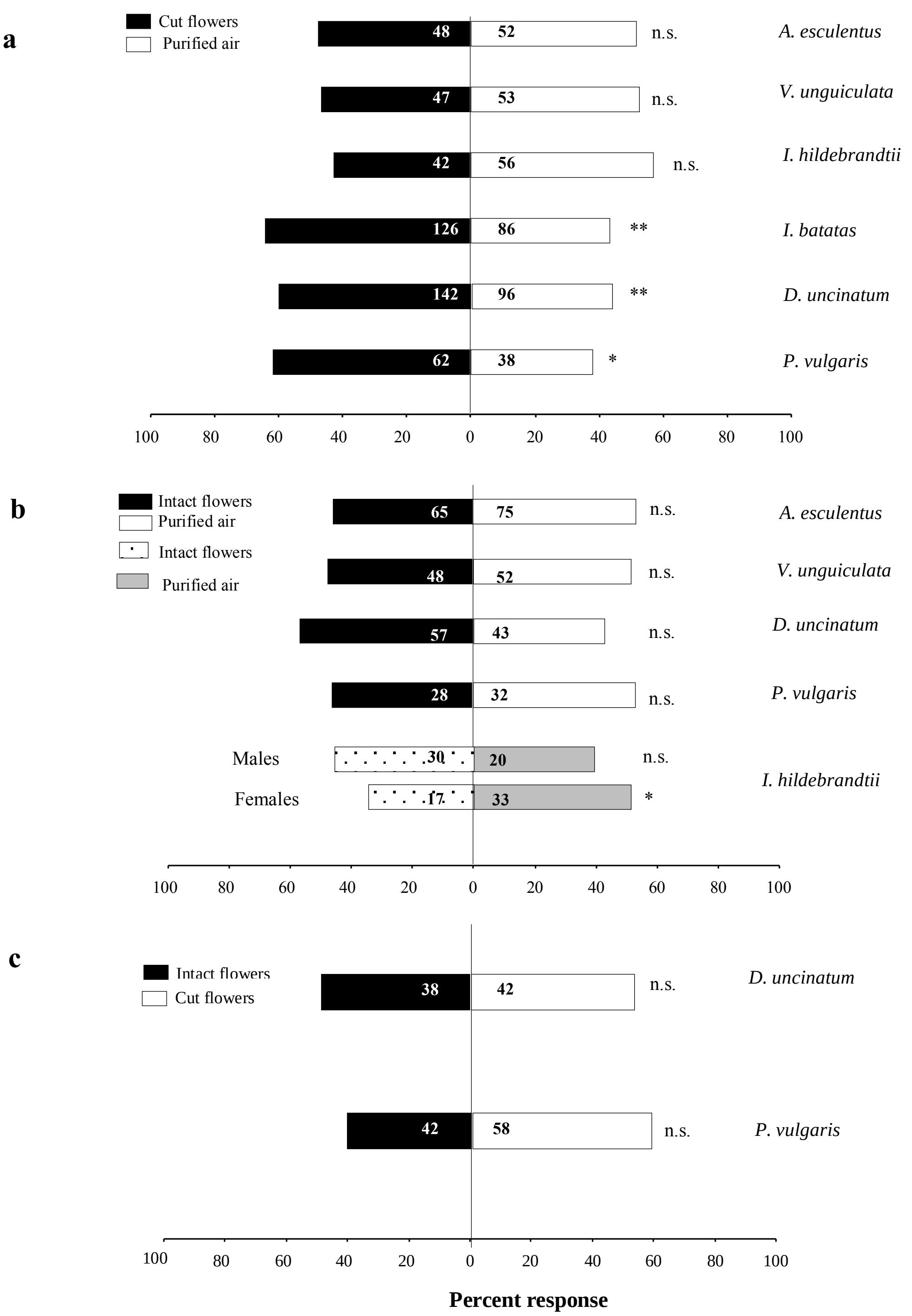


Figure 2. Response of blister beetles to odours of flowers of different host plants in Y-tube dual choice olfactometer tests; **a)** odours of cut flowers versus purified air, **b)** odours of flowers from intact plants versus purified air and **c)** odours of cut flowers versus odours of flowers of intact plants for each plant species. The number of beetles that responded to the

odours is shown inside the bar. Asterisks next to bars denote significant differences between left and right of each bar (two-tailed binomial test, * = $P < 0.05$, ** = $P < 0.01$ and n.s. = not significant).

Response to volatile extracts

Volatile extracts of flowers of *D. uncinatum, P. vulgaris* and *I. batatas*, i.e. those plant species that were preferred by *H. apicicornis* when compared with purified air, were used in the dual choice tests to determine attraction of beetles to the blends. When odours collected from flowers of intact plants were compared with the control solvent, dichloromethane (DCM), there were no significant differences between flower odours and control (Fig. 3a). When comparing odours from flowers of intact plants with cut flowers, beetles were significantly attracted to odour collections of cut flowers of *D. uncinatum* ($z = 2.055$, $P < 0.05$) For *P. vulgaris* on the other hand, beetles did not show a preference for either odours of flowers from intact plants or those of cut flowers (Fig. 3b).

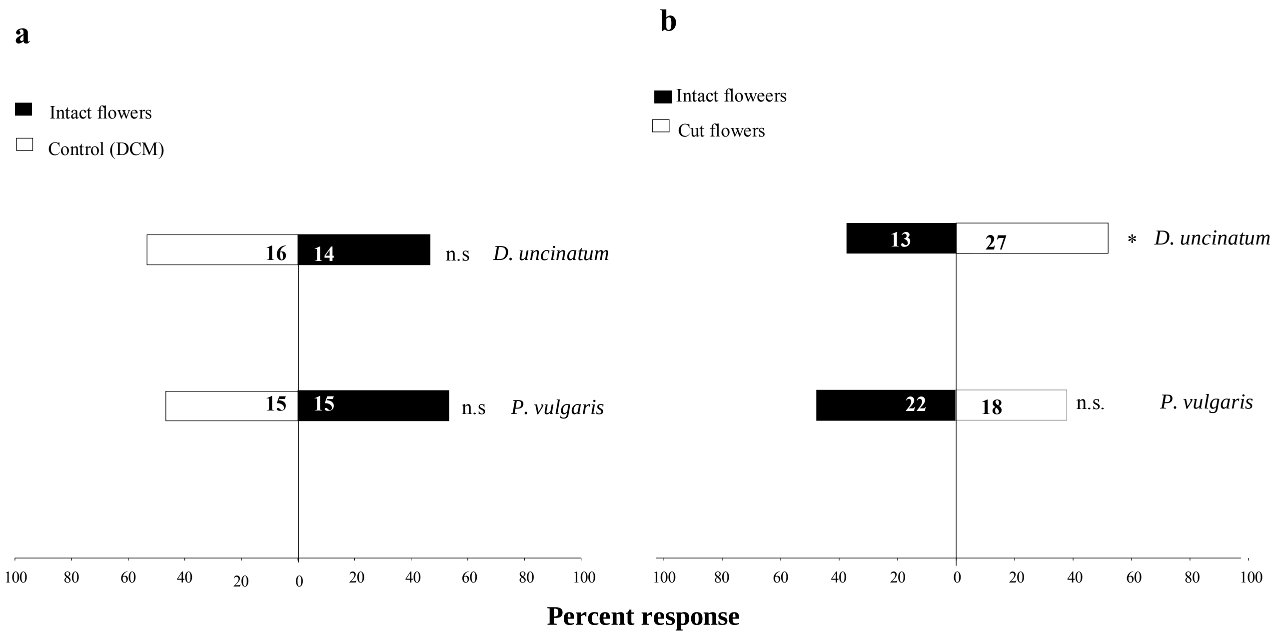


Figure 3. Response of blister beetles to odours of extracts of flowers of *Phaseolus vulgaris* and *Desmodium uncinatum* in the Y-tube dual choice bioassays; **a)** flowers of intact plants versus control (dichloromethane (DCM)), and **b)** odours of flowers of intact plants versus odours of cut flowers. The number of beetles that responded to each odour is shown inside the bar. Significant differences in preferences are shown for left and right of each bar (two-tailed binomial test, * = $P < 0.05$ and n.s. = not significant).

Response to authentic compounds

In the dual bioassay tests, both phenylacetaldehyde (z = 2.618, $P < 0.01$) and 2-phenylethanol (z = 3.650, $P < 0.001$) were found to be attractive to the beetles. When the odours from the authentic compounds were tested against each other 2-phenylethanol was more attractive to *H. apicicornis* than phenylacetaldehyde (z = 6.675, $P < 0.001$) (Fig. 4b).

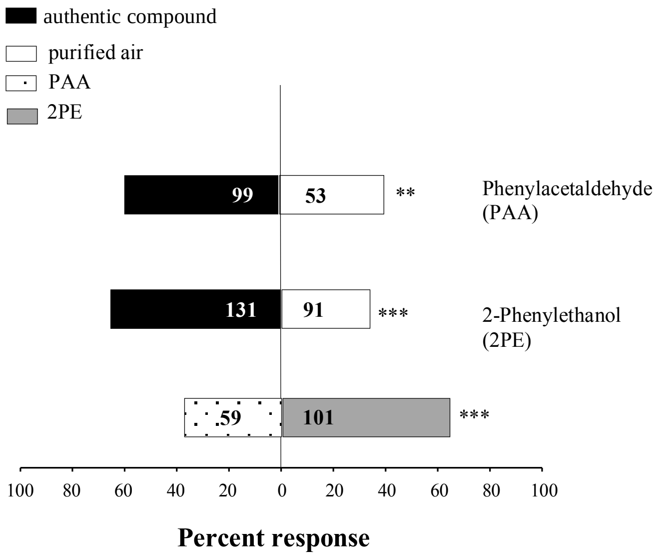


Figure 4. Response of blister beetles to odours of 2-phenylethanol and phenylacetaldehyde versus purified air and the two compounds against each other in Y-tube dual choice bioassays. The number of beetles that responded to each odour is shown inside the bar. PAA = phenylacetaldehyde and 2PE = 2-phenylethanol. Significant differences in preferences are shown for left and right of each bar (two-tailed binomial test, ** = *P* < 0.01, *** = *P* < 0.001 and n.s. = not significant).

Orientation and feeding response

Multiple-choice assays

Upon release from the centre of the cage, insects moved randomly towards different Petri dishes, although a preference towards flowers of *A. esculentus* was observed after approximately 5 min. The number of beetles settling on flowers was significantly affected by plant species (Wald statistic = 42.06, *d.f.* = 5, *P* < 0.001) and observation time (Wald statistic = 21.95, *d.f.* 3, *P* = 0.005). There was no significant interactive effect between observation time and plant species (Wald statistic = 2.57, *d.f.* = 15, *P* > 0.05). A significantly higher number of beetles was recorded on flowers of *D. uncinatum* and *A. esculentus* compared to *I. hildebrandtii* and *V. unguiculata.* The highest number of beetles settled on *D. uncinatum* and *A. esculentus* followed by *P. vulgaris*, *I. batatas* and *I. hildebrantii* and lastly *V. unguiculata,* which attracted a significantly fewer beetles than all other plant species with the exception of *I. hildebrandtii* (Fig. 5a). Significantly more beetles were recorded on flowers after 1.5 h and 2.5 h compared to 3.5 h.

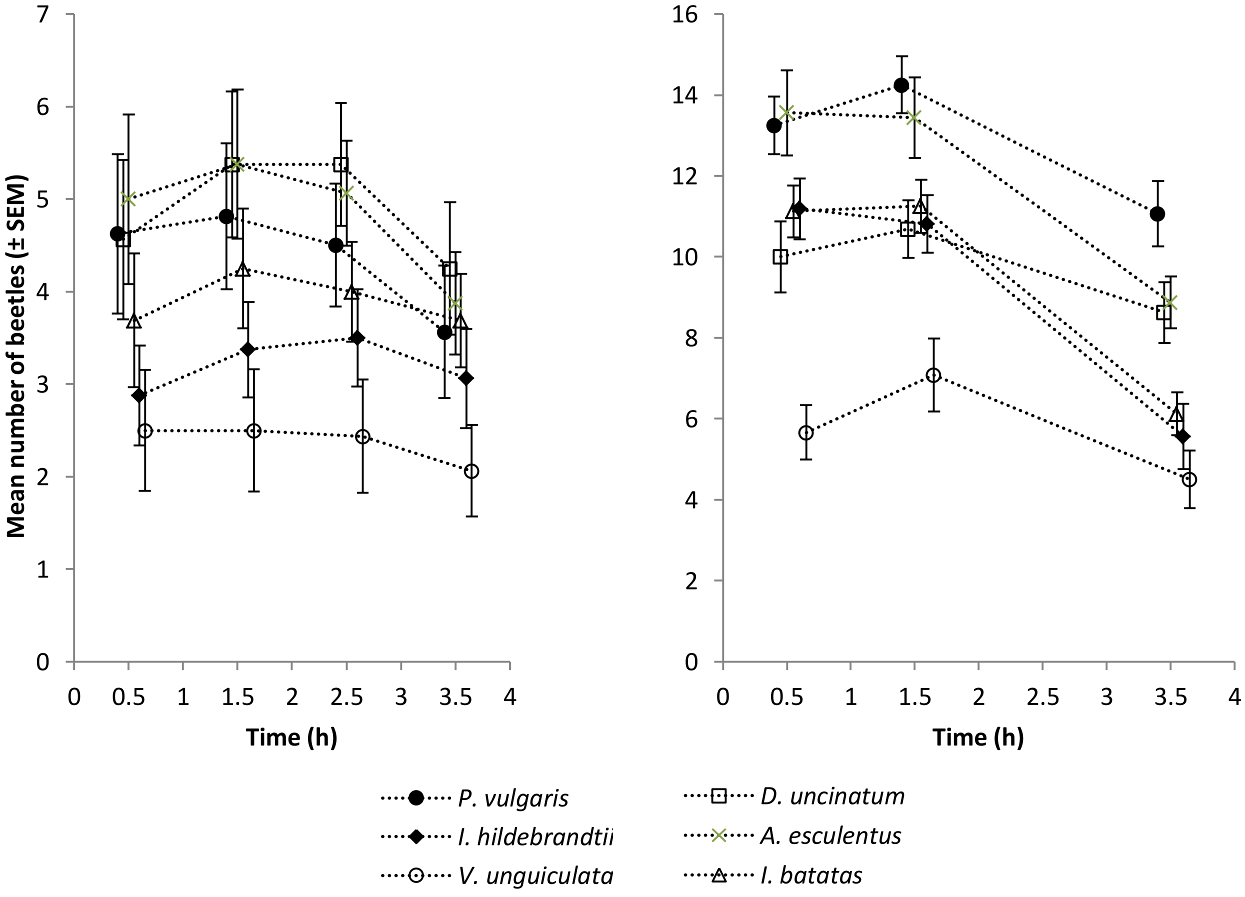


Figure 5. Orientation (0.5 h) and settling (1.5 to 3.5 h) preference of *H. apicicornis* adults on six host plant species in (a) multiple-choice and (b) no-choice assays.

No-choice assays

A comparison of all individual experiments showed that upon release (first 15 minutes of observation) more beetles were recorded on flowers of *D. uncinatum*. However, when observations made after 0.5, 2.5 and 3.5 h, the most preferred flowers were those of *P. vulgaris* (Fig. 5b).

The number of beetles settling on flowers was significantly affected by Day (Wald statistic = 16.87, *d.f.* = 3, $P < 0.001$), Time (Wald statistic = 96.97, *d.f.* = 2, $P < 0.001$) and Species (Wald statistic = 144.49, *d.f.* = 5, $P < 0.001$). The interactive effect between Time and Species was marginally not significant (Wald statistic = 18.71, *d.f.* = 10, $P = 0.05$).The number of beetles recorded on flowers was significantly higher after 0.5 h and 2.5 h than 4.5 h. After 0.5 and 2.5 h no differences in orientation and settling preference were observed among the five plant species, *P. vulgaris, A. esculentus, I. hildebrandtii*, *I. batatas* and *D. uncinatum.* In general, more beetles settled on flowers of *P. vulgaris, A. esculentus*, followed by *D. uncinatum, I. batatas* and *I. hildebrandtii.* Flowers of *V. unguiculata* were the least preferred for all sampling periods.

4.4 Discussion

Hycleus apicicornis adults were attracted to odours of mechanically damaged flowers but not to the odours of flowers of intact plants. Beetles were also attracted to cut flowers of *D. uncinatum*, the main host plant in this study, and two alternative host plants, *P. vulgaris* and *I. batatas.* Although *H. apicicornis* was not attracted to intact flowers, avoidance behaviour by females was observed towards flowers of *I. hildebrandtii*. Attraction to cut flowers could not be confirmed when odours from cut flowers and those of intact plants were compared; however, some attraction to cut flowers of *D. uncinatum* was observed when extracts of cut and intact flowers of the attractive plant species were evaluated. When evaluating orientation and settling preference using cut flowers, *H. apicicornis* showed a preference for *P. vulgaris, D. uncinatum* and *A. esculentus* in both multiple-choice and no-choice assays.

The preference noted for cut flowers could be due to volatiles being emitted in larger amounts, whereas for intact plants these can be very low (Agelopoulos *et al*. 1999b; Dicke & van Loon 2000). Similar observations were made in other studies where chrysomelid and curculionid beetles were more strongly attracted to damaged plants or plant parts emitting higher amounts of volatiles than non-damaged ones (Bolter *et al*. 1997; van Tol *et al*. 2002). During herbivore feeding or mechanical damage, a change was observed in the quality and/or quantity of volatiles emitted by flowers of the three host plants that were more attractive to beetles (Chapter 5). It is possible that one or more of the constituents of these volatiles were responsible for an enhanced attraction of *H. apicicornis* to the host plants. Carroll *et al*. (2006) also observed that an increased emission of linalool due to herbivory by *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae) resulted in enhanced attraction of conspecifics. However, changes in chemical profile depend on the plant species under attack and the type of insect species inflicting the damage (Arimura *et al*. 2009). Thus, each insect-plant interaction should be considered individually.

The attraction of *H. apicicornis* to *P. vulgaris*, *D. uncinatum* and *I. batatas* is consistent with the findings of a survey (Chapter 2, Lebesa *et al*. 2012), where farmers identified these three host plants as the most preferred among known host plants that also included *V. unguiculata*, *Desmodium* spp., and *Zea mays* L. Gas chromatography-mass spectrometry (GC-MS) analyses of the air-entrained volatile profile of *D. uncinatum* and *I. batatas* revealed the presence of 2-phenylethanol and phenylacetaldehyde among other compounds. These two compounds are amongst common plant volatiles attractive to various insects (Burguiere *et al*. 2001; Bruce *et al*. 2005), including some beetles (Bartlet *et al*. 2004; Bruce *et al*. 2005). Both were attractive to *H. apicicornis* in olfactometer tests in the current study. When the two compounds were compared with each other, the beetles preferred 2-phenylethanol. The attractiveness of both compounds may also be associated with polyphagy. Blister beetles are polyphagous and may employ common volatile compounds to locate their host plants although they may be able to detect appropriate combinations of common volatiles that make them orientate towards their host plants (Bruce *et al*. 2005; Webster *et al*. 2008).

A high preference for *A. esculentus* in both multiple and no-choice experiments, contradicted the results of olfactometer tests where *A. esculentus* volatiles were found to be less attractive to *H. apicicornis*. The high settling preference in cut flowers of *A. esculentus* may be due to visual attraction enhanced by the size of the flowers. Although the flowers of *A. esculentus* are larger than those of the other host plant species tested, size may not always be that critical under natural conditions because insects may view flowers of smaller size as a combined image (Ne'eman & Kevan 2001). The ability of insects to discriminate flowers by size suggests the mediation of combination of both visual and olfactory cues in their search for hosts. For example, Kalberer *et al*. (2001) noted that presence and visual access of plants in a wind tunnel enhanced attraction of *Oreina cacaliae* (Coleoptera: Chrysomelidae) to host plant odour.

The current study shows that *H. apicicornis* is attracted to the odours of cut flowers of the three host plants, *D. uncinatum*, *P. vulgaris* and *I. batatas* but not to intact flowers of the same plant species. Thus altering the volatile profile through mechanical damage enhanced attraction of flowers to *H. apicicornis*. To identify which volatile compounds are responsible for the difference in attractiveness, the qualitative and quantitative volatile profiles of intact and cut flowers need to be compared. It is further crucial to compare the volatile profiles of host plants found attractive with those found not attractive to determine which compounds are lacking, or occur in different relative amounts, between the two.

Attraction to cut flowers may explain the gregarious nature of *H. apicicornis* although the presence or absence of an aggregation pheromone was not investigated in the current study. Usually, the volatiles emitted from herbivore-damaged plants differ from the ones emitted by mechanically damaged plants (Turlings *et al*. 1995; Paré & Tumlinson 1999; De Moraes *et al*. 2001). However, in some studies responses of study insects to mechanically and herbivore-damaged plants showed no differences (Kalberer *et al*. 2001; Van Tol *et al*. 2002). It may thus be necessary to undertake further work comparing attraction of *H. apicicornis* to cut flowers (mechanically damaged) of host plants versus flowers damaged by conspecifics, also with a view to determine possible synergistic effects of host plant volatiles and aggregation pheromones, if any.

The ability of *H. apicicornis* to feed on flowers of different families suggests that both visual and olfactory cues are important for location of host plants by this species because these flowers have neither similar chemical profiles nor similar visual cues for each stimulus to work alone. Orientation towards host plants has been shown in some insect species to be due to detection of visual cues (especially colour) before olfactory cues are detected (Ômura & Honda 2005; Balkenius *et al*. 2006; Campbell & Borden 2006). When determining the response of *H. apicicornis* to visual cues (Chapter 3, Lebesa *et al*. 2011), a strong attraction to the blue targets was observed. To elucidate the role of these two cues it may also be necessary in the future studies to compare the attraction of *H. apicicornis* to scented and unscented plant models.

Acknowledgements

Research funds were provided by Gatsby Charitable Foundation (UK) and Kilimo Trust (East Africa) while living expenses for the first author were covered by DAAD - German Academic Exchange Service. The first author is also indebted to the University of Pretoria and the Lesotho Government for provision of some financial and logistical support. Our gratitude goes to the technical staff of the ICIPE - Habitat Management in particular, Paul Diela, Charles Oyugi and Jacob Odhiambo who assisted during the practical sessions. Prof L. Wadhams, Dr Sam Cook and Dr James Logan (Rothamsted Research) and Marie Smith, ARC-Biometry Unit are acknowledged for providing help. We are grateful also to Dr M. Liphoto, Mr S. Ratšiu and anonymous readers for making critical comments on this work.

References:

Abate, T. & Ampofo, J.K.O. (1996) Insect pests of beans in Africa: their ecology and management. *Annual Review of Entomology* **41**: 45-73.

Agelopoulos, N., Birkett, M.A., Hick, A.J., Hooper, A.M., Pickett, J.A., Pow, E.M., Smart, L.E., Smiley, D.W.M., Wadhams, L.J. & Woodcock, C.M. (1999a) Exploiting semiochemicals in insect control. *Pesticide Science* **55**: 225-235.

Agelopoulos, N.G., Hooper, A.M., Maniar, S.M., Pickett, J.A. & Wadhams, L.J. (1999b) A novel approach for isolation of volatile chemicals released by individual leaves of a plant in situ. *Journal of Chemical Ecology* **25**: 1411-1425.

Al Abassi, S. M. A., Birkett, M.A., Pettersson, J., Pickett, J.A. & Woodcock, C.M. (1998) Ladybird beetle odour identified and found to be responsible for attraction between adults. *Cellular & Molecular Life Science* **54**: 876-879.

Aldrich, J.R., Bartelt, R.J., Dickens, J.C., Knight, A.L., Light, D.M. & Tumlinson, J.H. (2003) Insect chemical ecology research in the United States Department of Agriculture – Agricultural Research Service. *Pest Management Science* **59**: 777-787.

Anderson, P. & Alborn, H. (1999) Effects on oviposition behaviour and larval development of *Spodoptera littoralis* by herbivore-induced changes in cotton plants. *Entomologia Experimentalis et Applicata* **92**: 45-51.

Arimura, G., Matsui, K., & Takabayashi, J. (2009) Chemical and molecular ecology of herbivore-induced plant volatiles: proximate factors and their ultimate functions. *Plant Cell Physiology* **50**: 911-923.

Balkenius, A., Rosén, W. & Kelber, A. (2006) The relative importance of olfaction and vision in a diurnal and a nocturnal hawkmoth. *Journal of Comparative Physiology A* **192**: 431-437.

Bartlet, E., Blight, M.M., Pickett, J.A., Smart, L.E., Turner, G. & Woodcock, C.M. (2004) Orientation and feeding responses of the pollen beetle, *Meligethes aeneus* to candytuft, *Iberis amara. Journal of Chemical Ecology* **30**: 913-925.

Bengtsson, M. Jaastad, G., Knudsen, G., Kobro, S., Bäckman, A., Pettersson, E. & Witzgall, P. (2006) Plant volatiles mediate attraction to host and non-host plant in apple fruit moth, *Argyresthia conjugella. Entomologia Experimentalis et Applicata* **118**: 77-85.

Birkett, M.A., Agelopoulos, N., Jensen, K.-M.V., Jespersen, J.B., J. A. Pickett, J.A., Prijs, H.J., Thomas, G., Trapman, J.J., Wadhams, L.J. & Woodcock, C.M. (2004) The role of volatile semiochemicals in mediating hostlocation and selection by nuisance and disease-transmitting cattle flies. *Medical and Veterinary Entomology* **18**: 313-322.

Bolter, C.J., Dicke, M.V., Loon, J.J.A., Visser, J.H. & Posthumus, M.A. (1997) Attraction of Colorado potato beetle to herbivore-damaged plants during herbivory and after its termination. *Journal of Chemical Ecology* **23**: 1003-1023.

Bruce, T.J.A., Wadhams, L.J. & Woodcock, C.M. (2005) Insect host location: a volatile situation. *Trends in Plant Science* **10**: 26-274.

Burguiere, L. Marion-Poll, F. & Cork, A. (2001) Electrophysiological responses of female *Helicoverpa armigera* (Hübner) (Lepidoptera; Noctuidae) to synthetic host odours. *Journal of Insect Physiology* **47**: 509-514.

Campbell, S.A. & Borden, J. (2006) Close-range, in-flight integration of olfactory and visual information by a host-seeking bark beetle. *Entomologia Experimentalis et Applicata* **120**: 91-98.

Carroll, M.J., Schmelz, E.A., Meagher, R.L. & Teal, P.E.A. (2006) Attraction of *Spodoptera frugiperda* larvae to volatiles from herbivore-damaged maize seedlings. *Journal of Chemical Ecology* **32**: 1911-1924.

Chamberlain, K., Khan, Z.R., Pickett, J.A., Toshova, T., & Wadhams, L.J. (2006) Diel periodicity in the production of green leaf volatiles by wild and cultivated host plants of stemborer moths, *Chilo partellus* and *Busseola fusca*. *Journal of Chemical Ecology* **32**: 565-577.

Chapman, R.F., Bernays, E.A. & Simpson, S.J. (1981) Attraction and repulsion of the aphid, *Cavariella aegopodii*, by plant odors. *Journal of Chemical Ecology* **7**: 881-888.

De Moraes, C.M., Mescher, M.C. & Tumlinson, J.H. (2001). Caterpillar-induced nocturnal plant volatiles repel conspecific females. *Nature* **410**: 577-580.

De Pury, J.M.S. (1968) *Crop pests of East Africa*. Oxford University Press, Nairobi.

Dicke, M. & Van Loon, J.A. (2000) Multitrophic effects of herbivore-induced plant volatiles in an evolutionary context. *Entomologia Experimentalis et Applicata* **97**: 237-249.

Dicke, M., Gols, R., Ludeking, D. & Posthumus, M.A. (1999) Jasmonic acid and herbivory differentially induce carnivore attracting plant volatiles in Lima bean plants. *Journal of Chemical Ecology* **25**: 1907–1922.

Evans, E.W. (1990) Dynamics of an aggregation of blister beetles (Coleoptera: Meloidae) attacking a prairie legume. *Journal of the Kansas Entomological Society* **63**: 616-625.

Gomez, K.A. & Gomez, A.A. (1984) *Statistical procedures for agricultural research*. 2nd edition. John wiley & Sons, New York.

Jackai, L.E.N. & Daoust, R.A. (1986) Insect pests of cowpeas. *Annual Review of Entomology* **31**: 95-119.

Kaiser, R. (2006) Flowers and fungi use scents to mimic each other. *Science* **311**: 806-907.

Kalberer, N.M., Turlings, T.C.J., & Rahier, M. (2001) Attraction of a leaf beetle (*Oreina cacaliae*) to damaged host plants. *Journal of Chemical Ecology* **27**: 647-661.

Kost, C. & Heil, M. (2006) Herbivore-induced plant volatiles induce an indirect defence in neighbouring plants. *Journal of Ecology* **94**: 619-628.

Lacey, E.S., Moreira, J.A., Millar, J.G. & Hanks, L.M. (2008) A male-produced aggregation pheromone blend consisting of alkanediols, terpenoids, and an aromatic alcohol from the Cerambycid beetle *Megacyllene caryae*. *Journal of Chemical Ecology* **34**: 408-417.

Lebesa, L.N., Khan, Z.R., Hassanali, A., Pickett, J.A., Bruce, T.J.A., Skellern, M., Krüger, K. (2011) Responses of the blister beetle *Hycleus apicicornis* to visual stimuli. *Physiological Entomology* **36**: 220-229.

Lebesa, L.N., Khan, Z.R., Krüger K., Bruce, T.J., Hassanali, A. & Pickett, J.A. (2012) Farmers' knowledge and perceptions of blister beetles, *Hycleus* spp. (Coleoptera: Meloidae), as pest herbivores of *Desmodium* legumes in western Kenya. *International Journal of Pest Management* **58**: 165-174.

Leitner, M., Boland, W. & Mithöfer, A. (2005) Direct and indirect defences induced by piercing-sucking and chewing herbivores in *Medicago truncatula*. *New Phytologist* **167**: 597-606.

Ne'eman, G. & Kevan, P.G. (2001) The effect of shape parameters on maximal detection distance of model targests by honeybee workers. *Journal of Comparative Physiology A* **187**: 653-660.

Ngi-Song, A.J., Overholt, W.A., Njagi, P.G.N., Dicke, M., Ayertey, J.N. & Lwande, W. (1996) Volatile infochemicals used in host and host habitat location by *Cotesia flavipes* (Cameron) and *Cotesia sesamiae* (Cameron) (Hymenoptera: Braconidae), larval parasitoids of stemborers on Graminae. *Journal of Chemical Ecology* **22**: 307-323.

Nikbakhtzadeh, M.R. (2004) Transfer and distribution of cantharidin within selected members of blister beetles (Coleoptera: Meloidae) and its probable importance in sexual behaviour. *PhD Dissertation*. Universität Bayreuth. Bayreuth, Germany.

Ômura, H. & Honda, K. (2005) Priority of color over scent during flower visitation by adult *Vanessa indica* butterflies. *Oecologia* **142**: 588-596.

Paré, P.W. & Tumlinson, J.H. (1996) Plant volatile signals in response to herbivore feeding. *Florida Entomologist* **79**: 93-103.

Paré, P.W. & Tumlinson, J.H. (1999) Plant volatiles as a defense against insect herbivores. *Plant Physiology* **121**: 325-331.

Payne, R.W., Murray, D.A., Harding, S.A., Baird, D.B. & Soutar, D.M. (2011) GenStat® *for Windows*™, Introduction. 14th Edition. VSN International, UK.

Pettersson, J. (1970) An aphid sex attractant. *Entomologica Scandinavica* **1**: 63-73.

Pichersky, E. & Gershenzon, J. (2002) The formation and function of plant volatiles: perfumes for pollinator attraction and defense. *Current Opinion in Plant Biology* **5**: 237-243.

Pinto, J.D. & Bologna, M.A. (1999) The new world genera of Meloidae (Coleoptera): a key and synopsis. *Journal of Natural History* **33**: 569-620.

Plimmer, J.R., Inscoe, M.N. & McGovern, T.P. (1982) Insect attractants. *Annual Review of Pharmacology and Toxicology* **22**: 297-320.

Rao, S., Cossé, A.A., Zilkowski, B.W. & Bartelt, R.J. (2003) Aggregation pheromone of the cereal leaf beetle: field evaluation and emission from males in the laboratory. *Journal of Chemical Ecology* **29**: 2165-2175.

Raguso, A. & Pellmyr, O. (1998) Dynamic headspace analysis of floral volatiles: a comparison of methods. *Oikos* **81**: 238-254.

Röse, U.S.R. & Tumlinson, J.H. (2004) Volatiles released from cotton plants in response to *Helicoverpa zea* feeding damage on cotton flower buds. *Planta* **218**: 824–832.

Sakuma, M. & Fukami, H. (1985) The linear track olfactometer: An assay device for taxes of the German cockroach, *Blattella germanica* (L.) (Dictyoptera: Blattellidae) toward their aggregation pheromone. *Applied Entomology and Zoology* **20**: 387-402.

Singh, S.R. & van Emden, H.F. (1979) Insect pests of grain legumes. *Annual Review of Entomology* **24**: 255-278.

Scholtz, C.H. & Holm, E. (1986) *Insects of Southern Africa*. Butterworth Publishers (Pty) Ltd Mayville, Durban.

Selander, R.B. (1964) Sexual behavior in blister beetles (Coleoptera: Meloidae). I. The genus *Pyrota*. *The Canadian Entomologist* **96**: 1037-1081.

Selander, R.B. (1986) Rearing blister beetles (Coleoptera, Meloidae). *Insecta Mundi* **1**: 209-220.

Smyth, R.R. & Hoffmann, M.P. (2003) A male-produced aggregation pheromone facilitating *Acalymma vittatum* [F.] (Coleoptera: Chrysomelidae) early-season host plant colonization. *Journal of Insect Behavior* **16**: 347-359.

Sokal, R.R. & Rohlf, F.J. (1969) *Biometry – the principle and practice of statistics in biological research*. 2nd edition. W.H. Freeman and Company, New York.

Stökl, J., Paulus, H., Dafni, A., Schulz, C., Francke, W. & Ayasse, M. (2005) Pollinator attracting odour signals in sexually deceptive orchids of the *Ophrys fusca* group. *Plant Systematics & Evolution* **254**: 105–120.

Turlings, T.C.J., Loughrin, J.H., McCall, P.J., Röse, U.S.R., Lewis, W.J. & Tumlinson, J.H. (1995) How caterpillar-damaged plants protect themselves by attracting parasitic wasps. *Proceedings of the National Academy of Sciences of the USA* **92**: 4169-4174.

Van Tol, R.W.H.M., Visser, J.H. & Sabelis, M.W. (2002) Olfactory responses of the vine weevil, *Otiorhynchus sulcatus*, to tree odours. *Physiological Entomology*. **27**: 213–222.

Watkins, E., Gianfagna, T.J., Sun, R. & Meyer, W.A. (2006) Volatile Compounds of tufted hairgrass. *Crop Science* **46**: 2575-2580.

Webster, B., Bruce, T.J.A., Dufour, S., Birkemeyer, C., Birkett, M.A., Hardie, J. & Pickett, J.A. (2008) Identification of volatile compounds used in host location by the black bean aphid, *Aphis fabae*. *Journal of Chemical Ecology* **34**: 1153-1161.

Wertheim, B., van Baalen, E.A., Dicke, M. & Vet, L.E.M. (2005) Pheromone-mediated aggregation in nonsocial arthropods: an evolutionary ecological perspective. *Annual Review of Entomology* **50**: 321-46.

Whitney, H.M. & Glover, B.J. (2007) Morphology and development of floral features recognised by pollinators. *Arthropod-Plant Interactions* **1**: 147-158.

CHAPTER 5

Volatile components of leaves and flowers of four host plants of *Hycleus apicicornis*

Abstract

Volatile chemicals were collected by air-entrainment from intact and cut flowers and leaves of four host plants (*Abelmoschus esculentus, Desmodium uncinatum, Phaseolus vulgaris* and *Vigna unguiculata)* of the blister beetle *Hycleus apicicornis*. Volatiles were analysed by gas chromatography coupled with mass spectrometry (GC-MS). Fifty-two compounds were identified although the number for each plant species differed, ranging from 21 for *P. vulgaris* to 35 for *D. uncinatum*. Both, qualitative and quantitative differences were observed among and within plant species. There were some similarities in the volatile profiles of *D. uncinatum* and *P. vulgaris,* two plant species that were previously found to be attractive to *H. apicicornis* in behavioural bioassays. Hexanal, 1-hexanol, (*E*)-β-ocimene-beta, 3-Δ-carene and methyl salicylate were found in both of these plant species but not in the headspace of *A. esculentus* and *V. unguiculata,* which were previously found not attractive in behavioural bioassays. Terpenes were the dominant group constituting more than 70% of volatiles of both intact and cut flowers of *D. uncinatum*. For the other three plant species, the dominant group was the aliphatics, which constituted more than 50% of identified volatiles. Of the five compounds detected in intact and cut flowers of *D. uncinatum* and *P vulgaris* alone, (*E*)-β-ocimene was the most abundant constituent. The results highlight similarities and differences between host plants attractive and unattractive to *H. apicicornis.* The results obtained in this study together with those on the behavioural responses of the blister beetle to odour blends from the plant species demonstrate the importance of olfactory cues in host searching by *H. apicicornis.* These findings form the basis for further identification of volatiles/blends that should prove suitable in developing a trapping system in the future.

Keywords: *Abelmoschus esculentus*; air-entrainment; blister beetles; *Desmodium uncinatum*; gas chromatography – mass spectrometry; olfactory cues; *Phaseolus vulgaris*; *Vigna unguiculata*; volatile compounds

5.1 Introduction

Plants emit numerous volatile organic compounds which send out different signals that attract or repel herbivorous insects (Maffei *et al*. 2010; Åhman *et al*. 2010). Plant volatiles may be useful in highlighting the status of a plant at the time of emission. For example, stressed plants may show a qualitative or quantitative increase of volatile emission (Paré & Tumlinson 1996, 1999; Dudareva & Pichersky 2000; Roeder *et al*. 2007; Parra *et al*. 2009) that differs from the volatile blend emitted by a healthy host plant (Paré & Tumlinson 1996). Such variation may result in increased attack by insect pests (Agelopoulos *et al*. 1999a; Rao *et al*. 2003, McCullough *et al*. 2009) while on the other hand it may lead to self-defence through repellence or attraction of natural enemies (Paré & Tumlinson 1999; De Moraes *et al*. 2001; Rodriguez-Saona *et al*. 2009; Bruce *et al*. 2010). Variation in the volatiles released may also be observed in different parts of a single host plant and serve various purposes to different insects (Agelopoulos *et al*. 1999b; Parra *et al*. 2009; Gouinguene & Turlings 2002; Dudareva *et al*. 2004).

Information on the volatile profiles of different host plants of the blister beetle *Hycleus apicicornis* (Guér.) (Coleoptera: Meloidae), a recent pest of *Desmodium* species (Fabaceae) in East Africa (Pickett *et al*. 2010; Lebesa *et al*. 2011), may be important for its management on desmodium and other hosts it attacks (Chapter 4, Abate & Ampofo 1996; Lale & Sastawa 2000). Blister beetles are generally polyphagous (Hill 1975; Zhu *et al*. 2005) and are considered minor pests except when they attack crops that are cultivated on smallholdings where they can cause substantial losses because of their gregarious nature (Hall 1984; Nikbakhtzadeh 2004). Adults of different species of blister beetles feed on the leaves of host plants (Clark *et al*. 1995), floral parts (flowers and pollen) (Evans 1990; Robinson 2005) or developing seeds/grains (Selander 1988; Evans *et al*. 1989; Evans 1990; Chidumayo 2002). Feeding on the floral parts or developing grains/seeds affects seed setting (Gahukar 1991; Lale & Sastawa 2000). This is a challenge that desmodium seed farmers are facing with *Hycleus* spp. in western Kenya (Lebesa *et al*. 2012).

Hycleus apicicornis was recorded as the most abundant species in various locations of western Kenya (Lebesa *et al*. 2012). It was observed feeding on flowers of several plant species belonging to several plant families (Lebesa *et al*. 2011). A study by Lebesa *et al*. (2011, Chapter 3) has shown that visual cues play a major role for host location in this species. However, the behavioural responses of *H. apicicornis* demonstrated mediation of volatiles of some of its host plants in attracting this species in the olfactometry study (Chapter 4). This suggests the olfactory cues may also be involved and thus play a complementary role to the visual cues in host finding of *H. apicicornis*. The importance of olfactory cues may be especially useful for *H. apicicornis* because the flowers it feeds on are different in colour and morphology and are therefore visually different. For example *Abelmoschus esculentus* (L.) Moench, (Malvaceae) has yellow flowers*;* *Phaseolus vulgaris* (L.) (Fabaceae) has white and pink flowers, *Desmodium* spp. (Fabaceae) and *Vigna unguiculata* (L.) Walp, (Leguminoseae) have violet and purple flowers (Lebesa *et al*. 2011).

As part of evaluating the role of olfactory cues in host finding of *H. apicicornis* the chemical profiles of four host plants, *A. esculentus, Desmodium uncinatum* (Jacq.) DC *P. vulgaris* and *V. unguiculata* were determined. Qualitative and quantitative similarities or differences among these plant species were determined. Results obtained could provide some insights into the behavioural differences observed in olfactometer tests (Chapter 4).

5.2 Material & Methods

Host plants

Of the six host plant species, *A. esculentus*, *D. uncinatum*, *I. batatas*, *Ipomoea hildebrandtii* Vatke (Convolvulacieae), P. *vulgaris* and V.*unguiculata*, tested for behavioural responses in Chapter 4, four, *A. esculentus*, *D. uncinatum*, *P. vulgaris* and *V. unguiculata*, were used for collection of volatiles in this study. The choice was based on the availability of seeds of each plant species and the ability to produce flowers when they are planted in pots. Based on the response of *H. apicicornis* in the olfactometer tests the four plants were divided into two groups. The first group was composed of plants species, *D. uncinatum* and *P. vulgaris* whose flowers were attractive and the second was composed of *A. esculentus* and *V. unguiculata* whose flowers were not attractive to *H. apicicornis* (Chapter 4).

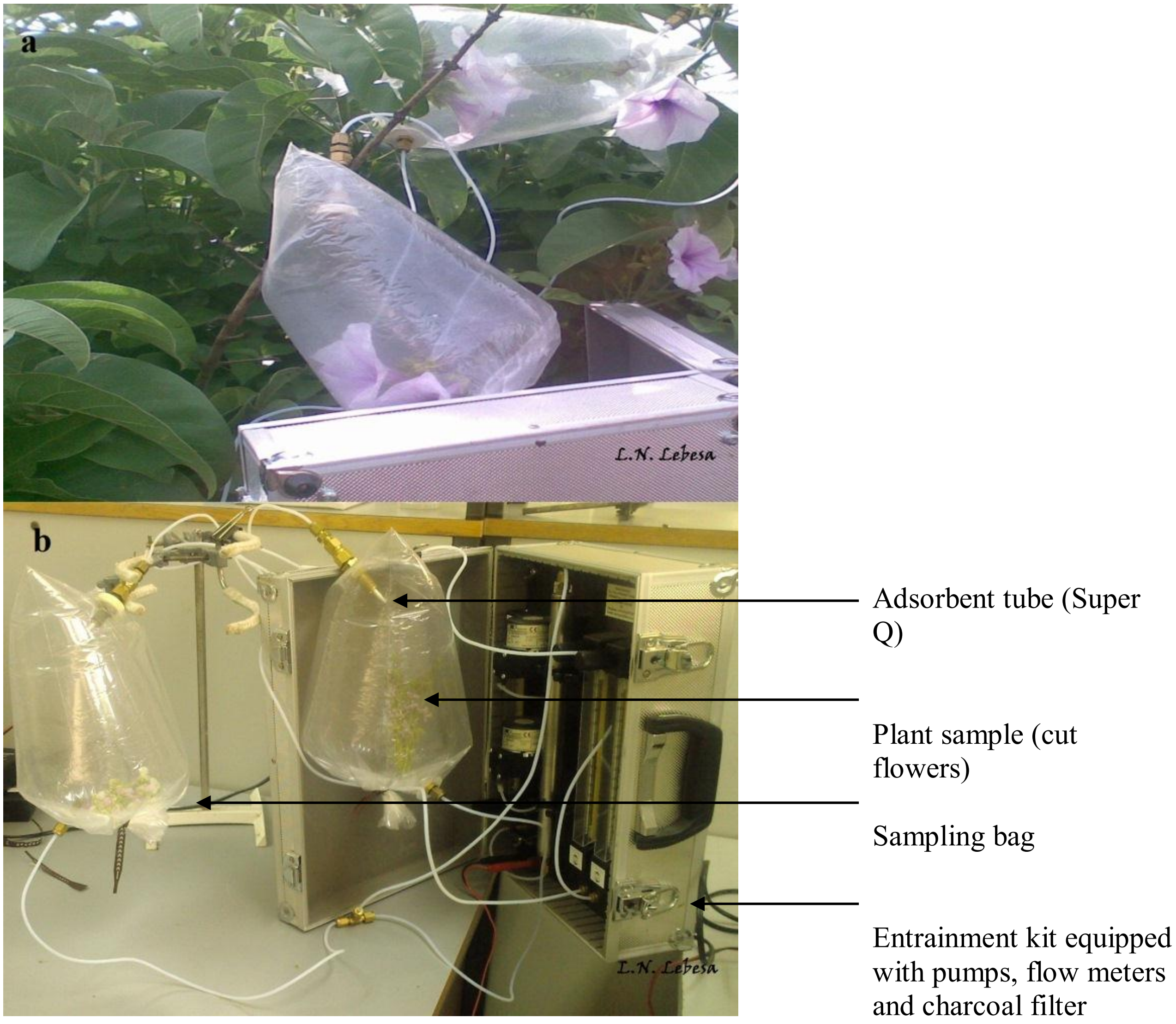
Plants were grown in 5 l pots in an insect-free greenhouse at Experimental Farm of the University of Pretoria (Pretoria, South Africa, 25º43'S 28º17'E), under natural lighting conditions from August to October (10L:14D) and November to December (11L:13D) 2009. Temperatures and relative humidity ranged from 24.0 °C to 32.4 °C and 94.2 % to 99.9 %, respectively. Planting and fertilization followed the normal recommendations for these crops.

Air entrainment of volatiles from plants

Adsorbent tubes, Super Q (Analytical Research Systems, Gainesville, FL), were cleaned with 600µl of GC/GC-MS–grade dichloromethane (Burdick and Jackson, Muskegon, Michigan, USA) under a gentle stream of nitrogen before use. Volatiles were collected from the following organs i) flowers of intact plants, ii) leaves of intact plants and iii) cut flowers (excised at the pedicel to exclude leaves and stems except for flowers of *D. uncinatum* that were attached to the stem) (Fig. 1).

The organs from which volatiles were collected were enclosed in cooking bags (Pick 'n Pay multi-purpose oven bags, South Africa) that were baked overnight in an oven at 180 °C to desorb any volatiles. A bag was attached to two tubes, one supplying clean incoming air and the other was used for outgoing air. Air was purified by passing through a charcoal tube and was then blown into the bottom of a glass chamber at 0.7 l/min and was drawn out at 0.4 l/min from the top. Volatiles were collected on Super Q adsorbent traps (Fig. 1). After removal, tubes were sealed with Teflon tape and stored in a freezer at -10 °C until elution. Floral volatiles from three individual plants of each host plant species were entrained at two intervals of three hours (0900-1200hrs) and seven hours (0900-1600hrs) each day for three consecutive days. Volatiles from leaves were collected from one plant of each host plant species each day for the three consecutive days. Air entrainments were also undertaken using four empty bags as controls.

Volatiles trapped on Super Q tubes were eluted with 200µl of GC/GC-MS–grade dichloromethane (Burdick and Jackson, Muskegon, Michigan, USA) under a gentle stream of nitrogen and stored in tightly screw-capped 2 ml vials (Agilent Technologies). Samples were stored at -10°C until needed for GC-MS analyses or further behavioural tests. The ability of the samples to be stored for a longer period for subsequent use is one of the advantages of the solvent desorption method (D'Alessandro & Turlings 2006).



Adsorbent tube (Super Q)

Plant sample (cut flowers)

Sampling bag

Entrainment kit equipped with pumps, flow meters and charcoal filter

Figure 1. Set up of an air-entrainment system. Collection of volatiles from a) intact flowers of *Ipomoea hildebrandtii* Vatke and b) cut flowers of *Phaseolus vulgaris* (left) *Desmodium uncinatum* (right).

Identification of Volatiles

The extracted volatiles were analysed with 7890A Gas Chromatograph (GC) equipped with an HP-5 column (30 x 250 µm i.d. x 0.25 µm film thickness) coupled to a 5975C mass spectrometer (MS) (Agilent Technologies, UK) at ICIPE, Kenya. The carrier gas used was helium. The initial oven temperature was 35 °C for 5 min and was then increased to 280 °C at 10 °C/min and held for 10.5 min. Compounds were identified through comparison of observed mass spectra (MS) with those of library data (NIST 05a, Adams 2 and chemecol). Identified compounds were quantified by comparing the peak area ratio with that of an internal standard (methyl octanoate). The chemical purity of the internal standards was 95% and serial dilutions were made with dichloromethane. Of each volatile sample containing an internal standard and volatile solution, 1 µl aliquot was injected into the port for GC-MS analysis.

Statistical analysis

A one-way analysis of variance (ANOVA) was used to determine significant differences between host plant species. T-tests were used to compare the levels of compounds in cut and intact flowers of individual plants (Sokal & Rohlf 1969). Data were analysed with Statistica (Version 9.0, StatSoft, Inc, 1984-2009).

5.3 Results

Volatile collection from flowers

Compounds that were found in the control samples (empty bags) were excluded from analyses in the plant samples. During the seven-hour volatile collection period 35 and 49 compounds were identified in the intact and cut flowers, respectively, from four host plant species (Table 1). Some compounds not listed in Table 1, were recorded from a single sample of a plant species only or occurred in trace amounts. Such compounds include ethyl acetophenone in *D. uncinatum,* (*E*)-2-decenal in *D. uncinatum* and *A. esculentus* and limonene in all plants. Apart from a greater number of compounds being trapped from cut flowers than their intact counterparts, most of the compounds were also emitted in greater quantities (Fig. 2).

Desmodium uncinatum emitted the largest number of the recorded compounds (35), followed by *A. esculentus* (24), *V. unguiculata* (23) and *P. vulgaris* (21). The aliphatics were dominant qualitatively and quantitatively in all host plant species except in *D. uncinatum* whose volatiles were dominated by terpenoids (Table 1). The aliphatics constituted more than 50% of the volatiles of the cut flowers of the plant species evaluated. The same trend was observed with intact flowers of these plant species, except for *P. vulgaris* where aromatic compounds were dominant (Table 1).

The compound emitted in the highest proportion was (*E*)-β-ocimene from both cut and intact flowers of *D. uncinatum*. The most abundant compounds per plant species differed among plant species. For example, (*Z*)-3-hexenol, acetophenone and (*Z*)-3-hexenyl acetate were the most abundant compounds in *P. vulgaris,* (*E*)-4,8-dimethyl-1,3,7-nonatriene (DMNT) in *V. unguiculata* and isopropyl myristate in *A. esculentus*.

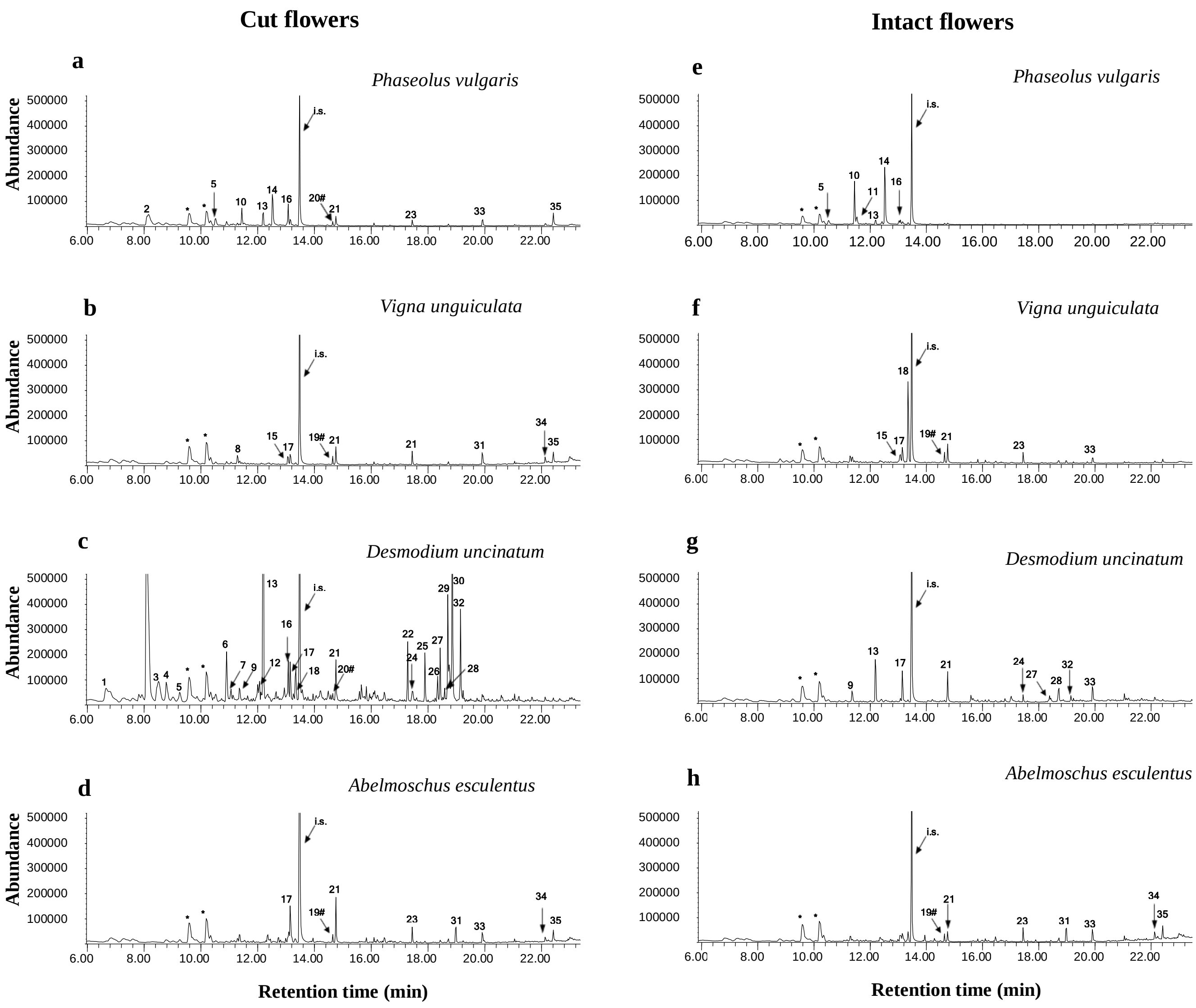


Figure 2. Chromatographic profile of volatile compounds collected for 7 hours from cut and intact flowers of four host plant species of *Hycleus apicicornis*. Asteriks denote some of the components observed also in the control chromatograms, # denotes the overlapping retention times (14.644 and 14.666) for dodecane and methyl salicylate respectively. For a description of labelled peaks, see Table 1.

Both qualitative and quantitative similarities were observed for the two groups, attractive and unattractive of host plant species. Five compounds, hexanal, 1-hexanol, ((*E*)-β-ocimene 3-Δ-carene and methyl salicylate, were common to cut flowers of *D. uncinatum* and *P. vulgaris*. Most of the compounds were emitted in larger amounts in cut than intact flowers of these two host plant species (Table 1). Compounds common to mostly cut flowers of *A. esculentus* and *V. unguiculata* were n-undecane, dodecane, butylated hydroxytoluene, pentadecane, and heptadecane. The latter two were also recorded from intact flowers of *D. uncinatum*. In contrast to *D. uncinatum* and *P. vulgaris*, most volatiles from *A. esculentus* and *V. unguiculata* were emitted in larger amounts from the intact than cut flowers of (Table 1).

More than ten compounds were present in the cut flowers of *D. uncinatum* alone. The majority of these compounds were terpenoids (mainly sesquiterpenes), a group that forms major constituents of its volatiles. Fig. 2 and Table 1). None of the flowers of other host plants emitted sesquiterpenes except flowers of *A. esculentus* which emitted low amounts of (*E*)-caryophyllene (Table 1). (Z)-3-Hexenol and hexenyl acetate were detected in the flowers of *P. vulgaris* only. In addition, acetophenone was found in relatively large amounts in cut and intact flowers of *P.* vulgaris. This aromatic compound was also detected, albeit in very low amounts, in cut and intact flowers of *V. unguiculata* and intact flowers of *A. esculentus* but not in intact or cut flowers of *D. uncinatum*. No unique compounds were collected from cut or intact flowers of *A. esculentus* and *V. unguiculata*.

Nine compounds, namely 2-penten-2-one (tentative identity), nonanal, decanal, tridecane, hexadecane, octadecane, isopropyl myristate, benzaldehyde and DMNT were common to all four host plant species. Of these only two, nonanal and decanal, were present in both cut and intact flowers of all plant species (Table 1). Among the compounds that were not recorded from intact flowers were hexanal, (*Z*)-3-hexenol, 1-hexanol, benzaldehyde and octadecane, and a number of terpenoids for *D. uncinatum*.

Table 1. Volatiles collected for 7 hours from intact and cut flowers of four host plant species of *Hycleus apicicornis.* Mean values (ng ± SEM) from three to four samples are presented. Undetected compounds indicated by a minus (-) sign. Compounds with superscript numbers denote labelled peaks in Figure 2.

Compounds	Retention time (min)	*Phaseolus vulgaris*		*Vigna unguiculata*		*Desmodium uncinatum*		*Abelmoschus esculentus*	
		cut flowers	intact flowers	cut flowers	intact flowers	cut flowers	intact flowers	cut flowers	intact flowers
Aliphatic compounds									
Hexanal^{1}	6.67	3.96 ± 0.20	–	–	–	31.23 ± 12.76	–	–	–
(*E*)-2-Hexenal^{2}	8.104		–	–	–	150.3 ± 51.80	–	–	–
(*Z*)-3-Hexenol	8.193	29.98 ± 13.24	–	–	–	2.82 ± 0.36	–	–	–
1-Hexanol^{3}	8.529	8.84 ± 0.03	–	–	–	22.16 ± 8.43	–	–	–
3-Penten-2-one**	8.798	5.50 ± 0.56	3.90	5.00 ± 0.76	5.42 ± 1.75	13.51 ± 2.91	5.00 ± 0.31	3.83 ± 1.03	6.25 ± 1.62
Heptanal	9.268	–	–	3.07 ± 0.47	4.76*	8.05 ± 0.89	3.90 ± 0.57	2.50 ± 0.69	–
1,3-Octadiene^{6}	10.903	6.00 ± 1.21*	–	2.66 ± 0.94	2.01*	23.70 ± 2.12	1.48 ± 0.08	–	–
3-Octanone^{7}	11.06	–	–	1.61 ± 0.20	3.39 ± 1.89	7.76 ± 0.54	–	1.13 ± 0.27	–
n-Decane^{8}	11.307	2.59 ± 0.32	–	5.68 ± 1.85	–	–	–	3.80 ± 1.60	5.18 ± 1.29
Octanal^{9}	11.374	–	–	3.86 ± 1.90	–	11.79 ± 1.38	5.54 ± 1.59	3.37 ± 1.14	–
(*Z*)-3-Hexenyl acetate^{10}	11.441	10.14 ± 2.19	23.54*	–	–	–	–	–	–
2-Nonanone	12.942	–	–	–	–	10.85 ± 0.38	–	–	–
n-Undecane^{15}	13.054	–	–	4.34 ± 0.42	7.71 ± 2.39	–	–	5.53 ± 1.09*	–
Nonanal^{17}	13.166	5.38 ± 1.34	4.02 ± 0.15	7.60 ± 1.83	9.93 ± 4.19	22.87 ± 3.06	12.70 ± 2.58	11.45 ± 2.26	8.86 ± 4.77
Dodecane$^{19\#}$	14.644	–	–	2.72 ± 0.51	4.17 ± 1.23	–	–	2.86 ± 0.77	2.89*
Decanal^{21}	14.756	6.08 ± 1.41	2.71*	9.10 ± 2.57	10.01 ± 4.07	25.23 ± 5.68	12.87 ± 2.69	10.97 ± 4.15	7.47*
Tridecane	16.01	1.69 ± 0.51*	–	1.95 ± 0.93	1.92 ± 0.74	11.12 ± 2.41	3.49 ± 1.70	2.05 ± 0.56	1.93 ± 0.27
Tetradecane^{23}	17.466	3.20 ± 1.19*	–	3.63 ± 0.97	4.65 ± 1.58	–	–	5.26 ± 1.46	–
Pentadecane	18.72	–	–	1.61 ± 0.48	2.25 ± 0.82	–	8.08 ± 4.43	2.21 ± 0.60	2.84 ± 0.89

Compounds	Retention time (min)	*Phaseolus vulgaris*		*Vigna unguiculata*		*Desmodium uncinatum*		*Abelmoschus esculentus*	
		cut flowers	intact flowers	cut flowers	intact flowers	cut flowers	intact flowers	cut flowers	intact flowers
Hexadecane^{33}	19.93	4.69 ± 1.64	—	4.95 ± 1.40	5.30 ± 1.55	7.68 ± 0.66	9.70 ± 2.94	7.44 ± 1.66	7.91 ± 1.24
Heptadecane	21.049	—	—	2.07 ± 0.04	2.68 ± 1.78	—	6.04 ± 1.77	6.75 ± 2.88	—
Octadecane^{34}	22.147	2.22 ± 1.01	—	3.23 ± 0.66	—	6.47 ± 2.36	6.03 ± 1.86	7.14 ± 2.78	—
Isopropyl myristate	22.416	7.54 ± 3.36	—	5.07 ± 1.56	6.44 ± 1.48	4.33 ± 0.65	2.90 ± 0.45	10.64 ± 3.61	15.41 ± 6.43
Aromatics									
Cyclooctane, methyl-	9.022	—	—	1.89 ± 0.20	3.13	5.67 ± 0.77	—	1.65 ± 0.33	—
Benzaldehyde^{5}	10.523	12.81 ± 2.11	9.16	1.92 ± 0.38	2.39 ± 0.69	5.64 ± 1.405	—	1.15 ± 0.20	—
Benzene, 1,4-dichloro-^{11}	11.531	4.56 ± 1.26	4.77 ± 2.58	1.56 ± 0.34	—	10.12 ± 6.12*	—	1.65 ± 0.53	2.01± 0.85
Acetophenone^{14}	12.538	29.92 ± 10.43	40.95 ± 17.43	1.10 ± 0.04	1.91	—	—	1.26 ± 0.22	—
Methyl benzoate	13.031	—	8.85						
Methyl salicylate$^{20\#}$	14.644	2.68 ± 0.37	1.84*	—	—	5.39 ± 1.75	16.18 ± 13.39	—	—
Butylated hydroxytoluene^{31}	18.989	—	—	1.74	2.35 *	—	—	5.30 ± 2.28	7.21 ± 3.69
Tepernoids									
β-Myrcene	11.15	3.68 ± 0.72*	3.89 ± 0.64*	—	—	5.72 ± 0.36	4.09 ± 0.95	1.27 ± 0.31	—
(*Z*)-β-ocimene^{12}	12.001	—	—	—	—	11.16 ± 2.28	7.59 ± 5.04	—	—
(*E*)-β-ocimene^{13}	12.203	10.18 ± 2.43	6.46 ± 2.14	—	1.84 *	329.86 ± 89.52	277.57 ± 182.30	—	—
3-Δ-Carene^{16}	13.076	13.13 ± 3.18	—	—	—	33.56 ± 8.84	20.04 ± 12.65	—	—
(*E*)-4,8-Dimethyl-1,3,7-nonatriene^{18}	13.345	—	1.93 ± 0.22	2.50 ± 1.09	26.35 ± 13.88	29.11 ± 6.91	22.35 ± 12.92	3.27 ± 1.74	10.33 ± 3.88*
Allo-ocimene	13.569	—	—	—	—	13.36 *	—	—	—
^{s}α-Copaene^{22}	17.287	—	—	—	—	26.16 ± 3.96	2.10 ± 0.21*	—	—
^{s}Elemene^{24}	17.466	—	—	—	—	10.29 ± 0.88	9.58 ± 4.79	—	—

Compounds	Retention time (min)	*Phaseolus vulgaris*		*Vigna unguiculata*		*Desmodium uncinatum*		*Abelmoschus esculentus*	
		cut flowers	intact flowers	cut flowers	intact flowers	cut flowers	intact flowers	cut flowers	intact flowers
[s](*E*)-Caryophyllene[25]	17.891	–	–	–	–	29.03 ± 7.30	–	0.95 ± 0.26	3.04 ± 0.81*
[s]Humulene[26]	18.339	–	–	–	–	13.66*	–	–	–
[s]α-Guaiene[27]	18.429	–	–	–	–	18.08 ± 1.77	9.16* ± 1.94	–	–
[s] γ-Muurolene[28]	18.587	–	–	–	–	9.02 ± 1.40	–	–	–
[s]β-Patchoulene[29]	18.698	–	–		–	65.58 ± 8.71	–	–	–
[s]α-Selinene[30]	18.877	–	–		–	65.71 ± 11.64	6.40 ± 3.61	–	–
[s]7- epi-α-Selinene [32]	19.146	–	–		–	52.60 ± 8.83	–	–	–
% Aliphatics		**(56.0)**	**(30.5)**	**(86.4)**	**(65.0)**	**(22.1)**	**(17.1)**	**(84.0)**	**(72.2)**
% Aromatics		**(28.6)**	**(50.6)**	**(10.4)**	**(9.0)**	**(2.8)**	**(3.6)**	**(10.6)**	**(11.3)**
% Terpenoids		**(15.4)**	**(18.9)**	**(3.2)**	**(26.0)**	**(75.1)**	**(79.3)**	**(4.4)**	**(16.5)**

Compounds in their respective groups are listed in the order of elution from an HP-5 GC column.
The peak area of an internal standard, methyl octanoate (109.515 ng) was used for comparison with peak areas of volatile compounds to estimate the amount of the detected compounds.
#denotes the overlapping retention times (14.644 and 14.666) for Dodecane and Methyl salicylate respectively.
*compounds were detected in two samples only.
[S] = sesquiterpenes, all other are monoterpenes
**tentative identity.

Of the volatile compounds collected during the seven-hour collection period, 34 compounds were observed to have prominent peaks (Fig. 2). Further analyses show some significant differences among host plant species and between cut and intact flowers of individual plants. Quantitatively, cut flowers of *D. uncinatum* had a significantly larger total amount of volatiles than cut flowers in all other host plant species ($F_{(3, 416)} = 15.170$, $P < 0.0001$). Although a general quantitative increase of compounds was observed in cut flowers of the different host plant species, significant differences were observed only for *D. uncinatum* and on four compounds only (3-penten-2-one: $t = 2.94$, df = 4, $P = 0.043$; 1,3 octadiene: $t = 10.44$, df = 4, $P < 0.001$; α-copaene: $t = 6.44$, df = 4, $P = 0.003$; α-guaiene: $t = 3.02$, df = 4, $P = 0.039$; α-selinene: $t = 4.90$, df = 4, $P = 0.008$).

Volatile collection from leaves of host plants

In total 27 compounds were detected from the leaves of all four host plant species (Table 2). The same compounds were also present in the flowers (Table 1). *Desmodium uncinatum* had the highest number of volatiles (18) followed by *A. esculentus* (17), *V. unguiculata* (14) and *P. vulgaris* (11). The aliphatic compounds were the most abundant in all four host plant species. *Desmodium uncinatum* had the highest number of terpenoids although, unlike in the floral volatiles, they were not the dominant group (Table 2). Although volatiles in the leaves were not quantified, (*E*)-β-ocimene similar to intact and cut flowers, was the most abundant compound in leaves of *D. uncinatum* based on the chromatographic profile (not presented). Sesquiterpenes were not detected in the leaves of any of the host plant species. Similar to the floral volatile profiles, (*E*)-4,8-dimethyl-1,3,7-nonatriene (DMNT) was among the seven common compounds present in the leaf profiles of all host four plant species (Table 2). The other six compounds common to the leaf volatile profiles, and which were recorded in at least one of floral profiles of the host plant species, were n-decane, nonanal, tetradecane, hexadecane, isopropyl myristate and butylated hydroxytoluene (Table 1 and 2).

Table 2. Volatiles collected for 7 hours from intact leaves of four host plant species of *Hycleus apicicornis*. (√ : present; – : not detected; T : traces; below 1.5 percent of the peak area or detected in only one sample per host plant).

Compounds	Retention time (min)	*Abelmoschus esculentus*	*Desmodium uncinatum*	*Phaseolus vulgaris*	*Vigna unguiculata*
Aliphatic compounds					
Hexanal	6.67	–	√	–	–
(*Z*)-3-Hexenol	8.193	–	√	–	–
3-Penten-2-one*	8.798	–	T	T	–
Heptanal	9.268	T	–	–	T
3-Octanone	11.06	–	T	–	T
n-Decane	11.307	√	T	T	√
Octanal	11.374	T	–	–	√
(*Z*)-3-hexenyl acetate	11.441	T	√	–	T
n-Undecane	13.054	√	–	–	√
Nonanal	13.166	√	√	T	√
Dodecane#	14.644	√	–	–	√
Decanal	14.756	√	√	–	√
Tetradecane	17.466	√	√	T	√
Hexadecane	19.93	√	√	T	√
Octadecane	22.147	T	–	–	–
Isopropyl Myristate	22.416	√	T	√	√
Aromatics					
Benzaldehyde	10.523	T	–	–	–
Benzene, 1,4-dichloro-	*11.531*	*T*	–	–	–
Acetophenone	12.538	T	–	–	–
Ethyl benzoate	14.263	–	T	–	–
Methyl salicylate#	14.644	–	√	–	–
Butylated hydroxytoluene	18.989	√	T	T	√

Compounds	Retention time (min)	*Abelmoschus esculentus*	*Desmodium uncinatum*	*Phaseolus vulgaris*	*Vigna unguiculata*
Tepernoids					
(*Z*)-β-ocimene	12.001	–	√	–	–
(*E*)-β-ocimene	12.203	–	√	T	–
3-Δ-Carene	13.076	–	√	T	–
(*E*)-4,8-Dimethyl-1,3,7-nonatriene	13.345	√	√	√	√
Allo-ocimene	13.569	–	√	–	–
No. (%) Aliphatics		**12 (70)**	**11 (58)**	**6 (60)**	**12 (86)**
No. (%) Aromatics		**4 (24)**	**3 (16)**	**1 (10)**	**1 (7)**
No. (%) Terpenoids		**1 (6)**	**5 (26)**	**3 (30)**	**1 (7)**

Compounds in their respective groups are listed in the order of elution from an HP-5 GC column.
# denotes the overlapping retention times (14.644 and 14.666) for dodecane and methyl salicylate respectively.
*tentative identity.

Volatile collection from the flowers of host plant species at 3 hour interval

Compared to the seven-hour collection period fewer compounds were detected in the three-hour volatile collection period for all plant species. However, all the green-leaf volatiles recorded during the seven-hour collection period, except hexenal and (*Z*)-3-hexenol, were present (Table 3). In comparison to the seven-hour collections most compounds were emitted in smaller amounts in the intact flowers than in the cut flowers for all plant species. (*E*)-β-ocimene was again the most abundant compound but at less than a quarter of the concentration observed in the seven-hour collections (Table 3). Similar to the seven-hour collections, (*E*)-β-ocimene was detected in the leguminous crops only. For both collection periods the amounts present in *V. unguiculata* samples were very small (Table 1 and 3). The aliphatics were dominant in flowers of all plants except the flowers of *D. uncinatum* whose most abundant group was terpenoids (Table 3).

Table 3. Total volatiles collected for 3 hours from the intact and cut flowers of four host plant species of *Hycleus apicicornis*. Mean values (ng ± SEM) from three to four replicates are presented. Undetected compounds indicated by a minus (-) sign.

Compounds	Retention time (min)	*Phaseolus vulgaris*		*Vigna unguiculata*		*Desmodium uncinatum*		*Abelmoschus esculentus*	
		cut flowers	intact flowers	cut flowers	intact flowers	cut flowers	intact flowers	cut flowers	intact flowers
Aliphatic compounds									
Hexanal	6.67	—	—	—	—	7.95 ± 1.85	—	—	—
(*E*)-2-Hexenal	8.104	—	—	—	—	38.28 ± 9.08	—	—	—
3-Penten-2-one*	8.798	4.87 ± 0.20	11.62 ± 5.45	4.36 ± 0.38	4.47 ± 0.15	5.17 ± 0.84	4.40 ± 0.44	5.94 ± 1.44	4.89 ± 0.30
Heptanal	9.268	—	—	—	—	—	2.08 ± 0.49	4.38 ± 0.85	—
1,3-Octadiene	10.903	—	—	—	—	4.06 ± 1.14	—	—	—
3-Octanone	11.06	0.99 ± 0.27	—	—	—	—	—	—	—
n-Decane	11.307	—	2.70 ± 1.14	—	—	—	—	4.40 ± 1.92	—
Octanal	11.374	2.07 ± 0.26	—	3.32 ± 1.21	2.65 ± 0.68	—	—	3.72 ± 0.45	2.35 ± 0.66
(*Z*)-3-hexenyl acetate	11.441	3.71 ± 1.01	—	—	—	—	—	—	—
2- nonanone	12.942	—	—	—	—	—	—	0.88 ± 0.18	—
Nonanal	13.166	—	3.49 ± 0.87	6.18 ± 3.04	6.06 ± 1.40	—	2.50 ± 0.85	11.08 ± 2.61	7.75 ± 1.91
Dodecane	14.644	—	—	—	0.72 ± 0.02	—	—	2.59 ± 0.51	—
Decanal	14.756	4.89 ± 0.55	—	4.55 ± 1.52	3.60 ± 0.11	2.15 ± 0.44	2.26 ± 0.31	7.64 ± 0.82	3.57 ± 1.76
Tridecane	16.01	—	—	—	—	—	—	1.59±0.15	—
Tetradecane	17.466	3.57 ± 0.59	—	—	—	—	—	—	—
Pentadecane	18.72	—	—	1.21 ± 0.32	—	—	1.44 ± 0.27	—	—
Hexadecane	19.93	5.65 ± 1.94	—	—	—	—	—	8.86 ± 5.49	—

	Retention	*Phaseolus vulgaris*		*Vigna unguiculata*		*Desmodium uncinatum*		*Abelmoschus esculentus*	
Compounds	time (min)	cut flowers	intact flowers	cut flowers	intact flowers	cut flowers	intact flowers	cut flowers	intact flowers
Heptadecane	21.049	2.90 ± 1.30	—	—	—	—	1.71 ± 0.97	—	—
Octadecane	22.147	2.50 ± 1.01	—	—	—	—	2.34 ± 0.37	—	—
Isopropyl myristate	22.416	2.08 ± 0.77	—	—	—	—	1.40 ± 0.44	—	—
Aromatics									
Cyclooctane, methyl-	9.022	—	—	5.54 ± 3.78	—	—	—	—	—
Benzaldehyde	10.523	4.97 ± 2.27	—	—	—	—	—	—	—
Benzene, 1,4-dichloro-	11.531	—	—	—	—	—	1.18 ± 0.11	2.96 ± 1.45	—
Acetophenone	12.538	22.83 ± 7.34	—	—	—	—	—	—	—
Methyl salicylate[#]	14.644	1.95 ± 0.73	—	—	—	—	—	—	—
Butylated hydroxytoluene	18.989	1.14 ± 0.38	—	—	—	—	—	—	—
Tepernoids									
β-Myrcene	11.15	—	—	—	—	—	1.18 ± 0.31	3.32 ± 1.73	—
(*Z*)-β-Ocimene	12.001	—	—	—	—	1.73 ± 0.16	—	—	—
(*E*)-β-Ocimene	12.203	7.22 ± 4.98	—	2.17 ± 0.76	—	79.45 ± 12.52	15.14 ± 7.97	—	—
3-Δ-Carene	13.054	5.53 ± 1.72	—	—	—	9.67 ± 3.52	—	—	—
(*E*)-4,8-Dimethyl-1,3,7-nonatriene	13.345	—	—	1.39 ± 0.24	—	3.05 ± 0.40	2.56 ± 1.92	3.34 ± 1.08	—
[s]α-Copaene	17.287	—	—	—	—	2.11 ± 0.81	—	—	—
[s] (*E*)-Caryophyllene	17.891	—	—	—	—	2.81 ± 0.74	—	—	—
[s]β-Patchoulene	18.698	—	—	—	—	7.19 ± 2.78	—	—	—
[s]α-Selinene < α ->	18.877	—	—	—	—	6.87 ± 2.94	—	—	—

	Retention	*Phaseolus vulgaris*		*Vigna unguiculata*		*Desmodium uncinatum*		*Abelmoschus esculentus*	
Compounds	time (min)	cut flowers	intact flowers	cut flowers	intact flowers	cut flowers	intact flowers	cut flowers	intact flowers
[s]7-Selinene-epi-α-	19.146	—	—	—	—	5.01 ± 1.80	—	—	—
% Aliphatics		**(43.2)**	**(100)**	**(68.3)**	**(100)**	**(32.8)**	**(47.5)**	**(84.1)**	**(100)**
% Aromatics		**(40.2)**	**(0.0)**	**(19.3)**	**(0.0)**	**(0.0)**	**(3.1)**	**(4.9)**	**(0.0)**
% Terpenoids		**(16.6)**	**(0.0)**	**(12.4)**	**(0.0)**	**(67.2)**	**(49.4)**	**(0.0)**	**(0.0)**

Compounds in their respective groups are listed in the order of elution from an HP-5 GC column.
T = traces (compounds found in three or more samples but were below 1.5% or were more than 1.5% but were present in only two samples).
# Methyl Salicylate and Dodecane were eluted at the same time and the first was found in beans and desmodium while the latter was found in cowpea and okra plants.
**tentative identity.

5.4 Discussion

The four host plant species, *A. esculentus, D. uncinatum, P. vulgaris* and *V. unguiculata*, whose floral and leaf volatiles were evaluated in this study, represented the plant species that were evaluated for their attraction to *H. apicicornis* in the previous chapter (Chapter 4). Due to responses of *H. apicicornis* to odours of host plant species in the olfactometer tests (Chapter 4), plant species were divided into two groups based on whether they were attractive or unattractive to adult beetles. In that study flowers of *D. uncinatum* and *P. vulgaris* were more attractive than other plants and these were more attractive as cut flowers than intact flowers. Therefore, the volatile constituents of the tested plant species would also be expected to fall into these two broad groups of attractive and unattractive plants as well as examining differences in the volatile profiles between cut and intact flowers.

For *D. uncinatum* and *P. vulgaris,* the more attractive host plants, a quantitative increase with most volatile compounds was observed in cut flowers. Whereas for *A. esculentus* and *V. unguiculata*, the non-attractive host plants, an unusual observation was made in that most compounds were emitted in larger amounts by the intact than by the cut flowers. Several studies have shown that damaging plants modifies their volatile composition quantitatively and/or qualitatively (Dicke & van Loon 2000; Van Tol *et al.* 2002; Jönsson & Anderson 2008) thus confirming differences between cut and intact flowers in this study.

The exclusive presence of five volatile compounds (hexanal, 1-hexanol, (*Z*)-3-hexenol, (*E*)-β-ocimene and 3-Δ-carene) in *D. uncinatum* and *P. vulgaris* were among the qualitative similarities observed between these two plant species. (*E*)-β-ocimene was the most abundant compound and was recorded almost exclusively in the attractive plant species, with minor quantities occurring in *V. unguiculata*. To determine whether these compounds are attractants behavioural bioassays are needed. (*E*)-β-ocimene was found to be repellent to the stemborer moths but attractive to their natural enemies (Khan *et al*. 2006; Khan *et al*. 2010), thus its influence on the blister beetle may be important.

N-undecane, dodecane and butylated hydroxytoluene were exclusively recorded from *A. esculentus* and *V. unguiculata,* the non-attractive plant species, pentadecane and heptadecane were also detected in both cut and intact flowers of *A. esculentus* and *V. unguiculata*. However, they were also recorded in the intact flowers of *D. uncinatum*. The exclusive presence of some compounds in the non-attractive plants suggests that either one or all of them may be associated with repellence or avoidance behaviour of *H. apicicornis* towards them. Again, behavioural assays are required to determine the role of these compounds in host plant selection of *H. apicicornis*.

Benzaldehyde, (*E*)-ocimene, α-pinene, myrcene and caryophyllene often occur in floral profiles (Knudsen *et al*. 2006; Maffei 2010). Knudsen *et al*. (2006) also highlight the fact that during headspace collection from flowers it is not easy to determine whether emissions are from the flowers themselves or from the nearest vegetative parts. *Desmodium uncinatum* may have the richest profile due to the position of its flowers which may have resulted in trapping of volatiles from other plant organs i.e. stems and buds. However, volatiles from vegetative parts are less diverse than floral volatiles (Dudareva *et al*. 2004; Knudsen *et al*., 2006). The absence of sesquiterpenes except for caryophyllene in leaves is in agreement with the fact that these terpenes are typical of floral volatile compositions (Chen *et al*. 2003; Stranden *et al*. 2003; Maffei 2010). This observation together with the results that fewer leaf volatiles compared to floral volatiles were recorded for all four plant species in this study suggests that the diversity of volatiles collected from flowers from *D. uncinatum* is largely due to floral volatiles.

Most plants release green-leaf volatiles in response to mechanical damage (Hatanaka 1993; Shiojiri *et al*. 2006; Jönsson & Anderson 2008). Usually, mechanical damage does not induce as great an increase in volatile emissions as herbivory (Van Den Boom *et al*. 2004) but still results in a modified quantitative or qualitative profile in the damaged plants compared to intact plants (Knudsen *et al*. 2006). In the current study, the presence of green-leaf volatiles such as (*Z*)-3-hexenol, hexenal and 1-hexenol in cut flowers only supports the observation that mechanical damage results in an increased emission of green-leaf volatiles (Hatanaka 1993; Van Den Boom *et al*. 2004; Jönsson & Anderson 2008).

A reduction in the number and quantities of some volatiles was observed during the three-hour collection period compared to the longer seven-hour collection period. Photoperiodic variation in plants may result in disparities in volatile emissions within or between different studies (Agelopoulos 2000; De Moraes *et al*. 2001; Chamberlain *et al*. 2006; Jönsson & Anderson 2008). According to Karlsson (2010), green leaf volatiles of potato foliage were released in larger amounts in the first five minutes after damage, and then declined after ten minutes. Agelopoulos *et al*. (2000) observed that sesquiterpenes were released in significantly larger amounts in the afternoon than in the morning hours. In this study, the three-hour collections were undertaken from 9.00 a.m. to 12.00 p.m while the seven-hour durations extended into the afternoon (4.00 p.m.). It is, therefore, possible that collections over a longer time period result in quantitatively and qualitatively increased volatile emissions. Similarly, Colazza *et al*. (2004) observed that fewer compounds were released when samples were collected over shorter intervals.

Most other studies done to profile volatile blends of the plant species used in this study were done on leaves only. For example, only eleven and eight of the compounds identified in the leaves of *P. vulgaris* by Birkett *et al*. (2003) and Colazza *et al*. (2004), respectively, were recorded in this study. These authors also observed that green-leaf volatiles were released in larger amounts from damaged leaves of *P. vulgaris* compared to the undamaged leaves. This is similar to this study where cut compared to intact flowers of

P. vulgaris released volatiles in larger amounts. In this study, however, only three of those compounds (nonanal, DMNT and (*E*)-β-ocimene) were observed in the leaves. The disparity may be due to the undamaged status of the plant tissue and the shorter period of volatile collection (3 hours) from the leaves in the current study.

Major volatile compounds in the studies on *V. unguiculata*, included hexanal, n-octane, (*Z*)-3-hexenol, (*Z*)-3-hexenyl acetate and nonyl acetate (Lwande *et al*. 1989; Whitman & Eller 1990) that were either found as traces in the leaves only or were not detected in this study. In other studies, DMNT is one of the commonly induced volatiles in many plants including cowpea (Van den Boom *et al*. 2004; Carroll *et al*. 2008). However, in this study it was detected in greater quantities in the intact flowers than in the cut flowers. As observed in the profiles of flowers of the different host plant species, the volatiles identified in this study are not necessarily species or family-specific because they were found in Fabaceae as well as in Malvaceae. This suggests that the ratio of volatile components believed to be crucial for host plant selection in some insects could be applicable also in the case of *H. apicicornis* (Bruce *et al*. 2005; Webster et al. 2008a).

Comparing the plant volatiles in the different host plant species used in this study does not automatically imply that compounds found in the attractive group are responsible for attraction of *H. apicicornis* to *D. uncinatum* and *P. vulgaris* because bioassays with synthetic compounds and electrophysiology studies are required to determine this. However, the presence of (*E*)-β-ocimene and 3-carene in cut and intact flowers of *D. uncinatum* and *P. vulgaris* may be associated with the differences observed in the behavioural bioassays (Chapter 4) where the two plant species were attractive in contrast to *A. esculentus* and *V. unguiculata*. Although some of the green leaf volatiles that were unique to the attractive group were not detected in the three-hour collections carried out in the morning hours, these two compounds (3-carene and (*E*)-β-ocimene) were detected.

The three-hour period also overlapped with the time recorded as one of the two peak activity periods, 09h00 - 11h00 in the morning and 15h00 - 17h00 in the afternoon, of *H. apicicornis* (Chapter 2; Lebesa *et al*. 2012). Therefore this result suggests that if olfactory cues are important for *H. apicicornis* in locating food resources, some key volatiles are likely to be released during these periods.

The exclusive presence of methyl salicylate in *D. uncinatum* and *P. vulgaris,* the attractive plant species, poses an interesting question because its production in a plant is usually associated with plant defence by attracting parasitoids (Hardie *et al*. 1994; De Boer & Dicke 2004; Van den Boom *et al*. 2004) and sometimes with repellence. However, Mithöfer *et al*. (2005) and Carroll *et al*. (2008) stress that although production of induced volatiles is usually for plant defence, such compounds may also result in further herbivory because their release from the damaged tissue increases apparency to the herbivore. Taking into consideration the aggregative nature of *Hycleus* spp. on their host plants, it is possible that initial damage inflicted on a host plant may lead to attraction of more conspecifics.

Generally, insects do not use a single compound for host location, rather each of the compounds may have different roles e.g. attraction over longer distance or shorter distance or synergism e.g. Birkett *et al*. (2003) and blends play an important role in attraction (Bruce & Pickett, 2011). Insects are unlikely to encounter a single compound in nature (Evans & Allen-Williams 1998; Webster *et al*. 2010b), thus exposure to single constituents in an experimental set-up may even lead to avoidance behaviour because an insect may associate it with non-hosts (Webster *et al*. 2010b; Bruce *et al*. 2011). Therefore, further behavioural studies that involve volatile blend reduction bioassays are necessary where *H. apicicornis* may be exposed to a full blend, a blend with reduced number of volatiles and single compounds to determine which volatiles play a key role in the attraction of this species to its host plants (Birkett *et al*. 2003; Webster *et al*. 2008a,b,c).

These findings set the foundation for follow-up behavioural studies using different blend combinations of synthetic compounds identified from each plant species in this study. Such experiments will allow for testing whether the differences in responses are associated with qualitative or quantitative differences in volatile profiles. These results are also expected to serve as the basis for identifying individual behaviourally active constituents and blends by electroantennography and further olfactometric assays that can be used for attraction in the future development of traps.

References

Abate, T. & Ampofo, J.K.O. (1996) Insect pests of beans in Africa: their ecology and management. *Annual Review of Entomology* **41**: 45-73.

Agelopoulos, N.G., Hooper, A.M., Maniar, S.M., Pickett, J.A. & Wadhams, L.J. (1999a) A novel approach for isolation of volatile chemicals released by individual leaves of a plant in situ. *Journal of Chemical Ecology* **25**: 1411–1425.

Agelopoulos, N., Birkett, M.A., Hick, A.J., Hooper, A.M., Pickett, J.A., Pow, E.M., Smart, L.E., Smiley, D.W.M., Wadhams, L.J. & Woodcock, C.M. (1999b) Exploiting semiochemicals in insect control. *Pesticide Science* **55**: 225-235.

Agelopoulos, N.G., Chamberlain, K. & Pickett, J.A. (2000) Factors affecting volatile emissions of intact potato plants, *Solanum tuberosum*: variability of quantities and stability of ratios. *Journal of Chemical Ecology* **26**: 497-511.

Åhman I., Glinwood R., Ninkovic V. (2010) The potential for modifying plant volatile composition to enhance resistance to arthropod pests. *CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources*. **5**: pp10. http://www.cabi.org/cabreviews/default.aspx?LoadModule=Review&ReviewID=126125&site=167&page=1486

Balkenius, A. & Kelber, A. (2006) Colour preferences influences odour learning in the hawkmoth, *Macroglossum stellatarum*. *Naturwissenschaften* **93**: 255-258.

Bartlet, E., Blight, M.M., Lane, P. & Williams, I.H. (1997) The responses of the cabbage seed weevil *Ceutorhynchus assimilis* to volatile compounds from oilseed rape in a linear track olfactometer. *Entomologia Experimentalis et Applicata* **85**: 257-262.

Bernays, E.A. & Chapman, R.F. (1994) *Host-plant Selection by Phytophagous Insects. Contemporary Topics in Entomology 2* Chapman & Hall, London.

Bicchi, C. & Joulain, D. (1990) Headspace-Gas Chromatographic analysis of medicinal and aromatic plants and flowers. *Flavour and Fragrance Journal* **5**: 131-145.

Bichão, H., Borg-Karlson, A.-K., Araújo, J. & Mustaparta, H. (2003) Identification of plant odours activating receptor neurons in the weevil *Pissodes notatus* F. (Coleoptera, Curculionidae). *Journal of Comparative Physiology A* **189**: 203-212.

Birkett, M.A., Chamberlain, K., Guerrieri, E., Pickett, J.A., Wadhams, L.J., & Yasuda, T. (2003) Volatiles from whitefly-infested plants elicit a host-locating response in the parasitoid, *Encarsia formosa*. *Journal of Chemical Ecology* **29**: 1589-1600.

Bruce, T.J.A. & Pickett, J.A. (2011) Perception of plant volatile blends by herbivorous insects – Finding the right mix. *Phytochemistry* **72**: 1605-1611.

Bruce, T.J.A., Wadhams, L.J. & Woodcock, C.M. (2005) Insect host location: a volatile situation. *Trends in Plant Science* **10**: 269-274.

Bruce, T.J.A., Midega, C.A.O., Birkett, M.A., Pickett, J.A. & Khan, Z.R. (2010) Is quality more important than quantity? Insect behavioural responses to changes in a volatile blend after stemborer oviposition on an African grass. *Biology Letters* **6**: 314-317.

Bruce, T.J.A, Martin, J.L., Smart, L.E. & Pickett, J.A. (2011) Development of semiochemical attractants for monitoring bean seed beetle, *Bruchus rufimanus*. *Pesticide Management Science* **67**: 1303-1308.

Carroll, M.J., Schmelz, E.A. & Teal, P.E.A. (2008) The Attraction of *Spodoptera frugiperda* neonates to cowpea Seedlings is mediated by volatiles induced by conspecific herbivory and the elicitor inceptin. *Journal of Chemical Ecology* **34**: 291-300.

Chamberlain, K., Khan, Z.R., Pickett, J.A., Toshova, T. & Wadhams, L.J. (2006) Diel periodicity in the production of green leaf volatiles by wild and cultivated host plants of stemborer moths, *Chilo partellus* and *Busseola fusca*. *Journal of Chemical Ecology* **32**: 565-577.

Chen, F., Tholl, D., D'Auria, J.C., Farooq, A. Pichersky, E. & Gershenzon, J. (2003) Biosynthesis and emission of terpenoid volatiles from *Arabidopsis* flowers. *The Plant Cell* **15**: 481-494.

Chidumayo, E.N. (2002) Population ecology of an afro-tropical savanna herb, *Lapeirousia rivularis*, in Zambia. *Plant Ecology* **165**: 275–286.

Clark, K.M., Bailey, W.C. & Myers R.L. (1995) Alfalfa as a companion crop for control of *Lygus lineolaris* (Hemiptera: Miridae) in Amaranth. *Journal of the Kansas Entomological Society* **68**: 143-148.

Colazza, S., McElfresh, J.S. & Millar, J.G. (2004) Identification of volatile synomones, induced by *Nezara Viridula* feeding and oviposition on bean spp., that attract the egg parasitoid *Trissolcus basalis*. *Journal of Chemical Ecology* **30**: 945-964.

Cook, S., Bartlet, E., Murray, D.A. & Williams, I.H. (2002) The role of pollen odour in the attraction of pollen beetles to oilseed rape flowers. *Entomologia Experimentalis et Applicata* **104**: 43-50.

Dafni, A. & Potts, S.G. (2004) The role of flower inclination, depth, and height in the preferences of a pollinating beetle (Coleoptera: Glaphyridae). *Journal of Insect Behavior* **17**: 823-834.

D'Alessandro, M. & Turlings, T.C.J. (2006) Advances and challenges in the identification of volatiles that mediate interactions among plants and arthropods *Analyst* **131**: 24-32.

De Boer, J.G. & Dicke, M. (2004) The role of methyl salicylate in prey searching behavior of the predatory mite *Phytoseiulus persimilis*. *Journal of Chemical Ecology*. **30**: 255-271.

De Moraes, C.M., Mescher, M.C. & Tumlinson, J.H. (2001) Caterpillar-induced nocturnal plant volatiles repel conspecific females. *Nature* **410**: 577-580.

Dicke, M. & Van Loon, J.A. (2000) Multitrophic effects of herbivore-induced plant volatiles in an evolutionary context. *Entomologia Experimentalis et Applicata* **97**: 237-249.

Dobson, H.E.M., Danielson, E.M. & Van Wesep, I.D. (1999) Pollen odor chemicals as modulators of bumble bee foraging on *Rosa rugosa* Thunb. (Rosaceae). *Plant Species Biology* **14**: 153-166.

Dudareva, N. & Pichersky, E. (2000) Biochemical and molecular genetic aspects of floral scents. *Plant Physiology* **122**: 627-633

Dudareva, N., Pichersky, E. & Gershenzon, J. (2004) Biochemistry of plant volatiles. *Plant Physiology* **135**: 1893-1902.

Evans, E.W. (1990) Dynamics of an aggregation of blister beetles (Coleoptera: Meloidae) attacking a prairie legume. *Journal of the Kansas Entomological Society* **63**: 616-625.

Evans, K.A. & Allen-Williams, L.J. (1998) Response of cabbage seed weevil (*Ceutorhynchus assimilis*) to baits of extracted and synthetic host-plant odor. *Journal of Chemical Ecology* **24**: 2101-2114.

Evans, E.W., Smith, C.C. & Gendron, R.P. (1989) Timing of reproduction in a prairie legume: seasonal impacts of insects consuming flowers and seeds. *Oecologia* **78**: 220-230.

Gahukar, R.T. (1991) Pest status and control of blister beetle in West Africa. *Tropical Pest Management* **37**: 415-420.

Gouinguene, S., & Turlings, T. (2002) The effects of abiotic factors on induced volatile emissions in corn plants. *Plant Physiology* **129**: 1296-1307.

Hall, M.J.R. (1984) Trap-oriented behaviour of red-banded blister beetles, *Mylabris designata* var. *hacolyssa* Rochebrune (Coleoptera: Meloidae), in the Sudan. *Bulletin of Entomological Research* **74**: 103-112.

Hardie, J., Isaacs, R., Pickett, J.A., Wadhams, L.J. & Woodcock, C.M. (1994) Methyl salicylate and (-)-(1R,5S)-myrtenal are plant-derived repellents for black bean aphid, *Aphis fabae* Scop (Homoptera, Aphididae). *Journal of Chemical Ecology* **20**: 2847-2855.

Hatanaka, A. (1993) The biogeneration of green odour by green leaves. *Phytochemistry* **34**: 1201-1218.

Heisswolf, A., Gabler, D., Obermaier, E. & Müller, C. (2007) Olfactory versus contact cues in host plant recognition of a monophagous Chrysomelid beetle. *Journal of Insect Behavior* **20**: 247-266.

Hill, D.S. (1975) *Agricultural insect pests of the tropics and their control*. 1st edition. Cambridge University Press, London.

Jönsson, M. & Anderson, P. (2008) Emission of oilseed rape volatiles after pollen beetle infestation; behavioural and electrophysiological responses in the parasitoid *Phradis morionellus*. *Chemoecology* **17**: 201-207.

Karlsson, M.F. (2010) Odours, potato and insects: hostfinding and behaviour. *Introductory paper for PhD thesis 'Safe control of Guatemalanpotato moth in household storage'*. Swedish University of Agricultural Sciences, Alnarp, Sweeden.

Khan, Z.R., Midega, C.A.O., Hassanali, A., Pickett, J.A., Wadhams, L.J. & Wanjoya, A. (2006) Management of witchweed, *Striga hermonthica*, and stemborers in sorghum, *Sorghum bicolor*, through intercropping with greenleaf desmodium, *Desmodium intortum*. *International Journal of Pest Management* **52**: 297-302.

Khan, Z.R., Midega, C.A.O., Bruce, T.J.A., Hooper, A.M. & Pickett, J.A. (2010) Exploiting phytochemicals for developing a 'push–pull' crop protection strategy for cereal farmers in Africa. *Journal of Experimental Botany* **61**: 4185-4196.

Knudsen, J.T., Eriksson, R., Gershenzon, J. & Ståhl, B. (2006) Diversity and distribution of floral scent. *The Botanical Review* **72**: 1-120.

Lale, N.E.S. & Sastawa, B.M. (2000) Evaluation of host plant resistance, sowing date modification and intercropping as methods for the control of *Mylabris* and *Coryna* species (Coleoptera: Meloidae) infesting pearl millet in the Nigerian Sudan savanna. *Journal of Arid Environments* **46**: 263-280.

Lebesa, L.N., Khan, Z.R., Hassanali, A., Pickett, J.A., Bruce, T.J.A., Skellern, M., Krüger, K. (2011) Responses of the blister beetle *Hycleus apicicornis* to visual stimuli. *Physiological Entomology* **36**: 220-229.

Lebesa, L.N., Khan, Z.R., Krüger K., Bruce, T.J., Hassanali, A. & Pickett, J.A. (2012) Farmers' knowledge and perceptions of blister beetles, *Hycleus* spp. (Coleoptera: Meloidae), as pest herbivores of *Desmodium* legumes in western Kenya. *International Journal of Pest Management* **58**: 165-174.

Lwande, W., McDowell, P.G., Amiani, H. & Amoke, P. (1989) Analysis of airborne volatiles of cowpea. *Phytochemistry* **28**: 421-423.

Maffei, M.E. (2010) Sites of synthesis, biochemistry and functional role of plant volatiles. *South African Journal of Botany* **76**: 612-631.

Mccullough, D.G., Poland, T.M., Anulewicz, A.C. & Cappaert, D. (2009) Emerald ash borer (Coleoptera: Buprestidae) attraction to stressed or baited ash Trees. *Environmental Entomology* **38**: 1668-1679.

Mithöfer, A., Wanner, G. & Boland, W. (2005) Effects of feeding *Spodoptera littoralis* on lima bean leaves. II. Continuous mechanical wounding resembling insect feeding is sufficient to elicit herbivory-related volatile emission. *Plant Physiology* **137**: 1160-1168.

Nikbakhtzadeh, M.R. (2004) Transfer and distribution of cantharidin within selected members of blister beetles (Coleoptera: Meloidae) and its probable importance in sexual behaviour. *PhD Dissertation*. Universität Bayreuth. Bayreuth, Germany

Ômura, H. & Honda, K. (2005) Priority of color over scent during flower visitation by adult *Vanessa indica* butterflies. *Oecologia* **142**: 588-596.

Paré, P.W. & Tumlinson, J.H. (1996) Plant volatile signals in response to herbivore feeding. *Florida Entomologist* **79**: 93-103.

Paré, P.W. & Tumlinson, J.H. (1999) Plant volatiles as a defense against insect herbivores. *Plant Physiology* **121**: 325-331.

Parra, L. Mutis, A., Ceballos, R., Lizama, M., Pardo, F., Perich, F. & Quiroz, A.S. (2009) Volatiles released from *Vaccinium corymbosum* were attractive to *Aegorhinus superciliosus* (Coleoptera: Curculionidae) in an olfactometric bioassay. *Environmental Entomology* **38**: 781-789.

Pickett, J.A., Hamilton, M.L., Hooper, A.M., Khan, Z.R. & Midega, C.A.O. (2010) Companion cropping to manage parasitic plants. *Annual Review of Phytopathology* 48: 161-177.

Raguso, R.A. & Willis, M.A. (2002) Synergy between visual and olfactory cues in nectar feeding by naïve hawkmoths, *Manduca sexta*. *Animal Behavior* **64**: 685-695.

Rao, S., Cossé, A.A., Zilkowski, B.W. & Bartelt, R.J. (2003) Aggregation pheromone of the cereal leaf beetle: field evaluation and emission from males in the laboratory. *Journal of Chemical Ecology* **29**: 2165-2175.

Robinson, J. (2005) Pests and integrated pest management in western Equatoria, southern Sudan. *International Journal of Tropical Insect Science* **25**: 224-235.

Rodriguez-Saona, C.R., Rodriguez-Saona, L.E. & Frost, C.J. (2009) Herbivore-induced volatiles in the perennial shrub, *Vaccinium corymbosum*, and their role in inter-branch signaling. *Journal of Chemical Ecology* **35**: 163-175.

Roeder, S., Hartmann, A-M., Effmert, U., & Piechulla, B. (2007) Regulation of simultaneous synthesis of floral scent terpenoids by the 1,8-cineole synthase of *Nicotiana suaveolens*. *Plant Molecular Biology* **65**: 107-124.

Schoonhoven, L.M., Jermy, T. & van Loon, J.J.A. (2005) *Insect-Plant Biology*. 2nd edition. Chapman & Hall, London.

Selander, R.B. (1988). An annotated catalog and uummary of bionornics of Blister Beetles of the Genus *Psalydolytta* (Coleoptera: Meloidae). *Insecta Mundi* **2**: 1-15.

Shiojiri, K., Kishimoto K., Ozawa, R., Kugimiya, S., Urashimo, S. Arimura, G., Horiuchi, J., Nishioka, T., Matsui, K., & Takabayashi, J. (2006) Changing green leaf volatile biosynthesis in plants: An approach for improving plant resistance against both herbivores and pathogens. *Proceedings of the National Academy of Science* **103**: 16672-16676.

Smith, R.M. (2003) Before the injection-modern methods of sample preparation for separation techniques. *Journal of Chromatography A* **1000**: 3-27.

Sokal, R.R. & Rohlf, F.J. (1969) *Biometry – the principle and practice of statistics in biological research*. 2nd edition. W.H. Freeman and Company, New York.

Stranden, M., Røstelien, T. Liblikas. I., Almaas, T.J., Borg-Karlson, A-K. & Mustaparta, H. (2003) Receptor neurones in three heliothine moths responding to floral and inducible plant volatiles. *Chemoecology* **13**: 143-154.

Tholl, D., Boland, W., Hansel, A., Loreto, F., Röse, U.S.R. & Schinitzler, J-P. (2006) Practical approaches to plant volatile analysis. *The Plant Journal* **45**: 540-560.

Van den Boom, C.E.M., Van Beek, T.A., Posthumus, M.A., De Groot, A. & Dicke, M. (2004) Qualitative and quantitative variation among volatile profiles induced by *Tetranychus urticae*. Feeding on plants from various families. *Journal of Chemical Ecology* **30**: 69-89.

Van Tol, R.W.H.M., Visser, J.H. & Sabelis, M.W. (2002) Olfactory responses of the vine weevil, *Otiorhynchus sulcatus*. *Physiological Entomology* **27**: 213-222.

Webster, B. Bruce, T.J.A., Dufour, S., Birkemeyer, C., Birkett, M.A., Hardie, J. & Pickett, J.A. (2008a) Identification of volatile compounds used in host location by the black bean aphid, *Aphis fabae*. *Journal of Chemical Ecology* **34**: 1153-61.

Webster, B., Bruce, T.J.A., Pickett, J.A. & Hardie, J. (2008b) Olfactory recognition of host plants in the absence of host-specific volatile compounds. *Communicative and Integrative Biology* **1**: 167-169.

Webster, B. Bruce, T. Pickett, J. & Hardie. J. (2008c) Olfactory recognition of host plants in the absence of host-specific volatile compounds - Host location in the black bean aphid, *Aphis fabae*. *Communicative & Integrative Biology* 1-2: 167-169.

Webster, B., Gezan, S.A., Bruce, T.J.A., Hardie, J. & Pickett, J.A. (2010a) Between plant and diurnal variation in quantities and ratios of volatile compounds emitted by *Vicia faba* plants. *Phytochemistry* **71**: 81-89.

Webster, B., Bruce, T., John Pickett, J. & Hardie, J. (2010b) Volatiles functioning as host cues in a blend become nonhost cues when presented alone to the black bean aphid. *Animal Behaviour* 79: 451-457.

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

Zhu, F., Lei, C.L. & Xue, F. (2005) The morphology and temperature-dependent development of *Mylabris phalerata* Pallas (Coleoptera: Meloidae). *The Coleopterist's Bulletin* **59**: 521-527.

CHAPTER 6

General discussion, conclusions and recommendations

This study was conducted as part of the Habitat Management Programme at *ICIPE*, Mbita, Kenya. In that programme the stimulo-deterrent diversionary strategy (Miller & Cowles 1990), commonly known as ‘push-pull’, plays a central role in the development of easy-to-use and affordable strategies for resource-poor farmers in Eastern Africa to enhance cereal production and productivity. The ‘push-pull’ strategy employs *Desmodium* spp. (Fabaceae) as a repellent crop for stemborers and a suppressant for witchweed, *Striga* spp. (Scrophulariaceae) (Khan *et al*. 2000, 2008). However, availability of *Desmodium* spp. appears to be seriously constrained by blister beetles, *Hycleus* spp. (Coleoptera: Meloidae), which feed on the flowers thus negatively affecting seed setting. This is especially destructive where seed is produced in plots of smallholder farmers. Therefore, my research was aimed at (a) determining the pest status of blister beetles, *Hycleus* spp., on *Desmodium* spp. and sweetpotato; and (b) elucidating the behavioural responses of *H. apicicornis* and how these findings can be used as a basis for devising control strategies for resource-poor farmers to control *Hycleus* spp. on *Desmodium* spp.

Currently, global trends are to move away from using harmful crop protection pesticides towards more user-friendly and environmentally safe products, thus the use of semiochemicals and other strategies seems to afford the best potential alternative in pest management. This study determined the pest status of *Hycleus* spp. through assessment of farmers’ knowledge and perceptions of *Hycleus* species as pests and field sampling of

Desmodium spp. and sweetpotato in order to compare the quantitative results with the responses received from farmers (Chapter 2). It also examined how the key insect pest (*H. apicicornis*) of *Desmodium* spp. responds to visual and olfactory cues in locating its food with the ultimate aim of laying down a foundation on which such behaviours can be manipulated against this pest (Chapters 3 & 4). Various studies have shown that insects use either one or both of these cues for locating their food and other resources (Raguso 2001; Schmera *et al*. 2004; Ômura & Honda 2005; Campbell & Borden 2006). *Hycleus apicicornis* gave very strong behavioural responses towards colour targets (Chapter 3), suggesting they may be using visual cues (especially colour) to orientate towards their food resources. While olfactory cues seem to play a major role for food location in most insects (Bolter *et al*. 1997; Agelopoulos 1999; Schoonhoven *et al*. 2005; Bruce *et al*. 2011), their use by *H. apicicornis* appears to be inconsistent as shown by my results in Chapter 4. The olfactometer studies showed some positive responses to some, but not all, of the host plants tested. It is not clear why there was a lack of response to some of the host plants that are known to be used in the wild by these beetles.

However, flowers of okra, although strongly differing in colour from flowers of most host plants for *H. apicicornis*, are larger, and therefore in the wild they may be more conspicuous thus this beetle may not need the olfactory cues to locate them. However, the same theory does not hold for cowpea whose flowers are quite small. Results of both the survey and the orientation and settling preference work in this study still places cowpea as the least preferred host plant species, therefore showing that it is not very attractive visually or through chemical attraction. It is possible that cowpea is one of the alternative host plants that are used as secondary host plants in the absence of the preferred host plants (Bernays & Minkenberg 1997; Bernays 1999).

Being an introduced crop in East Africa, not many pests have been recorded from *Desmodium* spp. The attacks by blister beetles, *Hycleus* spp., were mentioned to ICIPE mainly by its seed producers. Therefore, official documentation as well as establishing pest status of *Hycleus* spp. was a necessity to have a basis for formulating pest management strategies. Chapter 2 shows that, according to farmers, *Hycleus* spp. are not only threatening seed production of *Desmodium* spp. but may also be potential serious pests for other crops such as sweetpotato and beans in Kenya. Adult meloids are generally polyphagous, and many other genera have been documented as feeding on plants from different families, thus records of *Hycleus* spp. from several crops in Kenya should not appear unusual.

Often, polyphagous insects would commonly use odours for selecting their food resources and most likely the compounds ratio theory would apply to these beetles for identification of their resources (Bruce *et al*. 2005). One would expect that insect pests that feed on flowers of plants from different families that differ visually in colour, shape and pattern are most likely to employ olfactory cues to locate resources from distance. However, given the strong responses observed to visual cues, specifically colour, in this study colour maybe the main cue employed by *H. apicicornis* (Chapter 3). Apart from the study by Hall (1984) this is the second study done on colour preference by blister beetles Neither previous nor even the current study cannot confirm the presence of colour vision. However, wavelength-specific behaviour was observed for *H. apicicornis.* Adult beetles showed preference and attraction to the human sky blue colour targets whose spectral readings were in the (440 – 500 nm) ranges in this study. Similar observation was made by Hall (1984) on the adults of a related species, *Mylabris designata* var. *Hacolyssa*, which were attracted to the blue traps in his trapping study.

Although not commonly encountered, it is possible that *H. apicicornis* may be using visual cues for long range host location because there are some beetles that make use of visual cues as very crucial for host search; either exclusively for host location e.g. in *Altica engstroemi* J. Sahlberg (Coleoptera: Chrysomelidae) (Stenberg & Ericson 2007) or as a long range cue e.g. *Dendroctonus ponderosae* (Coleoptera: Scolytidae) (Campbell & Borden 2006). It is possible that for *H. apicicornis* colour as a visual cue is more important than the olfactory cue because this is a diurnal species that locates and feeds during the day as has been observed with other insect species such as Lepidopterans (Balkenius *et al*. 2006; Balkenius & Kelber 2006).

The results from the coloured targets helped to elucidate the role played by visual cues in the location of food by *H. apicicornis*, as to whether they are used as long or short range cues. Results obtained in this study suggest that *H. apicicornis* is very sensitive to light in the blue region especially the lighter hues of blue than the darker ones. In this study, results obtained showed that shapes may not be as crucial as the colour. This suggests that reflectance associated with colour could be a more important visual cue than shape for host location by *H. apicicornis.* While the study did not produce a proto-type trap as was initially hoped for, the information from responses to colour targets, however, forms the basis for further investigation into the role played by colour in host finding by this beetle, which will eventually lead to the development of a trap.

Although a stronger response was obtained with the visual cues compared to olfactory cues, positive behavioural responses by *H. apicicornis* adults to odours of some host plants (Chapter 4) led to the comparison of the chemical profiles of those plant species that were attractive with the ones that were not attractive (Chapter 5). Based on the observed differences among the different host plant species as well as between damaged and undamaged flowers, hexanal, 1-hexanol, (*E*)-β-ocimene, 3-Δ-carene, (*Z*)-3-hexenol, and benzaldehyde were identified as potentially responsible for attraction. However, further tests are required to evaluate individual compounds as well as blends that may mimic the host that is attractive to *H. apicicornis*. In this study, only mechanical wounding was investigated. However, the quantities and quality of a profile of a plant may differ considerably depending on what inflicted the damage. Usually herbivore damage results in a qualitatively and quantitatively higher increase in volatile emission than mechanical damage (Turlings *et al*. 1995; De Moraes *et al*. 2001; Mithöfer *et al*. 2005).

While (*E*)-β-ocimene was found in high quantities in damaged plants other compounds that are also usually known to be quantitatively modified by herbivore damage, such as 3-octanone and (*E*)-4,8-dimethyl-1,3,7-nonatriene (DMNT), were not as prominent as observed in similar plant species in other studies (Birkett *et al*. 2003; Carroll *et al*. 2008) or were absent possibly because the damage was only mechanically induced. This highlights the need for inclusion of herbivore damage in the identification of chemical profiles of each host in the future studies. Further behavioural assays that include evaluation of individual and blends of different key compounds are needed to determine the appropriate blend that can be used in the trapping studies.

Although the study has not produced a certain odour blend for the control of *H. apicicornis*, it has added to the knowledge of how this blister beetle responds to olfactory cues. This study has laid the foundation for further research into understanding visual and chemical ecology of *H. apicicornis.* For example, electrophysiology studies may determine the role of each of the compounds identified in the profiles of the different host plant species. The visual cue study can be used as the basis to determine whether the response observed was due to presence of true vision or whether it was only the wavelength behaviour, results of which may have impact on future pest control strategies that may involve trapping.

Measures to control *H. apicicornis* on *Desmodium* spp. and other crops in east Africa have not been documented before. An understanding of visual and olfactory cues and their effects on the behaviour of a pest are important in any attempt at developing pest control strategies that manipulate insect behaviours. Therefore, further development of the integration of visual and semiochemicals will provide a platform from which simple to use strategies for resource poor farmers could be developed. This would make such farmers be less dependent on the use of chemical pesticides that are often not easily accessible to them and may also bring detrimental side effects to them and the environment. Further research work and modification on findings of this study are necessary so that results could be adapted to daily use by an ordinary farmer. For example, development of traps of a sky-blue colour as the main visual attractant but involving different designs that take into consideration other shapes and patterns that were not tested in this study. Such traps can either be used as part of the integrated pest management strategy. Traps can be employed in sampling to monitor pest densities where they can be used without an appropriate chemical cue or with one to enhance efficiency of attractiveness. For a direct control their efficiency can further be increased through impregnation of an insecticide to kill the beetles upon landing.

References

Agelopoulos, N., Birkett, M.A., Hick, A.J., Hooper, A.M., Pickett, J.A., Pow, E.M., Smart, L.E., Smiley, D.W.M., Wadhams, L.J. & Woodcock, C.M. (1999) Exploiting semiochemicals in insect control. *Pesticide Science* **55**: 225-235.

Balkenius, A. & Kelber, A. (2006) Colour preferences influences odour learning in the hawkmoth, *Macroglossum stellatarum*. *Naturwissenschaften* **93**: 255-258.

Balkenius, A., Rosén, W. & Kelber, A. (2006) The relative importance of olfaction and vision in a diurnal and a nocturnal hawkmoth. *Journal of Comparative Physiology A* **192**: 431-437.

Bernays, E.A. (1999) When host choice is a problem for a generalist herbivore: experiments with the whitefly, *Bemisia tabaci*. *Ecological Entomology* **24**: 260-267.

Bernays, E.A., Minkenberg, O.P.J.M. (1997) Insect herbivores: different reasons for being a generalist. *Ecology* **78**: 1157-1169.

Birkett, M.A., Chamberlain, K., Guerrieri, E., Pickett, J.A., Wadhams, L.J., & Yasuda, T. (2003) Volatiles from whitefly-infested plants elicit a host-locating response in the parasitoid. *Encarsia formosa*. *Journal of Chemical Ecology* **29**: 1589-1600.

Bolter, C.J., Dicke, M.V., Loon, J.J.A., Visser, J.H. & Posthumus, M.A. (1997) Attraction of Colorado potato beetle to herbivore-damaged plants during herbivory and after its termination. *Journal of Chemical Ecology* **23**: 1003–1023.

Bruce, T.J.A., Wadhams, L.J. & Woodcock, C.M. (2005) Insect host location: a volatile situation. *Trends in Plant Science* **10**: 26-274.

Bruce, T.J.A, Martin, J.L., Smart, L.E. & Pickett, J.A. (2011) Development of semiochemical attractants for monitoring bean seed beetle, *Bruchus rufimanus*. *Pesticide Management Science* **67**: 1303-1308.

Campbell, S.A. & Borden, J.H. (2006) Close-range, in-flight integration of olfactory and visual information by a host-seeking bark beetle. *Entomologia Experimentalis et Applicata* **120**: 91-98.

Carroll, M.J., Schmelz, E.A. & Teal, P.E.A. (2008) The Attraction of *Spodoptera frugiperda* neonates to cowpea Seedlings is mediated by volatiles induced by conspecific herbivory and the elicitor inceptin. *Journal of Chemical Ecology* **34**: 291-300.

De Moraes, C.M., Mescher, M.C. & Tumlinson, J.H. (2001) Caterpillar-induced nocturnal plant volatiles repel conspecific females. *Nature* **410**: 577–580.

Hall, M.J.R. (1984) Trap-oriented behaviour of red-banded blister beetles, My*labris designata var. hacolyssa* Rochebrune (Coleoptera: Meloidae), in the Sudan. *Bulletin of Entomological Research* **74**: 103-112.

Khan, Z.R., Pickett, J.A., Van den Berg, J., Wadhams, L.J., Woodcock, C.M. (2000) Exploiting chemical ecology and species diversity: stem borer and striga control for maize and sorghum in Africa. *Pest Management Science* **56**: 957-962.

Khan, Z.R., Midega, C.A.O., Amudavi, D.M., Hassanali, A., Pickett, J.A. (2008) On-farm evaluation of the 'push-pull' technology for the control of stemborers and striga weed on maize in Western Kenya. *Field Crops Research* **106**: 224-233.

Miller, J.R. & Cowles, R.S. (1990) Stimulo-deterrent diversion: A concept and its possible application to onion maggot control. *Journal of Chemical Ecology* **16**: 3197-3212.

Mithöfer, A., Wanner, G. & Boland, W. (2005) Effects of feeding *Spodoptera littoralis* on lima bean leaves. II. Continuous mechanical wounding resembling insect feeding is sufficient to elicit herbivory-related volatile emission. *Plant Physiology* **137**: 1160-1168.

Ômura, H. & Honda, K. (2005) Priority of color over scent during flower visitation by adult *Vanessa indica* butterflies. *Oecologia* **142**: 588-596.

Raguso, R.A. (2001) Floral scent, olfaction, and scent-driven foraging behavior. In: L. Chittka, L. & Thomson, J. (Eds.), *Cognitive Ecology of Pollination* 83-105. Cambridge University Press, Cambridge.

Schmera, D., Tóth, M., Subchev, M., Sredkov, I. Szarukán, I. Jermy, T. & Szentesi, Á. (2004) Importance of visual and chemical cues in the development of an attractant trap for *Epicometis* (*Tropinota*) *hirta* Poda (Coleoptera: Scarabaeidae). *Crop Protection* **23**: 939–944.

Schoonhoven, L.M., Jermy, T. & van Loon, J.J.A. (2005) *Insect-Plant Biology*. 2nd edition. Chapman & Hall, London.

Stenberg, J.A. & Ericson, L. (2007) Visual cues override olfactory cues in the host-finding process of the monophagous leaf beetle *Altica engstroemi. Entomologia Experimentalis et Applicata* **125**: 81-88.

Turlings, T.C.J., Loughrin, J.H., McCall, P.J., Röse, U.S.R., Lewis, W.J. & Tumlinson, J.H. (1995) How caterpillar-damaged plants protect themselves by attracting parasitic wasps. *Proceedings of the National Academy of Sciences of the USA* **92**: 4169-4174.

Appendix 1: Assessing host range for Blister beetles, damage extent and practiced control methods

The purpose of this survey is to determine the following: i) whether blister beetles are perceived to be serious problem on Desmodium, ii) any control measures undertaken and their effectiveness and a range of alternate hosts.

Respondent's name:--------------------------- **Gender: --------------** **Date.-----------**

Enumerator:--------------------------District----------------- **Location:---------**

1. i) What crops do you grow? ii) Please indicate if you grow each of the crops mentioned on its own or in combination with other crops, iii) and the usual pattern? iv) Please estimate the acreage.

i) Crops	ii) Mono/Mixedcropping	iii) If mixing, how? e.g. A +B, A+B+C, C+E etc	iv) Acreage
A	i) Mono, ii) Mix		
B	i) Mono, ii) Mix		
C	i) Mono, ii) Mix		
D	i) Mono, ii) Mix		
E	i) Mono, ii) Mix		
F	i) Mono, ii) Mix		
G	i) Mono, ii) Mix		
H	i) Mono, ii) Mix		

2. i) What pests affect these crops? ii) Mention two main parts of the crop usually attacked and list in order of seriousness. iii) What control methods do you use against these pests?

Crop	**Pest**	**Parts**	**Method of Control**
	---------------------	a) leaves b) flowers c) other	i)Pesticide e.g. ------------------ ii) ------------------- iii) --------------------------- iv) ----------------------
	---------------------	a) leaves b) flowers c) other	i)Pesticide e.g. ------------------ ii) ------------------- iii) --------------------------- iv) ----------------------
	---------------------	a) leaves b) flowers c) other	i)Pesticide e.g. ------------------ ii) ------------------- iii) --------------------------- iv) ----------------------
	---------------------	a) leaves b) flowers c) other	i)Pesticide e.g. ------------------ ii) ------------------- iii) --------------------------- iv) ----------------------
	---------------------	a) leaves b) flowers c) other	i)Pesticide e.g. ------------------ ii) ------------------- iii) --------------------------- iv) ----------------------
	---------------------	a) leaves b) flowers c) other	i)Pesticide e.g. ------------------ ii) ------------------- iii) --------------------------- iv) ----------------------
	---------------------	a) leaves b) flowers c) other	i)Pesticide e.g. ------------------ ii) ------------------- iii) --------------------------- iv) ----------------------
	---------------------	a) leaves b) flowers c) other	i)Pesticide e.g. ------------------ ii) ------------------- iii) --------------------------- iv) ----------------------
	---------------------	a) leaves b) flowers c) other	i)Pesticide e.g. ------------------ ii) ------------------- iii) --------------------------- iv) ----------------------
	---------------------	a) leaves b) flowers c) other	i)Pesticide e.g. ------------------ ii) ------------------- iii) --------------------------- iv) ----------------------

NB: ***For those neither in Desmodium seed bulking nor Push-pull, continue to question 5***

3. If Desmodium is one of your crops, i) for how long have you been growing Desmodium? ii) Why do you grow Desmodium iii) What varieties do you use?

Experience with Desmodium	**Grown for what**	**Varieties grown**	**Acreage**
i) 1yr ii)2yrs iii) 3yrs iv) >3yrs	i) seed bulking ii) push-pull iii) other	i) Silverleaf ii) Greenleaf iii) both iv) other	i) 0.1 – 0.2 acre ii) 0.3 – 0.4 acres iii) 0.5 – 1.0 acres iv 1.1 – 2.0 acres v)>2 acres

4. List various pests of Desmodium in order of seriousness and ii) the type of damage iii) time of the year they occur, iv) the control measure you use v) and whether the methods are effective or not.

Pest	**Plant part damaged**	**Time (season when infestation is most serious)**	**Time of the day (estimate peak time)**	**Control measures and their effectiveness**	
				Type of control	**(***1=effective, 2=not effective, 3=somehow effective***)**
	i) leaves ii) roots iii) flowers iv) stem v) other	i) Short rainy season ii) Long rainy season iii) All the time iv) Not specific	i) mornings ----------------- ii) afternoons -----------------	i)Pesticide e.g. ------------------------ ii) iii) iv)	----------------- ----------------- ----------------- -----------------
	i) leaves ii) roots iii) flowers iv stem v) other	i) Short rainy season ii) Long rainy season iii) All the time iv) Not specific	i) mornings ------------------ ii) afternoons ------------------	i)Pesticide e.g. ------------------------ ii) iii) iv)	----------------- ----------------- ----------------- -----------------
	i) leaves ii) roots iii) flowers iv) stem v) other	i) Short rainy season ii) Long rainy season iii) All the time iv) Not specific	i) mornings ------------------ ii) afternoons ------------------	i)Pesticide e.g. ------------------------ ii) iii) iv)	----------------- ----------------- ----------------- -----------------
	i) leaves ii) roots iii) flowers iv) stem v) other	i) Short rainy season ii) Long rainy season iii) All the time iv) Not specific	i) mornings ------------------ ii) afternoons ------------------	i)Pesticide e.g. ------------------------ ii) iii) iv)	----------------- ----------------- ----------------- -----------------
	i) leaves ii) roots iii) flowers iv) stem v) other	i) Short rainy season ii) Long rainy season iii) All the time iv) Not specific	i) mornings ------------------ ii) afternoons ------------------	i) Pesticide e.g. ------------------------ ii) iii) iv)	----------------- ----------------- ----------------- -----------------

5. Have you seen any or all of these beetles before? (picture of beetles are shown)

i) yes ii) no

If yes, mention i) the crops they attack, ii) the parts attacked iii), severity of damage and iv) whether damage is severe in mono or in mixed cropping

Crops attacked	Which beetles	Which parts are damaged	Rate their damage (*1= low, 2 = moderate, 3= high*)	Severe on:
i) Desmodium	i) B_1 ii) B_2 iii) B_3	i) leaves ii) flowers iii) other	B_1= B_2 = B_3 =	B_1= i) mono, ii) mix, ii) same B_2 = i) mono, ii) mix, ii) same B_3 = i) mono, ii) mix, ii) same
ii) Sweetpotato	i) B_1 ii) B_2 iii) B_3	i) leaves ii) flowers iii) other	B_1= B_2 = B_3 =	B_1= i) mono, ii) mix, ii) same B_2 = i) mono, ii) mix, ii) same B_3 = i) mono, ii) mix, ii) same
iii) Cowpea	i) B_1 ii) B_2 iii) B_3	i) leaves ii) flowers iii) other	B_1= B_2 = B_3 =	B_1= i) mono, ii) mix, ii) same B_2 = i) mono, ii) mix, ii) same B_3 = i) mono, ii) mix, ii) same
v) Beans	i) B_1 ii) B_2 iii) B_3	i) leaves ii) flowers iii) other	B_1= B_2 = B_3 =	B_1= i) mono, ii) mix, ii) same B_2 = i) mono, ii) mix, ii) same B_3 = i) mono, ii) mix, ii) same
vi Maize	i) B_1 ii) B_2 iii) B_3	i) leaves ii) flowers iii) other	B_1= B_2 = B_3 =	B_1= i) mono, ii) mix, ii) same B_2 = i) mono, ii) mix, ii) same B_3 = i) mono, ii) mix, ii) same
vii)	i) B_1 ii) B_2 iii) B_3	i) leaves ii) flowers iii) other	B_1= B_2 = B_3 =	B_1= i) mono, ii) mix, ii) same B_2 = i) mono, ii) mix, ii) same B_3 = i) mono, ii) mix, ii) same
viii)	i) B_1 ii) B_2 iii) B_3	i) leaves ii) flowers iii) other	B_1= B_2 = B_3 =	B_1= i) mono, ii) mix, ii) same B_2 = i) mono, ii) mix, ii) same B_3 = i) mono, ii) mix, ii) same

6. Mention the i) time they occur (season and time of the day), ii) the control measures practiced. iii) Are they effective?

Pest	**Time (season when infestation is most serious)**	**Time of the day (estimate peak time)**	**Control measures and their effectiveness**	
			Type of control	**(***1=effective, 2=not effective, 3=somehow effective***)**
	i) Short rainy season ii) Long rainy season iii) No difference	i) mornings ------------------ ii) afternoons ------------------	i)Pesticide e.g. ------------------------ ii) iii) iv)	------------------ ------------------ ------------------ ------------------
	i) Short rainy season ii) Long rainy season iii) No difference	i) mornings ------------------- ii) afternoons -------------------	i)Pesticide e.g. ------------------------ ii) iii) iv)	------------------ ------------------ ------------------ ------------------
	i) Short rainy season ii) Long rainy season iii) No difference	i) mornings ------------------- ii) afternoons -------------------	i)Pesticide e.g. ------------------------ ii) iii) iv)	------------------ ------------------ ------------------ ------------------

7. If you are not practicing any form of control for these pests, give reasons why not.

____

____

8. If these beetles affect your seed production, can you estimate how much you lose (in terms of kilos) compared to the time you do not have beetles or the time you apply any control measures.

9. Any additional comments about these beetles or any other pest especially on Desmodium?

These beetles:

____

____

Other pests:

____

____

General remarks:

____

____