MORPHOLOGY, BIOLOGY AND SEMIOCHEMICAL MEDIATED BEHAVIOUR OF THE COREID BUG *PSEUDOTHERAPTUS WAYI* BROWN 1955, A MAJOR PEST OF CASHEW IN EAST AFRICA

BY

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DECLARATION AND APPROVAL

Declaration by the candidate

I, **Egonyu James Peter** (Registration Number: **I80/80504/2010)**, hereby declare that this Thesis is entirely mine, and to the best of my knowledge and belief, it has not been submitted for a degree in any other University.

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Approval by supervisors

We, the undersigned, hereby assert that the candidate conducted this study under our supervision.

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DEDICATION

To my dear wife Rose and our children.

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ABBREVIATIONS AND ACRONYMS

AC	Alternating current
ARPPIS	African Regional Postgraduate Programme in Insect Science
BHE	Bug hour equivalents
BMZ	German Federal Ministry for Economic Cooperation and Development
CABI	Centre for Agricultural Biosciences International
CHE	Cashew hour equivalents
DAAD	German Academic Exchange Service
DC	Direct current
EAD	Electroantennographic detection
EAG	Electroantennogram
EI	Electron impact
eV	Electron volts
FAO	Food and Agricultural Orgnaisation
FID	Flame ionisation detection
GC	Gas chromatograph
GC-EAD	Gas chromatography-electroantennographic detection
GC-MS	Gas chromatography-mass spectrometry
HP	Hewlett-Packard
icipe	International Centre of Insect Physiology and Ecology
ID	Internal diameter
Inc.	Incorporated
IPM	Integrated pest management
IS	Internal standard
KARI	Kenya Agricultural Research Institute
MT	Metric ton
SEM	Standard error of the mean
TIC	Total ion concentration
UK	United Kingdom
USA	United States of America

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ABSTRACT

The coreid bug, *Pseudotheraptus wayi* Brown 1955 (Heteroptera: Coreidae), is a major pest of cashew in East Africa. Effective environmentally sound management strategies for this pest are still lacking. This study aimed at elucidating the chemical communication system of *P. wayi* and, identifying semiochemicals that can potentially be developed into lures for use in integrated management of this pest. To achieve this, pre-requisite studies on the morphology of immatures to facilitate effective identification of this pest in the field, evaluation of French bean pods for suitability for mass rearing of the insects and diel behavioural patterns of the species to guide semiochemical bioassays were also carried out. Morphometric measurements were carried out using a Leica® Microsystems EZ4D microscope connected to a computer using the recommended Leica® Application Suite Software. The suitability of French beans for mass rearing of *P. wayi* and its diel behavioural patterns were determined by observation of the biology and behaviour of the insects on French bean pods in the laboratory. Candidate semiochemicals were identified by olfactometry, EthoVision XT digital video recording of insect behaviour, gas chromatography-electroantennographic detection and gas chromatography-mass spectrometry. The study has shown that immatures of this pest have unique morphological features, the knowledge of which can facilitate effective identification of the insects in the field. It has also demonstrated that French beans are more suitable for mass rearing of this species than coconut and cashew which have been used previously but were unavailable in Nairobi, Kenya where this study was carried out, and can be scarce and too costly. In addition, the study has revealed that peak mating in this species occurs at mid scotophase, and more mating incidences take place during scotophase than photophase; peak oviposition occurs at late photophase with another minor peak at late scotophase, and more eggs are laid during photophase than scotophase; peak nymphal feeding occurs between 18:00 and 0:00 hours, with more nymphs feeding during scotophase than photophase; and adult feeding pattern is unaffected by time or light and darkness. This knowledge was useful for proper timing of behavioural bioassays, and may also be applied in proper timing of control interventions in the field. Finally and most importantly, three chemicals namely (*Z*)-*β*-ocimene, (*E*)-*β*-ocimene and allo-ocimene were identified from cashew volatiles as attractants for male *P. wayi;* while hexanal, hexyl acetate and hexyl hexanoate in the natural ratio as in male volatiles facilitate recognition of males by either sex, eliciting attraction in females but repellence in males. These candidate semiochemicals offer opportunities for trapping *P. wayi* in the field.

CHAPTER ONE

INTRODUCTION

1.1 General background

The coreid bug, *Pseudotheraptus wayi* Brown 1955 (Heteroptera: Coreidae), is a major pest of cashew (*Anacardium occidentale* L.) in Eastern Africa, contributing up to 80% cashew nut yield loss (Martin *et al.*, 1997; Mitchell, 2000; CABI, 2005; Maniania, 2009; Nyambo, 2009). Both nymphs and adults suck sap from their hosts and inject toxins into the host tissues during feeding, causing wilting and necrosis of young stems, leaves, inflorescences and fruits (Mitchell, 2000; Hill, 2008). The adults breed continuously throughout the year (Mitchell, 2000); therefore, the pest is likely to have a profound effect on its host.

Management of this pest currently relies on use of the predatory weaver ant, *Oecophylla longinoda* Lattreille and application of insecticides such as cypermethrin (200 g/l EC) and lambda-cyhalothrin (50 g/l EC), but both approaches are faced with challenges (Martin *et al.*, 1997; Mitchell, 2000; Nyambo *et al.*, 2003; CABI, 2005). The predatory weaver ant is not only eliminated by broad spectrum insecticides, but it also faces competition and predation from other ants such as *Pheidole megacephala* (Fabricius), *P. punctulata*, *Anoplolepsis custodiens* (Smith) and *A. longipes*. (Mitchell, 2000; Nyambo *et al.*, 2003). On the other hand, chemical insecticides can be hazardous to human and environmental health, and are difficult to apply on tall trees (Nyambo *et al.*, 2003; Steinemann, 2004).

Anoplolepsis custodiens is not only a predator of the weaver ant, but it also preys on nymphs and adults of *P. wayi;* while three species of parasitoids namely

Ooencyrtus sp. from Eastern Africa, and *Anastatus* sp. and *Trissolcus* sp. from Southern Africa have been isolated from *P. wayi* eggs (CABI, 2005). *Anastatus* sp. and *Trissolcus* sp. parasitised 58% and 26% eggs, respectively, but no success reports of deliberate application of these natural enemies in the management of *P. wayi* are available.

Because effective and sustainable methods of managing *P. wayi* are lacking, there is an urgent need for new methods to reduce the devastating effects of this pest. The current study was therefore designed to generate knowledge which could lead to the development of semiochemical lures against *P. wayi.*

1.2 Semiochemicals as pest management tools

Plant odours play a key role in guiding insect pests to suitable hosts for foraging and oviposition (Bruce *et al.*, 2005; De Bruyne and Baker, 2008), and this interaction has been exploited in developing kairomone lures against crop pests. The American ball worm, *Helicoverpa* sp. and the corn rootworm, *Diabrotica* sp. for instance have been effectively managed in Australia USA respectively using plant derived attractants laced with insecticides (Witzgall *et al.*, 2010). Several other studies have been conducted to identify and/or develop host kairomone lures against insect pests such as *Plutella xylostella* (L.) (Reddy and Guerrero, 2000), *Manduca sexta* (L.) (Fraser *et al.*, 2003), *Hylastinus obscurus* Marsham (Quiroz *et al.*, 2005; Manosalva *et al.*, 2011), *Astylus atromaculatus* Blanchard (Van den Berg *et al.*, 2008), *Eurysacca melanocampta* (Costa *et al.*, 2009) and 49 species of bark and ambrosia beetles (Miller and Rabaglia, 2009).

On the other hand, insects emit pheromones for intraspecific communication, which have similarly been exploited in developing lures for monitoring and

managing crop pests (Witzgall *et al.*, 2010). Among these are the fruit and shoot borer, *Leucinodes orbonalis* Guen, the bark beetle, *Ips duplicatus* (Sahlberg), the palm weevils, *Rhynchoporus palmarum* (L.) and *R. ferrugineus* Olivier, the banana weevil, *Cosmopolites sordidus* Germar, the cotton boll weevil, *Anthonomus grandis* Boh. and the housefly, *Musca domestica* L. Other potential pheromone lures include aggregation pheromones produced by the male adult gregarious desert locust, *Schistocerca gregaria* (Forskal) comprising phenylacetonytrile, benzaldehyde, anisole, guaiacol and phenol (Torto *et al.*, 1994). A synthetic blend of these components in the natural proportions in adult male volatiles induces aggregation behaviour of the adult locusts, and the most dominant component, phenylacetonytrile, singly elicits the strongest aggregation response comparable to crude extracts. This indicates sex differentiation in production and activity of the pheromone, and superiority of the activity of some components. On the other hand, female *S. gregaria* produce pentanoic acid as a sex pheromone (Njagi and Torto, 2002). Female-produced sex pheromones are also reported in the heteropteran families Miridae (Groot *et al.*, 1999; Groot *et al.*, 2001; Zhang *et al.*, 2007) and Lygaeidae (Marques *et al.*, 2000); but sex pheromones in most heteropterans are produced by males (Aldrich, 1988; Aldrich *et al.*, 1993; Aldrich *et al.*, 1997; Borges *et al.*, 2006; Roth *et al.*, 2008; Raška, 2009).

In spite of the great potential of managing insect pests using semiochemicals, the same has not yet been exploited in a number of notorious pests such as *P. wayi*.

1.3 Importance of cashew

Cashew is an important cash crop in over 30 countries (FAO, 2011). It is mainly grown for its nuts from which edible kernels are extracted (Figure 1.1). The kernels are highly nutritious, with 49% fat, 36% protein, 1.4% carbohydrates and minerals (Table 1.1) (Akinhanmi *et al.*, 2008). Processed kernels are eaten direct or processed further into butter, nut milk and edible oil or used in baking and confections (FAO, 1992; Davis, 1999; Orwa *et al.*, 2009). The seedcake after oil extraction from kernels is consumed by both humans and livestock. Seed coats from kernels also serve as fodder. Extracts from seed coats have antifungal properties (Kannan *et al.*, 2009).

Cashew nut shell liquid is used for manufacturing paints, plastics, varnishes, surface coatings, automobile brake linings and pharmaceutical products (Bhunia *et al.*, 1998; Guilherme *et al.*, 2009; Kannan *et al.*, 2009). Shell residues are used as fuel in cashew processing plants and household cooking, thereby conserving fuel wood. The energy content of these shell residues is 24 MJ/kg, which is higher than wood pellets with 20 MJ/kg (Tsamba *et al.*, 2006).

The fleshy pedicle, commonly called the "cashew apple", is consumed as a fruit or squeezed into juice. The juice contains 231 mg of vitamin C and 12 mg of sugars per ml, in addition to being rich in minerals (Table 1) (Lowor and Agyente-Badu, 2009). The cashew apple juice is used to produce wine, strong alcohols, sweets and jams (FAO, 1992; Mohanty *et al.*, 2006; Orwa *et al.*, 2009).

Cashew wood is useful for firewood, charcoal, timber and pulp for fabrication of boxes (Orwa *et al.*, 2009). The bark produces gum or resin which is used to make

adhesives, varnish, flux for solder metals and indelible ink (FAO, 1992; Davis, 1999; Owusu *et al.*, 2005; Orwa *et al.*, 2009). The gum has insecticidal properties thus useful for preservation (Orwa *et al.*, 2009). The bark is ~ 9% tannin, which is employed in the tannin industry (Davis, 1999).

Tender shoots are used as vegetable, while various parts serve medicinal purposes for a range of diseases (Orwa *et al.*, 2009). The tree is used for forestation and reforestation to prevent desertification and soil erosion. It is also commonly used for roadside buffering and shades in homesteads.

Cashew therefore, has great potential for food security, income generation, environmental conservation and employment for people engaged in its production, processing and marketing. Exports of raw cashew nuts and processed kernels earned Africa over US$ 0.6 billion in 2008 (FAO, 2011). The products are also traded locally in the producing countries.

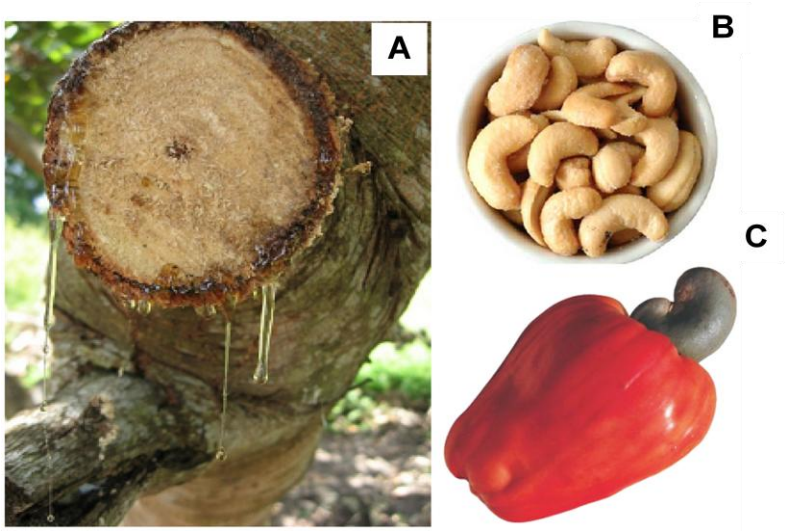

Figure 1.1 Economic products of cashew

A, gum oozing from the stem; **B**, processed kernels; **C**, cashew nut attached to the 'cashew apple'. Photos: **A**, Egonyu J.P.; **B**, Garden Enterprises (2012); **C**, Food and Beverage Online (2011).

Table 1.1 Mineral compositions of cashew kernels and "apple" juice

Cashew product	Nutrient content (mg/100g of kernel or 100ml of juice)				
	potassium	calcium	magnesium	phosphorus	sodium
Kernels	28	23	19	4	8
"Apple" juice	76	43	11	0.8	0.4

Sources: Akinhanmi *et al.* (2008) and Lowor and Agyente-Badu (2009) for kernels and "apple juice", respectively.

1.4 Constraints to cashew nut production

Despite its high economic value for Africa, low yield of cashew nuts with shells of 0.7 MT/ha was estimated in the continent in 2009, which was about 6 times lower than the yield in Vietnam (FAO, 2011). Pests and diseases, poor processing and marketing infrastructure, lack of high yielding varieties, poor agronomic practices, limited credit and extension services, inadequate and low quality planting materials, low prices, ageing trees and bush fires are the major constraints to cashew production in Africa (Martin *et al.*, 1997; Davis, 1999; Horus, 2005; Kenya Horticultural Developement Program, 2005). A number of efforts by many stakeholders to address these constraints and revive the subsector in the continent are currently underway. One such effort is the development of sustainable integrated pest management strategies at *icipe* under the Cashew IPM project (*icipe*, 2009; Maniania, 2009).

Cashew is susceptible to over 60 insect pest species including coreid bugs, mirids, stem borers, thrips, mealy bugs, weevils and leaf miners (Martin *et al.*, 1997; Davis, 1999; CABI, 2005; Dwomoh *et al.*, 2008; Maniania, 2009; Orwa *et al.*, 2009). According to CABI (2005), there are 36 major insect pests of cashew

and 24 minor ones. The most damaging pests of the crop vary with geographical location. For instance, *Helopeltis antonii* Signoret (Heteroptera: Miridae) is the most important pest of cashew in India with yield losses of 30 – 40% (Stonedahl, 1991); while in Ghana, a related species, *H. schoutedeni* Reuter (Heteroptera: Miridae) is the most prominent pest of the crop (Dwomoh *et al.*, 2008); and in East Africa, the coreid bug *P. wayi* and the mirid bug, *H. schoutedeni* are the key insect pests attacking cashew (Martin *et al.*, 1997; Maniania, 2009; Nyambo, 2009).

Infestation of cashew by *P. wayi* and *H. schoutedeni* can result in more than 75% shoot damage, 98% flower drop at early flowering stage and 80% cashew nut yield loss (Nyambo, 2009). Their feeding also causes shrivelling of kernels, lowering their market value.

1.5 Problem Statement

Terpenes are previously reported to be the most prominent constituents of odour from cashew leaves (Maia *et al.*, 2000; Moronkola *et al.*, 2007; Kossouoh *et al.*, 2008). However, the role of host volatiles in mediating attraction of cashew pests, including *P. wayi*, has not yet been established.

Defensive roles of coreid odour i.e., deterrence of natural enemies and alarm signalling in conspecifics, is quite well elucidated (Burger *et al.*, 1986; Blatt *et al.*, 1998; Millar, 2005; Prudic *et al.*, 2008). Other studies suggest that coreid males produce aggregation pheromones (Yasuda, 1990; Blatt and Borden, 1996; Khrimian *et al.*, 2012), and sex pheromones (Aldrich *et al.*, 1993; Wang and

Millar, 2000; Millar, 2005; Soldi *et al.*, 2012). In *P. wayi,* however, constituents and behavioural roles of conspecific odour have not yet been elucidated.

For semiochemical studies to succeed, sufficient numbers of insects of pre-determined reproductive stages and age, and a good understanding of the insect behavioural patterns is required. However, depending on the location of the study, hosts such as coconut and cashew fruits which were previously used for rearing *P. wayi* may not be readily available and can be too costly to obtain (Wheatley, 1961; Mitchell, 2000); and no previous reports on diel behavioural patterns of the pest is available. Since the insect is polyphagous, finding an alternative readily available food substrate for its mass rearing should be possible. French bean pods (*Phaseolus vulgaris* L.), which are readily available throughout the year in markets around Nairobi, Kenya where the current study was located were considered a good alternative food for rearing *P. wayi* because this pest also attacks wild legumes (Hill, 2008).

To be able to collect sufficient numbers of insects for rearing, an efficient identification procedure for all stages is essential. However, identification of *P. wayi* currently relies only on adult morphology which was described by Brown (1955). It is therefore important to describe immatures of *P. wayi* as well.

1.6 Justification

Cashew is one of the most important cash crops in Africa, supporting the livelihoods of more than 5 million households and worth over US$ 0.6 billion in foreign exchange annually (Maniania, 2009; FAO, 2011). However, its production in the continent has sharply fallen from about 70% of the global share

in 1970's to 36% in 2009 (FAO, 2011). East Africa which produced about 90% of the continent's cashew nuts in the 1970's is the worst affected resulting into the same share currently coming from West Africa (FAO, 2011).

The decline in cashew production is attributed to a number of factors among which is losses to insect pests (Martin *et al.*, 1997; Davis, 1999; Horus, 2005; Kenya Horticultural Developement Program, 2005). *Pseudotheraptus wayi* is one of the most damaging insect pests of cashew in East Africa, contributing to 80% nut yield loss (Martin *et al.*, 1997; Maniania, 2009; Nyambo, 2009).

Management of this pest is currently reliant on application of conventional pesticides, which although effective, can be very destructive to the environment, provoking loss of biodiversity and contamination of soil, water and air. They may also intoxicate consumers of cashew products and the farmers while applying the pesticides, and are difficult to apply on tall trees. Environmentally sound management options for this pest are therefore highly desired. The current study was conducted to elucidate morphology, biology and semiochemical mediated behaviour of *P. wayi* with a view to identifying natural attractants from *P. wayi* and cashew, for developing lures as an alternative option of managing the pest.

1.7 Objectives

1.7.1 Main objective

The main objective of this study was to elucidate the chemical communication system of *P. wayi* and identify semiochemicals that can potentially be developed into lures for use in management strategies for the pest.

1.7.2 Specific objectives

This study was specifically undertaken to**:**

1. Elucidate morphological differences of immatures of *P. wayi.*
2. Evaluate the suitability of French bean pods as an alternative food for rearing *P. wayi.*
3. Determine diel patterns of mating, oviposition and feeding in *P. wayi.*
4. Determine if *P. wayi* is attracted to cashew volatiles.
5. Identify components of cashew volatiles which attract *P. wayi.*
6. Determine if conspecific volatiles attract *P. wayi.*
7. Identify components of conspecific volatiles which attract *P. wayi.*

1.8 Hypotheses

The following hypotheses were tested in this study:

1. Immatures of *P. wayi* possess unique morphological features.
2. *P. wayi* can develop and reproduce successfully on French bean pods.
3. Diel patterns of oviposition, mating and feeding in *P. wayi* are affected by time of the day and light or darkness.
4. Cashew volatiles attract *P. wayi.*
5. Only some components of cashew volatiles attract *P. wayi.*
6. Conspecific volatiles attract *P. wayi.*
7. Only some components of conspecific volatiles attract *P. wayi.*

CHAPTER TWO

LITERATURE REVIEW

2.1 Cashew production and trade

World cashew production has increased almost 5-fold over the last 2 decades from 0.7 million metric tons (MT) to 3.3 million MT of nuts with shells produced in 1989 and 2009, respectively (FAO, 2011). India has been the world's leading cashew producer (Horus, 2005) but Viet Nam has emerged and surpassed her with 958,000 MT of nuts with shells in 2009 compared to India's 695,000 MT (FAO, 2011).

Asia has emerged the leading cashew producing continent with over 60% of world production in 2009 compared to Africa with 36%, yet almost 70% of world cashew production originated from Africa in 1970. In the 1970's, East Africa produced over 90% of the continent's cashew output but currently West Africa has emerged as the leading producing region with 90% of the continent's share in 2009. Nigeria is currently Africa's leading producer followed by Ivory Coast with 580,761 and 246,383 MT of cashew nut with shells produced in 2009, respectively. Other top cashew producing countries in Africa include Tanzania, Mozambique, Guinea-Bissau, Benin, Ghana and Kenya with 79,100, 69,846, 64,653, 49,487, 45,647 and 8,381 MT of cashew nut with shells produced in 2009, respectively (FAO, 2011).

Following increasing production trends, raw cashew nut exports have also increased by over 10 fold from 69,903 MT of cashew nut with shells in 1989 to 708,844 MT in 2008. Exports of raw cashew nuts originate almost exclusively from Africa for

instance over 90% in 2008, reflecting lack of processing facilities in the continent (Horus, 2005; FAO, 2011). India is the leading importer of raw cashew nuts with 97% of the world share in 2008. Almost all cashew nut processing is performed in India, Vietnam and Brazil which are also the leading exporters of processed kernels (Horus, 2005). Processed cashew nut kernels are almost exclusively imported by developed countries for example of the 362,706 MT imported in 2008, 37% went to Europe, 34% to North America, 7% to China, 5% to the United Arab Emirates and 2% to the Russian Federation. Africa on the other hand imported only 1% of processed cashew nut kernels during that year.

2.2 Geographic distribution of cashew

Cashew originated from Brazil, and was introduced to Central America by Spanish sailors and to East Africa and Asia by Portuguese colonists (The International Nut and Dried Fruit Foundation, 2011). The crop is presently cultivated in over 30 tropical countries (Figure 2.1) (CABI, 2005; FAO, 2011). In Africa, cashew is cultivated in Angola, Benin, Burkina Faso, Ivory Coast, Ghana, Guinea, Guinea-Bissau, Kenya, Madagascar, Mali, Mozambique, Nigeria, Senegal, Togo and Tanzania; while in Asia, Bangladesh, China, India, Indonesia, Kyrgyzstan, Malaysia, Philippines, Sri Lanka, Thailand and Vietnam are the cashew producing countries. In South America, the crop is cultivated in Brazil, Peru and Guyana; while Belize, El Salvador, Honduras, Mexico, Dominican Republic and Guadeloupe are cashew cultivating countries in Central America and the Caribbean (CABI, 2005; FAO, 2011).

Figure 2.1 World distribution of cashew and *Pseudotheraptus wayi*

Black spots represent distribution of cashew while hollow circles represent distribution of *Pseudotheraptus wayi;* source: CABI (2005).

2.3 Biology and ecology of cashew

Cashew is a medium sized evergreen branched tree with a prominent tap root and an extensive network of lateral and sinker roots (Figure 2.2) (Orwa *et al.*, 2009). It is mainly propagated using seeds, although vegetative propagation through layering or grafting is also feasible. Cashew seeds can also be planted directly in 5 – 8 cm deep and 30 cm wide holes where emergence occurs 3 weeks after sowing (Orwa *et al.*, 2009). The radicle ruptures the pericarp at its stalk end and as it grows downwards, the hypocotyl and the cotyledons emerge. The root grows fast, maintaining a depth of 1.5 times the height of the shoot. The juvenile phase lasts 3 – 4 years.

A fully grown up cashew shoot can reach 12 m tall while the tap root can reach 3 m deep. Pruning may be needed to attain a trunk height of 0.5 – 1.5 m. The lower limbs reach a length of 6 m or more and may be torn off during storms. The shoots grow in flushes. A major flush follows onset of the rainy season and on many shoots an inflorescence appears within 3 – 4 months. Anthesis of the first flowers occurs about 5 weeks later, and the rest of the flowers open over the next 5 – 6 weeks (Orwa *et al.*, 2009).

Cashew flowers are mainly pollinated by honey and wild bees, but flies, butterflies, beetles and ants also carry out pollination (Grundon, 1999; Freitas *et al.*, 2002; Bhattacharya, 2004; Kwapong, 2007; Orwa *et al.*, 2009). Orwa *et al.* (2009) listed wind as one of the pollinating agents but Grundon (1999) and Freitas *et al.* (2002) demonstrated otherwise owing to the sticky nature of cashew pollen making it

irremovable from the anthers by wind. The stigma is receptive for one day, starting a few hours before anther dehiscence. Both cross- and self-pollination occur with hermaphroditic flowers comprising 12 – 16%. The fruit develops for 2 months. Later in the wet season, flushing becomes less regular, usually from lateral buds of the earlier flush, and the occurrence of two distinct dry seasons results into two flowering periods. Out-of-season flowering is also fairly common.

Cashew requires high temperatures, and frost is deleterious. Whereas distribution of rainfall is important, quantity is less important. Cashew is adaptable to very dry conditions as long as its extensive root system has access to soil moisture. In very dry areas with annual rainfall of 800 – 1000 mm, a deep and well drained soil without impervious layers is essential. Better cashew fruiting occurs with low rainfall during the flowering season, and better storage quality is achieved when nut maturity coincides with a dry period (Orwa *et al.*, 2009).

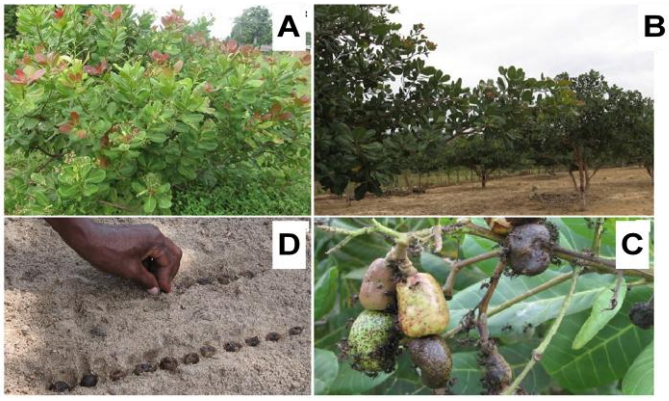

Figure 2.2 Various life stages of cashew

A, A young flushing tree with flowers and young fruits; **B**, A garden of mature trees; **C**, An inflorescence bearing fruits infested with predacious ants; **D**, Demonstration of pre-germination. Photos: Egonyu J. P.

2.4 Chemistry of cashew volatiles

Terpenes are the most prominent chemicals in odour of cashew leaves and flowers (Maia *et al.*, 2000; Moronkola *et al.*, 2007; Kossouoh *et al.*, 2008). Fruit odours are dominated by palmitic and oleic acids, furfural, 4-hydroxydodecanoic acid lactone, (*E*)-hex-2-enal, (*Z*)-hex-3-enol and hexadecanol. Fruits also release a large number of other aliphatic hydrocarbons, alcohols, aldehydes and esters, comprising 26 - 30% of total oils. Most volatiles of cashew 'apple' juice comprise 42% esters followed by aldehydes (14%) (Garruti *et al.*, 2003). All these studies focussed on the composition of cashew volatiles but not its odour mediated interaction with pests such as *P. wayi*.

2.5 Host range and geographical distribution of *Pseudotheraptus wayi*

Pseudotheraptus wayi is a polyphagous pest of cashew (*Anacardium occidentale* L.), coconut (*Cocos nucifera* L.), macadamia (*Macadamia integrifolia* Maiden & Betche), carambola (*Averrhoa carambola* L.), pecan (*Carya illinoinensis* (Wangenh.) K. Koch), cinnamon *(Cinnamomum verum* J. S. Presl), loquat (*Eriobotrya japonica* (Thunb.) Lindl.), mango (*Mangifera indica* L.), avocado (*Persea americana* Mill.), guava (*Psidium guajava* L.), cocoa (*Theobroma cacao* L.) and various wild legumes (Martin *et al.*, 1997; Mitchell, 2000; CABI, 2005; Hill, 2008; Maniania, 2009; Nyambo, 2009).

This insect species is hitherto reported only in Eastern and Southern Africa (CABI, 2005), thereby making it a quarantine pest in most parts of the world.

2.6 Economic importance of *Pseudotheraptus wayi*

Pseudotheraptus wayi can cause different levels of losses to various crops, for example, up to 52.4% damage on guava fruits (Van Der Meulen, 1992), 76.2% damage on avocado fruits (Van Der Meulen and Schoeman, 1994), 99.8% coconut fruit abortion (Way, 1953) and 80% cashew nut yield loss (Nyambo, 2009).

2.7 Biology of *Pseudotheraptus wayi*

Identification of *P. wayi* currently relies only on adult morphology which was described by Brown (1955), yet the five instars are also equally destructive (Mitchell, 2000; Hill, 2008). Description of immature stages of *P. wayi* is therefore required for its effective identification in the field.

Briefly, according to Brown (1955), adult *P. wayi* are dorsally red-brown but lighter ventrally, and possess a raised shiny black spot on the metapleura. Their heads are porrect (extended horizontally) with reddish eyes. There are four antennomeres, the first three red, and the distal, brown with a pale tip. Male antennae are slightly longer than the combined length of body and head, their elytra are 4 – 4.5 mm wide and their total length is 12 – 13 mm. Males possess a characteristic sculptured lateral process on each side of the pygophore (9^{th} abdominal segment). Female antennae are slightly shorter than combined length of body and head, their elytra are 4.5 – 5 mm wide and their total length is 14 – 15.5 mm.

Development of *P. wayi* from egg through five instars to the adult stage on coconut and cashew takes 31 – 41 days under different temperature regimes, while adult

females survive on coconut at 24.6 °C for 45 – 66 days and the males for 83 – 84 days (Way, 1953; Mitchell, 2000; CABI, 2005). Pre-oviposition period is 9 – 13 days; and eggs are laid singly at a rate of 2 to 3 per day, for a total lifetime fecundity of 74 – 100 eggs per female (Way, 1953; Mitchell, 2000).

2.8 Chemistry of *Pseudotheraptus wayi* volatiles

The chemistry of *P. wayi* odour has not yet been elucidated. However, previous work on other coreids shows that their scent chemistry are similar, comprising C_6 and C_8, α, β-unsaturated aldehydes from nymphal dorsal abdominal glands, and mixtures of saturated or α, β-unsaturated aldehydes, alcohols, acetates or butyrate esters of these alcohols, and C_2, C_4, or C_6 acids from adult metathoracic glands (Aldrich, 1988). Most if not all of the scent constituents of coreid bugs are synthesised rather than sequestrated as inferred from the similarity in odour constituents from eight coreid species namely *Amorbus rubiginosus* Guer., *A. alternatus* Dallas, *A. rhombifer* Westwood, *Mictis profana* F., *M. caja* Stål, *Aulacosternum nigrorubrum* Dallas, *Pachycolpura manca* Breddin and *Agriopocoris froggatti* Miller which were fed on different hosts (Waterhouse and Gilby, 1964).

In most coreids, hexanal and hexyl acetate are the most dominant compounds constituting over 90% of volatile components (Waterhouse and Gilby, 1964; Aldrich, 1988). Variations in the proportions of the two dominant components is partly attributed to compartmentalised biosynthesis in which hexyl acetate is secreted by the primary accessory gland into the metathoracic scent gland where esterase and

dehydrogenase enzymes which are apparently secreted by secondary accessory glands cleave the ester and oxidise the resulting alcohol (Aldrich, 1988). This is followed by spontaneous reactions to produce hexanoic acid, hexanal trimer, and an aldol condensation product. This hypothesis is corroborated by ~ 90% hexyl acetate in secretions of *A. nigrorubrum* in which secondary accessory glands are absent; and virtually pure hexanal in *Hyocephalas* sp. from a related family, Hyocephalidae, with an extra pair of accessory glands (Waterhouse and Gilby, 1964; Aldrich, 1988).

CHAPTER THREE

DESCRIPTION OF IMMATURES OF *PSEUDOTHERAPTUS WAYI*

3.0 Introduction

Laboratory mass rearing of insects is essential to facilitate semiochemical studies, thus requiring collection of sufficient numbers of insects in the field to establish the colony. This could only be achieved with efficient identification of all life stages of this species. However, identification of *P. wayi* at the onset of this study relied only on adult morphology which was described by Brown (1955). Only adults could therefore be collected during the first field trip, and only 25 of them were gathered. The morphology of immatures from these adults was described for use in subsequent field visits to collect all stages of the pest to boost the colony.

This chapter presents the description of immatures of *P. wayi;* and for completeness, adult images and previously un-described features of adults that relate to the instars. The descriptions are reported in 'telegraphic' style used in taxonomy, and not the past tense style normally used for results.

3.1 Materials and methods

3.1.1 Insect rearing

An initial stock culture of *P. wayi* originated from adults collected in June 2010, and later boosted with all stages in September the same year, from cashew trees at the Kenya Agricultural Research Institute (KARI)-Mtwapa Research Centre, located at $3^{o}55$'S, $39^{o}44$'E and 15 m above sea level, Kilifi county, Kenya (KARI-Mtwapa,

2004). The colony was reared in a laboratory maintained at 24.6 ± 1^{o}C, 80 ± 1.3% r.h. and photoperiod: L12:D12, at *icipe*, Nairobi, Kenya, where this study was carried out. The room was heated using an electric heater (Vortice, Italy) and humidified using a Defensor® 505 humidifier fitted with a Defensor® hygrostat (Walter Meier, Switzerland). The temperature and r.h. were monitored using a digital hygro-thermometer (Forestry Suppliers, Inc., USA). The room was uniformly illuminated during photophase using six well distributed white fluorescent tubes (~ 1.2 m, 36 W) (Osram, China) which were programmed to turn on at 6:00 hours and off at 18:00 hours using an analogue time switch (Orbis Technologia Electrica, Spain).

Figure 3.1 shows images of cages used in rearing the insects. Adults were reared in wooden cotton drill cages (61 x 46 x 46 cm) and the eggs were incubated in glass vials (2.5 cm ID x 7.5 cm high) according to Wheatley (1961). An attempt to rear nymphs in the wooden cotton drill cage (23 x 30 x 30 cm) designed by Wheatley (1961) was unsuccessful because most of the newly hatched first instars were unable to timely detect the food and initiate feeding probably due to excessive space in the cage, and starved to death. These cages were also unessential for the nymphs unlike adults, which preferred to lay eggs on the cotton, therefore, a relatively cheaper cage could suffice. To avoid these challenges, all first instars were transferred from the glass vials into small cylindrical plastic bottles (3.5 cm ID x 6 cm high) using a camel-hair brush within 0 – 12 hours of hatching. The emerging nymphs had less space to wander around and were maintained in these bottles for 3 – 4 days. At this

age, most nymphs had acclimatized to the food substrate and could search for it even in relatively more spacious cages. They were therefore transferred to a ventilated plastic basket (24 cm ID x 16 cm high), lined at the bottom with filter paper (24 cm diameter) that was replaced weekly. The nymphs were maintained in these plastic baskets until the emergence of adults which were transferred into the cotton drill cages described above.

All the rearing cages accommodated up to 50 individuals of the respective stages. In all cases, nymphs and adults fed *ad libitum* on fresh French bean pods which were replaced twice a week.

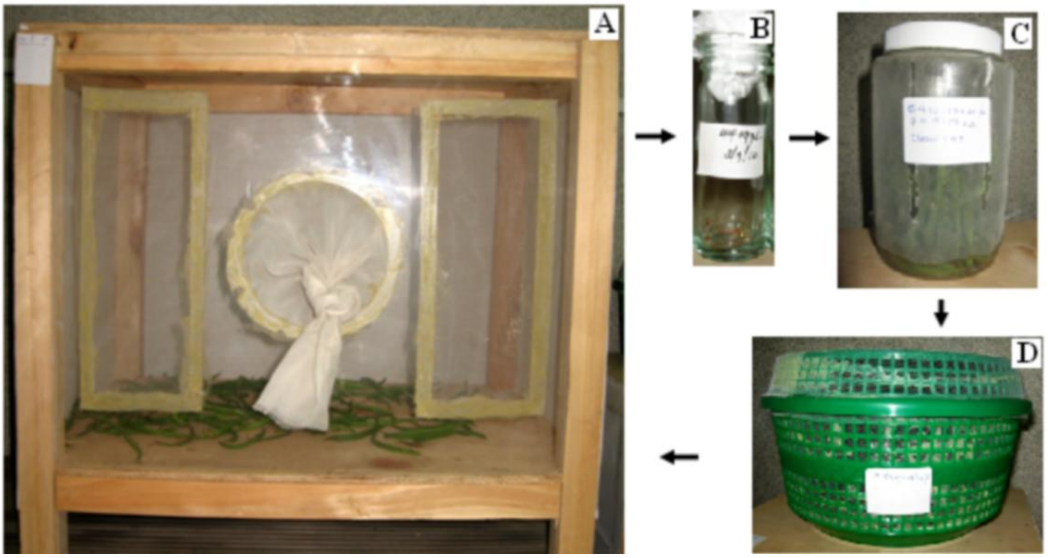

Figure 3.1 A pictorial summary of the procedure for laboratory mass rearing of *Pseudotheraptus wayi* on French bean pods

A, An adult rearing cotton drill cage; **B**, An egg incubation glass vial; **C**, A plastic bottle for rearing nymphs for the first 3 – 4 days; and **D**, A plastic basket for rearing nymphs till adult emergence. Photos: Egonyu J.P.

3.1.2 Measurements and images

Specimens were obtained from the colony described in section 3.1.1. The studies were conducted at the Biosystematics Support Unit laboratory at *icipe*. Images of five specimens of each instar preserved in 80% ethanol and of five live eggs were photographed and measured using a Leica® Microsystems EZ4D Microscope connected to a computer using the recommended Leica® Application Suite Software, version 1.5 (Leica Microsystems Limited, Switzerland), for imaging and measurement of specimens. Egg length and width were measured from two tips and widest point, respectively. Nymphal and adult pronotal length and width were measured from the medial longitudinal axis and the widest posterior end, respectively.

Synthlipsis (shortest inter ocular distance) was also measured. For antennae, both left and right antennomeres were measured. Wet weights of eggs, nymphs and adults were determined using 10 individual live specimens, and the specimens were photographed. Voucher specimens of nymphs and adults were deposited in the Biosystematics Support Unit collection at *icipe*.

3.1.3 Data analysis

Means and standard errors of the means (SEM) were computed for lengths, widths and weights.

3.2 Results

3.2.1 Egg

Egg oval, smooth, cream but turning reddish-brown prior to hatching (Figure 3.2).

Figure 3.2 An egg of *Pseudotheraptus wayi* deposited on a cashew leaf

Length 1.85 ± 0.05 mm, width 1.26 ± 0.02 mm, weight 1.27 ± 0.07 mg; Photo: Fabian Haas; scale bar: 1 mm.

3.2.2 First instar

Body lanceolate, generally reddish-brown with head-thorax region darker and abdominal terga with whitish patches (Figure 3.3; Table 3.1). Ratio of length of head : thorax : abdomen approximately 1 : 1 : 2. Reddish-brown punctation dorsally on the head. Tylus porrect, protruding well forward between the antennal tubercles. Antennae non-cylindrical but rather flattened laterally. Antennomeres uniformly reddish-brown, with 2nd clavate and 3rd conspicuously widest. Eyes and ocelli reddish-pink. Rostrum four-segmented, basal segment thickest and with reddish-brown punctation, 2nd and 3rd sub-equal in length and both shorter than basal segment, 4th longest with a dark brown tip. About one-fifth of rostrum extending posteriorly beyond metacoxae when at rest. An orange Y-shaped ecdysial line present dorsally through head and thorax.

Pronotum broader than long, its anterior and posterior width sub-equal, and medial length sub-equal to that of mesonotum. A pair of prominent brown spines on either side of pronotum. Mesonotum wider posteriorly with minor constrictions between scutellum and forewing buds, the former extending farther posteriorly than the latter and acute to medial axis. Hind wing buds obscured. Metanotum less distinct than pro- and mesonota but also wider posteriorly; and conjoining first abdominal sclerite with a gentle constriction. Fore, mid and hind legs approximately same length. Ratio of length of femora : tibiae : tarsi approximately 4 : 3 : 1 in fore legs and 2 : 1 : 1 in mid and hind legs. Femora and tarsi brown, tibia with reddish-brown and white bands.

Ten abdominal terga with 1 – 3 not easily discernible and 4 or 5 the widest; margin serrated. Posterior half of abdominal terga with more whitish patches at centre than the rest. A pair of dome-like scent glands with posteriorly curving sutures present at mid posterior ends of 4^{th} and 5^{th} abdominal terga, about equal in size, each with a brown spot on either side. A pair of sub-equal dark brown spots laterally on either sides of 5^{th} abdominal tergite.

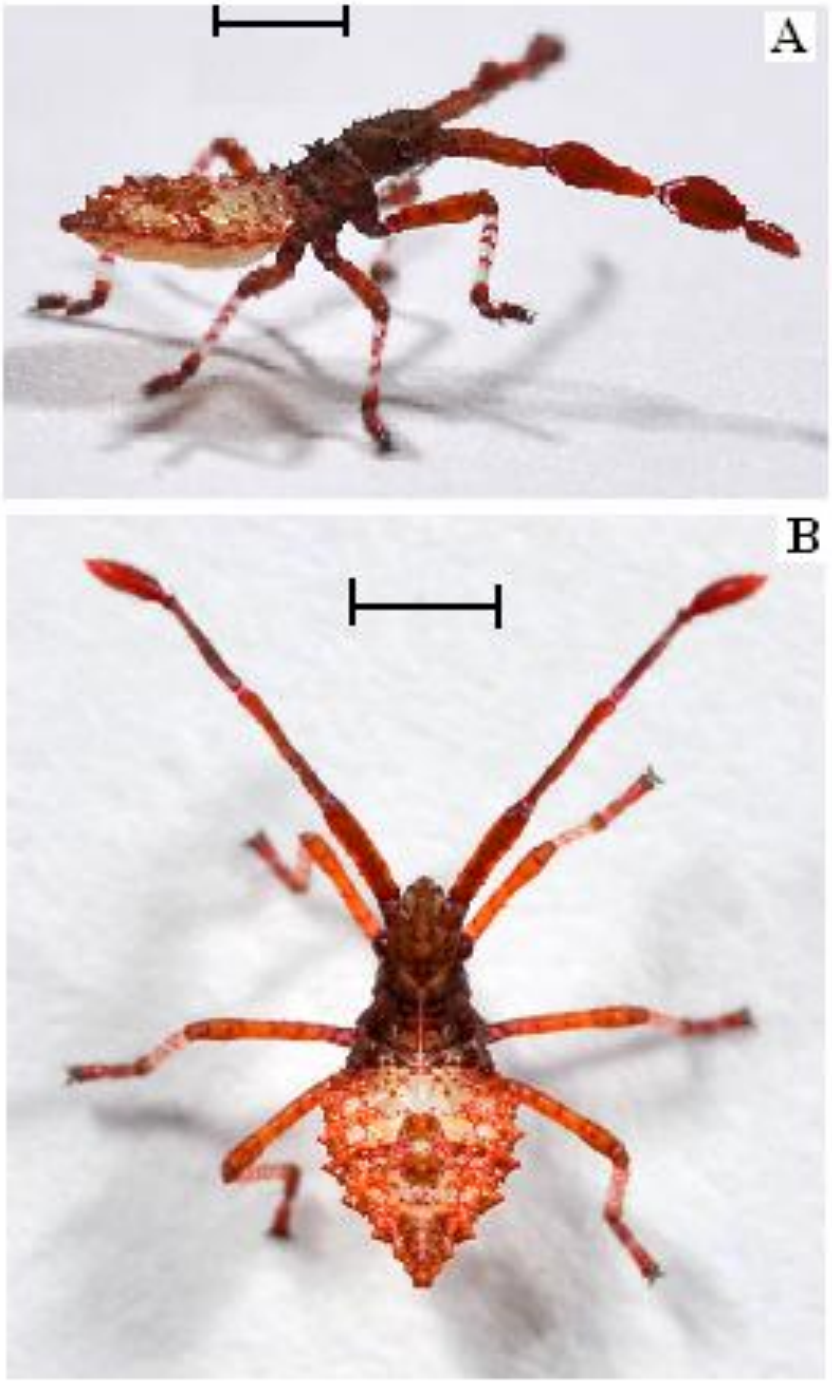

Figure 3.3 First instar of *Pseudotheraptus wayi*

Weight 1.09 ± 0.05 mg; antennomeres (from proximal to distal) 0.81 ± 0.03 mm, 0.95 ± 0.02 mm, 0.77 ± 0.01 mm, 0.60 ± 0.01 mm; synthlipsis 0.46 ± 0.01 mm; pronotum 0.25 ± 0.0 mm long, 0.88 ± 0.14 mm wide; Photos: Fabian Haas; scale bars: 2 mm; A, lateral view and B, dorsal views.

3.2.3 Second instar

Body oblanceolate, generally brown with head-thorax region and 3rd antennomere dark brown to black (Figure 3.4). Antennal shape and 3rd antennomere as in 1st instar. Ocelli, dorsal head punctation and pronotal spines obscured. A well marked median longitudinal impression on vertex between eyes. Rostral tip black and punctation on basal segment dark brown, ecdysial line reddish-brown.

Scutellar-wing bud constrictions sharper than in 1st instar, fore wing buds and scutellum sub-equal in length posteriorly. Posterior end of scutellum approximately perpendicular to medial axis. Metanotum distinct, about half the length of pro- and mesonota, with reddish-brown longitudinal bands at hind wing buds, and conjoining 1st abdominal sclerite with a sharper constriction than in 1st instar. Hind wing buds not covered by fore wing buds. Fore and mid legs approximately same length but shorter than hind legs. All legs with same ratio of length of femora : tibiae : tarsi which is approximately 2 : 2 : 1. Femora pinkish-brown with reddish-brown punctation, tibia as in 1st instar, tarsi reddish-brown.

Abdominal terga 1 – 3 more distinct than in 1st instar; an additional brown spot laterally on either sides of 3rd abdominal tergite and closer to the medial axis than their counterparts on 5th abdominal tergite; and all 6 spots as well as those on dorsal abdominal scent glands darker brown than in 1st instar. Abdominal margins as in 1st instar**.**

Figure 3.4 Second instar of *Pseudotheraptus wayi*

Weight 3.37 ± 0.64 mg; antennomeres 1.27 ± 0.02 mm, 1.59 ± 0.02 mm, 1.29 ± 0.02 mm, 0.74 ± 0.01 mm; synthlipsis 0.61 ± 0.01 mm; pronotum 0.38 ± .02 mm long, 0.85 ± 0.02 mm wide; Photo: Fabian Haas; scale bar: 1 mm.

3.2.4 Third instar

Body ovate, generally pinkish-brown with head-thorax region and 3rd antennomere lighter than in 2nd instar (Figure 3.5). Dorsal head punctation dark brown. Tylus as in 1st and 2nd instars. Eyes and ocelli as in 1st instar. Antennal shape as in 1st and 2nd instars, basal antennomere widest and rest sub-equal in width. Ecdysial line and dorsal head impression as in 2nd instar. Basal rostral punctation smaller and lighter than in 2nd instar but rostral tip black as in 2nd instar.

Pronotal spines dark brown. All thoracic terga wider posteriorly. Constriction between scutellum and forewing buds more distinct than in 1st and 2nd instars with fore wing buds longer than scutellum posteriorly. Posterior end of scutellum as in 2nd instar. Length of fore, mid and hind legs as in 2nd instar. Ratio of length of femora : tibiae : tarsi approximately 3 : 2 : 1 in fore and mid legs, and 4 : 2 : 1 in hind legs. Patterns and colour of legs as in 2nd instar.

Dorsal abdominal spots on terga and scent glands darker than in 1st and 2nd instars. Pair of dark brown spots barely visible on 4th abdominal sternum.

Figure 3.5 Third instar of *Pseudotheraptus wayi*

Weight 6.4 ± 0.22 mg; antennomeres 1.72 ± 0.02 mm, 2.11 ± 0.02 mm, 1.52 ± 0.53 mm, 0.79 ± 0.09 mm; synthlipsis 0.68 ± 0 mm; pronotum 0.52 ± .01 mm long, 1. 14 ± 0.01 mm wide; Photo: Fabian Haas; scale bar: 1 mm.

3.2.5 Fourth instar

Body shape and colour as in 3rd instar, with head-thorax region and 3rd antennomere lighter than in 2nd and 3rd instars (Figure 3.6). Ratio of length of head : thorax : abdomen approximately 1 : 1 : 3. Antennal shape as in 1st – 3rd instars, basal and 2nd antennomeres light brown, 3rd dark brown, distal with reddish-brown tint, and width as in 3rd instar. Dorsal head punctation as in 3rd instar, tylus as in 1st – 3rd instars. Eyes and ocelli as in 1st and 3rd instars. Dorsal head impression and ecdysial line as in 2nd and 3rd instars. Rostrum terminating at metacoxal area, punctation on basal segment sparsely visible and tip black as in 2nd and 3rd instars.

Pronotal spines lighter than in 2nd and 3rd instars. Fore and hind wing buds sub-equal and reaching 1st abdominal tergum. Posterior terminal of scutellum acute to medial axis as in 1st instar. Length of fore, mid and hind legs as in 2nd and 3rd instars. Ratio of length of femora : tibiae : tarsi in fore and mid legs as in 3rd instar and approximately 3 : 3 : 1 in hind legs. Patterns on legs as in 2nd and 3rd instars but femora and tibial white bands turn light pink.

More smaller dorsal abdominal spots present in some specimens, these and, other dorsal abdominal and scent gland spots darker than in 1st – 3rd instars. Spots on 4th abdominal sternum distinctly visible and dark brown to black.

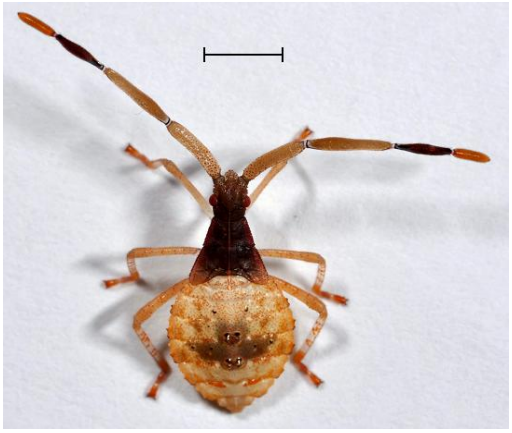

Figure 3.6 Fourth instar of *Pseudotheraptus wayi*

Weight 25.18 ± 2.78 mg; antennomeres 2.21 ± 0.04 mm, 2.67 ± 0.07 mm, 1.81 ± 0.04 mm, 1.41 ± 0.06 mm; synthlipsis 0.8 ± 0.01 mm; pronotum 0.79 ± 0.04 mm long, 1.81 ± 0.04 mm wide; Photo: Fabian Haas; scale bar: 2 mm.

3.2.6 Fifth instar

Body elliptical, with colour of head-thorax region and 3rd antennomere not as distinct from the rest as in 1st – 4th instars (Figure 3.7). Ratio of length of head : thorax : abdomen approximately 1 : 2 : 3. Antennal shape and tylus as in 1st – 4th instars, width of antennomeres and dorsal head punctation as in 3rd and 4th instars. Eyes and ocelli as in 1st, 3rd and 4th instars. Dorsal head impression as in 2nd – 4th instars. Rostrum as in 4th instar but without punctation at basal segment. Ecdysial line whitish and less pronounced than previous instars. Pronotal anterior width shorter than its medial length as well as that of mesonotum. Metanotal medial length less than a quarter those of meso- and pro- nota. Pronotal spines absent. Scutellar terminal acute to the medial axis as in 1st and 4th instars. Fore wing buds reaching 2nd or 3rd abdominal terga and longer than hind ones. Length of fore, mid and hind legs as in 2nd – 4th instars. Ratio of length of femora : tibiae : tarsi approximately 4 : 2 : 1

in fore legs, 4 : 4 : 1 in mid legs and same as 4th instar in hind legs. All legs lack femoral punctation and tibial bands, femora light pink and darker distally, tibiae and tarsi brown. Dorsal abdominal spots as in 4th instar and are black.

Ventral sclerites more distinct and smoother than in previous instars, and are light pink with a pair of black spots on metapleura and 4th abdominal sternum.

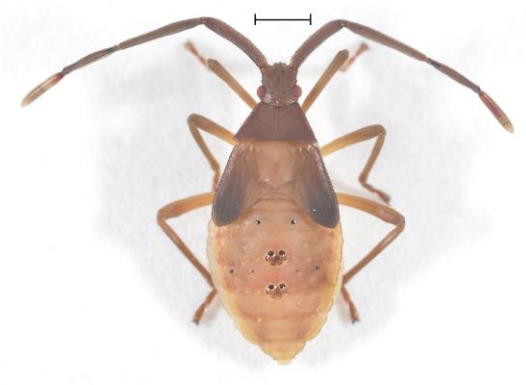

Figure 3.7 Fifth instar of *Pseudotheraptus wayi*

Weight 47.96 ± 3.52 mg; synthlipsis 0.92 ± 0.02 mm; antennomeres 2.82 ± 0.05 mm, 3.12 ± 0.08 mm, 2.12 ± 0.05 mm, 2.17 ± 0.051 mm; pronotum 1.25 ± 0.07 mm long, 3.06 ± 0.03 mm wide; Photo: Fabian Haas; scale bar: 2 mm.

3.2.7 Adults

Adults lack the spots on 4th abdominal sternum found on 3rd – 4th instars, and dorsal abdominal spots (Figures 3.8 and 3.9). Dorsal abdominal scent glands not easily discernible.

Length of fore, mid and hind legs as in 2nd – 5th instars. Ratio of length of femora : tibiae : tarsi in ♀s approximately 1 : 1 : 1 in fore and mid legs, and 2 : 2 : 1 in hind legs; while that in ♂s approximately 2 : 2 : 1 in all legs. Colour of legs as in 5th instar.

Figure 3.8 A couple of *Pseudotheraptus wayi* mating

Left is ♀; weight ♀: 54.68 ± 1.87 mg, ♂: 44.02 ± 1.78 mg; antennomeres ♀: 2.79 ± 0.07 mm, 3.21 ± 0.03 mm, 2.1 ± 0.04 mm, 2.32 ± 0.09 mm, ♂: 2.72 ± 0.1 mm, 3.27 ± 0.07 mm, 2.21 ± 0.03 mm, 3.04 ± 0.06 mm; synthlipsis ♀: 1.08 ± 0.04 mm, ♂: 1.04 ± 0.05 mm; length of pronotum ♀: 2.2 ± 0.12 mm, ♂: 2.5 ± 0.06 mm; width of pronotum ♀: 4.19 ± 0.19 mm, ♂: 4.26 ± 0.11 mm; Photo: Egonyu James Peter; scale bar: 2 mm.

Figure 3.9 Side view of a female *Pseudotheraptus wayi*

Photo: Fabian Haas; scale bar: 2 mm.

Table 3.1 Key qualitative features of *Pseudotheraptus wayi* instars

Character	Instars				
	1st	**2nd**	**3rd**	**4th**	**5th**
Dorsal head punctation	Reddish-brown	Obscured	Dark brown	Dark brown	Dark brown
Vertex impression	Absent	Present	Present	Present	Present
2nd antennomere	Clavate	Clavate	Rectangular	Rectangular	Rectangular
3rd antennomere	Widest, Reddish-brown	Widest, Dark brown to black	Width as in 2nd & 4th, dark brown	Width as in 2nd & 4th, dark brown	Width as in 2nd & 4th, dark brown
Colour of rostral tip	Dark brown	Black	Black	Black	Black
Terminal of rostral tip	Beyond metacoxae	Beyond metacoxae	Beyond metacoxae	At metacoxae	At metacoxae
Basal rostral punctation	Reddish-brown	Dark brown	Brown	Obscured	Absent
Pronotal spines	Present, brown	Obscured	Present, dark brown	Present, brown	Absent
Scutellum	Acute to medial axis	Perpendicular to medial axis	Perpendicular to medial axis	Acute to medial axis	Acute to medial axis
Wing buds	Hind obscured	Fore not covering hind	Fore partly covering hind	Fore & hind sub-equal	Fore covering hind
Bands on hind wing buds	Absent	Present	Absent	Absent	Absent
Tibial bands	Present	Present	Present	Present	Absent
3rd abdominal tergite spots	Absent	Present	Present	Present	Present
Dorsal abdominal spots	Dark brown	Dark brown	Dark brown to black	Dark brown to black	Black
Colour of head & thorax	Dark brown	Dark brown to black	Dark brown	Dark brown	brown
4th abdominal sternal spots	Absent	Absent	Dark brown, obscured	Dark brown to black, distinct	Black, distinct
Black metapleural spots	Absent	Absent	Absent	Absent	Present

3.3 Discussion

The length of an egg of *P. wayi* was previously estimated at 1.5 mm (De Villiers 1992, cited by CABI 2005) which is slightly shorter than ~ 1.9 mm determined in this study; but literature on weight and width is not available.

Instars of *P. wayi* had a number of distinct morphological features as opposed to a previous assertion that they are morphologically very similar (De Villiers 1992, cited by CABI 2005). The distinct features of each stage of *P. wayi* can be of great help in effective identification of the pest in the field. Similar studies on other species of the genus would be of value in developing a dichotomous key for all stages. Furthermore, the revelation of morphological transformations in the development stages may be a recipe for identification of juvenile hormones triggering the transformations with a view to using them in managing the pest through growth regulation.

All the instars possessed dorsal abdominal scent glands opening between tergites 4/5 and 5/6 as is reported in other coreid nymphs (Cobben 1978, cited by Aldrich, 1988). Each nymph possessed a porrect tylus and reddish pink eyes and ocelli (except the second instars where eyes and ocelli were obscured) as in adults (Brown, 1955), but their antennae were flattened laterally as opposed to those of adults which are cylindrical. Apart from first instars, the rest possessed the median longitudinal impressions on their vertices as in adults (Brown, 1955). The metapleural spots used in diagnosis of adult *P. wayi* (Brown, 1955) were only present in the fifth instar.

In most coreids, dorsal abdominal scent glands though present often do not function in adults, and the metathoracic scent glands opening laterally on metapleura, become functional (Staddon, 1979; Aldrich, 1988). This probably explains why the dorsal abdominal scent glands were not easily discernible in adult *P. wayi.*

CHAPTER FOUR

DEVELOPMENT AND REPRODUCTION OF *PSEUDOTHERAPTUS WAYI* ON FRENCH BEANS

4.0 Introduction

Semiochemical studies require a large number of insects of pre-determined reproductive stages and age. However, hosts such as coconut and cashew fruits which were previously used to rear *P. wayi* are not readily available in Nairobi, Kenya where this study was carried out and can be too costly to obtain (Wheatley, 1961; Mitchell, 2000). An alternative readily available host for mass rearing of *P. wayi* was therefore required. French bean pods, which were readily available throughout the year in markets around Nairobi, were considered a good alternative food for rearing *P. wayi* because this pest also attacks wild legumes (Hill, 2008).

This chapter presents the biology of *P. wayi* on French bean pods to evaluate its suitability for mass rearing of the insects. The development and reproduction of pest French beans is discussed in relation to data reported previously on coconut and cashew.

4.1 Materials and methods

4.1.1 Biology on French beans

The colony described in section 3.1.1 was maintained for one generation before commencement of studies on the biology. A cohort of 100 eggs (0 – 12 hours old) was obtained from the colony and monitored daily for two weeks for hatching in

glass vials. Similarly, a cohort of 100 newly emerged first instars (0 – 12 hours old) was obtained and reared singly in the ventilated plastic bottles until adult emergence. Each nymph was monitored daily for molting to the successive stages, confirmed by the presence of exuviae which were carefully removed using a camel hair brush.

Ten pairs (female and male) of newly emerged adults (0 – 12 hours old) were each reared until death in wooden cotton drill cages measuring 30 x 30 x 30 cm. Sexing was based on the characteristic sculptured lateral process on each side of the 9th abdominal segment which is only present in males (Brown, 1955). Each couple was observed daily at 3 hour intervals during photophase throughout their lifespan for mating. Since it was not possible to observe the couples for mating over 24 hours throughout their lengthy lifespan, photophase was used to estimate pre-mating period, mating frequency and mating period. The eggs laid by each female from the test couples were enumerated and removed from the cages daily. Pre-oviposition period, oviposition period and post-oviposition period were computed.

4.1.2 Data analysis

Means and SEM were computed for the durations of various life stages, fecundity (number of eggs laid by each female in its lifetime) and daily egg production (eggs per female per day). Survival of immatures (percentage of individuals at a specific stage that progress to the next stage), sex ratio (proportion of female adults) and median adult survival age (age at which 50% of adults are still alive) were computed.

4.2 Results

Table 4.1 shows durations of various life stages of *P. wayi* and percentages of its immatures surviving to subsequent stages on French beans. Total developmental duration (egg – adult emergence) was approximately 43 days. Sex ratio was 0.44. Fifty percent of females lived up to the age of 154 days while the median adult survival age for males was 183 days. Mean fecundity was 171 ± 39.2 (ranging from 2 – 339) eggs per female. Mean mating frequency was 9.1 ± 2.3 (ranging from 4 - 21) times. Daily egg production ranged from 0 – 14 eggs per female per day. Figure 4.1 shows mean daily egg production, with a polynomial relationship between number of eggs per female per day and age.

Table 4.1 Durations of various life stages of *Pseudotheraptus wayi* and survival of its immatures on French beans

Stage	Sample size	Minimum (days)	Maximum (days)	Mean ± SEM (days)	Survival (%)
Egg	91	3	9	7.3 ± 0.1	91.0
First instar	87	1	7	3 ± 0.1	87.0
Second instar	77	4	15	7.5 ± 0.3	87.0
Third instar	67	3	25	8 ± 0.4	98.5
Fourth instar	66	4	15	7.6 ± 0.3	98.5
Fifth instar	65	6	28	9.9 ± 0	98.5
Adult male	10	43	374	199.6 ± 28.7	-
Adult female	9	57	223	156.9 ± 16.3	-
Pre-oviposition	9	12	53	27.3 ± 8.7	-
Oviposition period	9	2	161	118.1 ± 16.3	-
Post-oviposition	9	0	35	11.4 ± 4.0	-
Pre-mating period	8	9	113	35.3 ± 3.1	-
Mating period	7	31	204	108 ± 20.1	-

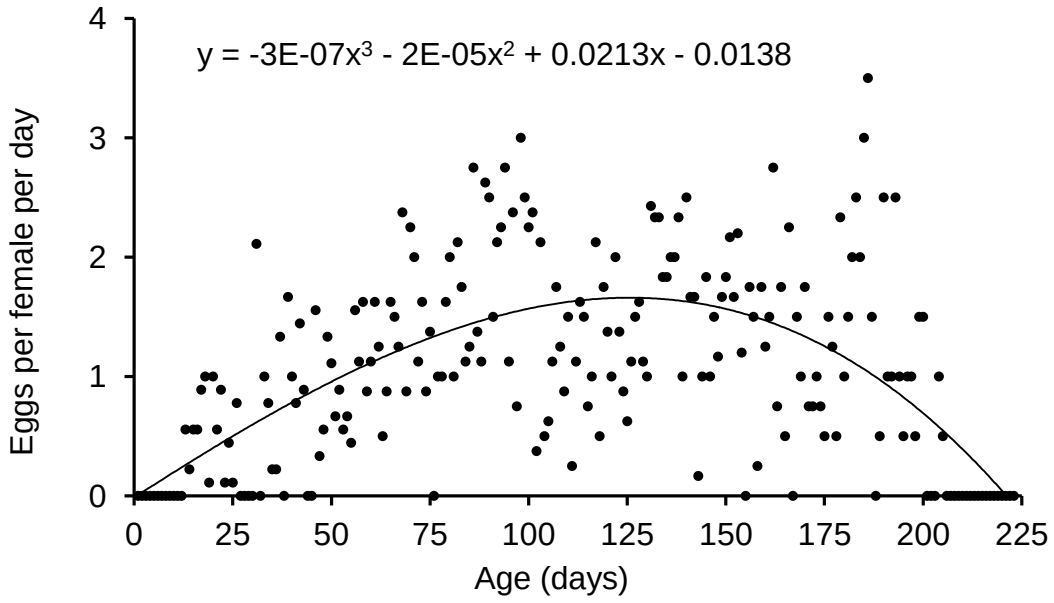

Figure 4.1 Age-specific oviposition rate of *Pseudotheraptus wayi*

4.3 Discussion

Development of *P. wayi* from an egg to adult emergence on French beans compares favourably with those reported on coconut and cashew (Way, 1953; Wheatley, 1961; Mitchell, 2000; CABI, 2005). Wheatley (1961) reported 10 – 15% mortality of 1st – 2nd instars reared on coconut and described the mortality of later instars as very low, which corroborates findings on French beans. This suggests that the first two instars are the most vulnerable stages which require more attention to minimize their mortality during mass rearing.

In the current study, males lived longer than females, which agrees with the findings of Way (1953). Longevities of both sexes were however approximately thrice longer than those reported by Way (1953), which indicates that French beans support a longer lifespan of the species. Although data from this study show pre-oviposition

period which is more than twice the range (9 – 13 days) reported in literature, indicating delayed sexual maturity on French beans, fecundity on French beans was almost twice that previously reported on coconut and cashew (Way, 1953; Mitchell, 2000; CABI, 2005). The maximum number of eggs laid per female per day on French beans was more than four times that which is reported on coconut and cashew (Way, 1953; Mitchell, 2000). The trend of daily egg production indicates that the number of eggs laid by each female increases with age to a peak at ~ 100 – 150 days then declines with the age until the death of the female. This study shows that *P. wayi* ensures fertility of eggs throughout the reproductive period by having comparable mating and oviposition periods.

This is the first report of median adult survival ages for this species. This parameter was longer in males than females, probably because the males lived longer than females. The median adult survival ages may also be shorter on other hosts such as coconut which support shorter longevities of adult *P. wayi*.

CHAPTER FIVE

DIEL PATTERNS OF MATING, OVOPISITION AND FEEDING IN *PSEUDOTHERAPTUS WAYI*

5.0 Introduction

Semiochemical studies require a good understanding of the insect throughout the 24 hours of the, i.e., diel behavioural patterns. However, no previous reports on the diel behavioural patterns of *P. wayi* are available. The studies presented in this chapter were therefore carried out to elucidate peak mating, oviposition and feeding times of this pest, and compare incidences of these activities between photophase and scotophase.

5.1 Materials and methods

5.1.1 Diel patterns of mating, oviposition and feeding

Approximately 25 females and 25 males (~ 1 month old) obtained from the colony described in section 3.1.1 and maintained for one generation prior to this study were put in a wooden cotton drill cage (61 x 46 x 46 cm) provided with fresh French bean pods as food for the insects. The insects were presumably mated because they were mixed since their emergence into adults. The cages were placed under the rearing conditions described in section 3.1.1. The experiments were replicated five times. Visual observations for mating, oviposition and feeding were made at 3 hour intervals over 24 hours for one day. At each inspection time, eggs on the wall of the cage were counted and removed. The number of couples that were mating (Figure 3.8) and that of insects (both sexes) either feeding or not at each inspection time

were recorded for each cage. Seven couples were timed during the day from initiation to termination of mating, to estimate mean mating duration. An insect was deemed to be feeding if its stylet was inserted into a bean pod (Figure 5.1). Observation of the insects as well as data recording during scotophase was achieved using a portable 9 W red lamp (Illumatt, China).

Nymphal feeding was determined using the procedures described above for adults. A total of 10 nymphs (2^{nd} – 5^{th} instars) were put in ventilated red plastic fruit baskets (No 1, 24 cm x 16 cm) (Kenpoly Manufacturers Limited, Nairobi, Kenya). Lids of these baskets were gently removed at each inspection time to allow visualization of the nymphs for feeding. First instars were not considered because they spent most of their time at the top of the rearing container rather than on the food. The nymphal trial was replicated three times.

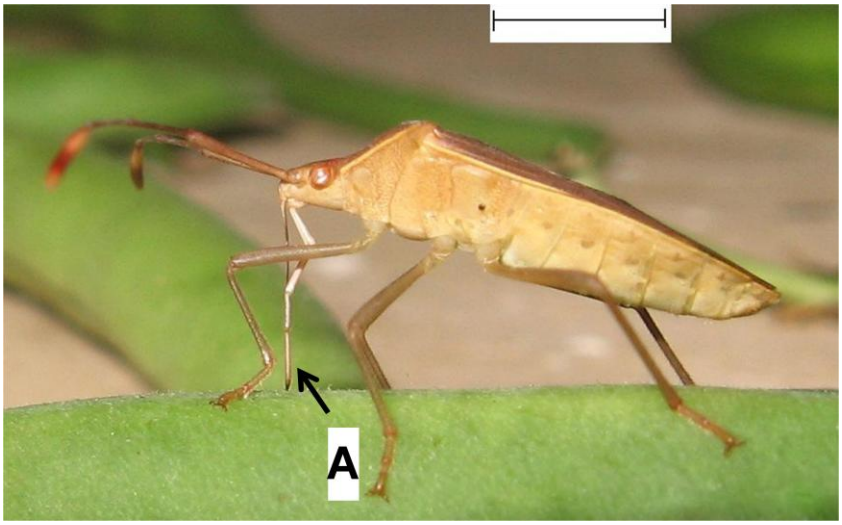

Figure 5.1 *Pseudotheraptus wayi* feeding on a French bean pod
A, the stylet inserted into the pod. Photo: Egonyu J.P.; Scale bar: 5 mm.

5.1.2 Data analysis

Means and SEM were computed for the number of couples mating, number of eggs laid and proportion of insects feeding per cage as well as for mating duration. The proportions of couples mating, eggs laid and insects feeding during photophase and scotophase were compared using a χ^2 test at $\alpha = 0.05$ in R-statistical software, version 2.15.0. (R Development Core Team, 2012). Only the insects which were alive during the data collection time were considered in the analyses for feeding pattern.

5.2 Results

Peak mating incidences occurred at mid scotophase (Figure 5.2) and a higher percentage of mating incidences occurred during scotophase than photophase (Figures 5.3). Mean copulation duration was 3.6 ± 0.8 h. Diel oviposition curve had a major peak at late photophase and a minor one at late scotophase; and a higher number of eggs was laid during photophase than scotophase. Nymphs had extended feeding peaks between 18:00 – 0.00 hours (Figure 5.4). The percentage of nymphs which were feeding during scotophase was higher than that of photophase (Figure 5.5). Conversely, the feeding pattern of adults was unaffected by time or light and darkness.

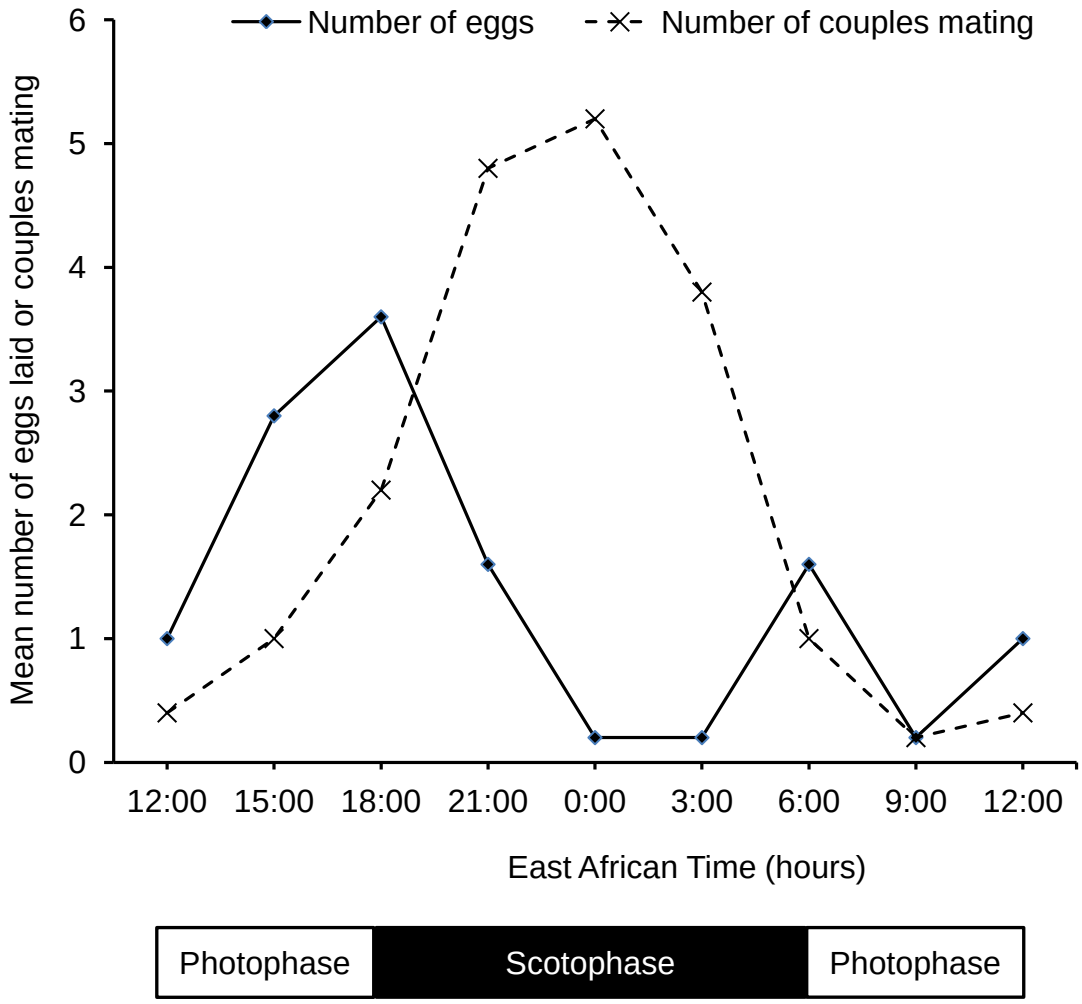

Figure 5.2 Diel patterns of mating and oviposition by *Pseudotheraptus wayi*

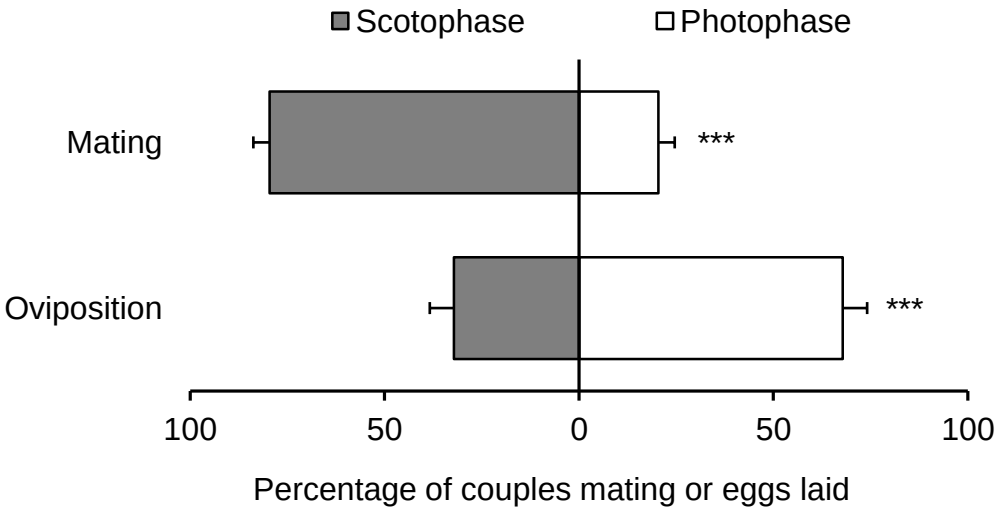

Figure 5.3 Comparison of mating and oviposition by *Pseudotheraptus wayi* during photophase and scotophase

Three *asterisks* indicate significant differences ($P < 0.001$ for both activities) in percentages between photophase and scotophase; χ^2 = 12.90 and 62.71 for oviposition and mating, respectively.

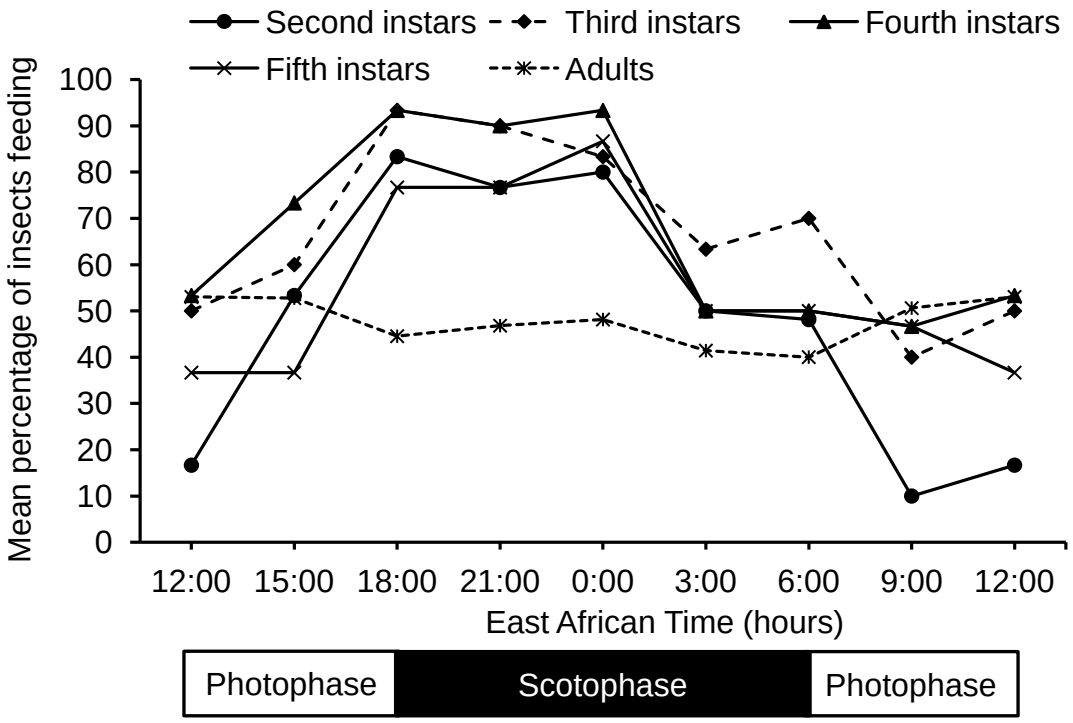

Figure 5.4 Diel patterns of feeding by *Pseudotheraptus wayi*

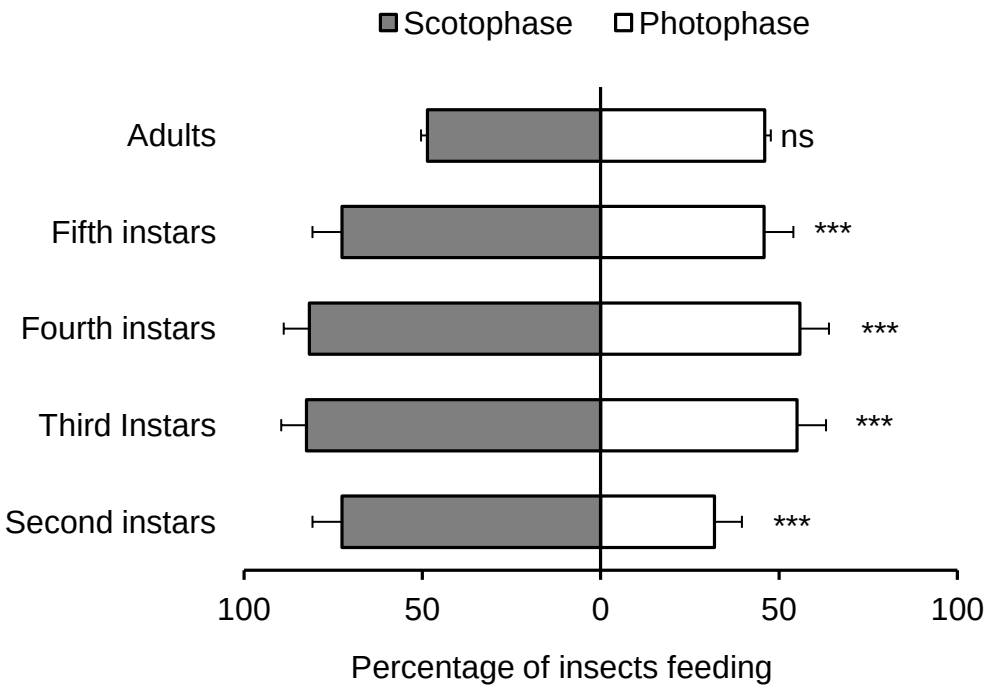

Figure 5.5 Comparison of feeding by *Pseudotheraptus wayi* during photophase and scotophase

Three *asterisks* indicate significant differences ($P < 0.001$ for all the instars) in percentages of the insects feeding during photophase and scotophase. $\chi^2 = 37.81$, 19.86, 17.45 and 16.57 for $2^{nd} – 5^{th}$ instars, respectively.

5.3 Discussion

This study has revealed for the first time, diel behavioural patterns in *P. wayi*. Mating incidences were approximately four times more prevalent during scotophase than photophase with a peak at mid scotophase and a mating duration of ~ 4 h. These observations concur with those of another coreid bug, *Leptoglossus clypealis* Heidemann (Wang and Millar, 2000).

Oviposition peaked at late photophase with another minor peak at late scotophase, and approximately twice more eggs were laid during photophase than scotophase. The two peaks of oviposition in *P. wayi* observed in this study suggest that peak

oviposition occurs in synchronization of change in light intensity, as is reported in moths and beetles (Keil *et al.*, 2001; Omkar and Singh, 2007). Literature on diel oviposition pattern is scarce for coreids, but that of *Thyanta pallidovirens* (Stal) from a related family, Pentatomidae, is similar to that of *P. wayi* with major and minor peaks (Schotzko and Okeeffe, 1990).

Nymphal feeding patterns had extended peaks between 18:00 – 0:00 hours, with approximately twice more nymphs feeding during scotophase than photophase. Although diel feeding patterns of instars were similar, it is evident from Figures 5.4 and 5.5 that feeding by second instars, most especially during off peaks, was relatively less than the rest probably due to poor acclimatization to feeding or lower nutritional requirements compared to their older counterparts. The nymphal feeding pattern contrasts with that of the rice coreid bug, *Leptocorisa chinensis* Dallas which feeds more during photophase than scotophase (Kainoh *et al.*, 1980), suggesting variable feeding patterns in coreids. However, unlike nymphs, diel adult feeding pattern was unaffected by time of the day or light and darkness. This may be because the sexually mature adults had to balance feeding with mating and oviposition. Reproduction presumably alters insect diel feeding rhythms (Socha and Zemek, 2007). Further studies on the effect of adult physiological state on diel feeding rhythm in *P. wayi* are therefore essential.

In most insects, causes of periodicity in activities whether environmentally controlled or internally driven by biological clocks, are largely unknown (Omkar and Singh, 2007). Daily light and darkness are the main environmental cues organisms

use to synchronize diel rhythms to the 24 hours of the day (Greenberg *et al.*, 2006). Similarly, diel mating, oviposition and feeding patterns in *P. wayi* described in this study can only be explained in terms of light or darkness but more work is needed to explain why the specific activities peak at certain times of the day.

Besides being useful for studies on *P. wayi* that may be influenced by mating, oviposition and feeding, the knowledge on its diel behavioural patterns may be useful for proper timing of control interventions against the pest in the field.

CHAPTER SIX

BEHAVIOURAL AND ELECTROPHYSIOLOGICAL RESPONSES OF *PSEUDOTHERAPTUS WAYI* TO CASHEW VOLATILES

6.0 Introduction

Plant volatiles play a crucial role in host location by phytophagous insects (Bruce *et al.*, 2005; De Bruyne and Baker, 2008). However, before this study, odour mediated interaction of *P. wayi* with its hosts had not been elucidated. Recent work showed that terpenes are the most prominent constituents of odour from cashew leaves (Maia *et al.*, 2000; Moronkola *et al.*, 2007; Kossouoh *et al.*, 2008). However, the exact function of the leaf odour in the ecology of cashew pests including *P. wayi* was hitherto unknown. Identification of kairomones of the coreid bug may offer opportunities for their complementary use in existing management tools for this pest. In this chapter, laboratory behavioural assays, electrophysiology and chemical analysis were carried out to establish if *P. wayi* is attracted to cashew volatiles and, if so, to identify the components of the volatiles responsible for the attraction.

6.1 Materials and methods

6.1.1 Insects

The insects used for these studies were obtained from the colony described in section 3.1.1. Behavioural assays were conducted with virgin insects which were ~ 1 month old and starved for 24 hours prior to the experiments. Virginity was ensured through sexing newly emerged adults and rearing either sex separately.

6.1.2 Behavioural responses to volatiles from live cashew seedlings

Bioassays were conducted in a four-arm olfactometer previously described by Suazo *et al.* (2003) (Figure 6.1). Charcoal filtered, humidified air was passed through two glass chambers measuring 14 cm ID x 55 cm high, the treatment one containing a potted cashew seedling (~ 16-wk-old) as the odour source and the other serving as a blank control. The air was thereafter split into two equal parts using a glass T-junction and delivered into the olfactometer at ~ 170 ml min^{-1} $quadrant^{-1}$. Air plumes from the treatment and control odour sources were delivered into two opposite halves of the olfactometer arena. Air was drawn from the center of the olfactometer (vacuum line) at ~ 680 ml min^{-1}. Initially, white smoke generated by the Wizard Stick (Zero Toys, Spaceship Earth, Massachusetts, USA) was passed into the olfactometer until two well-defined odour zones were formed in each half. The glass ware were washed with Teepol odourless detergent and hot water, rinsed with acetone and distilled water, and heated overnight in an oven at 150 °C prior to use.

Individual test females and males were gently released into a plastic vial (2.5 cm ID x 2.5 cm high) connected to the vacuum line, and monitored for 10 min. The bottom of the release vial was perforated to allow air movement. A test insect which responded by moving from the release vial to the odour arena was observed for 120 seconds and the time spent in each zone was recorded using a stopwatch; while the insects that failed to respond during this time were considered non-respondents and removed. Each treatment was replicated with 30 female and 30 male respondents. Each insect was tested only once. To minimize possible positional effects, locations

of odour zones of the olfactometer were switched after obtaining 15 responses. The bioassays were conducted during the day in a room maintained at conditions similar to those in the rearing room.

6.1.3 Collection of volatiles

Cashew headspace volatiles were trapped *in situ* from flushing cashew leaves on intact trees at KARI-Mtwapa Research Centre in September 2011 using a portable battery powered pump (Figure 6.2). Five leaves at the apex of a branch were enclosed in a transparent airtight oven-conditioned polyethene bag (Baco®, Wrap Film Systems Limited, UK) through which charcoal filtered air was passed at 348 ml min^{-1}, then on to Super-Q traps (30 mg, Alltech, Nicholasville, Kentucky) for 12 hours during the day. This was repeated thrice on different trees, and each Super Q trap was eluted with 200 µl of dichloromethane (99%) (Sigma Aldrich, Chemie, Steinhem, Germany) into the same glass vial (Agilent, USA). The sample was concentrated to 500 µl under a gentle stream of nitrogen gas. This sample contained 180 cashew hour equivalents (CHE), where 1 CHE = volatiles emitted by 1 cashew leaf h^{-1}. Volatiles were also trapped repeatedly as above from blank polyethene bags as controls and each Super Q trap was eluted with 200 µl of dichloromethane into a separate glass vial.

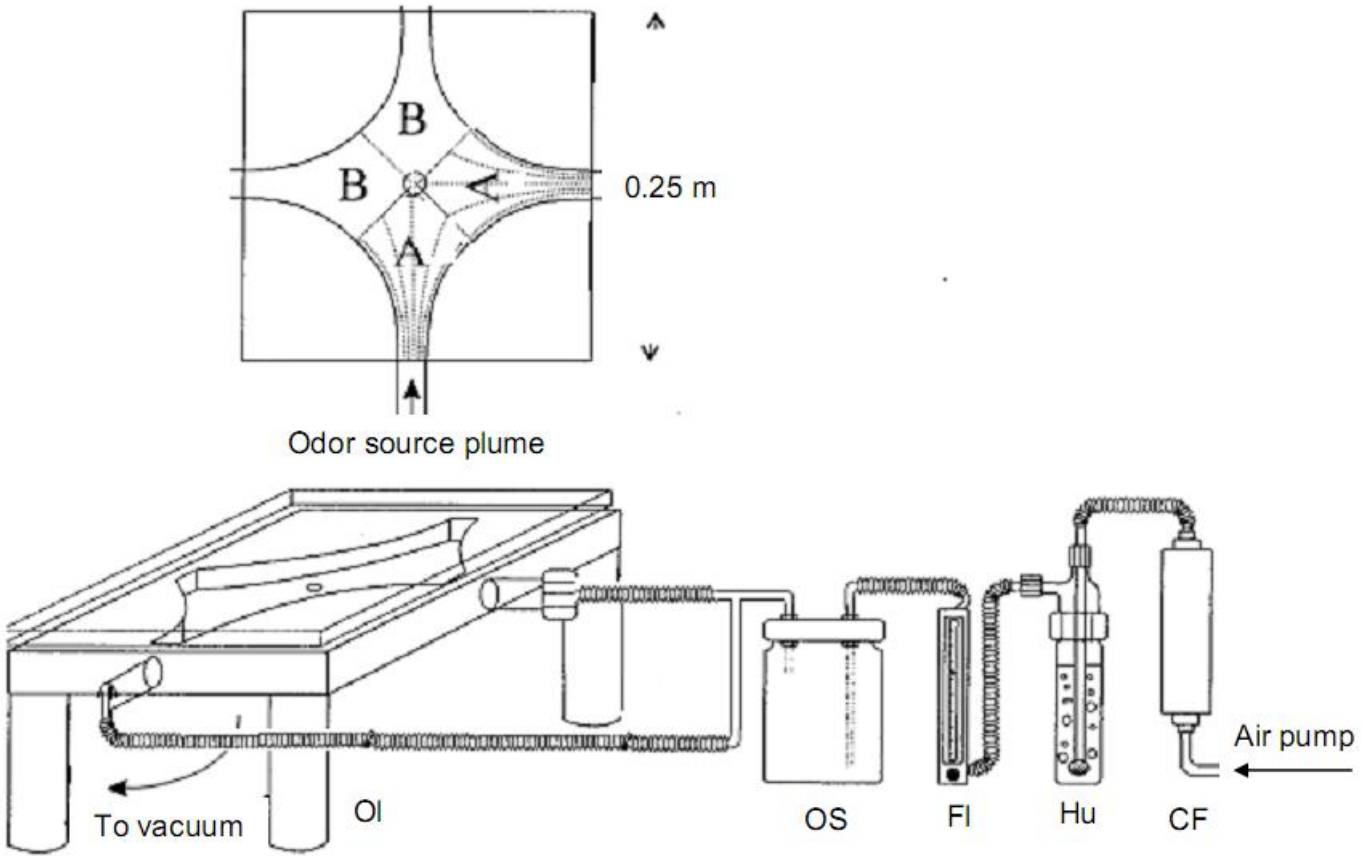

Figure 6.1 A four-arm olfactometer

"A"s = odour zone; "B"s = control zone; Ol = Olfactometer; OS = Odour source; Fl = Flowmeter; Hu = Humidifier; CF = Charcoal Filter; modified from Suazo *et al.* (2003).

Figure 6.2 A battery powered pump used for trapping volatiles in the field

The pump in this photo is being used to trap from another tree other than cashew; Photo: Egonyu J.P.

6.1.4 Analysis of volatiles

Coupled gas chromatography-electroantennographic detection (GC-EAD) analysis was carried out using an Hewlett-Packard (HP) 5890 Series II gas chromatograph equipped with an HP-1 column (30 m x 0.32 mm ID x 0.25 μm, Agilent, Palo Alto, California, USA) in the splitless mode (Figure 6.3). Nitrogen was used as the carrier gas at a flow rate of 1.2 ml min^{-1}. Oven temperature was programmed at 35 °C for 5 min and then increased by 10 °C min^{-1} to 280 °C, and held at this temperature for 5 min. The GC injector temperature was 250 ºC, with FID detection at 270 ºC. The column effluent was combined with nitrogen as make-up gas and then split 1:1 to the FID and EAD using a splitter (Scientific Glass Engineering splitter, Scientific Glass Engineering Inc., Austin, Texas, USA). The reference and recording electrodes were

silver coated wires contained in glass capillaries filled with Ringer's solution (NaCl, 7.5 g; KCl, 0.35g, $CaCl_2$, 0.21 g l^{-1} of H_2O). Antennae of both females and males were cut off at the base of the second antennomere and their tips were clipped off before use. The antennal base was inserted into the reference electrode, and the recording electrode was sleeved over the tip of the antenna. The microelectrodes were connected to an AC/DC amplifier in DC mode (Syntech, Hilversum, The Netherlands). A GC-EAD program (Syntech GC-EAD 2000, Hilversum, The Netherlands) was used to simultaneously record and analyse EAD and FID signals on a computer. An aliquot of 5 µl of the crude sample and the blend of synthetic chemicals was analysed, and this was replicated with fresh antennae of three females and three males.

Coupled gas chromatography-mass spectrometric (GC-MS) analysis of the cashew volatiles was carried out on an HP-7890A GC (Agilent Technologies, Inc., Beijing, China) coupled to an HP-5975 mass spectrometer (EI, 70 eV, Agilent Technologies, Inc., Santa Clara, California, USA) equipped with an HP-5 MS column (30 m × 0.25 mm ID × 0.25 µm) (Restek, Bellefonte, Pennsylvania, USA) (Figure 6.4). The oven temperature was programmed using conditions described for the GC-EAD analysis above. An aliquot of 1 µl of the volatile extract was analysed, and the components were identified by comparison of their retention times and mass spectral data with library data (NIST, 2005) and authentic standards.

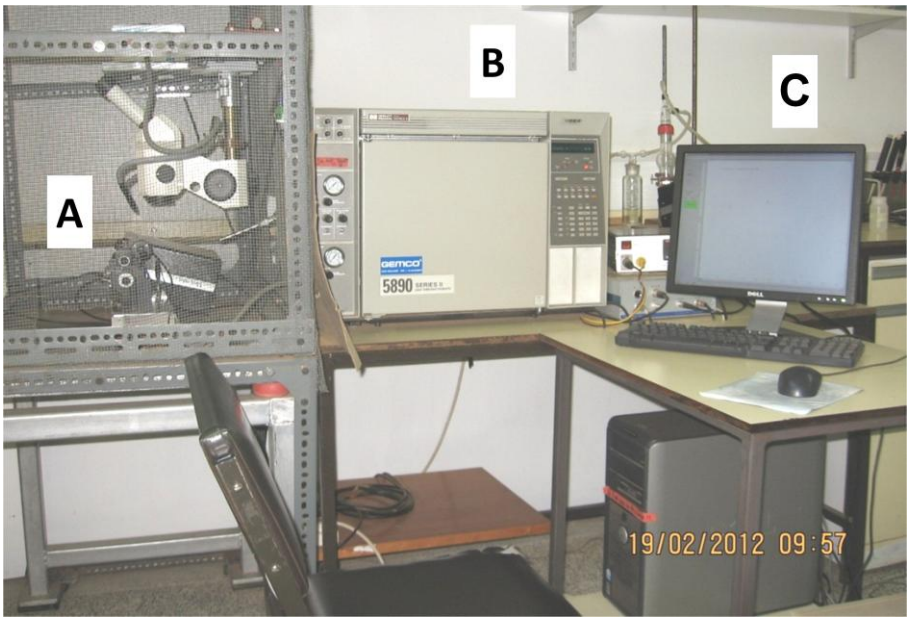

Figure 6.3 Gas chromatography-electroantennographic detection apparatus

A, Electroantennographic detector; B, Gas chromatograph, Computer screen. Photo: Egonyu J.P.

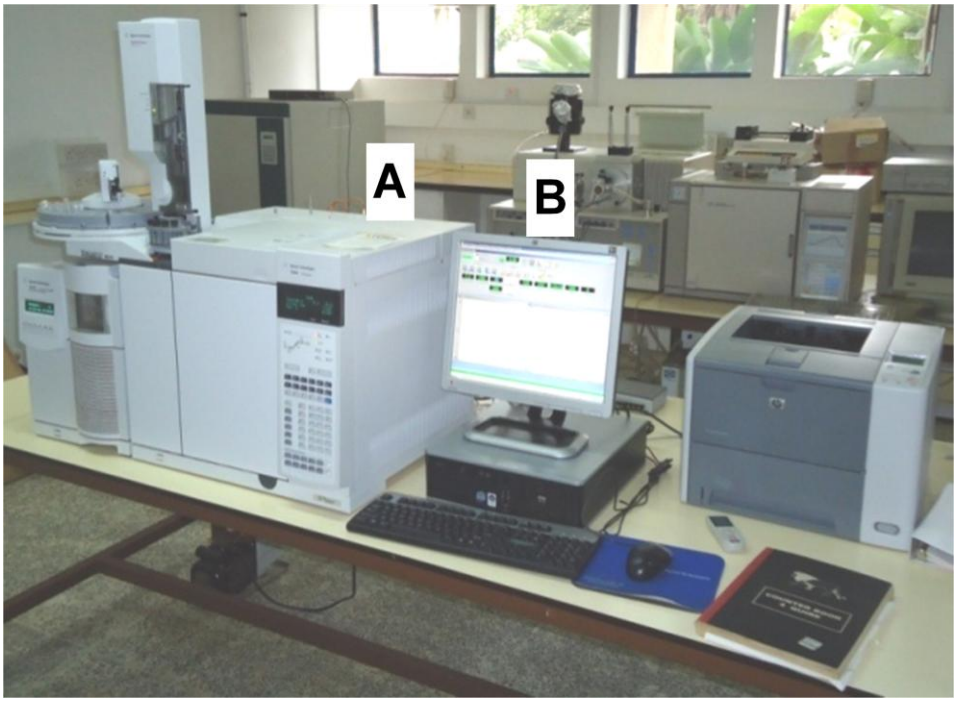

Figure 6.4 Gas chromatography-mass spectrometry apparatus

A, Gas chromatograph, Computer screen. Photo: Egonyu J.P.

6.1.5 Chemicals

A synthetic mixture of ocimene isomers (90%) which contained (*Z*)-*β*-ocimene, (*E*)-*β*-ocimene and allo-ocimene in the ratio of 4:10:1, and butanoic acid (99%) were purchased from Sigma Aldrich (Milwaukee, Wisconsin, USA); (*Z*)-3-hexen-1-ol (98%) was purchased from Sigma Aldrich (Gillingham, England); while (*Z*)-3-hexenyl butanoate was synthesised in the laboratory by esterification of (*Z*)-3-hexen-1-ol and butanoic acid.

6.1.6 Behavioural responses to crude volatiles and synthetic chemicals

Several trials with crude volatiles and synthetic chemicals using the olfactometer described in section 6.1.2 were unable to yield significant differences in responses of the insects to the odour sources compared to controls. Trapping and analysis of the chemicals presented in the olfactometer indicated loss of most components in the airflow system suggesting that the insects were not perceiving the whole blend. This necessitated an alternative bioassay system. Dual choice bioassays were therefore carried out in a glass Petri dish (14 cm ID x 1.5 cm high) (Figure 6.5) according to Yoneya *et al.* (2010). The base and lid of the Petri dish were separated by a cylindrical wire mesh (~ 14 cm ID x 3.5 cm high) to provide enough vertical space for navigation and ventilation for the insects. The Petri dishes (and the wire mesh) were washed with Teepol odourless detergent and hot water, rinsed with acetone and distilled water, and heated for 24 hours in an oven at 150 °C prior to use.

Two pieces of filter paper (~ 1 cm^2), one loaded with an aliquot of 10 µl of the solution of the test crude cashew or synthetic EAD-active components in

dichloromethane, and the other loaded with the same volume of the solvent only as a control were placed at opposite sides of the Petri dish. The solvent was allowed to evaporate for ~ 2 min. Different solutions containing 0.1, 0.2 and 0.4 CHE of crude cashew extract per 10 µl, and 0.25, 0.5 and 1 µg of the blend of synthetic EAD-active cashew chemicals per 10 µl were tested. In preliminary assays, these doses were observed to elicit responses from the adult insects. The ocimene mixture and (*Z*)-3-hexenyl butanoate were blended in the ratio of 25:1, representing the natural ratio of (*E*)-*β*-ocimene (the major constituent of the ocimene mixture) and (*Z*)-3-hexenyl butanoate in crude cashew volatiles.

The movement of an individual virgin adult female or male insect (~ 1 month old; starved for 24 hours prior to the bioassays) released at the centre of the Petri dish was video recorded for 10 min, using EthoVision XT version 8.0 video tracking system (Grieco *et al.*, 2010). Positions of the test insect were sampled every 0.5 seconds and the mean distances to either odour sources were generated using the EthoVision computer software. The trial was replicated ten times with each insect used only once. To minimize possible positional effects, locations of odour and control filter papers were switched between replicates with a fresh clean Petri dish.

After obtaining the optimal dose (0.5 µg) of activity with the blend of the synthetic EAD-active chemicals from cashew, 0.48 µg of the ocimene mixture and 0.02 µg of (*Z*)-3-hexenyl butanoate representing the same amounts in the blend, were also tested

against blank controls. These bioassays were also carried out under similar conditions described in section 6.1.2 for olfactometer bioassays.

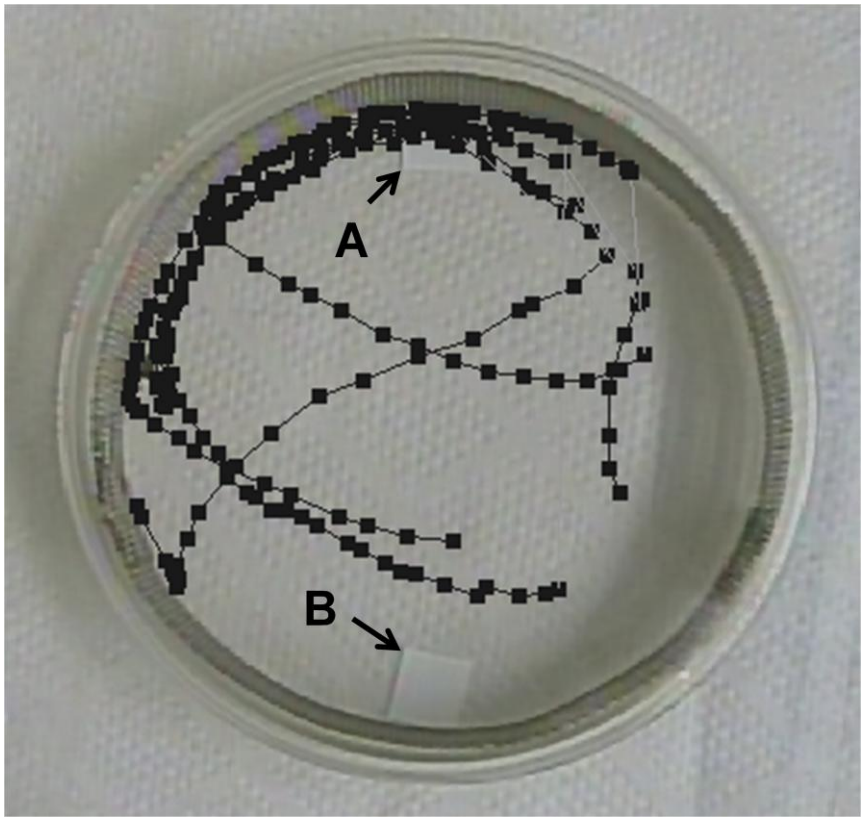

Figure 6.5 Track of the movement of a female *Pseudotheraptus wayi* in a Petri dish arena

A, filter paper loaded with 10 BHE of hexyl hexanoate; B, control filter paper loaded with dichloromethane only; black squares represent positions of the insect.

6.1.7 Data analysis

Attraction of insects to the odour sources in the olfactometer and Petri dish bioassays was analysed using the two sample *t*-test in R-statistical software version 2.15.0 (R Development Core Team, 2012). A significantly higher time in the odour zone of the olfactometer than the control zone implied attraction of the insects to the odour; while a significantly shorter time in the odour zone than the control zone implied

repulsion. On the other hand, a significantly shorter distance to the odour point than the control point in the Petri dish implied attraction of the insects to the odour; while a significantly longer distance to the odour point than the control point implied repulsion.

6.2 Results

6.2.1 Behavioural responses to volatiles from live cashew seedlings

Only males were attracted to odour from cashew seedlings (Figure 6.6).

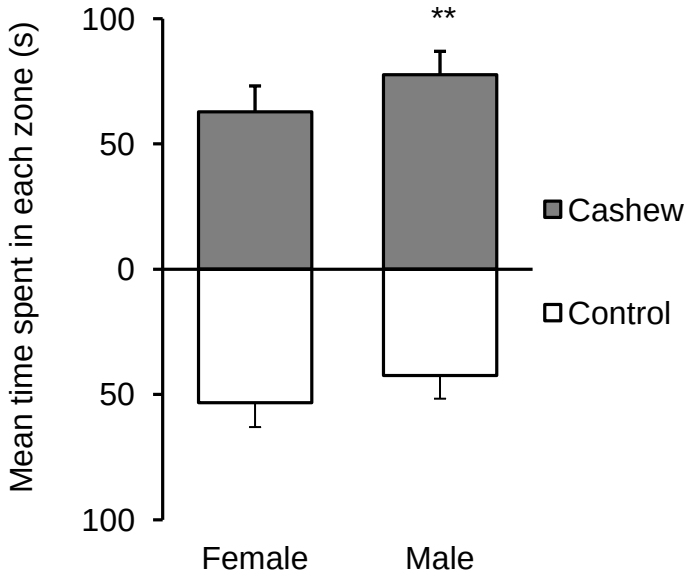

Figure 6.6 Responses of *Pseudotheraptus wayi* to volatiles from live cashew seedlings

Two *asterisks* indicate a significant difference between the treatment and the control at $P < 0.01$; error bars represent SEM.

6.2.2 Analysis of volatiles

GC-EAD analysis revealed four components of crude cashew odour which consistently generated antennal responses from male antennae, but female antennae were seldomly weakly sensitive to only two of these chemicals (Figures 6.7 and 6.8). The EAD-active components were identified by GC-MS as (*Z*)-*β*-ocimene, (*E*)-*β*-ocimene, allo-ocimene and (*Z*)-3-hexenyl butanoate (ratio 1:25:3:1) (Figures 6.9 and 6.10; Table 6.1), and confirmed with authentic standards.

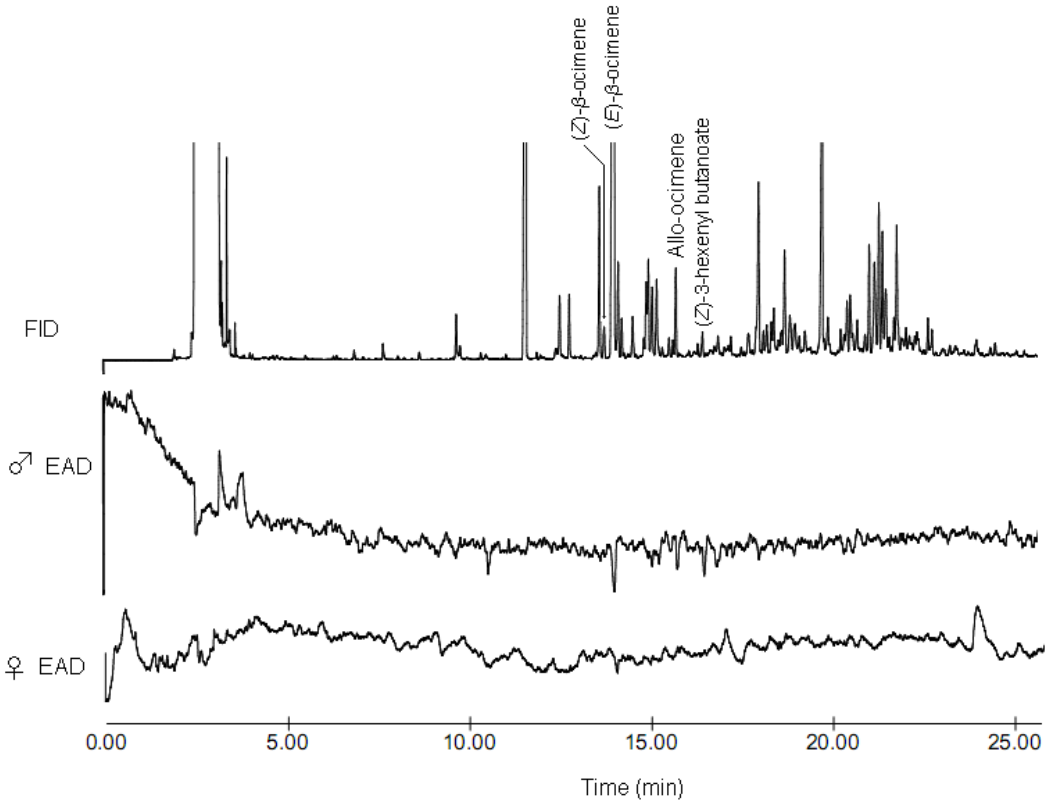

Figure 6.7 Coupled gas chromatography-electroantennographic detection responses of male and female *Pseudotheraptus wayi* to crude cashew volatiles

FID = flame ionisation detector; EAD = electroantennographic detection; the weak EAD responses to peaks which were also present in the GC-MS profile of the blank control extract were not considered.

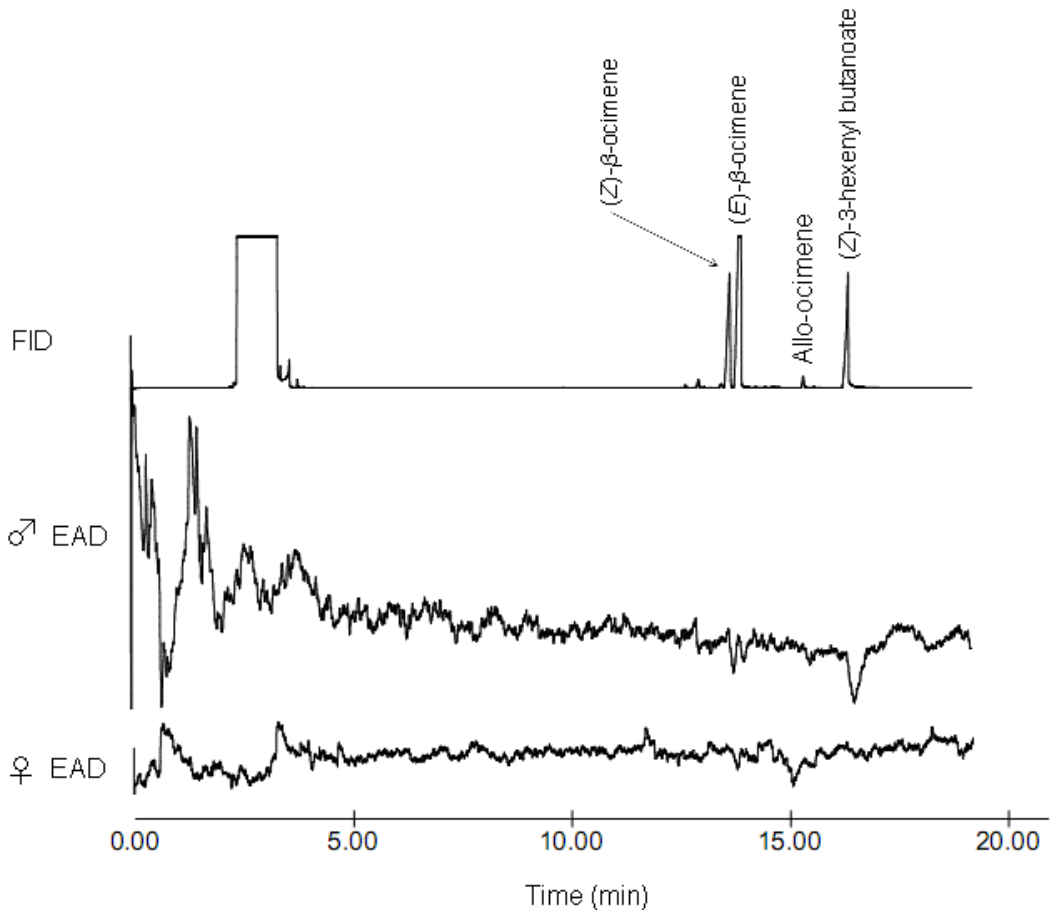

Figure 6.8 Coupled gas chromatography-electroantennographic detection responses of female and male *Pseudotheraptus wayi* to synthetic EAD-active cashew chemicals

FID = flame ionisation detector; EAD = electroantennographic detection.

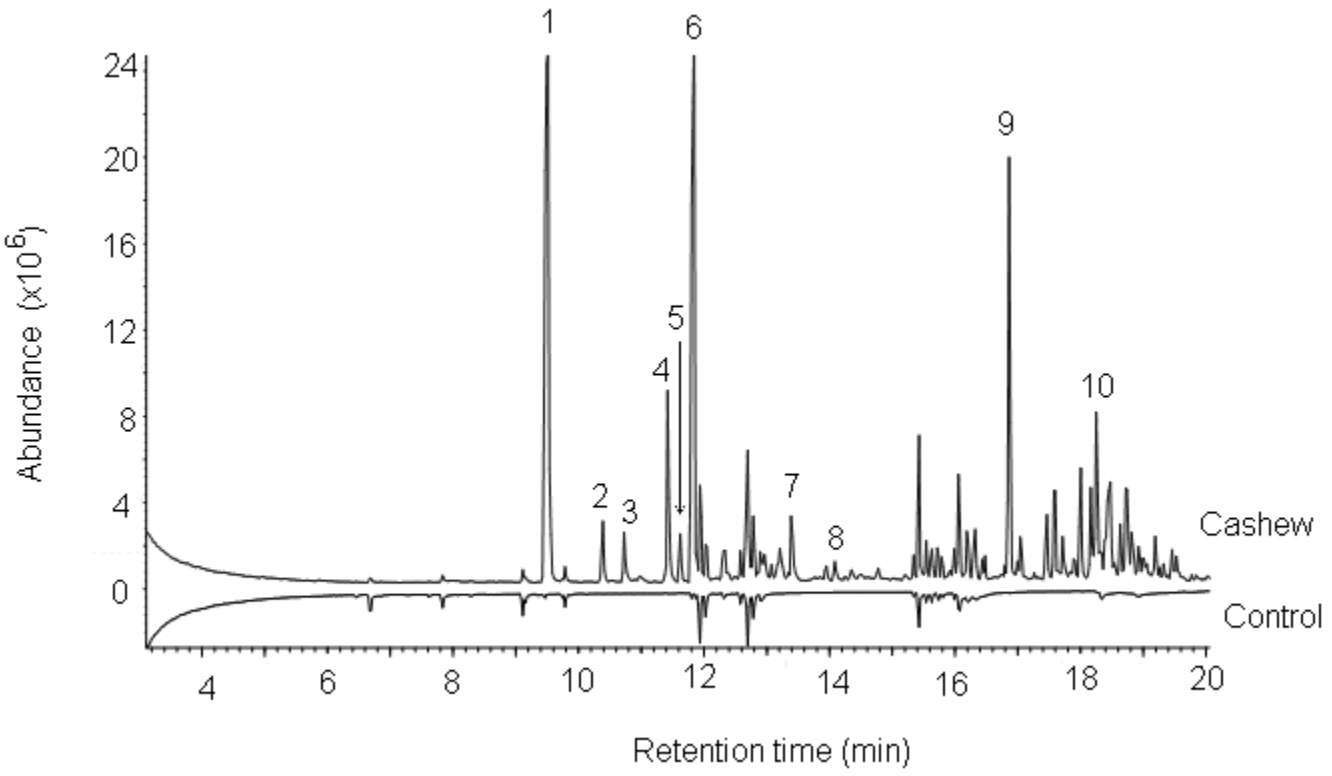

Figure 6.9 Total ion concentrations of components of cashew leaf volatiles

Peaks 1 – 10 represent *α*-pinene, *β*-pinene, myrcene, limonene, (*Z*)-*β*-ocimene, (*E*)-*β*-ocimene, allo-ocimene, (Z)-3-hexenyl butanoate, *α*-copaene and germacrene D, respectively; some of the un-numbered peaks were contaminants which were also present in the sample from the blank control polyethene bag; while others were unidentified.

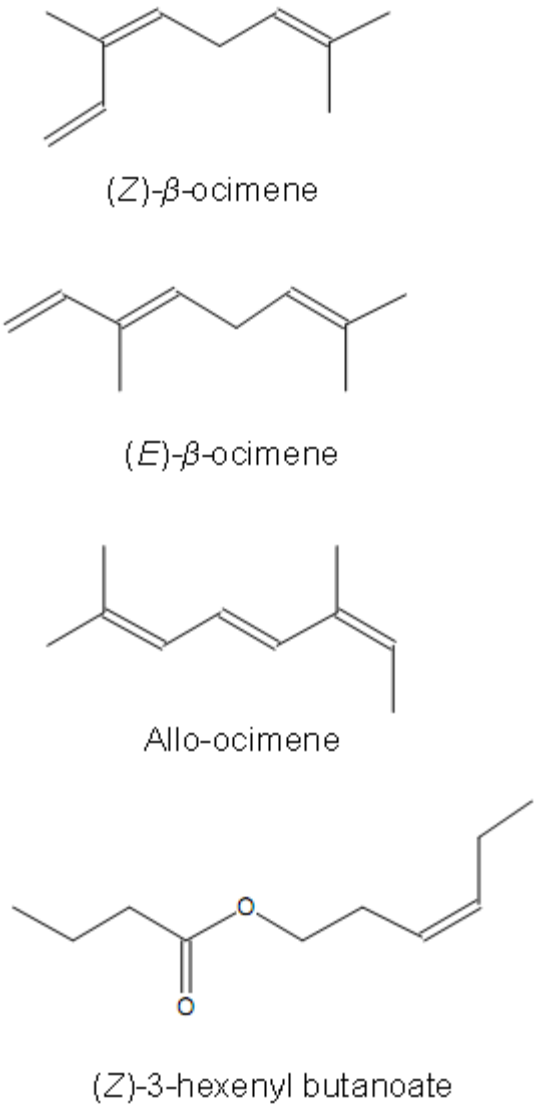

Figure 6.10 Chemical structures of four components of cashew volatiles that are detected by antennae of *Pseudotheraptus wayi*

Table 6.1 Mass spectral data and ratio of cashew leaf volatiles

Compound	Formula	Molecular weight	Retention time (min)	Ratio
1. α -pinene	$C_{10}H_{16}$	136.1	9.5	25
2. β-pinene	$C_{10}H_{16}$	136.1	10.4	2
3. Myrcene	$C_{10}H_{16}$	136.1	10.7	2
4. Limonene	$C_{10}H_{16}$	136.1	11.4	5
5. (*Z*)-β-ocimene	$C_{10}H_{16}$	136.1	11.6	1
6. (*E*)-β-ocimene	$C_{10}H_{16}$	136.1	11.8	25
7. Allo-ocimene	$C_{10}H_{16}$	136.1	13.4	3
8. (*Z*)-3-hexenyl butanoate	$C_{10}H_{18}O_2$	170.1	14.1	1
9. α-copaene	$C_{15}H_{24}$	204.2	16.9	11
10. Germacrene D	$C_{15}H_{24}$	204.2	18.3	9

6.2.3 Behavioural responses to crude and synthetic cashew volatiles

Only males were attracted to crude cashew volatiles at 0.2 and 0.4 CHE with 0.2 CHE eliciting optimum attraction, while 0.1 CHE were unable to evoke behavioural responses in either sex (Figure 6.11).

Furthermore, only males were attracted to 0.5 μg and 1 μg of the blend of the ocimene mixture and (*Z*)-3-hexenyl butanoate, with 0.5 μg eliciting optimum attraction, while 0.25 μg were unable to evoke behavioural responses in either sex (Figure 6.12). The ocimene mixture alone at 0.48 μg, corresponding to the same

amount as in the 0.5 μg of the blend, also attracted males, while (*Z*)-3-hexenyl butanoate alone at 0.02 μg evoked no attractive response of the males (Figure 6.13).

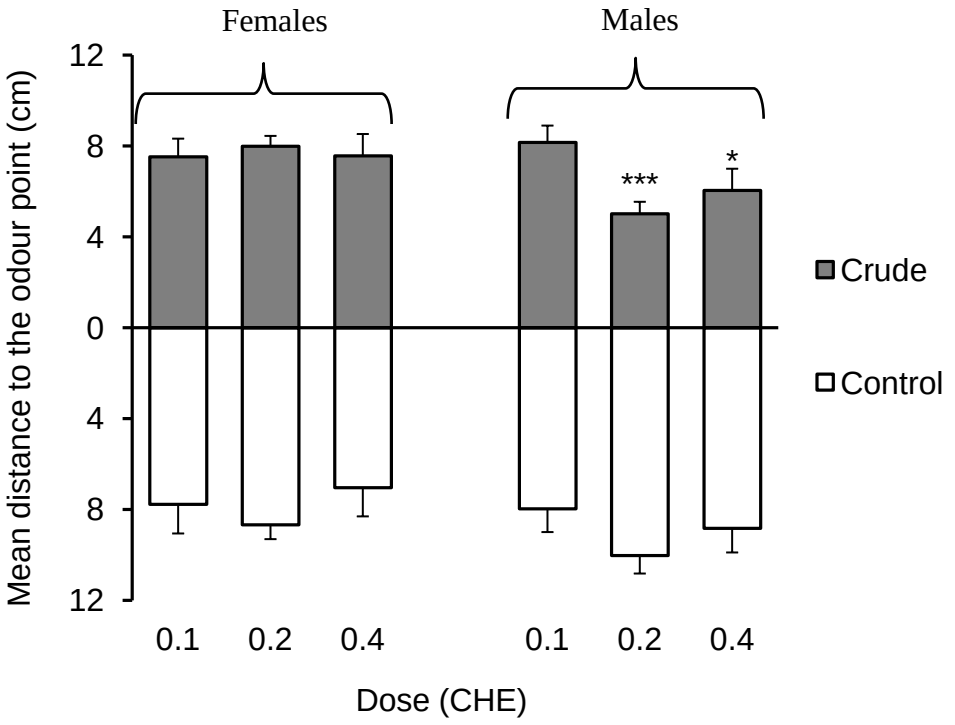

Figure 6.11 Responses of *Pseudotheraptus wayi* to crude cashew volatiles

Three and one *asterisks* indicate significant difference of the treatment from the control at $P < 0.001$and $P < 0.05$; error bars represent SEM.

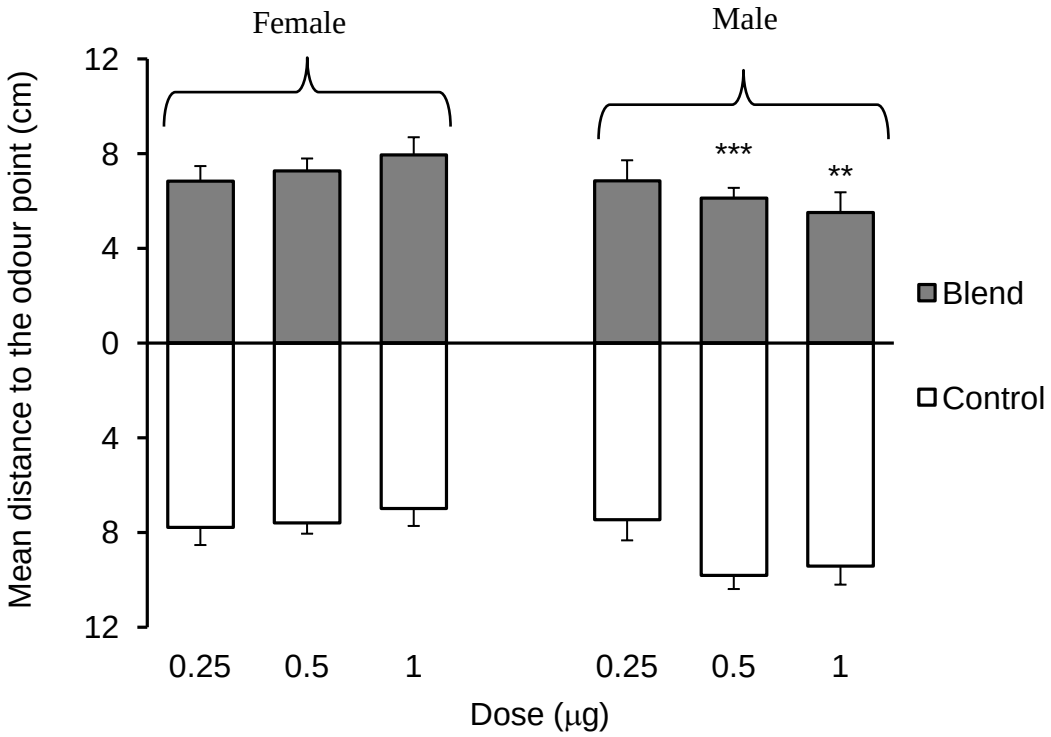

Figure 6.12 Responses of *Pseudotheraptus wayi* to a blend of the ocimene mixture and (*Z*)-3-hexenyl butanoate

Two and three *asterisks* indicate significant differences of the treatments from the controls at $P < 0.01$ and $P < 0.001$, respectively; error bars represent SEM.

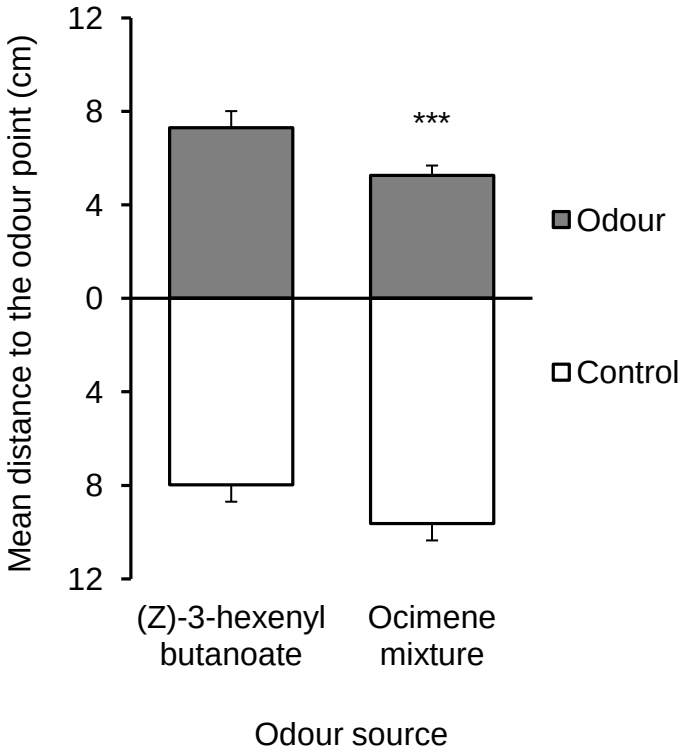

Figure 6.13 Responses of *Pseudotheraptus wayi* males to 0.02 μg of (*Z*)-3-hexenyl butanoate and 0.48 μg of the ocimene mixture

Three *asterisks* indicate a significant difference of the treatment from the control at $P < 0.001$; error bars represent SEM.

6.3 Discussion

Behavioural assays with live cashew seedlings and crude cashew volatiles show that only males are attracted to the host odour. Literature on olfactory responses to host odour by coreids is scarce. Xiao and Fadamiro (2009) suggested that attraction of the leaf footed coreid bug, *Leptoglossus zonatus* (Dallas) to its hosts tomato and santsuma may be mediated by host odour, but olfactory studies are required to verify this. Reports on related families namely Miridae and Alydidae show that males are more sensitive to both plant and conspecific odours than females (Chinta *et al.*, 1994; Groot *et al.*, 1999; Niiyama *et al.*, 2007; Watanabe *et al.*, 2009; Williams *et al.*,

2010). Furthermore, the first ever odorant binding protein characterized in heteropterans from the genus *Lygus* was also more abundant in males than females (Dickens *et al.*, 1998), thus corroborating the findings in this study. However, some reports on responses of mirids to host odour deviate from the trend discussed above depending on the plant and/or insect physiological states. In the leaf mirid, *Trigonotylus caelestialium* (Kirkaldy), although males are more attracted to whole rice odour than females, females are more attracted to the plant odour at panicle formation and flowering stages than males (Niiyama *et al.*, 2007; Fujii *et al.*, 2010), probably due to the demand for grains by the females for oviposition (Ishimoto and Sato, 2006). In the western tarnished plant bug, *Lygus hesperus* Knight, on the other hand, only females are attracted to volatiles produced by their host, alfalfa, presumably to locate oviposition sites (Blackmer *et al.*, 2004); while in a related species, *L. rugulipennis* Poppius, both sexes are attracted to odour from *Vicia faba* L. and the responses are dependent on the presence of conspecifics and whether or not the plant is damaged (Frati *et al.*, 2008; Frati *et al.*, 2009). Behavioural responses of *P. wayi* to odour from different stages of the host as well as host odour in combination with that of conspecifics, therefore, need further investigations.

Analysis of the crude cashew volatiles by GC-EAD and GC-MS isolated four EAD-active components including three terpenoids namely (*Z*)-*β*-ocimene, (*E*)-*β*-ocimene and allo-ocimene, and an ester i.e., (*Z*)-3-hexenyl butanoate which were consistently detected by only male antennae, with the females being only weakly sensitive to (*Z*)-*β*-ocimene and (*E*)-*β*-ocimene. This confirms higher responsiveness of males to

cashew volatiles than females, and suggests that males possess more plant odour receptors than females. This is supported by the findings in section 3.2.1 that the most hairy distal antennomere of adult male *P. wayi* is approximately 1.5 times longer than that found in females. Although sexual dimorphism is not apparent in antennae of some heteropteran species (Henry, 1989; Henry, 2006; Stehlík and Jindra, 2008), it is evident in others. Male nabids, *Nabicula (Limnonabis) propinqua* (Reuter) have disproportionately longer antennae than females (Asquith and Lattin, 1990); while the second antennomere of the male gerrine water striders, *Limnometra tiomanensis* Mayr, is twice as long as that in females (Zettel *et al.*, 2009). In other heteropterans, particularly mirids, male antennae are thicker than those of females (Schwartz and Schuh, 1999; Schuh and Schwartz, 2004; Schwartz, 2005; Soto and Weirauch, 2009). Furthermore, the first ever odorant binding protein characterized in heteropterans from the genus *Lygus* was also more abundant in males than females (Dickens *et al.*, 1998), thus corroborating our findings on antennal responses of *P. wayi* to cashew leaf volatiles. The weak responses of females to some of the cashew chemicals further indicates that these volatiles could be attractive to the females under certain conditions such as presence of conspecifics (Frati *et al.*, 2008; Frati *et al.*, 2009).

Behavioural assays with synthetic chemicals also revealed that only males were attracted to the blend of the ocimene mixture and (*Z*)-3-hexenyl butanoate, with 0.5 μg eliciting optimum attraction. The ocimene mixture alone at 0.48 μg, corresponding to the same amount as in the 0.5 μg of the blend, also attracted males.

These results indicate that at least one of the isomers of ocimene is critical for attraction of males. This concurs with an earlier suggestion that monoterpenes may be important in attraction of odour guided insects because of their relatively high volatility (Fraser *et al.*, 2003). It is also interesting to note that at least one of these ocimene isomers (order of prominence: (*E*)-*β*-ocimene > (*Z*)-*β*-ocimene > allo-ocimene) is a component of odour profiles of most alternative hosts of *P. wayi* e.g., guava (Da Silva *et al.*, 2003; Ogunwande *et al.*, 2003; Rouseff *et al.*, 2008), cocoa (Quijano and Pino, 2009), mango (Ollé *et al.*, 1998; Jayanthi *et al.*, 2012), pecan (Corella-Madueño *et al.*, 2011), carambola (Mahattanatawee *et al.*, 2005) and French beans (Barra *et al.*, 2007). This supports our data indicating that these terpenoids may mediate kairomonal signaling in *P. wayi*. Although (*Z*)-3-hexenyl butanoate was EAD-active with male antennae its behavioural role has not been revealed in this study.

(*Z*)-*β*-ocimene, (*E*)-*β*-ocimene, allo-ocimene and (*Z*)-3-hexenyl butanoate have previously been reported to mediate behaviour of some phytophagus insects. (*Z*)-*β*-ocimene, (*E*)-*β*-ocimene and allo-ocimene, for instance, attract larvae of the leaf beetle, *Plagiodera versicolora* Laich. (Yoneya *et al.*, 2010), and gravid female oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Jayanthi *et al.*, 2012); while (*Z*)-*β*-ocimene and (*E*)-*β*-ocimene attract the male tea weevil, *Myllocerinus aurolineatus* Voss (Sun *et al.*, 2012). Furthermore, (*E*)-*β*-ocimene is EAD-active with antennae of the sphinx moth, *Manduca sexta* and the codling moth, *Cydia pomonella* L*;* and was attractive to the former in a blend with seven other components of tomato volatiles

(Fraser *et al.*, 2003; Witzgall *et al.*, 2005). (*Z*)-3-Hexenyl butanoate elicits antennal responses in some insects including parasitic wasps namely *Cotesia marginiventris* (Cresson), *Microplitis rufiventris* Kok. and *Campoletis sonorensis* (Carlson) (Gouinguené *et al.*, 2005), a mirid bug, *Lygocoris pabulinus* (Groot *et al.*, 1999), and the codling moth, *C. pomonella* (Witzgall *et al.*, 2005; Casado *et al.*, 2006). In behavioural assays, (*Z*)-3-hexenyl butanoate repelled the European corn borer, *Ostrinia nubilalis* (Hübner) (Solé *et al.*, 2010). These reports support the findings in this study that the EAD-active cashew chemicals play a role in the interaction of the host with *P. wayi.*

CHAPTER SEVEN

BEHAVIOURAL AND ELECTROPHYSIOLOGICAL RESPONSES OF *PSEUDOTHERAPTUS WAYI* TO CONSPECIFIC VOLATILES

7.0 Introduction

Intra-specific odour mediated communication in *P. wayi* was hitherto unknown. Studies in other coreids show that these insects use their odour as allomones to deter natural enemies and as alarm pheromones to disperse conspecifics (intra-specific counterparts) during danger (Burger *et al.*, 1986; Blatt *et al.*, 1998; Millar, 2005; Prudic *et al.*, 2008). Other behavioural studies suggest that coreid males produce aggregation pheromones (Yasuda, 1990; Blatt and Borden, 1996), and sex pheromones (Aldrich *et al.*, 1993; Wang and Millar, 2000; Millar, 2005; Soldi *et al.*, 2012). Identification of a sex pheromone for the coreid bug could offer an opportunity for development of lures to complement the existing management tools for this pest. These pheromones could also complement the candidate kairomones identified in chapter 6 of this study.

This chapter presents results based on laboratory behavioural assays, electrophysiology and chemical analysis, demonstrating that odour of *P. wayi* serve as sex and alarm pheromones.

7.1 Materials and methods

7.1.1 Insects

Sexually mature virgin insects (~ 1 month old) used in this study were obtained from the colony described in section 3.1.1. The age of the insects for the bioassays was

selected based on results in section 3.2.2 that sexual maturity (initiation of mating and egg laying) occurred ~ 1 month after adult emergence.

7.1.2 Behavioural responses to volatiles from live insects

Olfactometer bioassays were conducted as described in section 6.1.2. Glass chambers used to contain insect odour sources and as controls measured 7 cm ID x 6 cm high. The number of insects tested as odour sources against blank controls was 1, 5 and 10 females or males. Since results in section 5.2 indicated that most coupling occurred during late photophase to mid scotophase, bioassays were also conducted during the same period.

7.1.3 Collection of volatiles

Headspace volatiles were trapped separately from 60 females and 60 males in batches of 30 insects per trapping. The insects were gently enclosed in clean glass chambers measuring 10 cm ID x 20 cm high (Figure 7.1). Charcoal filtered humidified air was passed through the chambers at ~ 400 ml min^{-1}, then through Super-Q adsorbents (30 mg, Alltech, Nicholasville, Kentucky) for 12 hours (mid photophase to mid scotophase). Prior to onset of trapping, the air was allowed to pass through glass chambers containing the insects for ~ 30 min to flash out defensive compounds which may have been released during handling. Each adsorbent from the same sex was eluted with 200 µl of dichloromethane (Sigma Aldrich, Chemie, Steinhem, Germany) into the same glass vial (Agilent, USA) to obtain ~ 400 µl containing 720 bug hour equivalents (BHE), where 1 BHE = volatiles emitted by 1 insect h^{-1}. The trapping was replicated three times with the same number of fresh

females and males as above. Volatiles were also trapped and eluted repeatedly as above from blank glass chambers as controls. The samples were stored at -80 ^{o}C prior to use.

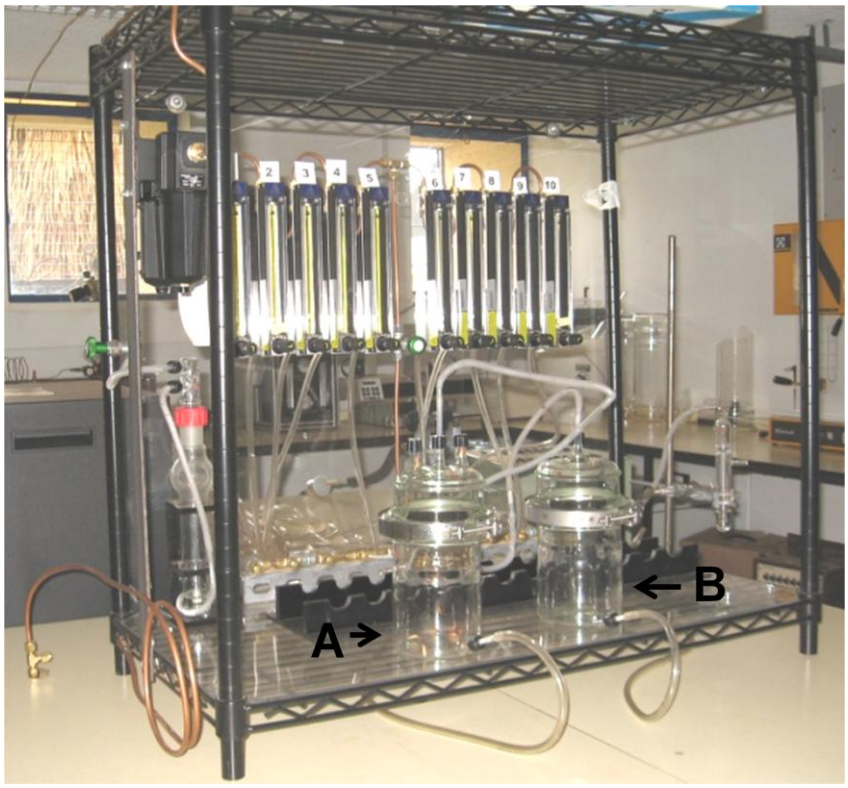

Figure 7.1 Trapping of odour from the insects

Glass chamber A contained living insects while B was a blank control. Photo: Egonyu J.P.

7.1.4 Analysis of volatiles

Crude samples of male and female volatiles were analysed by GC-EAD and GC-MS following the procedures in section 6.1.4. For quantification of components, 3 samples (40 µl) were prepared separately from the 3 replicates of female and male crude volatiles, and an internal standard, ethyl nonanoate, was added to each

replicate at ~ 40 ng μl^{-1} prior to GC-MS analysis. Quantities of components were calculated by comparing their corrected areas with that of the internal standard.

7.1.5 Chemicals

Hexanal, hexanol, hexyl acetate, nonanal, decanal, ethyl nonanoate and hexanoic acid (98 – 99.5%) were purchased from Aldrich (Milwaukee, Wisconsin, USA), while hexyl hexanoate was synthesised in the laboratory by esterification of hexanol and hexanoic acid.

7.1.6 Behavioural responses to crude male volatiles and synthetic chemicals

Because volatiles from live females were unable to evoke behavioural responses in either sex in olfactometer assays, bioassays with crude volatiles and synthetic chemicals were based on only male odour. Dual choice bioassays were carried out following the procedure described in section 6.1.6. Two pieces of filter paper (~ 1 cm^2), one loaded with an aliquot of 10 µl of the crude male extract or a blend of synthetic chemicals in the natural ratio as in male odour (Table 7.1) in dichloromethane and the other loaded with the same volume of the solvent only as a control were placed at opposite sides of the Petri dish. Three doses of the crude sample, the blend of synthetic chemicals and individual synthetic chemicals i.e., 5, 10 and 20 BHE per 10 µl were tested.

7.1.7 Data analysis

Attraction of insects to the odour sources in the olfactometer and Petri dish bioassays was analysed using the two sample *t*-test as described in section 6.1.7. The quantities of the chemicals released by females and males were also compared using the *t*-test.

7.2 Results

7.2.1 Behavioural responses to volatiles from live insects

Only male odour evoked significant behavioural responses in either sex. Females were attracted to odour from 10 males, while odour from 1 and 5 males were unable to evoke a significant behavioural response from the females (Figure 7.2). The males on the other hand were repelled by odour from 5 and 10 males, while odour from 1 male failed to evoke a significant behavioural response from the males.

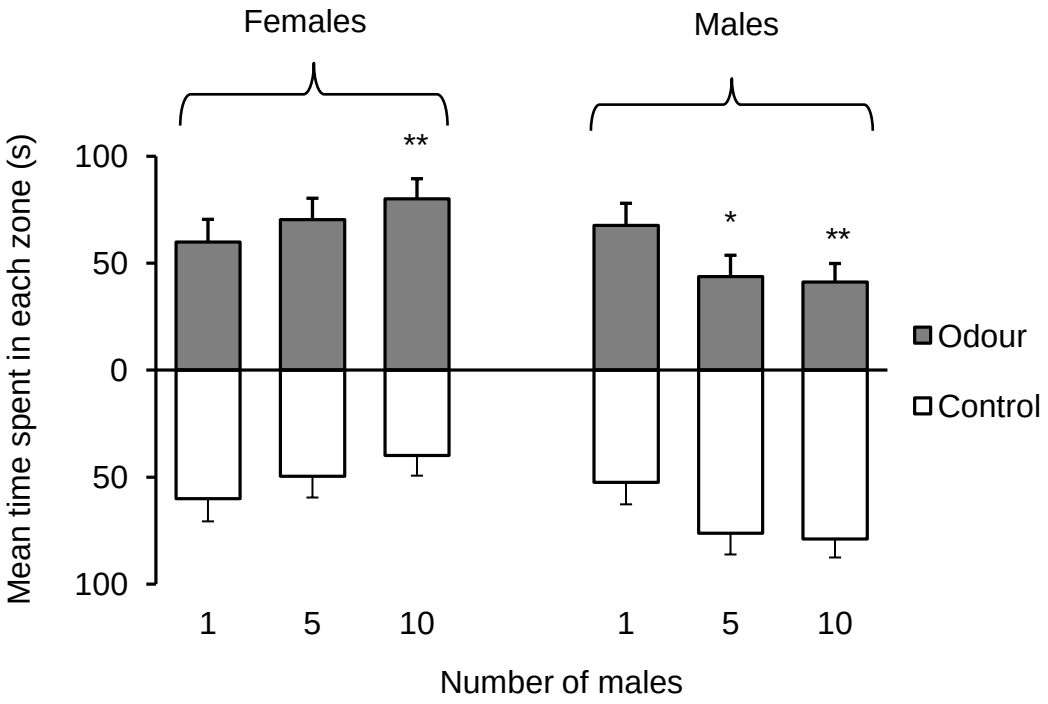

Figure 7.2 Responses of *Pseudotheraptus wayi* to volatiles from live males

One and two *asterisks* indicate significant differences of treatments from the control at $P < 0.05$ and $P < 0.01$, respectively; error bars represent SEM.

7.2.2 Analysis of volatiles

Analysis of crude volatiles by GC-EAD using antennae of both sexes revealed six consistently EAD-active components in both female and male odour (Figures 7.3, 7.4 and 7.5). These compounds were identified by GC-MS as hexanal, hexanol, hexyl acetate, nonanal, decanal and hexyl hexanoate (Figures 7.6 and 7.7), and confirmed with authentic standards. Whereas quantities of hexanol, decanal and hexyl hexanoate were not significantly different in crude volatiles of females and males, males produced ~ 6 – 7-fold more hexanal and hexyl acetate than females, while females produced twice as much nonanal as males (Table 7.1).

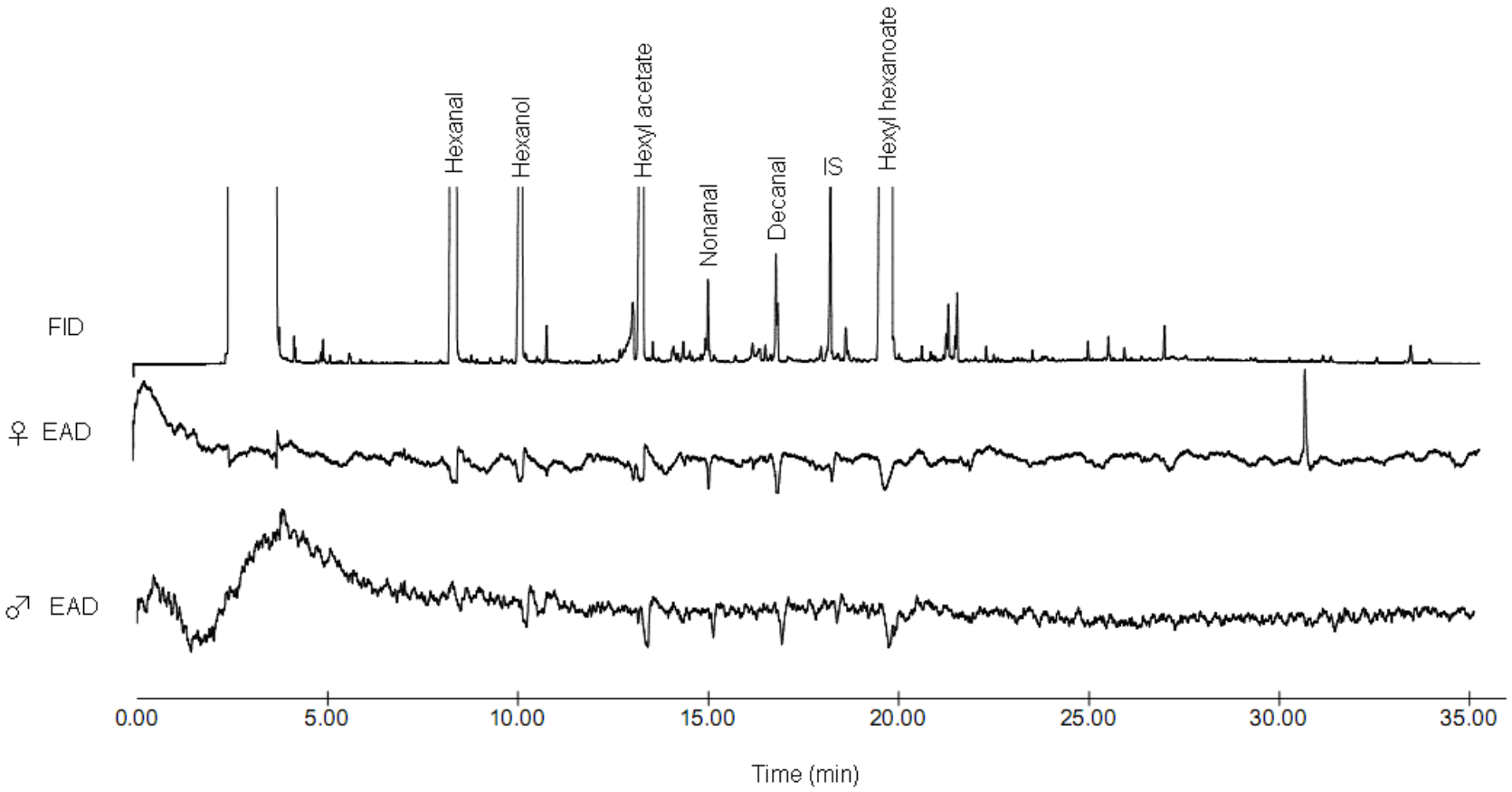

Figure 7.3 Coupled gas chromatography-electroantennographic detection responses of female and male *Pseudotheraptus wayi* to crude male volatiles

IS = internal standard, ethyl nonanoate; FID = flame ionisation detector; EAD = electroantennographic detection.

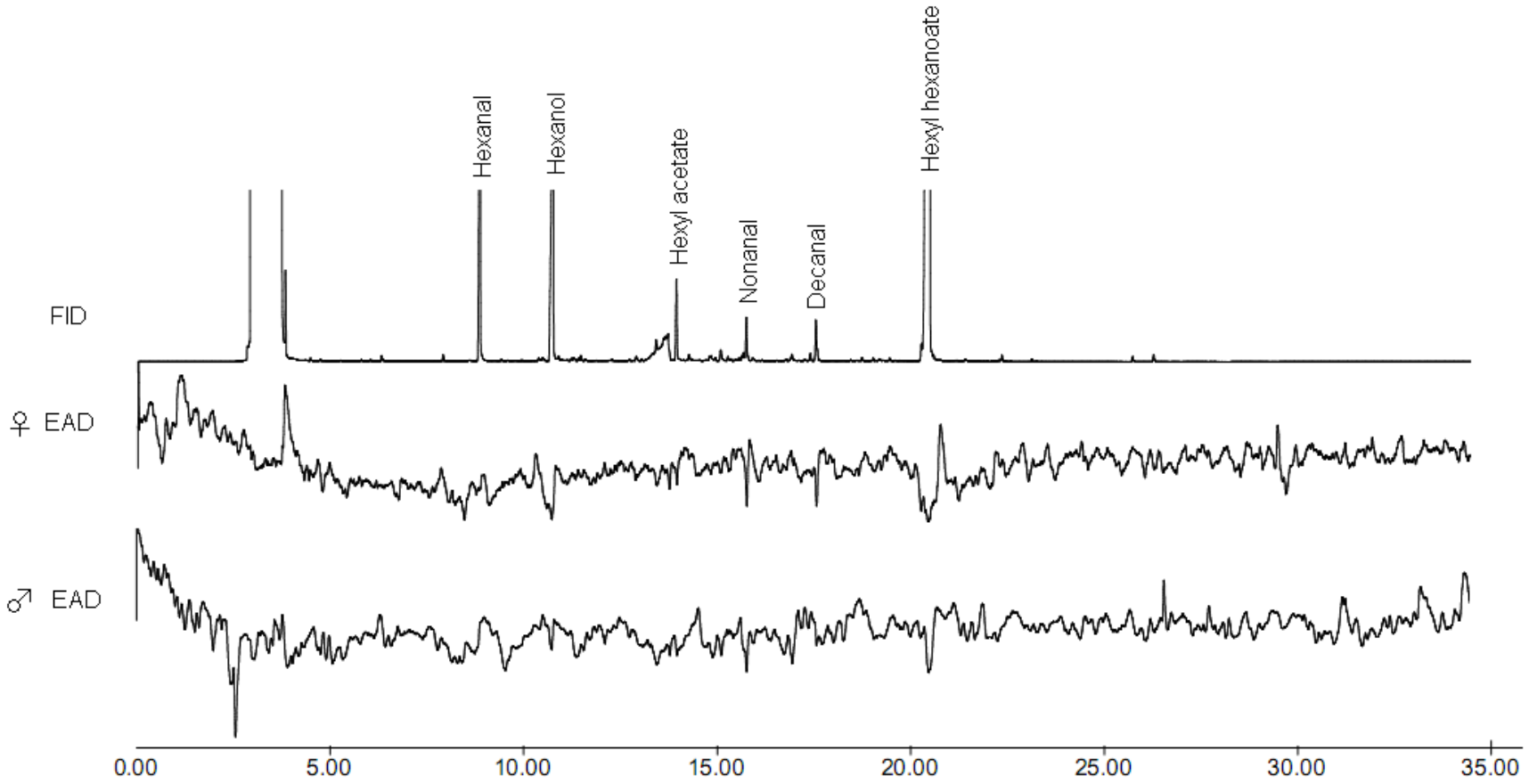

Figure 7.4 Coupled gas chromatography-electroantennographic detection responses of female and male *Pseudotheraptus wayi* to crude female volatiles

FID = flame ionisation detector; EAD = electroantennographic detection.

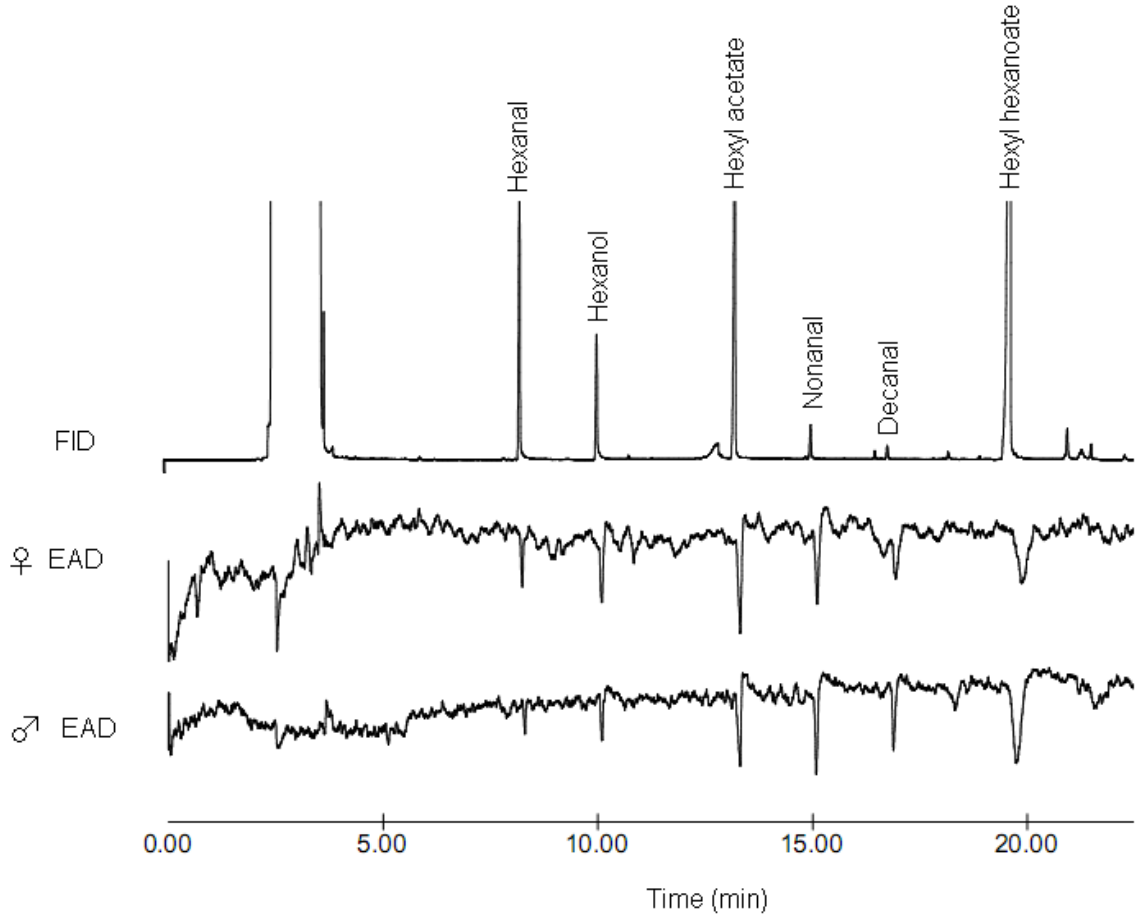

Figure 7.5 Coupled gas chromatography-electroantennographic detection responses of female and male *Pseudotheraptus wayi* to synthetic components of conspecific volatiles

FID = flame ionisation detector; EAD = electroantennographic detection.

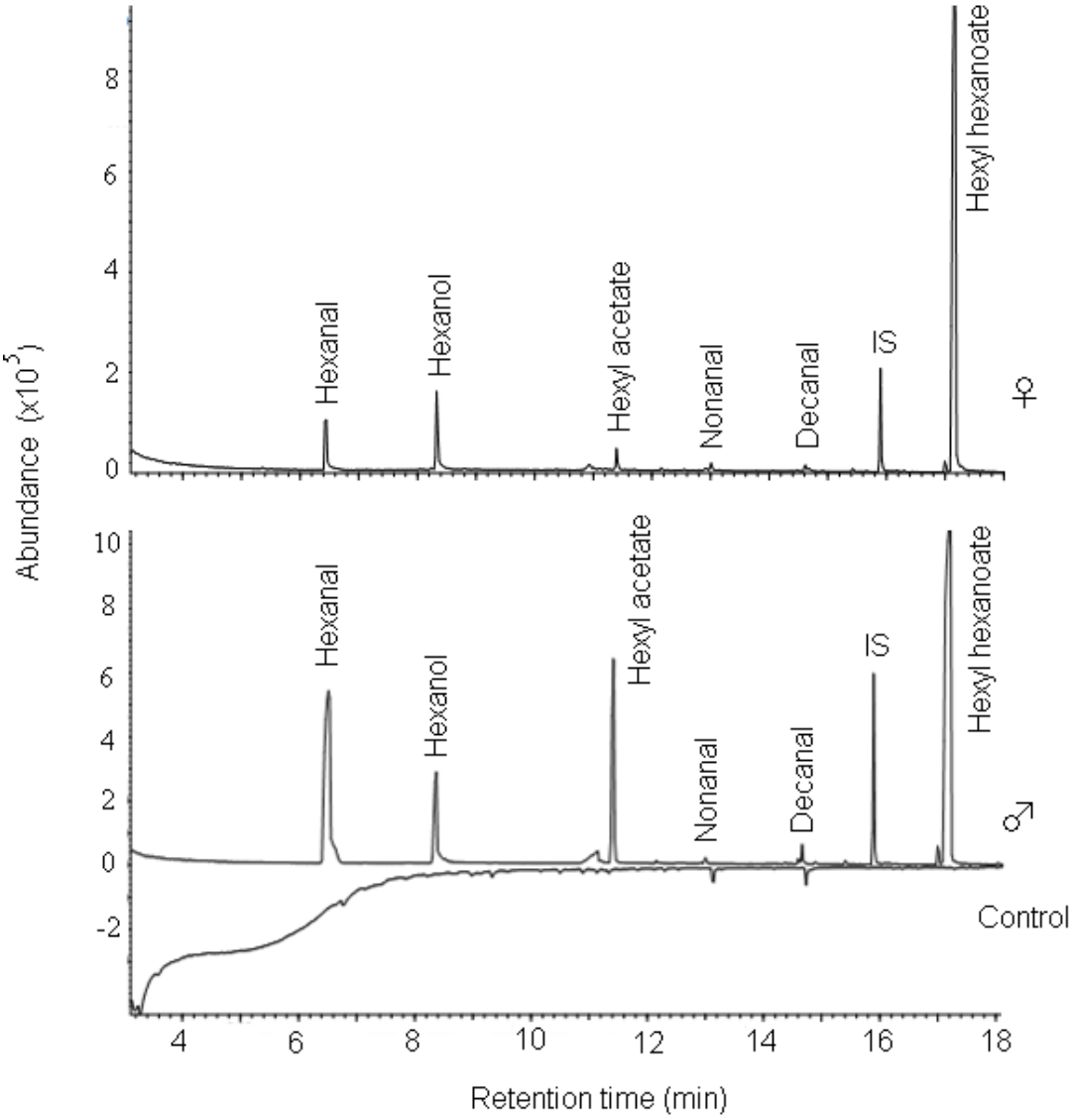

Figure 7.6 Total ion concentrations of EAD-active compounds in *Pseudotheraptus wayi* volatiles

IS = internal standard, ethyl nonanoate.

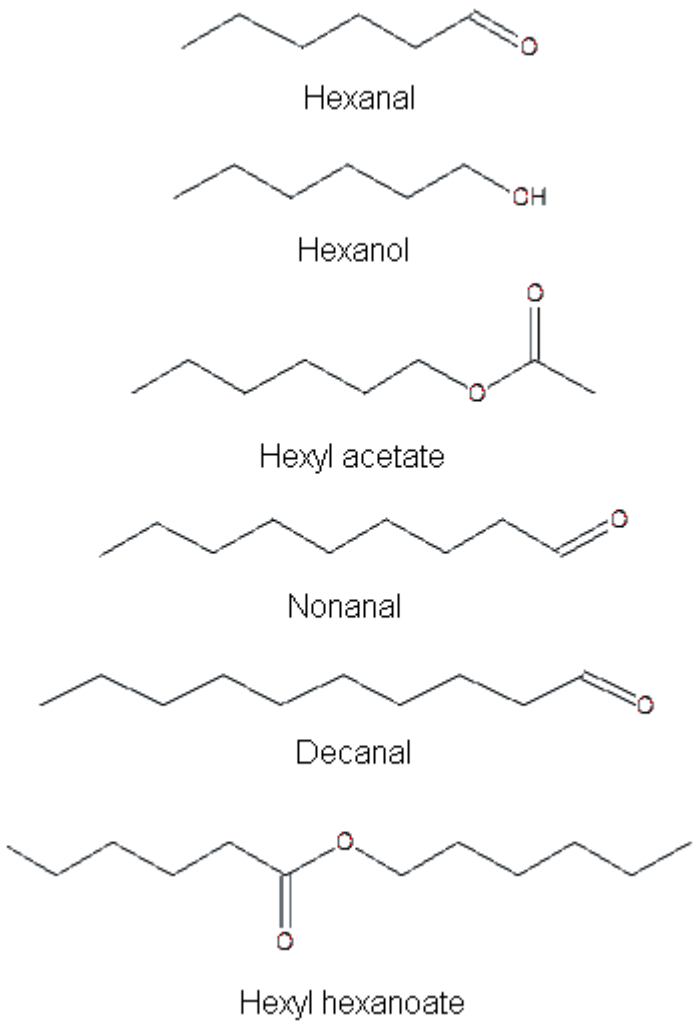

Figure 7.7 Chemical structures of EAD-active compounds in *Pseudotheraptus wayi* volatiles

Table 7.1 Mass spectral data, quantities and ratios of EAD-active components of *Pseudotheraptus wayi* volatiles

Compound	Formula	Molecular weight	Retention time (min)	Females (ng/insect/h)	Males (ng/insect/h)	Ratio (Female)	Ratio (Male)
1. Hexanal	$C_6H_{12}O$	100.1	6.4	9.6 ± 0.3	54.0 ± 9.1**	5	35
2. Hexanol	$C_6H_{14}O$	102.1	8.2	12.2 ± 1.3	18.1 ± 3.3	7	12
3. Hexyl acetate	$C_8H_{16}O_2$	144.1	11.3	3.7 ± 0.2	26.2 ± 5.0*	2	17
4. Nonanal	$C_9H_{18}O$	142.1	12.9	3.4 ± 0.1	1.5 ± 0.4*	2	1
5. Decanal	$C_{10}H_{20}O$	156.2	14.5	1.8 ± 0.1	1.9 ± 0.0	1	1
6. Hexyl hexanoate	$C_{12}H_{24}O_2$	200.2	17.1	116.7 ± 3.02	107.1 ± 18.6	64	70

One and two *asterisks* indicate significant differences in quantities of male volatiles from those of females at $P < 0.05$ and $P < 0.01$, respectively.

7.2.3 Behavioural responses to crude male volatiles and synthetic chemicals

Crude male volatiles and a blend of synthetic chemicals at 20 BHE repelled both sexes, while 5 and 10 BHE were unable to evoke behavioural responses in either sex (Figures 7.8 and 7.9). Hexyl hexanoate tested singly at 5 and 10 BHE attracted only females, and repelled only males at 20 BHE. On the other hand, hexanal at 20 BHE, and hexyl acetate at 5 BHE repelled only males, while hexanal at 5 and 10 BHE, and hexyl acetate at 10 and 20 BHE were unable to evoke detectable behavioural responses in either sex. Hexanol, nonanal and decanal singly were unable to evoke significant behavioural responses in either sex.

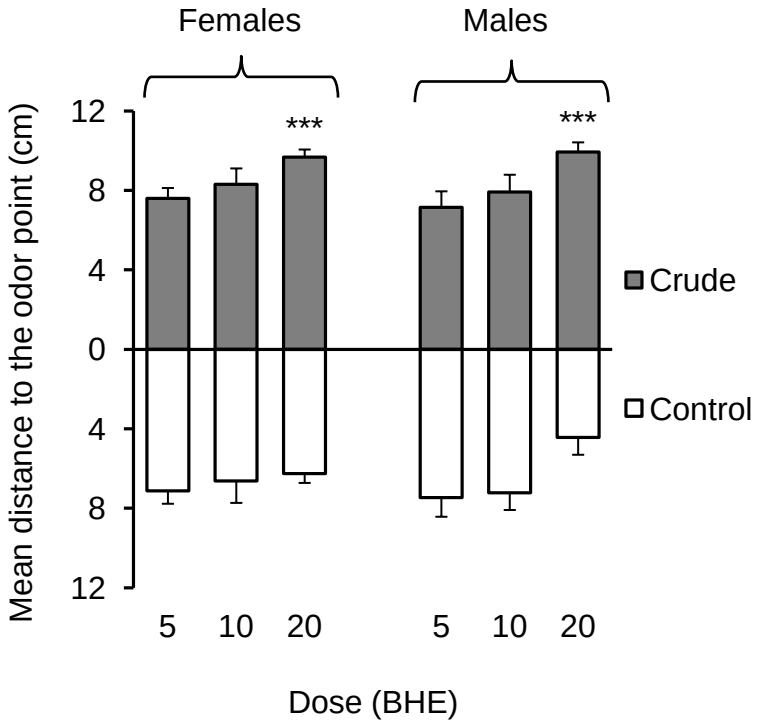

Figure 7.8 Responses of *Pseudotheraptus wayi* to crude male volatiles

Three *asterisks* indicate a significant difference of treatments from the controls at $P < 0.001$; error bars represent SEM.

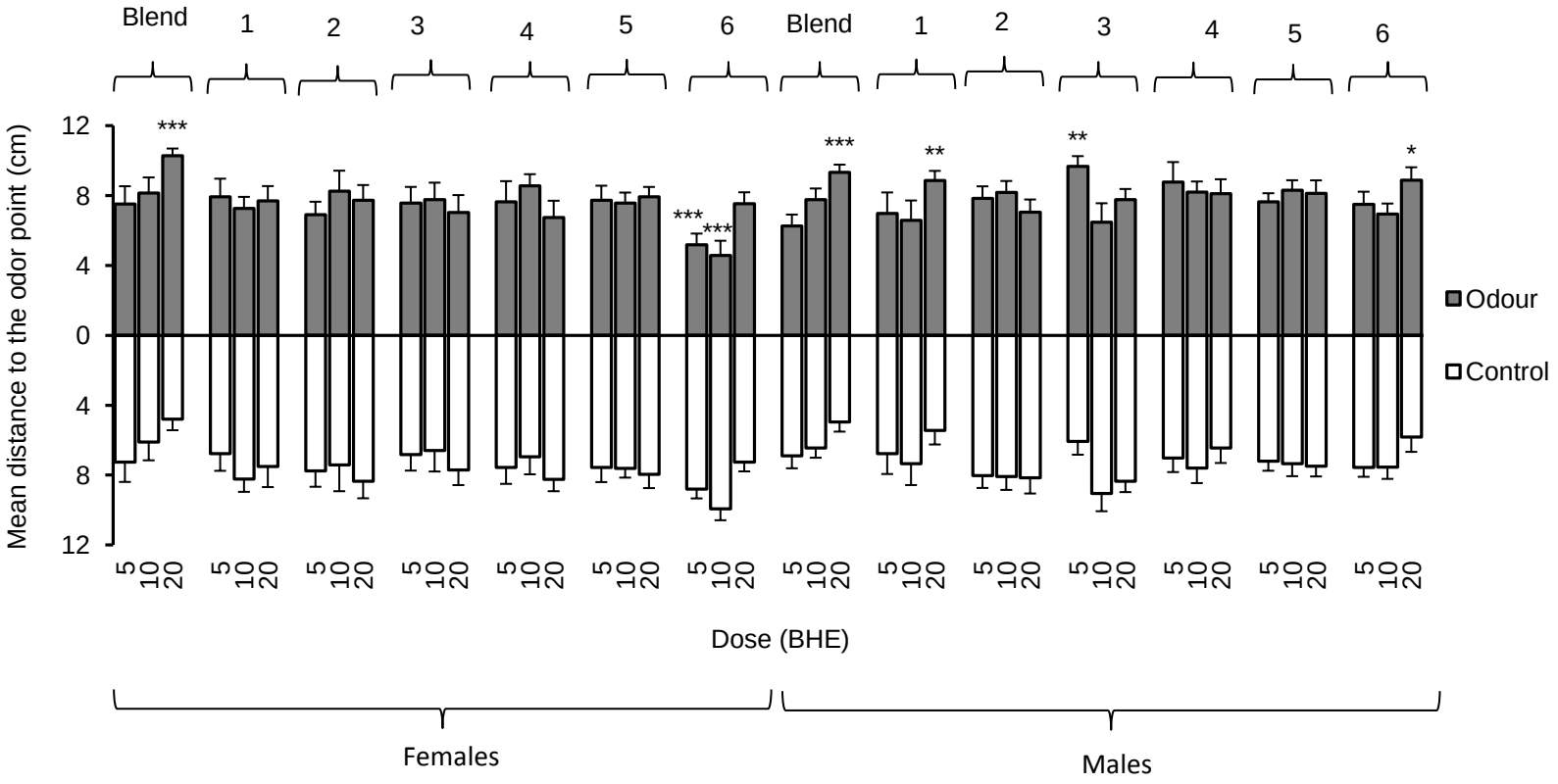

Figure 7.9 Responses of *Pseudotheraptus wayi* to EAD-active synthetic components mimicking quantities in crude male volatiles

One, two and three *asterisks* indicate significant differences of treatments from the controls at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively; error bars represent SEM; Numbers 1-6 represent hexanal, hexanol, hexyl acetate, nonanal, decanal and hexyl hexanoate, respectively.

7.3 Discussion

Only odour from live males evoked behavioural responses in either sex, attracting females and repelling males. Evidence of sex-pheromonal activity of male volatiles is also reported in other coreids such as *L. clypealis* (Wang and Millar, 2000) and *Phthia picta* (Drury) (Soldi *et al.*, 2012). Furthermore, aggregation behaviour in coreids is mediated by male volatiles (Yasuda, 1990; Aldrich *et al.*, 1993; Blatt and Borden, 1996; Khrimian *et al.*, 2012), indicating that this sex is the chief mediator of intraspecific chemical communication in the family. Similarly, males are the chief mediators of intraspecific sexual and/or aggregation chemical communication in a number of other families of Heteroptera for example, Pentatomidae (McBrien *et al.*, 2002; Millar, 2005; Borges *et al.*, 2006), Alydidae (Numata *et al.*, 1990; Higuchi and Nakamori, 1999; Millar, 2005), Lygaeidae (Aldrich *et al.*, 1997; Aldrich *et al.*, 1999; Millar, 2005), Ruduvidae (James *et al.*, 1994; Millar, 2005), Gerridae (Millar, 2005) and Veliidae (Millar, 2005). However, in mirids (Aldrich, 1988; Millar *et al.*, 1997; Millar and Rice, 1998; Millar, 2005; Zhang *et al.*, 2007), and, a lygaeid *Geocoris punctipes* (Say) (Marques et al., 2000), an alydid *Riptortus linearis* (Fabricius) (Higuchi and Nakamori, 1999) and an anthocorid *Orius sauteri* (Poppius) (Nakashima and Hirose, 1999), sex pheromones are produced by females. Data from this study showed that male odour repelled males, suggesting that although only females are attracted to odour from male *P. wayi,* males also use the odour to recognise same sex and avoid them.

GC-EAD and GC-MS analyses revealed that hexanal, hexanol, hexyl acetate, nonanal, decanal and hexyl hexanoate are components of odour of both sexes of *P. wayi,* and are consistently detected by antennae of either sex. These are common constituents of coreid odours (Aldrich *et al.,* 1993; Steinbauer and Davies, 1995; Blatt *et al.*, 1998; El-Sayed, 2011; Khrimian *et al.*, 2012), but this is the first report of odour profile in the genus, *Pseudotheraptus*. Hexanal, hexanol and hexyl acetate are also EAD-active components of volatiles of the western conifer seed bug, *Leptoglossus occidentalis* (Blatt *et al.,* 1998), but literature on EAD activity of nonanal, decanal and hexyl hexanoate in coreids is scarce. Qualitative similarity in profiles of male and female coreid odour is also previously reported (Prestwich, 1976; Steinbauer and Davies, 1995; Blatt *et al.,* 1998), but other coreids contrastingly produce male specific compounds which are in most cases presumed to be sex and/or aggregation pheromones (Aldrich *et al.,* 1993; Wang and Millar, 2000; Millar, 2005, Khrimian *et al.,* 2012; Soldi *et al.,* 2012).

The study revealed that both sexes produce similar quantities of hexanol, decanal and hexyl hexanoate, but males produce ~ 6 – 7 fold more hexanal and hexyl acetate than females, while females produce twice as much nonanal as males. These differences may contribute to sex recognition and subsequently, differential behavioural activity observed above for odour from live females and males. Whereas hexanal and hexyl acetate are dominant components of most coreid odour (Waterhouse and Gilby, 1964; Aldrich, 1988; Blatt *et al.,* 1998), this study has revealed that hexyl hexanoate is the most abundant component in *P. wayi* volatiles. Recently, chemicals other than

hexanal and hexyl acetate were reported to be most dominant in some coreid males for example, (*R,E*)-nerolidol in *Amblypelta lutescens* Distant (Aldrich *et al.*, 1993; Khrimian *et al.*, 2012) and 5,9,17-trimethylhenicosane in *P. picta* (Soldi *et al.*, 2012). These dominant chemicals were involved in aggregation and sexual attraction, respectively, suggesting that hexyl hexanoate may also play a crucial role in the semiochemical communication system of *P. wayi.*

Crude male volatiles and a blend of synthetic chemicals in the natural ratio as in male odour at 20 BHE repelled both sexes, while 5 and 10 BHE were unable to evoke behavioural responses in either sex, suggesting an alarm pheromonal role of the odour at high concentrations. Similar results were reported on the dispersal of the western conifer coreid bug, *Leptoglossus occidentalis* Heidemann following doubling of a synthetic blend of its alarm pheromone (Blatt *et al.*, 1998). Mirids are also known to use their odour for defence at high doses (Millar, 2005).

Behavioural responses to individual synthetic chemicals showed that hexyl hexanoate alone attracted only females at 5 and 10 BHE, and repelled only males at 20 BHE; while hexanal at 20 BHE, and hexyl acetate at 5 BHE repelled only males, but a half and a quarter of the dose of hexanal, and twice and four times the dose of hexyl acetate were unable to evoke behavioural responses in either sex. These responses concur with the observed attraction of females and repulsion of males by volatiles from live males, suggesting that volatiles released by a calling male which are rich in the three male repellents and a female attractant may be critical for attraction of only females. It appears that the insects manipulate the concentrations of

these behaviourally active chemicals to achieve sex and alarm pheromone functions. A similar dual role of coreid secretions was observed in the rice bug, *Leptocorisa oratorius* Fabricius, where the insects were first attracted and subsequently dispersed, and later revisited the odour source from time to time (Gunawardena and Bandumathie, 1993). Mirids also use their odour as sex pheromones at low doses and defence pheromones at high doses (Millar, 2005). Hexanol, nonanal and decanal singly were unable to evoke behavioural responses in either sex, therefore, their functions remain unknown.

CHAPTER EIGHT

CONCLUSIONS, IMPLICATIONS AND RECOMMENDATIONS

8.1 Conclusions

The following conclusions have been drawn from this study:

1. Immature stages of *P. wayi* have a number of unique morphological features.
2. Development of *P. wayi* on French beans takes a similar duration as on coconut and cashew; adults live longer on French beans than on coconut; sexual maturity is delayed on French beans compared to coconut and cashew, but fecundity is higher on French beans than the two hosts.
3. Peak mating occurs at mid scotophase, and more mating incidences occur during scotophase than photophase. Diel oviposition pattern has a major peak at late photophase and a minor one at late scotophase; and more eggs are laid during photophase than scotophase. Peak nymphal feeding occurs between 18:00 and 0:00 hours, with more nymphs feeding during scotophase than photophase; while adult feeding pattern is unaffected by time or light and darkness.
4. Only males are attracted to cashew volatiles.
5. Four components of cashew volatiles namely (*Z*)-*β*-ocimene, (*E*)-*β*-ocimene, allo-ocimene and (*Z*)-3-hexenyl butanoate are consistently detected by antennae of only male *P. wayi*, with the three terpenoids alone, and in a blend with the ester demonstrating attraction of the males. Females on the other

hand only weakly detect (*Z*)-*β*-ocimene and (*E*)-*β*-ocimene, and are not attracted to the synthetic chemicals.

6. Only male volatiles mediate intraspecific behavioural responses in *P. wayi*. Females are attracted to volatiles from live males but the same volatiles repel other males. A high concentration of crude male volatiles serves as an alarm pheromone, repelling either sex.
7. Six chemicals which are present in volatiles of both sexes of *P. wayi* namely hexanal, hexanol, hexyl acetate, nonanal, decanal and hexyl hexanoate elicit strong antennal responses in either sex. Of these, hexanol, decanal and hexyl hexanoate are produced in similar quantities by both sexes, but males produce a lot more hexanal and hexyl acetate than females, while females produce more nonanal than males. A blend of the six chemicals in the natural ratio as in male crude sample also repels either sex at a high concentration. Hexanal and hexyl acetate which are dominant in male volatiles and hexyl hexanoate singly repel only males, while hexyl hexanoate singly attracts only females. These three chemicals therefore facilitate male recognition, thus provoking attraction of females and repulsion of males by male volatiles.

8.2 Implications of the study

The unique morphological features of immature *P. wayi* can facilitate effective identification of the insects in the field. The protocol used in this study for mass rearing the insects on French beans will reduce reliance on coconut and cashew which have been used previously. Owing to their short lifespan, French beans are

easier to raise and maintain than perennial crops such as coconut and cashew. The suitability of French beans as an alternative host, however, raises concerns on the likely devastating effect of this pest on the crop which has previously not been listed as a host of *P. wayi*. Furthermore, the knowledge on diel behavioural patterns can facilitate timing of behavioural studies as well as application of control interventions against *P. wayi* in the field.

Finally and most importantly, three chemicals namely (*Z*)-*β*-ocimene, (*E*)-*β*-ocimene and allo-ocimene have been identified from cashew volatiles as attractants of male *P. wayi;* while hexanal, hexyl acetate and hexyl hexanoate in the natural ratio as in male volatiles facilitate recognition of the males by either sex. These findings suggest that male *P. wayi* colonise new habitats with the aid of host odour then guide the females using sex pheromones, a hypothesis which is proposed for heteropterans (Aldrich *et al.,* 1999). The results offer prospects of trapping *P. wayi* in the field using kairomone and pheromone based lures.

8.3 Recommendations

The following are recommended for further research:

1. Determination of juvenile hormones which regulate morphological transformations in immatures of *P. wayi* for use as growth regulators for the management of the pest.
2. Description of morphological features of immatures of other species of *Pseudotheraptus* to develop a dichotomous key for all stages in the genus.
3. Evaluation of other legumes as alternative hosts of *P. wayi.*

4. Determination of the effect of the physiological state of adult *P. wayi* on its diel feeding pattern.
5. Determination of factors causing mating, oviposition and nymphal feeding peaks to occur at mid scotophase, late photophase and 18:00 – 0:00 hours, respectively.
6. Determining if all plants containing (*Z*)-*β*-ocimene, (*E*)-*β*-ocimene and allo-ocimene in their odour are suitable hosts of *P. wayi*.
7. Determination of the effect of host and/or insect physiological stage on responses of *P. wayi* to cashew odour.
8. Evaluation of candidate kairomones and pheromones in attracting *P. wayi* in the field.
9. Determining the effect of blending the candidate kairomones and pheromones on the behaviour of *P. wayi*.

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LIST OF PUBLICATIONS

Journal articles

1. **Egonyu JP**, 2013. Diel patterns of mating, oviposition and feeding in the coconut bug, *Pseudotheraptus wayi* (Heteroptera: Coreidae). *African Entomology*, 21(1): 103-107.
2. **Egonyu JP**, J Kabaru, L Irungu and F Haas, in press. Description of pre-adult stages of the coconut bug, *Pseudotheraptus wayi. Journal of Insect Science*.
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4. **Egonyu JP**, S Ekesi, J Kabaru, L Irungu and B Torto, under review. Electrophysiological and behavioural responses of the coconut bug, *Pseudotheraptus wayi* to cashew and conspecific volatiles. *Journal of Chemical Ecology*.

Conference abstracts/posters

5. **Egonyu JP**, S Ekesi, J Kabaru, L Irungu and B Torto, 2011. Identification of sex pheromones of the coconut bug, *Pseudotheraptus wayi*. *A poster presentation during the icipe science day, 16th November 2011, icipe, Nairobi, Kenya*.
6. **Egonyu JP**, S Ekesi, J Kabaru, L Irungu and B Torto, 2011. Inter and intraspecific olfactory behaviour of the coconut bug, *Pseudotheraptus wayi*: do males search for the food then invite females? *In: Book of abstracts, Semio 11 workshop, 12th to 15th November 2011, icipe, Nairobi, Kenya,* 26pp.
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