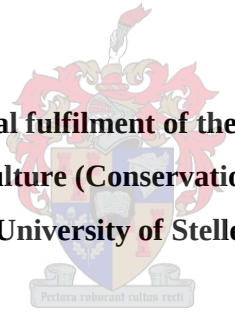
ASSESSING THE CHEMICAL ECOLOGY AND SHELTER-SEEKING BEHAVIOUR OF THE GRAIN CHINCH BUG, *Macchiademus diplopterus* (HEMIPTERA: LYGAEIDAE) FOR OPTIMISATION OF TRAPPING DURING AESTIVATION

By

Masimbaashe Ngadze



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DECLARATION

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ABSTRACT

The grain chinch bug (GCB), *Macchiademus diplopterus* (Distant) (Hemiptera: Lygaeidae) is a key quarantine pest of South African export fruit and is endemic to the Western Cape Province. The pest is troublesome in the drier wheat growing areas where it disperses from wheat in summer to find sheltered sites in which to aestivate. Aestivating adults can end up contaminating export fruit. The aim of the study was to gather more knowledge on the chemical ecology and shelter-seeking behaviour of the GCB. The involvement of pheromones in the aggregation behaviour of GCBs is yet to be fully elucidated. Further investigating the chemical ecology of the GCB in order to optimize its pheromone trapping was the primary focus of the first research chapter in this study. Headspace volatile compounds were identified from active bugs through gas chromatography-mass spectrometry (GC-MS) analysis. A total of 14 volatile compounds were identified from males and females in varying concentrations. For both sexes pooled, tridecane, *(E)*-2-hexanal and *(E)*-2-octenal were the three main components; *(E)*-2-hexenol, *(E)*-2-octenol, decanal and pentadecane were in medium amounts, while decanoic acid, dodecane, hexadecanal, hexanal, icosane, nonanal and tetradecanoic acid were minor components. The efficacy of synthetic lures using previously identified aggregation pheromone components, and sex pheromone volatile components (identified in present study) was studied in combination with modified traps using rubber septa dispensers in a field trial. There was no significant difference ($P > 0.05$) between insects caught in the sex pheromone baited traps and the aggregation pheromone baited traps. Traps caught low numbers of GCBs compared to the level of orchard infestation indicated by the amount of bugs that were found sheltering in corrugated cardboard bands tied around tree trunks. The corrugated cardboard bands showed a significant difference in the number of bugs sheltering between bands placed at bottom and top positions (0.5m and 1.5m above ground respectively) on the trees, at site 1 ($P = 0.0058$), site 2 ($P < 0.0169$) and site 4 ($P < 0.0496$) with the exception of site 3 ($P > 0.4115$). Cardboard band position influenced catches, as more bugs were found in bottom bands. This can be used

advantageously in optimising innovative trap placements in the future in order to improve catches. In the second research chapter investigations into the behavioural responses of GCBs to visual objects were conducted. This was done to increase knowledge on how this behaviour can lead to the development of control measures such as the use of coloured traps of different shapes. Behavioural responses of GCBs to different shapes presented in their visual space indicated that there was a significant difference ($P = 0.0001$) in the choice of shape. Vertical/upright rectangular shapes had the highest number of GCB visits. GCBs responded to upright rectangles of different colours.Black and red rectangles were not significantly different ($P > 0.05$) from each other but were both significantly different ($P = 0.0001$) from green and yellow rectangles, off-target and sedentary insects. Vertical rectangles of two different colour patterns (black & white) and (red & white) did not show any significant difference ($P > 0.153$) in the number of GCB visits. Both black & white and red & white vertical stripes were significantly different ($P = 0.0001$) from off-target and sedentary insects. This indicates that GCBs were equally responsive to both colour patterns. These results indicate that GCBs exhibit a positive scototactic reaction towards dark upright surfaces. Information generated from this study will facilitate the development of pre-harvest monitoring and management measures against GCBs, using pheromone traps and physical barriers that prevent GCBs from dispersing into fruit orchards at the wheat to fruit orchard interface. This can help to reduce fruit contaminations, ultimately lowering the rejection risk of export fruit from South Africa.

OPSOMMING

Die graanstinkluis, *Macchiademus diplopterus* (Distant) (Hemiptera: Lygaeidae), is ’n belangrike kwarantynplaag van Suid-Afrikaanse uitvoervrugte en is endemies aan die Wes-Kaapprovinsie. Die plaag is ’n probleem in die droër graanbougebiede waar dit in die somer van graan versprei om skuilplekke te vind om in ’n somerrusperiode in te gaan. Volwasse insekte in hierdie somerrusperiode kan uitvoervrugte besmet. Die doel van hierdie ondersoek was om meer kennis oor die chemiese ekologie en skuilpleksoekende gedrag van die graanstinkluis te versamel. Daar moet nog afdoende bewys van die betrokkenheid van feromone by die aggregasiegedrag van graanstinkluise gevind word. Verdere ondersoek van die chemiese ekologie van die graanstinkluis om die feromoonlokval te optimaliseer was die primêre fokus van die eerste navorsingshoofstuk van hierdie studie. Vlugtige organiese verbindings in die bodamp van saamgetrosde stinkluise is deur gaschromatografie-massaspektrometrie (GC-MS)-ontleding geïdentifiseer. Altesaam 14 vlugtige verbindings is van mannetjies en wyfies in wisselende relatiewe konsentrasies geïdentifiseer. Vir albei geslagte was tridekaan, *(E)*-2-heksanaal en *(E)*-2-oktenaal die drie hoofkomponente; *(E)*-2-heksenol, (E)-2-oktenol, dekanaal en pentadekaan was in mediumhoeveelhede teenwoordig terwyl dekanoësuur, dodekaan, heksadekanal, heksanaal, ikosaan, nonanal en tetradekanoësuur mindere komponente was. Die doeltreffendheid van sintetiese lokmiddels deur gebruik van voorheen geïdentifiseerde aggregasie-feromoonkomponente en seksferomoon vlugtige komponente (in die huidige studie geïdentifiseer) is in ’n praktiese toets bestudeer in kombinasie met gemodifiseerde lokvalle deur gebruik van rubberseptahouers. Daar was geen beduidende verskil ($P > 0.05$) tussen insekte wat in die lokvalle met seksferomoon-lokmiddels en lokvalle met aggregasieferomoon-lokmiddels gevang is nie. Lokvalle het klein getalle stinkluise gevang in vergelyking met die vlak van boordinfestering wat aangedui word deur die hoeveelheid luise wat gevind is in riffelkartonstroke wat om boomstamme gebind is. Daar was ’n beduidende verskil tussen die aantal luise wat in die riffelstroke onderom en bo-om die bome gebind is (0.5m en 1.5m bo die grond), in terrein 1 ($P = 0.0058$),

terrein 2 ($P < 0.0169$) en terrein 4 ($P < 0.0496$), met die uitsondering van terrein 3 ($P > 0.4115$). Die posisie van die riffelkartonstroke het die vangste beïnvloed aangesien meer luise in die onderste stroke gevind is. Dit kan voordelig aangewend word deur in die toekoms innoverende lokvalplasings te optimaliseer ten einde vangste te verbeter. In die tweede navorsingshoofstuk is gedragsresponse van graanstinkluise op visuele voorwerpe ondersoek. Dit is gedoen om kennis uit te brei oor hoe hierdie gedrag tot die ontwikkeling van beheermaatreëls soos die gebruik van gekleurde lokvalle in verskillende vorms kan lei. Gedragsreaksies van stinkluise op verskillende vorms wat in hulle gesigsveld aangebied word het getoon dat daar ’n betekenisvolle verskil ($P = 0.0001$) in die keuse van vorm was. Vertikale/regop reghoekige vorms het die grootste aantal besoeke gehad. Stinkluise het teenoor regop reghoeke van verskillende kleure gereageer. Die reaksie op swart en rooi reghoeke was nie beduidend verskillend ($P > 0.05$) van mekaar nie, maar albei het aansienlik verskil ($P = 0.0001$) van dié van groen en geel reghoeke, buiteteiken- en sedentarye insekte. Vertikale reghoeke van twee verskillende kleurpatrone (swart & wit) en (rooi & wit) het geen beduidende verskil ($P > 0.153$) in die aantal besoeke getoon nie. Swart & wit sowel as rooi & wit vertikale strepe het aansienlik verskil ($P = 0.0001$) van buiteteiken- en sedentarye insekte. Dit dui daarop dat graanstinkluise ewe goed op albei kleurpatrone gereageer het. Hierdie resultate dui daarop dat graanstinkluise ’n positiewe skototaktiese reaksie teenoor donker, regop vlakke toon. Inligting uit hierdie studie sal die ontwikkeling van vooroes-monitering en -bestuursmaatreëls teen die graanstinkluis fasiliteer deur gebruik van feromoon-lokvalle en fisieke grense wat stinkluise verhinder om na vrugteboorde by die graan-tot-vrugteboord-koppelvlak te versprei. Dit kan help om vrugtebesmettings te verminder, wat uiteindelik die afkeuringsrisiko van uitvoervrugte uit Suid-Afrika sal verminder.

DEDICATION

This dissertation is dedicated to my lovely wife Nyaradzo Ngadze. You are exceptionally supportive, I love you and I celebrate you.

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I thank my creator, God Almighty for the life that He has given me.

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CHAPTER 1

LITERATURE REVIEW

1.1 Introduction

The grain chinch bug (GCB), *Macchiademus diplopterus* (Distant) (Hemiptera: Lygaeidae) is an important quarantine pest of deciduous fruit in South Africa. It mainly affects the South Western Cape Province which is the centre of commercial fruit production in the country. It is a pest that does not feed on fruit but due to a migratory shelter-seeking behaviour seen in adults during aestivation, the GCBs can be found sheltering in fruit commodities destined for export. GCB is endemic to South Africa and consequently, a pest of phytosanitary concern that if left uncontrolled can negatively impact the export fruit market of the Western Cape.

GCB populations fluctuate between seasons in affected areas, such as Ceres (Addison 2004). This is linked to prevailing weather conditions and is dependent on photoperiod, temperature and humidity levels. High GCB populations are more prevalent in drier areas experiencing low minimum temperatures and low relative humidity levels (Johnson & Addison 2008). The pest feeds and reproduces in winter on grasses and small grain crops, such as wheat (Slater & Wilcox 1973; Sweet 2000). It feeds on host plants through sucking sap, a trait common to other Lygaeidae species (Solbreck 1979; Dingle et al. 1980; Solbrech & Sillen-Tullberg 1981; Schuh & Slater 1995).

The seasonal life cycle of the GCB includes a period of aestivation, a state of dormancy entered at adult stage in early summer. At the onset of aestivation the insect seeks out shelter sites in which to aestivate (Giliomee 1959; Annecke & Moran 1982; Sweet 2000). This stage in the life cycle of the GCB coincides with the ripening and harvesting of many deciduous fruit cultivars. Orchards and vineyards near to wheat

fields, become infested with GCBs during the migration period. This begins at the onset of summer from around October to November.

The harvesting of wheat is a major cause of GCB dispersal from wheat to fruit orchards where they coincidentally find shelter in fruit cavities of ripening fruit (Annecke & Moran 1982). Adult GCBs are known to hide in concealed fruiting structures such as in the calyx and stalk end of pome, stone and citrus fruit. The insects have inadvertently been exported within various fruit commodities to international markets causing consignment rejection problems for the local fruit producers.

1.2 Pest history on host plants

The GCB is a sap sucking pest of grain crops such as wheat, barley, oats and other wild grasses (Matthee 1974; Annecke & Moran 1982). According to Sweet (2000) the natural host plants for this pest are within the plant family *Poaceae* and these include longflowered veldtgrass *Ehrharta longiflora*, panic veldtgrass *E. erecta*, common wild oat *Avena fatua*, and annual meadow grass *Poa annua*. When feeding on preferred host plants they normally aggregate and cause wilting of the plants, before the plants dry and die (Sweet 2000; Summers et al. 2010; pers. obs.).

In South Africa, reports of severe economic losses in wheat fields as a result of GCB damage dates back as far as the late 19th century in the Touws River area in the Western Cape (Smit 1964). The arrival of the agricultural revolution in the past century brought with it a breakthrough in the management of GCBs in wheat using synthetic pesticides. This was attributed to the availability and registration of systemic insecticides that were adopted from European countries and chemical companies that extended their markets into Africa. Currently there are several pesticides available for use on grain crops and are being used in wheat pest control programmes. GCBs are now considered an occasional pest of wheat in the Western Cape (ARC 2014).

1.3 Pest history on fruit commodities

The GCB has for some time presented serious economic challenges for South African fruit exporters. There are numerous consignment rejection reports for fruit exported to the United Kingdom dating back to the 1920's (Malumphy et al. 2012). Initially it was thought that GCBs were feeding on the fruit, but later it became clear that bugs were only sheltering on the fruit and did not feed (Annecke & Moran 1982). GCBs have no specific fruit targets, but can be found on all fruit types including stone and pome fruit, table grapes and citrus within the Western Cape area (Johnson & Addison 2008).

As a result of fruit rejections and the concurrent loss of income to the fruit industry, research is presently focused on finding effective and reliable monitoring and management methods as a solution to the GCB pest problem. This is crucial for reducing the risk of fruit carton contaminations with GCBs in the future. This mitigates the rejection that would result when South African fruit consignments are refused and/or destroyed by the receiving export markets. Innovative and effective control measures will also be required to stop the spread of the insect into new areas where it does not occur. Care should thus be taken to ensure that potential pests are not spread to new areas through exportation and importation of agricultural produce.

1.4 Quarantine status and interception history of pest

There are strict trade requirements and regulations governing the export of fruit that need to be adhered to in order to achieve successful business with international markets (Wakgari & Giliomee 2004). The high risk of exporting GCB contaminated fruit to export markets has led to the classification of the insect as a key phytosanitary pest of South African export fruit. One of the first positive interception incidences of GCBs on South African export fruit was recorded in England on peaches in 1923, followed by an interception on nectarines at Newcastle docks in 1960 (Malumphy et al. 2012). On one occasion, GCBs reportedly survived cold storage treatment of -0.5º C for 8

weeks on stone fruit in transit from South Africa, and live insects were intercepted in England (Myburgh & Kriegler 1967; Malumphy 2011).

GCB has been detected in England on more than 14 occasions in association with fresh produce imported from South Africa, especially on peaches (Malumphy et al. 2012). One of the most significant findings to date was in February 2011 where hundreds of live bugs were found in a shipped consignment of fresh peaches from South Africa (Malumphy 2011; Malumphy et al. 2012). The consignment was destroyed soon after detection. Other reports also mention that in the 2006/07 season more than 50% of locally produced table grapes were rejected at several international markets due to GCB infestation (Johnson & Addison 2008). The GCB can easily hide in crevices and cavities, a factor which increases the demand for the development of novel species specific control methods for this pest. At present, the pest continues to affect South African export fruit thereby increasing the quarantine concern (Malumphy & Reid 2007).

1.5 Classification of the grain chinch bug, *Macchiademus diplopterus*

The GCB, *Macchiademus diplopterus* belongs to order Hemiptera, one of the largest insect orders and a very important group in agriculture as many insects of economic importance occur within this group (Smit 1964; Sweet 2000). Members in this order are terrestrial or aquatic and they pass through incomplete metamorphosis with their nymphs developing wing pads without pupating (Scholtz & Holm 2008). The GCB goes through five nymphal stages of development in its life cycle before turning into an adult (Fig. 1.1).

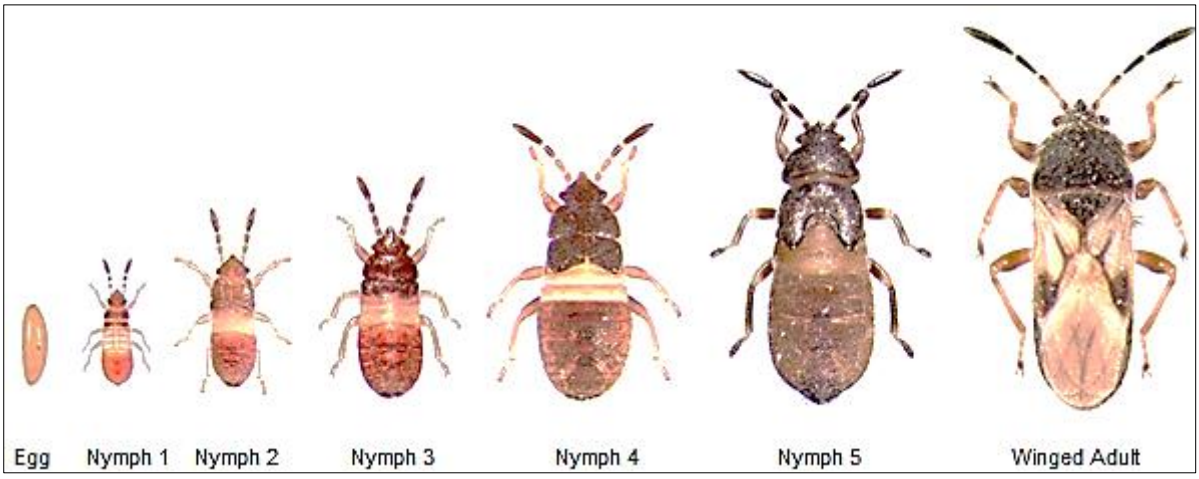

Fig. 1.1. The developmental stages of the grain chinch bug (Insecta: Hemiptera: Heteroptera: Lygaeidae) showing the five nymphal stages before reaching adult stage (Source: Shetlar & Andon 2011).

As a result of the many different morphological forms of insects in this order, its classification is complex (Scholtz & Holm 2008). The various hemipteran species possess piercing and sucking mouthparts, enabling them to extract plant sap from plants or blood from animals (Hansell 1984; Schuh & Slater 1995; Scholtz & Holm 2008). The GCB belongs to suborder Heteroptera which has members that feed on green plants (phytophagous) by the use of stylet shaped mandibles and maxillae also called juice extracting mouth parts (Sweet 1979). Their fore wings consist of a basal hardened portion and a distal membranous portion, making it a Hemiptera (Scholtz & Holm 2008). Members of suborder Heteroptera possess two paired wings, the forewings being different in texture and venation than the larger hind wings, hence the term ‘Hetero’ which means different (Smit 1964; Burdfield-Steel & Shuker 2014).

All species of the Heteroptera have compound eyes, but often have, two or no ocelli. The GCB only has compound eyes. A segmented antenna is also a common feature for all the Heteroptera species with the number of segments varying within the group. One of the common characteristics of these insects is their ability to reproduce prolifically. They have attained pest status in agriculture by virtue of huge numbers which results in enormous crop damage (Smit 1964; Sweet 2000; Summers et al. 2010). The GCB is classified in the family Lygaeidae which are the ‘true bugs’ and

sometimes are incorrectly referred to just as ‘bugs’ (Smit 1964). Members of this family are generally called seed bugs, stilt bugs, ground bugs or milkweed bugs because of where they live and feed (Burdfield-Steel & Shuker 2014). These insects have a rich evolutionary background but many attributes of their ecology are not fully known. Members of family Lygaeidae are generally small measuring between 1 mm to 12 mm average size (Aldrich et al. 1997). These are sometimes called lygaeids and are all infamous for causing substantial economic losses to wheat and other grain crops (Smit 1964; Sweet 2000).

Distinguishing lygaeids from other Heteroptera using morphological features is very challenging since they are highly polyphyletic exhibiting a complex morphology (Weirauch & Schuh 2011). Initially the GCB was classified by Slater (1977) in the genus *Atrademus*. Prior to this, the GCB belonged to the genus *Blissus* which was its former name used in old literature (Schaefer & Panizzi 2000) before Slater & Wilcox (1973) erected the genus *Macchiademus* in which it is placed at present. The GCB is classified as an indigenous South African species. Four other closely related species were also placed in the genus *Macchiademus* (Schaefer & Panizzi 2000). All five species are considered indigenous to the South Western Cape of South Africa. Herring (1973) once described the GCB as similar to the chinch bug *Blissus leucopterus* found in North America. However, the GCB was found to be distinctively thinner and longer than *B. leucopterus* (Schaefer & Panizzi 2000). Malumphy (2011) stated that it resembled *Ischnodema sabuleti* a British blissid species.

The GCB is the most economically important among the five species grouped in genus *Macchiademus*. It is known to be locally distributed in the Touws River, Citrusdal, Porterville, Piketberg and Ceres areas. The GCB attacks wheat, barley, oats and wild grasses as the main hosts. Furthermore, the GCB is macropterous with long wings capable of flight which gives it a migrating advantage over the other four brachypterous species with reduced forewing length (Slater & Wilcox 1973; Schuh & Slater 1995). The GCB has the potential to find new host plants with ease. In summer it can be found sheltering out of sight under loose bark of trees or on fruit (Fig. 1.2a & b). The

ability to fly and crawl enables the species to migrate readily into new areas, including the ability to disperse to distant aestivation sites (Slater & Wilcox 1973). This characteristic, in addition to the cold hardiness of the GCB, exacerbates the quarantine concern of the pest.

Fig. 1. 2. GCBs aggregating under loose bark of blue gum tree (Eucalyptus globulus) during aestivation (a), and GCB on the shoulder depression of a nectarine fruit while seeking shelter for aestivation during early summer season (b).

1.6 Basic biology and seasonal life cycle

The GCB is a small black insect between 4 mm to 8 mm in size and has shiny white wings when fully mature. It goes through five wingless nymphal stages before reaching adulthood by gaining wings. When fully mature the adult develops four to five membranous markings (veins) on the forewings. The female GCB lays its eggs either in ground crevices or in host plant leaf sheaths (Matthee 1974). The eggs are laid in clusters and a female produces not less than 100 eggs in a lifetime (Sim 1965; Matthee 1974; McLain 1989). Egg development takes on average one and half months before nymphs emerge. The nymphs mature into adults within 6 weeks. When fully grown and mature, the female GCB is usually a few millimetres larger than the male (Slater & Baranowski 1978) (Fig. 1.3).

Fig. 1.3. Adult GCBs, female (above) and male (below) with distinctive light brown markings (veins) on the membranous portion of the hind wings. The female has a larger, bulging abdomen than the male which is slender throughout its body length.

Although the female has a wider, more rounded and larger abdomen it also has a well-defined depression running down along the centre on the ventral side of the abdomen (Schaefer & Panizzi 2000). The depression contains the ovipositor which protrudes at an angle from the body with its pivot at the distal tip of the abdomen when laying eggs, as shown in Fig. 1.4a & b.

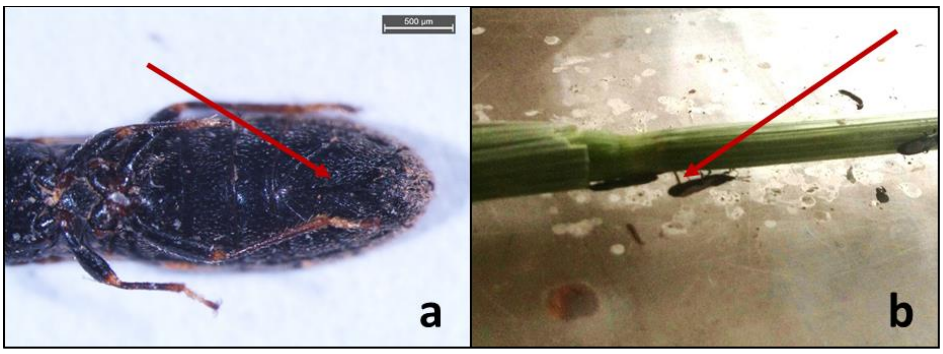

Fig. 1.4. (a) Adult female GCB ventral view of abdomen with arrow indicating the position of the ovipositor depression. (b) Female GCB penetrating grass leaf sheath with ovipositor during the egg laying season in winter.

The GCB has thrived for a long time under the Mediterranean climate characterised by a short rainy and cold winter in South Africa, feeding and reproducing on wheat and grass host plants (Sweet 2000; Malumphy et al. 2012). The females lay eggs during the winter time, from early May to late August in the Western Cape Province of South Africa (Sweet 2000). It is this new generation that will seek aestivation sites and migrate from the wheat into nearby fruit orchards after the harvest of wheat from October to November each year, coinciding with fruit ripening (Annecke & Moran 1982).

1.7 Aestivation as a survival strategy

Insects survive resource limited seasons by aestivating. They do so in either a quiescent or diapaused mode depending on their ecology and physiology. These modes of dormancy are triggered by changes in environmental factors such as temperature, moisture and photoperiod length which facilitates entry into dormancy in many arthropods (Morris 1976; Taylor & Taylor 1977; Eber & Brandl 1994). These factors differ significantly between summer and winter seasons thereby inducing diapause in due course (Tauber & Tauber 1970; Masaki 1980; Tauber et al. 1986; Garcia et al. 1990; Hodek & Okuda 1997; Narung & Merritt 1999; Zhu & Tanaka 2004). The three environmental factors mentioned above work in combinations, but temperature alone sometimes controls aestivation in many insects (Lamb et al. 2007).

Quiescence: When insects are in a quiescent state, they can tolerate extreme high or low temperatures and water scarcity, and are able to survive the adverse conditions that become a hindrance to the insect's normal life cycle (Dingle 1972). They arrest their own metabolic functions in order to survive the adverse conditions. Insects may in some instances do this over very long periods of time (Dingle et al. 1980). Quiescence is common in insects that occur in arid regions that sometimes have to go for several seasons without water. When the hindrance is removed the insects are able to immediately resume metabolic functions and development. They start from where they were physiologically, before they experienced the limiting factors.

Diapause: This mode of dormancy is similar to hibernation where the insects prepare themselves by reducing metabolic rates and increasing protection by covering their bodies. Some insect species such as the blackfly *Prosimulium mysticum* larvae protect themselves by spinning cocoons during their pupal stage (Mansingh & Steele 1973). They prepare for the upcoming unfavourable seasonal conditions allowing them to survive throughout dormancy. Some insects migrate to special sites were they aestivate. This is a form of diapause common in hemipteran and lepidopteran species (Resh & Carde 2003).

Some insect species such as the black and red bug, *Lygaeus equestris* and the seed bug, *Lygaeus simulans* undergo reproductive diapause in which they only go into the state of diapause as adults (Solbreck & Kugelberg 1972). The timing and pattern of their migratory flights is strongly influenced by weather conditions such as wind speed, temperature and length of photoperiod in autumn. They migrate into hibernation sites of favourable conditions where they can survive the winter (Solbreck 1979; Dingle et al. 1980). The adult Bogong moth *Agrotis infusa* of Australia migrates from the flat lands to mountains where they aggregate in cracks found on rocks (Resh & Carde, 2003). Similarly, the GCB migrates from wheat and grass to find shelter sites. This may be under loose bark of shrubs and trees, or in instances where fruit orchards are close by, inside fruit cavities. Understanding the migratory and aggregating behaviour of the GCB, and the signalling communication behind these behaviours may shed light on ways towards development of management options to control this pest.

1.8 Major signaling modes in insects

There are various channels of signal communication in insects and the most dominant pathways are olfactory, auditory and visual (Kerkut & Gilbert 1985). Many insects achieve communication by investing in the use of smells or odours as much as they would depend on sound and vision. The use of odour, sound and visual channels of signal communication in insects contributes towards the diverse behaviour systems demonstrated across many arthropod species. Both flying and crawling insects are

mainly known to use odour signals in orienting themselves towards resources essential for their survival (Otte & Cade 1976; Kennedy 1977; Payne et al.1986; Law et al. 2004). In general, insects also release odorous pheromone compounds in various forms such as alcohols, aldehydes or hydrocarbons that emanate from different parts of the insect body (Otte 1977; Rockstein 1978). The released chemical compounds play a major role as part of the olfactory communication operations in insects and are mediated through wind diffusion thereby relying on wind currents.

These chemical compounds transmit pheromone signal responses which are attained by organisms through accurately sensing discrete chemical components from suitable sources (Pureswaran et al. 2004; Wright & Smith 2004; Pureswaran & Borden 2005). The attraction functions of pheromones rely on the central nervous system which is the main pathway through which insects regulate their behaviour, but the endocrine system also plays a major role as an assisting pathway (Johnston et al. 1965; Demirel 2007). These two systems work together especially in instances where pheromones transmit their characteristics for a long period of time. An example is illustrated by the fire ant *Solenopsis Invicta* that lays a trail by depositing scented chemicals on the ground from a food source towards the nest. By so doing it leaves a trail of long lasting pheromones for other fellow workers to follow until they reach the food source (Weaver 1978).

Insect pheromones can either be species specific or may work across different species (Cox 2004), in which case they are known as allelochemicals, such as kairomones and allomones (Howse 1998). Aldrich (1988) found that Heteroptera release certain odours through metathoracic and dorsal abdominal scent glands that they use as anti-predator pheromones. Insects have relied on releasing such types of pheromones when facing danger (Haynes & Birch 1985; Demirel 2007). In some cases these same pheromones are used for social communication within species (Moraes et al. 2008). Pheromones can also be released as isolates or mixtures of several compounds and are of numerous benefits to the insects as they provide an energy efficient communication channel (Shorey & McKelvey 1977).

Mixtures of several components provide the insect with pheromones of different attractive characteristics which are utilised within insect mating activities and also other social schemes across all ecological systems (Byers 2012). Some pheromone chemical components are highly volatile and are extremely difficult to isolate and to identify (Weaver 1978). Despite the isolation challenges, researchers have taken advantage of sex and aggregation pheromones by adopting them into Integrated Pest Management (IPM) strategies whereby traps incorporating active chemicals are used to catch insects for surveillance and monitoring (Grout et al. 1998). Due to analytical techniques that were introduced in the late 20th century such as gas chromatography, isolating pheromone components became more manageable, mostly requiring less than 100 individuals to isolate sex or aggregation pheromone compounds (Blum et al. 1971; Klun et al. 1973).

The term ‘sex pheromone’ is widely used to describe the active volatile compounds that animals use in initiating mating, which also act as aphrodisiacs (Beroza 1970). Sex pheromones are released from female sternal glands in *Macrotermes annandalei*, *Pseudacanthotermes spiniger* and *Reticulitermes* termite species (Buchli 1960; Stuart 1969; Clement 1982; Bordereau et al. 1991). Sex pheromones are used to attract males for copulation by females (Tamaki 1972). They are also equally used by the males to prepare the female for mating and such behavior can be classified into mating partner search models. Search models depend on the gender of the insect releasing the sex pheromone to attract the opposite sex. Male search models are the most common among numerous insect species (Jacobson et al. 1970; Silverstein 1970; Roelofs et al 1975; Read & Haines 1976; Kerkut & Gilbert 1985). In the male search models, the female members are the ones that release sex pheromones thereby attracting males for mating (Byers 2006; Kerkut & Gilbert 1985). An example of a male search model is that of the female Lygaeidae predatory species *Geocoris punctipes* that produces pheromones that stimulate searching behaviour in males (Miller 2005). In female search models, males release pheromones attracting females for mating. An example of a female search model is that of the dried bean beetle, *Acanthoscelides*

obtectus which releases the sex pheromone that attracts the females (Halstead (1973).

In some instances attraction pheromones have an effect on both the male and the female sexes (Borden 1985). When this happens they are called aggregation pheromones because they do not only attract the opposite sex but both sexes are attracted (Byers 2012). Also, apart from aggregation pheromones there also exists another chemical signalling system in Lygaeidae species which depends on cuticular hydrocarbons (Burdfield-Steel & Shuker 2014). The entire chemical communication system may provide more insights into the diversity of chemical compounds that are at work in these insect species in different seasons. Aggregation and communication pheromones of Lygaeidae have been suggested to play a key role in initiating and maintaining a number of social behaviors in many of the various species in this family (Solbreck & Kugelberg 1972; Aller & Caldwell 1979; Solbreck & Sillen-Tullberg 1990; Miller 2005). Hibernating and aggregating groups of insects across many Lygaeidae insects such as *Oncopeltus fasciatus* and *Spilostethus pandurus* that feed on host plants in groups are all social behaviours kept together by aggregation pheromones (Root & Chaplin 1976; Aller & Caldwell 1979; Dingle et al. 1980). A list of the various pheromone components of Lygaeidae which are released for attraction and defence are listed in Table 1.1.

Understanding insect chemical compounds gives us the opportunity to manipulate different behavioural activities in insects (Aldrich et al. 1999). Advantageously, sex and aggregation pheromones can be utilised in pest control agroecosystems for monitoring, trapping, or mating disruption practices (Aldrich 1988; Grout et al. 1998). Olfactory communication studies therefore provide insights into manipulating insect behaviour through the use of pheromones.

Table 1.1. Compounds detected in the pheromones of several species of Lygaeidae.

Species	Compounds from Metathoracic scent glands	Defence substances	References
Lygaeus kalmii	*(E)*-2-Hexenyl acetate, *(E)*-2,4-Hexadienyl acetate, *(E)*-2,5-Hexadienyl acetate, *(E)*-2-Heptenyl acetate, *(E)*-2-Octenyl acetate, *(E)*-2,7-Octadienyl acetate, *(E)*-2-Hexenyl butyrate, (E,E)-2,4-Octadienyl acetate, *(E)*-2-Hexen-1-ol, *(E)*-2-Hexenal, *(E)*-2-Octenal, *(E)*-4-oxo-2-Hexenal, *(E)*-4-oxo-2-Octenal		(Aldrich et al. 1999)
Oncopeltus cingulifer	*(E)*-2-Hexenyl acetate, (*E,E*)-2,4-Hexadienyl acetate, *(E)*-2,5-Hexadienyl acetate, *(E)*-2-Heptenyl acetate, *(E)*-2-Octenyl acetate, (*E,Z*)-2,6-Octadienyl acetate, (*E,E*)-2,6-Octadienyl acetate		(Aldrich et al. 1999)
Oncopeltus fasciatus	*(E)*-2-Hexenyl acetate, (*E,E*)-2,4-Hexadienyl acetate, *(E)*-2,5-Hexadienyl acetate, *(E)*-2-Heptenyl acetate, *(E)*-2-Octenyl acetate, *(E)*-2,7-Octadienyl acetate, (*E,Z*)-2,6-Octadienyl acetate, (*E,E*)-2,6-Octadienyl acetate, *(E)*-2-Hexenal, (*E,E*)-2,4-Hexadienal, *(E)*-2-Octenal, *(E)*-2,7-Octadienal, (*E,Z*)-2,6-Octadienal, (*E,E*)-2,6-Octadienal,2-Octenal	2-Isobutyl-3-methoxypyrazine	Aldrich et al. (1999, 1997), (Games & Staddon 1973)
Oncopeltus unifasciatellus	*(E)*-2-Hexenyl acetate, (*E,E*)-2,4-Hexadienyl acetate, *(E)*-2,5-Hexadienyl acetate, *(E)*-2-Heptenyl acetate, *(E)*-2-Octenyl acetate, *(E)*-2,7-Octadienyl acetate, *(E,Z)*-2,6-Octadienyl acetate, (E,E)-2,6-Octadienyl acetate, *(E)*-2-Hexenal, (*E,E*)-2,4-Hexadienal, *(E)*-2-Octenal, *(E)*-2,7-Octadienal, (*E,Z*)-2,6-Octadienal, (*E,E*)-2,6-Octadienal,		(Aldrich et al. 1999)
Spilostethus rivularis	*(E)*-2-Octenyl acetate, *(E)*-2-Hexenyl acetate, 3-Methylbutyl acetate, 3-Methyl-2-butenyl acetate, 2-Phenylethanol acetate, (*E,E*)-2,4-Hexadienyl acetate		(Staddon et al. 1985)
Geocoris punctipes	*(E)*-2-Octenyl acetate, *(E)*-2-Hexenyl acetate, *(E)*-2-Octenal, *(E)*-2-Hexenal, *(E)*-4-oxo-2-Hexenal, *(E)*-2-Decenal		(Marques et al. 2000)
Geocoris varius	*(E)*-2-Hexenal, *(E)*-2-Decenal, Tridecane		(Yamashita & Kanehisa 1979)
Neacoryphus bicrucis	(*E,E*)-2,4-Hexadienyl acetate, *(E)*-2-Octenyl acetate, 2-Phenylethanol acetate, *(E)*-2-Hexenal, *(E)*-2-Octenal, *(E)*-4-oxo-2-Hexenal, *(E)*-4-oxo-2-Octenal, (*E,E*)-2,4-Hexadienyl acetate, 2-Phenylethanol acetate		Aldrich et al. (1999, 1997)
Oxycarenus hyalinipennis	(*Z,E*)-3,7,11-Trimethyl-1,3,6,10-dodecatetraene, *(E)*-2-Octenyl acetate, *(E)*-2-Octenal, 2,6,6-Trimethylbicyclo [3.1.1]hept-2-ene, 1-Methyl-4-(1-methylethenyl)-cyclohexene, 2-Hexenal, 1,3,3-Trimethyl-2-oxabicyclo [2.2.2.]octane, *(E)*-2-Hexenyl acetate, 2-Octenal, *(E)*-4-oxo-2-Hexenal, 2-Octenyl acetate, *(E)*-4-oxo-2-Octenal		(Knight et al. 1984), (Olagbemiro & Staddon 1983)
Tropidothorax cruciger	*(E)*-2,7-Octadienyl acetate, *(E)*-2-Octenyl acetate		(Aldrich et al. 1997)

(Extracted from Burdfield-Steel & Shuker 2014).

Manipulating insect behaviour does not solely rely on the use of pheromones, but on visual signal functions as well. Insect visual signals are therefore an important channel of communication to consider when developing trapping systems against insect species that possess sight. Vision in insects depends on the type of eyes that the insect carries. The insect's head may carry two compound eyes or sometimes three simple eyes called, ocelli. (Smithers 1982). The ocelli are regarded as organs that detect changes in light intensity as they consist of a single lens and would provide very poor images if they were to be used for sight. The compound eye is composed of many single standing units within it called, ommatidia (Smithers 1982). These are made up of an outer lens and inner light receptors that form a complex called a facet. Each facet carries its own image and several facets converge images on one part of the eye enabling the eye to focus and depict a single image. (Smithers 1982; Chapman 1998).

Insects have easily been associated with good colour vision because of the way they interact with the inflorescence and other colourful parts of plants. On the contrary, many insects simply differentiate variances in reflected light rather than discriminating actual colours (Smithers 1982; Segura et al. 2007). True colour vision has only been demonstrated in very few insect species as it demands the use of complicated methods that require training of the animal (Menzel & Backhaus 1991). Insect vision is mostly focused and concentrated at the far left of the spectrum closer to violet and ultra violet colours (Fig. 1.5).

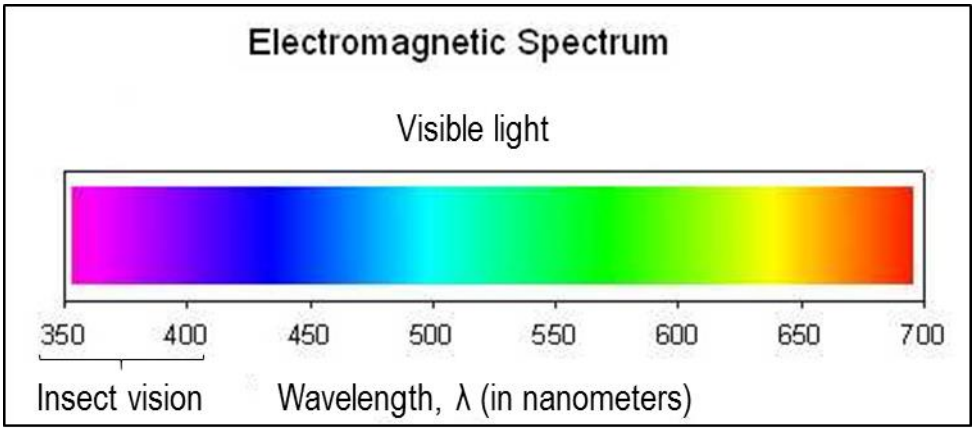

Fig. 1.5. Electromagnetic spectrum showing the range where visible light is perceived, measured in nanometres. (Source: www.euhou.net 2015).

Insects do not see red and other colours that are on the far right of the spectrum (650-700 nm) and usually associate colours close to red with the dark contrast colours on the far left of the spectrum (350 nm-400 nm) (Chapman 1998). This is because their vision is limited in that range. The ecological significance of colour attraction and avoidance is very crucial in creating trapping tools for pest control purposes.

There is lack of literature on colour vision and attraction in true bugs in general. There has not yet been enough research on the subject of visual perception and orientation behaviour in Lygaeidae species except for other insect species. One example of studies involving visual perception is the case of the striped ambrosia beetle, *Trypodendron lineatum* which is known to become photopositive to blue and green light before they select for hosts during the dispersal season (Atkins 1966). Other bark beetles are attracted to traps resembling host trees according to the perceived hue or form during dispersal (Atkins 1966; Lindgren et al. 1983).

1.9 Orientation behaviour in insects

There exists for insects, an action that involves the movement of the insect body or head towards the direction of objects presented in the local visual field. Such expressed arrangement of body and head is called orientation reaction (Jeanrot et al. 1981). Many insects express this orientation behaviour when detecting cues

associated with the location of hosts, facilitating the catching of prey, finding shelter or escaping danger. The Southern Hawker dragonfly larvae, *Aeschna cyanea* fixes its eyes on its prey by turning the body and head towards moving small objects until a complete full view of the prey is achieved (Baldus 1926; Friedrichs 1931). The spider, *Arctosa variana* exhibits an escape behaviour northwards finding shelter towards all dark objects while guided by the sun's position (Papi & Tongiorgi 1963).

A few external stimuli may produce huge behavioural responses in insect species more than in larger animals because they lack an equivalent physiological sophistication (Hansell 1984). Other neurological and physiological mechanisms undoubtedly exist in the insect physiology enabling them to regulate or modify sensory stimuli to give various complex behavioural responses (Davis 1976; Turlings et al. 1993). Several insect species exhibit complicated behavioural reactions by orienting towards volatile substances secreted by plant species as well (Kerkut & Gilbert 1985). Some insects with efficient foraging abilities learn and locate food sources by following complex species specific signals from different hosts (Papaj & Prokopy 1989; Dempster et al. 1995; Stireman 2002; Dudareva et al. 2004).

At a given time an insect may be found to physically orient towards a source of stimuli and the term 'taxis' can be added to the name of the source of signal, giving rise to the nomenclature of several taxis reactions. The response in which insects would move towards light for example, would be recognised as positive phototaxis and when they move against the light, negative phototaxis (Fraenkel & Gunn 1961). In other cases the insect moves towards dark areas (positive scototaxis) or against dark (negative scototaxis) (Atkins et al. 1987). Phototactic and scototactic behavioural studies in juice-sucking insects Culicidae and Muscidae (Allan et al. 1987) and Glossinidae (Green & Cosens 1983) revealed more about their visual ecology, thereby improving their control (Green 1986; Allan et al. 1987). Insects such as cockroaches *Periplaneta americana* make use of thermoreceptors on their antenna to locate food and shelter, this reaction is known as thermotaxis (Gordh & Headrick 2001). The temperature receptors of the hemipteran *Rhodnius prolixus* are a vital tool for its

survival in finding food, shelter hosts. Host finding is also assisted through thermotaxis in the braconid wasp *Coeloides brunneri*, which positively moves towards the source of heat to find a host (Gordh & Headrick 2001).

Signalling and modes of communication in the GCB, as well as orientation behaviour towards profiles in finding shelter resources essential for its survival, need to be investigated. Better understanding of these aspects of GCB biology may provide us with pathways of manipulation leading to the adoption of innovative and efficient management and control strategies against the pest in the future.

1.10 Study objectives

The overall aim of the project was to gain a better understanding of GCB chemical ecology and visual perception associated with the shelter-seeking behaviour exhibited during aestivation. Ultimately, pheromone-based monitoring and trapping strategies, as well as visual attraction mechanisms were assessed with the focus of developing pre-harvest management techniques aimed at reducing the risk of infestations in export fruit orchards.

The specific objectives were:

1) To isolate and identify the sex pheromone compounds in both sexes of *M. diplopterus* during the active season.

2) To evaluate methods for trapping GCBs using a previously identified aggregation pheromone lure, as well as a sex pheromone lure in field trials.

3) To evaluate the orientation behaviour of the shelter-seeking GCBs towards shapes of different colours in a localised visual field.

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CHAPTER 2

DEVELOPMENT AND EFFICACY OF SYNTHETIC LURES USING AGGREGATION AND SEX PHEROMONE CONSTITUENTS FOR TRAPPING THE GRAIN CHINCH BUG, *Macchiademus diplopterus*

2.1 INTRODUCTION

The grain chinch bug (GCB), *Macchiademus diplopterus* (Distant) (Hemiptera: Lygaeidae) seeks shelter and aggregates in large numbers during aestivation, suggesting that individuals release an aggregation pheromone that attracts their kin. The shelter-seeking behaviour leads them to finding shelter on different kinds of fruit that are in close proximity to wheat and other host plants. The presence of this endemic insect pest on export fruit poses a serious phytosanitary concern for countries importing fruit from South Africa. The use of pesticides is not recommended especially during the period of fruit harvest when the GCB is seeking shelter. Since pheromone mediated techniques are used in Integrated Pest Management (IPM) to control pests in orchards, it would be worthwhile considering the same approach against the GCB.

Developing a pheromone based GCB monitoring and management program is a vital strategy of control that anchors on the principle of using insect pheromones. Examples of successful IPM strategies include lure mediated monitoring and trapping mechanisms which are crucial in reducing the use of environmentally unfriendly chemicals (Jones 1998). Sex pheromone facilitated monitoring techniques are common for assessing insect populations for quarantine pests such as false codling moth, *Thaumatotibia leucotreta*, codling moth, *Cydia pomonella* and vine mealybug, *Planococcus ficus,* among others (Pringle et al. 2003; Walton 2003; Walton & Pringle 2004).

Insect mating systems are known to be driven by pheromones that are laden with attractive utilities, which may be characterised as sex pheromones if they act only on the opposite sex or aggregation pheromones if they act on both sexes (Byers 2012). Identified sex attractant pheromones of the Spanish moon moth, *Graellsia isabellae* assisted in the development of field tools for detecting and monitoring the populations of this endangered species. The highly attractive female sex pheromone was a breakthrough that allowed detailed assessments of this species' conservation status (Millar et al. 2010).

Attraction pheromones from stink bug families such as Pentatomidae have been used for developing general lures as they elicit responses across several insect species. Synthetic analogues based on Pentatomidae family pheromone structures have been used as bait for capturing several tachinid parasitoids of stink bugs such as *Euclytia flava* and *Gymnosoma par* flies (Aldrich & Zhang 2002). This provided more knowledge on how tachinids utilise sex pheromones of the stink bugs to orient to potential hosts in a predator and host relationship that was not yet understood (Dietrick & Van Den Bosch 1957; Pickett et al. 1996; Aldrich & Zhang 2002). Cross attraction of brown marmorated stink bug, *Halyomorpha halys* to the sex pheromone of another stink bug, *Plautia stali* has also been reported (Sugie et al. 1996; Tada et al. 2001).

Research by Addison (2004) focused on the potential of using seven different general stink bug aggregation pheromones including those from Pentatomidae, for the attraction of GCBs in orchards. These pheromones were used in wing traps with the aim of eliciting attraction in trapping GCBs, but was unsuccessful. This supports the assumption that there is probably species specificity when it comes to pheromone functions in GCBs. It is suspected that in several species of Lygaeidae the variety of chemicals produced attract conspecifics only (Games & Staddon 1973; Aldrich 1988; Aldrich et al. 1997; Aldrich et al. 1999).

Oliver et al. (1996) extracted three major chemical compounds from the scent glands of GCBs. These were tridecane, *(E)*-octenal and *(E)*-1-hexenel. The authors described

these chemical compounds as defence secretions without pheromonal attraction capabilities. Okosun (2012) found that aggregation pheromone components of GCBs isolated from aestivating females, showed some attraction in both sexes during laboratory bioassays. Aggregation of the large milkweed bug, *Oncopeltus fasciatus* and the small milkweed bug, *Lygaeus kalmia* nymphs, are examples where such behaviour is known to be mediated by aggregation pheromones in Lygaeidae (Aller & Caldwell 1979). In addition, cuticular hydrocarbons such as tridecane have also exhibited attraction in many Lygaeidae species (Kather & Martin 2012).

The aggregation pheromone compounds for some true bug species play two different roles as they are attractive at low concentrations, but are repellent at high concentrations. Examples include the three compounds *(E)*-2-hexenal, *(E)*-2-octenal and tridecane which stimulate alarm behaviour at high concentrations and attractive behaviour at low concentrations in *Nezara viridula* (Lockwood & Story 1986), *Dysdercus fasciatus* and *Dysdercus cingulatus* (Farine 1993). These insects use the same pheromone compounds but in different concentrations for either aggregation attraction or for alarm behaviour.

The observed behaviour of GCB in feeding on plants in aggregated groups during their reproductive phase, and then aggregating in shelter sites during their aestivation phase, may be based on the use of the same attractive volatile compounds for communication, in both winter and summer, but in different concentrations and ratios. The optimisation of a GCB pheromone trapping system has the potential to assist in the management of GCBs, as a pre-harvest control tool in fruit orchards. If developed, such a system may be implemented within an IPM program for monitoring and controlling the pest effectively. No identified sex pheromones have so far been reported in the GCB. In this study, the aim was, firstly, to isolate and identify volatile compounds produced by active female GCBs during winter, for the purpose of formulating a synthetic pheromone lure referred to as the ‘sex pheromone lure’ henceforth. Secondly, the efficacy of this lure was tested for eliciting the attraction of GCBs into traps.

2.2 MATERIALS AND METHODS

2.2.1 Sex pheromone lure experiments

2.2.1.1 Collection of active GCBs from host plants

Adult GCBs were collected from the foliage of wheat plants using scoop nets. Collections were carried out once every fortnight during the 2014 winter season (June to September) in Ceres (34.16ºS, 19.05ºW) and Piketberg (32.52ºS, 18.47ºE) areas in the Western Cape. The insects were collected between 8h00 and 13h00, as they were observed moving from the bottom of wheat plants to the top foliage during this time (Fig. 2.1). The contents of the scoop nets were transferred into sterilized 500 ml glass jars with aluminium foil covered screw caps. The foil was used to prevent the absorption of pheromones from the insects into the rubber seal on the jar lid. The insects were transported to the laboratory immediately after collection for sex determination.

Fig. 2.1. Grain chinch bugs scattered on top foliage of wheat plants during the mid-day period in the late winter season.

2.2.1.2 Sex determination of bugs in the laboratory

Sex determination was done by examining the ventral side of the abdomen under a microscope (Leica L2, model /PN MDG33 /10 450 123, Leica Microsystems, Singapore). Distinct morphological differences between male and female adult GCBs can be seen on the abdomen (Fig. 2.2). Females have a 'Y' shaped mark at the distal end of a bulging abdomen on the ventral side. Males have a crescent shaped mark at the distal tip of a slender abdomen on the ventral side.

Fig. 2.2. Image showing the abdominal markings (highlighted in black) on the ventral side of adult grain chinch bugs, which are unique to each gender and are used in combination with body size to distinguish males (above) from females (below).

2.2.1.3 Volatile organic compound sample collection

Males and females were placed in separate glass vials containing 20 insects each and used for qualitative and quantitative headspace analysis of the volatile organic compounds (VOCs) trapped from the insects' effluvium or gaseous emissions. The VOCs that make up the putative sex pheromone were collected using a high capacity (high sensitivity) sample enrichment probe (SEP) (Burger et al. 2011), consisting of a thin rod of inert material carrying a 30 mm sleeve of 0.64 mm i.d x 1.19 mm o.d

polydimethylsiloxane (PDMS) rubber tubing at its lower end. The upper, sharpened, end of the inert rod was furnished with a vial cap and septum in such a way that the vial could be closed for sample collection. The lower end of the SEP, carrying the rubber sleeve, was introduced into the vial with insects, the vial was closed and the sample collection (enrichment) was allowed to proceed for 2 h at 30 ºC.

2.2.1.4 Gas chromatograph mass spectrometry (GC-MS) analysis

After sample collection, the vial's septum and cap were installed on the SEP with the injector cap and septum of the Carlo-Erba QMD 1000 GC-MS instrument (Milan, Italy) equipped with a ZB-5MS column (Phenomenex, USA). The PDMS sleeve of the SEP was introduced into the instrument's injector, following the directions in Burger et al. (2011), where the VOCs were desorbed from the rubber sleeve and were flushed into the capillary column, for separation and subsequent analysis by low resolution electron ionization mass spectrometry (GC-EIMS). The flow rate of helium gas was kept at 1 ml/min. The column oven temperature was programmed from 40 ºC to 280 ºC at 4 ºC/min. Mass spectra were recorded at 70eV.

The data were used to plot reconstructed total ion chromatograms (TICs) from the mass spectra of the constituents of the putative pheromone. The identity of the compounds was assigned by comparison of their respective mass spectral data, with data from the reference libraries of National Bureau of Standards (1990) and National Institute of Standards and Technology (2005), by calculating Retention Index (RI) in relation to *n*-alkanes from C11-C18, considering the EI-MS mass fragmentation pattern, and by comparison with authentic synthetic reference compounds previously identified from other organisms in this group. The quantitative data were obtained by integration peaks in the TICs using the Laboratory-Base software of the GC-MS instrument.

2.2.1.5 Sex pheromone lure formulation

In the present study, the female VOCs were chosen for lure formulation. The choice of using female extracted putative pheromones was supported by a previous study carried out with live GCBs in behavioural bioassays. The study revealed that both GCB sexes showed attraction to odours emitted by females more than those from males in a four-arm olfactometer (Okosun 2012). All putative female pheromone constituents identified from the GC-MS analysis were commercially available and were mixed according to the concentration of the major constituent, tridecane. A 3-µl solution of the mixed synthetic compounds was put in a 2 ml glass vial before exposing a natural elastic rubber strip (32 mm x 3 mm x 1 mm) to the mixture for a 24 h period to take up these compounds by dissolution in the rubber.

To determine the rate of desorption of these compounds from the rubber strip, it was again placed in a glass vial (140 mm x 20 mm) with a Teflon-faced screw top and this time exposed to a SEP for 18 h at 25 ºC, as was described before for collections from insects. The volatile compounds taken up (enriched) in the SEP's rubber sleeve were analyzed as described above to ensure uptake and ultimate release of the volatiles from the rubber strips. For field use, rubber strips (10 mm x 10mm x 2 mm) were prepared and impregnated as before using 1-µl solution of the mixture of synthetic compounds. The impregnated rubber strips were then kept under airtight conditions in glass vials until they were used in field experiments. As in the previous study by Okosun (2012), who formulated a synthetic aggregation pheromone lure, all data were normalized with respect to tridecane = 100 for the sex pheromone formulation.

2.2.2. Field trapping experiments

Four field sites were chosen in historically high GCB areas (Johnson & Addison 2008). The aggregation pheromone lure formulated by Okosun (2012), as well as the sex pheromone lure formulated in the present study, were tested in modified delta traps using rubber dispensers. Since the field experiments were started during the transitional phase before the bugs enter into full aestivation, the potential of attraction

by the sex pheromone was assessed as well. The delta traps were modified using cardboard strips placed inside traps to increase the opportunity for GCB to shelter. Previously, Okosun (2012) tested the formulated aggregation pheromone using delta traps and discovered that the bugs were sheltering in the walls of the traps. Their need for shelter may be useful in improving pheromone trap catches. Regrettably, no behavioural bioassays were carried out in the present study before testing the formulated sex pheromone in the field experiments. This was due to the limited time between lure formulation and the season of GCB migration from host plants into orchards.

2.2.2.1 Study area

All field experiments were conducted in the Ceres valley located almost 170 km north east of Cape Town. Two farms located within historically high GCB areas were chosen in the Warm Bokkeveld area in Ceres (Fig. 2.3). The two farms, Vadersgawe and Eselfontein, were separated by a 12 km distance.

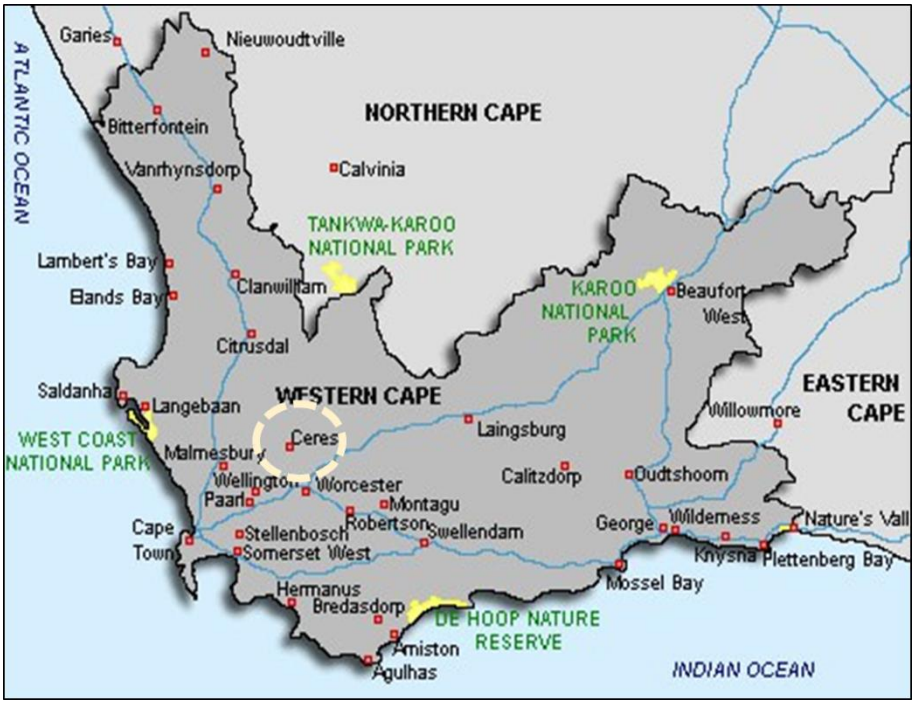

Fig. 2.3. Map of the Western Cape of South Africa showing where the field study was conducted during the 2014/15 trapping season in the Ceres area, encircled with yellow dash lines on map. (Source: sacarrental.com 2015).

All the pheromone lure trapping experiments were carried out in orchards that were adjacent to wheat fields. Two sites where allocated per farm: site 1 and site 2 where on Vadersgawe and site 3 and site 4 on Eselfontein. Site 1 was a block of 'Forelle' and 'Packham' pears, and site 2 was a block of 'Summer fire' nectarines and 'Sweet December' peaches next to each other. Site 3 was a block of 'Sweet December' peaches, and site 4 was a block of 'Western sun' peaches.

2.2.2.2 Experimental lay out and monitoring of traps in the field

Yellow delta traps (210 mm x 180 mm x 100 mm) (Chempack®, Simondium, Paarl, South Africa) with corrugated cardboard strips (for extra shelter) or with sticky pads were used to test the response of GCBs to the formulated aggregation pheromone and sex pheromone lures in the field. Either one of the two lures was applied per trap modified with cardboard strips (Fig. 2.4a) as well as per trap with sticky pad (Fig. 2.4b). Four different trap and lure combinations were applied during the field trial and were

replicated three times. A total of 12 yellow delta traps were hung at each site which were combinations of the following; aggregation pheromone lure and sticky pads (AS), aggregation pheromone lure and corrugated cardboard strips (AC), sex pheromone lure and sticky pads (SS), sex pheromone lure and corrugated cardboard strips (SC). The traps were hung every third tree, approximately 10 m apart from each other in the peripheral row facing wheat fields at each of the four sites. Trap placements were randomized in each row at each site and were positioned at shoulder height approx. (1.5 m) above ground.

Fig. 2.4. (a) Modified delta trap incorporating corrugated cardboard strips and (b) delta trap with sticky pad. The rubber dispensers were hung on the roof of each delta trap.

A single pheromone baited rubber dispenser was secured to the top interior of each delta trap, hanging free from walls, allowing the dissemination of lure by the wind in all directions. The pheromone baited dispensers created from white natural rubber were attached to nickel plated heavy duty 30 mm paper clips (Bantex (Pty) Ltd, South Africa) (Fig. 2.5). Dispensers with different lures were handled separately to avoid cross contamination before being hung inside delta traps on trees.

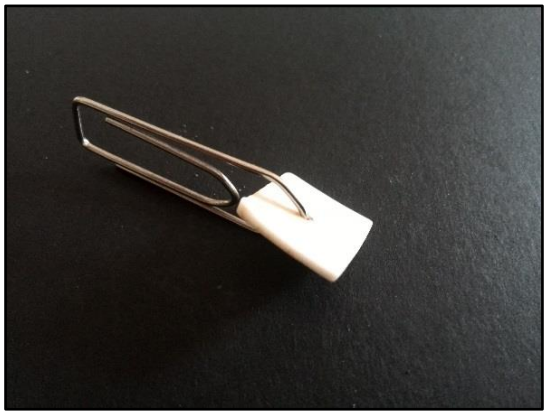

Fig. 2.5. Pheromone baited rubber dispenser attached to paper clip used in the delta traps.

In addition to using corrugated cardboard bands in modified delta traps, bands were also tied around tree trunks within the same experimental rows used to hang traps. Bands tied around tree trunks have previously been used in GCB monitoring surveys (Addison 2004; Johnson & Addison 2008). In this study, bands were tied at bottom and top positions in the trees to assess potential positional effect. The bottom bands were 50 cm above the ground (Fig. 2.6) and the top bands were at shoulder height approx. 1.5 m above the ground. The monitoring of GCBs through the use of corrugated cardboard bands was carried out to ascertain the level of GCB infestation in the test orchards. It was also important for determining the distribution of bugs along tree height when searching for sheltering sites.

Fig. 2.6. Corrugated cardboard band tied around tree trunk at bottom position for inspecting GCB numbers in orchards during the 2014/15 trapping period in Ceres.

Inspection of traps and bands were carried out after every 10 days from October 2014 to January 2015 yielding 8 collections in total. Cardboard strips, sticky pads, pheromone lures and corrugated cardboard bands tied around tree stems were inspected and the number of GCBs caught in traps and bands recorded. Components were also replaced at each inspection day.

2.2.3 Statistical analysis

All data collected from field experiments was analysed using SAS Enterprise Guide 2014. Shapiro-Wilk test was used to test for normality on all data. Bartlett's or Levene's test was used to test for differences in variance across all applied treatments throughout the four sites. In the case where data did not follow a normal distribution, non-parametric data analysis was done using the non-parametric equivalent of the t-test for two independent samples (Wilcoxon rank-sum test, the Mann-Whitney U test). Significant differences in the average catch between the two different band positions (bottom and top) throughout all four sites for the 2014/15 trapping period was tested using the same tools.

2.3 RESULTS

2.3.1 GCB sex pheromone lure composition

A number of volatile organic compounds, represented by peaks on total ion chromatograms (TICs) produced during GC-MS analysis, were identified from the headspace samples collected from active female (Fig. 2.7) and active male (Fig. 2.8) GCBs. Identities were only assigned to a few compounds on the TICs shown here to simplify the graph and avoid congestion. These two examples portray the typical TICs that were generated during the active season. The highest peak (100%) on the TICs for both active females and active males represented the volatile organic compound, tridecane. A total of 14 volatile compounds were identified from both genders, with substantial quantitative variations between male and female compositions (Table 2.1). Based on relative concentrations, tridecane, *(E)*-2-hexenal and *(E)*-2-octenal were present as main components (>120). Tridecane was high in both sexes, while the other two compounds were only main components in males. *(E)-2*-Hexenol, *(E)*-2-octenol, decanal and pentadecane were present in median relative concentrations (50 to 120).

Of the four median compounds, only decanal was substantially higher in females. Hexanal, hexadecanal, nonanal, dodecane, decanoic acid, tetradecanoic acid and icosane were minor compounds (<50), slightly higher in males as well, except for nonanal, decanoic acid and icosane which were marginally higher in females.The identified volatile components from the female gender blended together in formulating the lure were mixed according to their natural relative concentrations with respect to tridecane = 100 (Table 2.1). The sex pheromone lure formulated from female secretion was composed of known major chemical irritants (*(E)*-2-hexenal and *(E)*-2-octenal) in low amounts. No male sex pheromone lure was formulated.

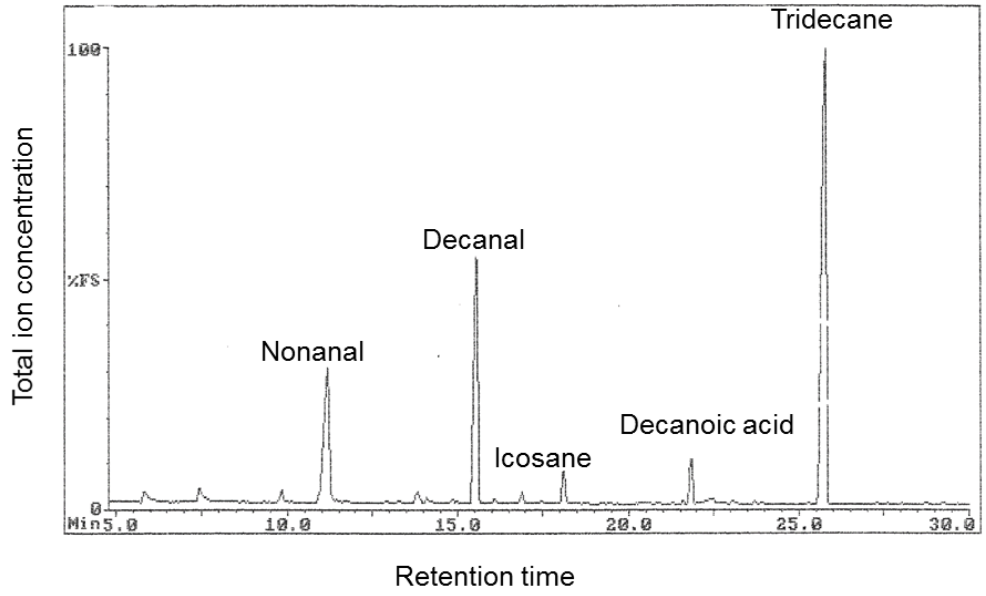

Fig. 2.7. Total ion chromatogram of identified compounds extracted from headspace samples of active female grain chinch bugs. The peaks are numbered according to the retention time in seconds at the apex of each peak. These numbers divided by 60, thus correspond to the retention time of the peaks in minutes.

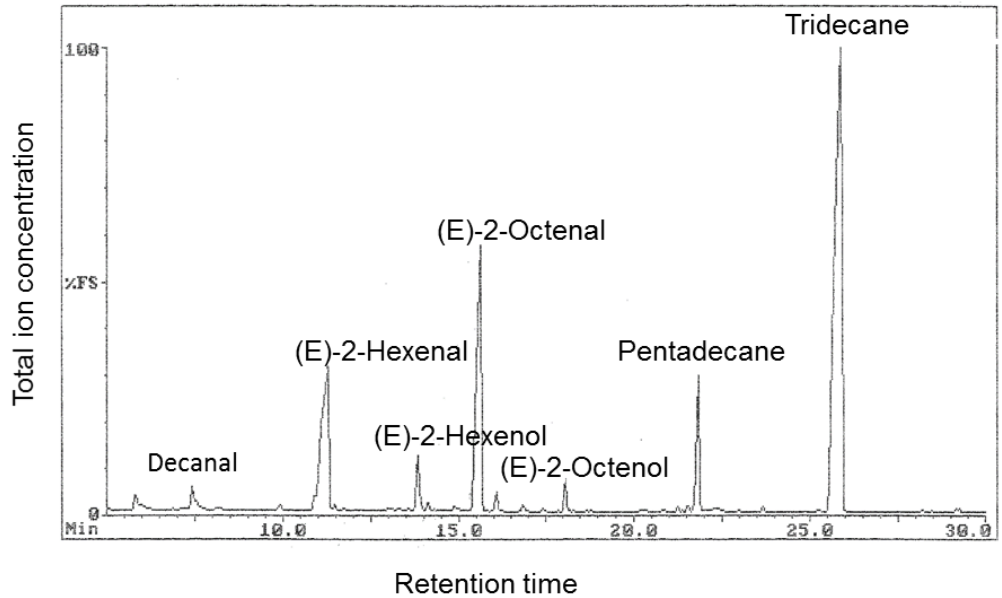

Fig. 2.8. Total ion chromatogram of identified compounds extracted from headspace samples of active male grain chinch bugs. The peaks are numbered according to the retention time in seconds at the apex of each peak. These numbers divided by 60, thus correspond to the retention time of the peaks in minutes.

Table 2.1. Quantitative composition of the secretions of female and male GCBs obtained by GC-MS analysis during the active winter season, and the composition of lure formulated using synthetic analogues of the natural compounds.

Volatile compounds	Relative composition of secretions [a]		Quantitative composition of lure (µl)
	Female	Male	Female
Hexanal	15 *	26 *	3.38
(E)-2-Hexenal	24 *	144 * * *	5.41
(E)-2-Hexenol	11 *	56 * *	2.47
(E)-2-Octenal	9 *	278 * * *	2.03
(E)-2-Octenol	7 *	53 * *	1.58
Nonanal	42 *	32 *	9.46
Dodecane	6 *	42 *	1.35
Decanal	87 * *	45 *	19.59
Tridecane	444 * * *	455 * * *	100
Decanoic acid	38 *	5 *	8.56
Pentadecane	24 *	116 * *	5.41
Hexadecanal	11 *	20 *	2.48
Tetradecanoic acid	23 *	27 *	5.18
Icosane	28 *	9 *	6.31

[a] The relative compositions of the constituents identified in the effluvium of the insects were obtained by integration of the peaks in the TICs of the secretions. Relative composition, * * *, main compound (>120); * *, median compound (50 to 120); *, minor compound (<50).

2.3.2 Field trapping trial

Throughout the trapping period the highest total number of bugs caught at any particular site, irrespective of trap type, was 561. This occurred at site 2, which also yielded the highest number of bugs per trap type with 160 recorded for AS traps followed by 155, 125 and 115 for SS, AC and SC traps, respectively (Fig. 2.9). Site 1, which was on the same farm, (Vadersgawe), as site 2, yielded the lowest total number of bugs caught throughout the trapping period – only 53. Of these, 38 were caught in SS traps. Total numbers of GCBs caught throughout the trapping season at site 3 and 4, on Eselfontein farm, were 140 and 335 respectively. At both these sites the highest numbers of GCBs were caught in AC traps (80 bugs at site 3 and 110 at site 4).

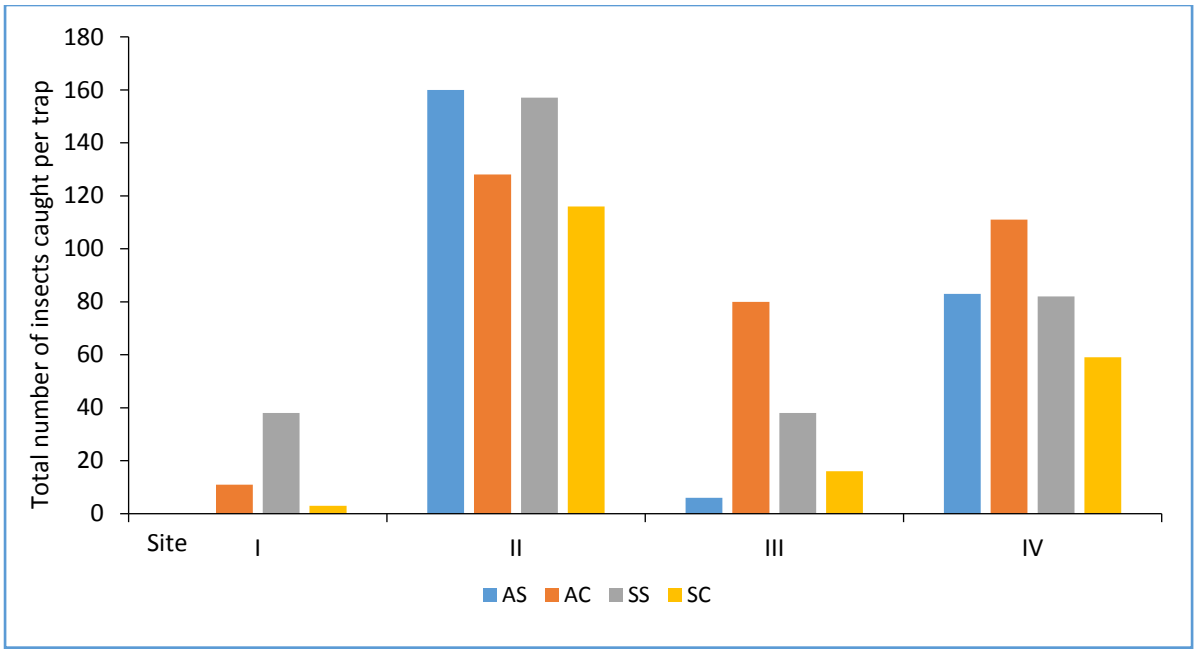

Fig. 2.9. Total number of grain chinch bugs caught per trap per site during the 2014/15 trapping period in Ceres. The trap and lure combinations applied were; aggregation pheromone lure + cardboard strips (AC) and aggregation pheromone lure + sticky pad (AS), sex pheromone lure + cardboard strips (SC) and sex pheromone lure + sticky pad (SS).

Although there was a difference in the total number of bugs present at the different sites, there was no significant difference ($F_{(4; 4)} = 2.18$, $P > 0.05$) between the mean number of GCBs caught per trap type, irrespective of the site, throughout the 2014/15 trapping period (Table 2.2). All trap types yielded a mean of less than 100 bugs per trap throughout the trapping period.

Table 2.2. The mean GCB trap catches for aggregation pheromone lure + sticky pad (AS), aggregation pheromone lure + cardboard strips (AC), sex pheromone lure + sticky pad (SS) and sex pheromone lure + cardboard strips (SC) collected from the four experimental field sites during the 2014/2015 trapping period in Ceres.

Trap & lure combination	Mean trap catch	±Std. error
AS	62.25 a	37.67
AC	82.50 a	25.82
SS	78.75 a	30.89
SC	48.50 a	25.48

* Figures followed by the same letter are not significantly different at ($P > 0.05$).

The cardboard bands tied around the trunks of trees in the same orchards as those with traps, indicated that there were much higher numbers of GCBs present in orchards at the time of the trial, than was indicated by the traps, suggesting that the traps were very poor at attracting GCBs. The total number of bugs found sheltering in the cardboard bands positioned at the top and bottom of sample trees at each site are shown in Fig. 2.10.

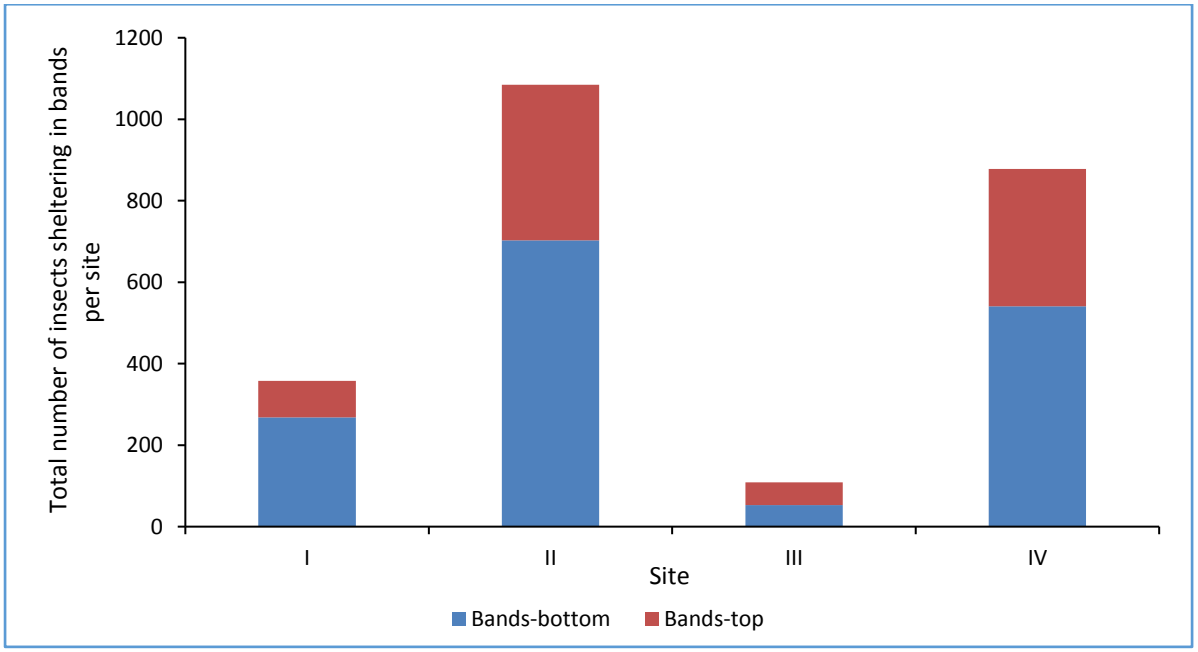

Fig. 2.10. Total number of grain chinch bugs sheltering in the corrugated cardboard bands placed at the top and bottom positions on sample tree trunks at each site tested during the 2014/15 trapping period in Ceres.

Site 2 had the highest number of GCBs sheltering in corrugated cardboard bands tied around tree trunks for the entire trapping period. A total of 1085 bugs were collected from bands at this site, while 358 were collected at site 1, which was on the same farm. Site 4 also yielded high numbers of GCBs in bands with a total of 878, while site 3 which was on the same farm, only yielded 109 GCBs in total. This was the lowest number recorded for total band counts at any particular site.

The total numbers of bugs found sheltering in bands at the different sites is an indication of the potential trap catch that was exposed to the pheromone baited traps at each site. At site 1, traps caught a total of 52 compared to 358 bugs found in bands, which was 6 times less than what the traps could potentially have caught during the trapping period. Half the potential catch was recorded in traps at site 2, with 561 bugs compared to 1085 found in bands. At site 3 trap catches were slightly more than cardboard band numbers, with a difference of just 31 bugs. Site 4 traps caught 335

while bands had 878 bugs, indicating more than double the number of bugs in bands than in pheromone traps.

A preference for sheltering position on trees was observed from the numbers of bugs sheltering in cardboard bands tied at the top and bottom of tree trunks (Table 2.3). Significantly more bugs were found sheltering in cardboard bands positioned at the bottom of the tree trunk in Site 1 ($P = 0.0058$), Site 2 ($P < 0.0169$) and Site 4 ($P < 0.0496$), compared to those tied on top. There was no significant difference ($P > 0.4115$) in the number of bugs found sheltering in cardboard bands at the top and bottom of the tree trunk in Site 3. This indicates that bugs preferred sheltering in bands on bottom of trees more than they preferred sheltering in bands on top positions.

Table 2.3. Median scores of grain chinch bugs sheltering in corrugated cardboard bands at two tree trunk positions in each of the four experimental sites tested during the 2014/15 trapping period in Ceres.

Site	Band position on tree	Median catch
1	Top	13.66a
	Bottom	23.33b
2	Top	14.31a
	Bottom	26.69b
3	Top	19.92a
	Bottom	17.08a
4	Top	15.06a
	Bottom	21.94b

*Figures followed by the same letters are not significantly different ($P > 0.05$).

2.4 DISCUSSION

Tridecane was the most abundant volatile compound identified in aestivating GCBs from previous studies by Oliver et al. (1996) and Okosun (2012), as well as in the active bugs used in this study. Tridecane is a waxy cuticular hydrocarbon that has been reported to play an important role in water retention physiology and preventing desiccation of the insect body during aestivation (Burdfield-Steel & Shuker 2014). Tridecane has also been reported to work synergistically with other volatile compounds such as *(E)*-2-hexenal, undecane and dodecane as irritants in many true bugs (Whitman et al. 1990; Gunawardena & Herath 1991; Aldrich 1996; McBrien & Millar 1999) or deterrents for chemical defense in the stink bug, *Cosmopepla bimaculata* (Krall et al. 1999). The cuticular components tridecane, hexyl acetate and *(E)*-2-decanal also have fungicidal roles (Jackson 1983; Surender et al. 1987; Sosa-Gomez et al. 1997).

Hexanal, *(E)*-2-hexenal and tridecane were identified by Okosun (2012) as the most attractive aggregation pheromone compounds to both males and females, *(E)*-2-hexenal being the most attractive to males during behavioural bioassays. Oliver et al. (1996) looked for attraction chemicals in the GCB and discovered the same three compounds, among a few others, but could not prove their attraction capabilities. The insects displayed behaviour synonymous with alarm responses when exposed to these compounds and the authors concluded that they were not attraction pheromones, but defensive pheromones.

There were common volatile compounds in the present study that have previously been identified in aestivating males and females. However, these compounds were not retested in sex attraction bioassays, which would have helped in their characterization in the present study as was done in the previous study by (Okosun 2012). The compounds in their varying compositions identified by Okosun (2012) were, tridecane in the highest amounts, *(E)*-2-hexenal, *(E)*-2-octenol and *(E)*-2-octenal in median amounts, *(E)*-2-hexenol in low amounts and hexanal in trace concentrations.

In the present study, four compounds were identified in median amounts and these were *(E)*-2-hexenol, *(E)*-2-octenol, decanal and pentadecane. Out of these four compounds only decanal was higher in females than in the males. The majority of the compounds identified in females were minor components, supposedly of the putative sex pheromone which are hexanal, nonanal, dodecane, hexadecanal, tetradecanoic acid, decanoic acid and icosane.

The main components, other than tridecane, found in active GCBs in this study, were *(E)*-2-hexenal and *(E)*-2-octenal, which were both high in males compared to females. These two compounds are known to be main components of stink bug defensive secretions, and their role as defensive chemicals has been revealed in several other studies involving defense secretions in Heteroptera species (Farine et al. 1993; Aldrich et al. 1993; Leal et al. 1994; Krall et al. 1999; Baldwin et al. 2014). These two aldehydes are of an acrid nature and are known to be major components of stink bug defensive secretions with increased efficiency when blended with tridecane (Farine et al. 1993; Krall et al. 1999).

Everaerts et al. (2010) suggested that tridecane is probably a wetting agent that facilitates the dissolving and evaporation of other compounds, and therefore plays an important role in aggregation. This means that tridecane may play a synergistic role in the efficient functioning of either sex, aggregation or even defense pheromones. This school of thought disqualified the use of male volatile compounds in formulating the putative sex pheromone lure leading to the use of female volatile compounds instead in this study. Also, since sex pheromones are produced by one gender to attract the other (Farine et al. 1993), leading to the assumption that the behaviour was a result of a pheromone used by females for attraction of males for mating.

The two aldehydes *(E)*-2-hexenal and *(E)*-2-octenal are major pheromone components of the defensive scent of the black stink-roach, *Platyzosteria novaeseelandiae* (Benn et al. 1977). They have also been reported by Jacobs et al. (1989) as major components of the metathoracic scent glands in the bugs,

Thaumastella namaquensis and *Thaumastella elizabethae.* Interestingly, *(E)*-2-hexenal was found to have a dual role in the southern green stink bug, *Nezara viridula* were it was attractive at low concentration and repellent at high concentration (Lockwood & Story 1986). In the present study, *(E)*-2-hexenal was found in low concentration in females and could have contributed as a trace component of the female sex pheromone. According to Okosun (2012), *(E)*-2-hexenal alone attracted the most males in aggregation pheromone bioassays conducted on GCBs and it was in median to low relative concentrations.

Lofstedt et al. (2008) reported that a synthetic bait containing two alcohols heptanol and nonanol extracted from female caddisfly, *Molanna angustata,* were attractive to males and gave the highest trap catches in the field. However, trap catches reduced when nonanol which was the main component of the pheromone was mixed with non-alcohol compounds of the same secretion. Hexadecanal is an unsaturated aldehyde and is also a component of the sex pheromones of many moth species (Daimon et al. 2012; McElfresh & Millar 1999; Uehara et al. 2016). These two compounds may contribute to the sex pheromone in GCBs, but might have been masked by other compounds present. According to Farine et al. (1993) insect secretions are continually inclusive of trace or minute components and the drawback with such components is that their significance may always be neglected even though their effects may still be detected. These compounds may potentially contribute to the discovery of attractive volatile compounds from the female GCBs and may be candidates for future work on GCB response to attraction pheromones.

In the present study, four compounds were identified for the first time in various proportions in active adult GCBs. There were nonanal, decanal, decanoic acid and icosane. Nonanal and decanal have been identified before as airborne aggregation pheromone components mediating aggregation in the common bed bug, *Cimex lectularius* (Siljander et al. 2008). Torto et al. (1996) identified hexanal, octanal, nonanal, decanal and decanoic acid as components of an aggregation pheromone in fifth instar nymphs of the desert locust, *Schistocerca gregaria* after testing a synthetic

mixture in laboratory bioassays. The authors also proved that the pheromone was produced by both males and females supporting previous work by Obeng-Ofori et al. (1993).

There was an increased relative concentration of volatile compounds noticed in males more than in females during the active season. The reason for this could have been that the male gender produces a strong emission of the defensive volatile compounds during the active season. This is highly likely as most of the compounds found in higher proportions in males have been reported to play alarm and defensive roles in other Hemiptera insects (Farine et al. 1993). The compounds *(E)*-2-hexenal and *(E)*-2-octenal have been reported to be the major constituents of scent gland secretions causing alarm and defensive behaviour in the bedbug, *Cimex lectularius* (Levinson et al. 1974). Eight volatile compounds were discovered in the secretions of a Hemipteran bedbug, *Dysdercus intermedius* which also displays aggregation behaviour. The mixture had dodecane, tridecane, pentadecane, hexanal, *(E)*-2-hexenal, 4-keto-2-hexenal, *(E)*-2-octenal and 4-keto-2-octenal (Calam & Youdeowei 1968). This mixture is common in other Hemiptera and Pentatomidae species secretions comprising mainly of the irritants, hexanal, and octenal in high amounts. These irritants are common in stinkbug species such as *Eurydema rugosa*, *Eurydema pulchra* and *Nezara viridula* (Ishiwatari 1974).

Regrettably no individual volatile compounds isolated from active GCBs were tested under laboratory conditions in behavioural bioassays in the present study unlike in the previous aggregation studies by Oliver et al. (1996) and Okosun (2012). This was a result of limited time between formulating the lure and running the laboratory bioassays, before conducting the field trials. This would have assisted in finding the most attractive compounds from females, prior to formulating the sex pheromone lure and testing it under field conditions. Characterisation of individual compounds identified from GCB headspace samples would have been beneficial if it had been carried out beforehand as it is vital in improving the attractiveness of a formulated lure. Chemical ecology research has shown that either gender in some insect species can

make use of several blended compounds in creating sex pheromones (Roelofs & Carde 1977; Kochansky et al. 1989; Carde 1990).

There is a possibility that the chemical compounds isolated from GCBs may also be contact or short range pheromones that only elicit attraction at very close range. The efficacy of GCB volatile compounds may have diminished at this time as a result of weak or contact pheromones with poor diffusion propensity in the field, thereby reducing the effective insect response to lured traps. This will need to be reviewed in future trials. An example of contact pheromones is that of the fruit fly, *Drosophila melanogaster* whose attractive hydrocarbons work as short distance pheromones (Everaerts et al. 2010) and are only effective at close range for mating. Lygaeidae are known to recognize their kin using either aggregation compounds or cuticular hydrocarbons in different seasons (Aller & Cardwell 1979).

Rigorous trials need to be carried out preferably for more than two seasons using the two synthetic pheromone lures in order to comprehensively test their efficacy. Analyses of male and female secretions carried out over a total period of 5 years from a previous investigation and the present study, varied greatly regarding the quantitative compositions of the volatile emissions trapped from the headspace of the active insects. This is a result of the different methods of extracting volatile compounds used, and also the different periods in which the investigations were carried out. Of the 14 components identified in the active GCB secretions in the present study, four compounds have not been described before as either aggregation or defense pheromone constituents in Lygaeidae. Quantities of nonanal, decanoic acid and icosane varied insignificantly between males and females except for decanal which was higher in females. This suggests that the GCBs secrete a mixture of volatile compounds that may always be present in both gender but, in various concentrations according to the season, as expected under normal pheromone function dynamics. If one or all compounds identified here are found to be attractive components of the sex pheromone, they may be used in various ways to control and monitor GCBs. One

method would be to divert the bugs into traps using the sex pheromone lure during their reproductive phase, before they start migrating to aestivation sites.

In the present study not more than 2500 bugs were found sheltering in bands. The total number of GCBs in orchards was relatively low during the 2014/15 trapping season in Ceres as compared to other previous studies. Addison (2004) used corrugated cardboard bands to inspect the seasonal occurrence of GCBs in Ceres for three years in pear orchards and caught more than 1000 GCBs per band per site on average during the investigation period. Johnson & Addison (2008) recorded a total number of more than 6000 bugs in a nectarine orchard in Ceres caught during a single season in bands. In another study an average number of more than 8000 bugs per site were caught in bands in Ceres (Okosun 2012). On this occasion, the number of bugs caught during the recent field trial indicated a generally low GCB orchard infestation level, in comparison to what has been found in other studies also using cardboard bands.

The number of bugs in bands tied around the tree trunks showed there was a sheltering preference, as more bugs sheltered in bottom bands as compared to top bands. This indicates that the shelter seeking behaviour of GCBs is influenced by their preference for sheltering sites on the bottom of trees. However, this does not mean they do not seek shelter on higher positions in the tree canopy since a considerable number were caught in the top bands. This could only be an indicator of the conditions that attract the bugs to stay at the bottom of tree canopy. One challenge is that insects may not only be attracted to chemical compounds but may also respond to other sources of stimuli under field conditions. Insects may respond to temperature and humidity among other environmental factors which may influence where they go. One of the reasons could be the cool, moist and shady conditions that are usually associated with the bottom of tree canopy in orchards. Since more GCBs were caught in bottom bands in orchards it would be useful to consider placing baited traps on the bottom parts of trees rather than on top in order to catch more bugs in the future. This

is crucial to consider when designing traps in the future for efficient placement of traps to catch more bugs.

A complex mixture of volatiles have so far been identified from analyzing secretions from *M. diplopterus* carried out over the past few years during both winter and summer seasons. The previous studies and the present investigation have provided widely comprehensive results as far as the quantitative compositions of the secretions of male and female insects are concerned. Future studies must now focus on characterizing individual components identified from the female secretion. This will bring us closer towards a full understanding of the individual compounds and the roles they play during the active season. Modifying the concentration of both lures in future investigations may improve GCB trapping. This can be achieved by increasing lure concentrations and retesting what distance the pheromones would be carried by the wind. From this study we saw that the abundance of GCBs was higher at lower levels of the tree trunk in the cardboard bands. In this study, delta traps were hung at 1.5m, the same height as the higher bands on the tree trunks and we now know that the potential catch is lower at that height. Hanging the lured traps lower down the tree trunk could increase trapping of GCBs.

To conclude, this investigation into the volatile compounds produced by active GCBs, and their potential in trapping adult bugs in the field has contributed to our growing knowledge of the chemical ecology of this agricultural pest, and highlighted important considerations that must be taken into account as we endeavour to develop a practical monitoring system and ultimately provide pre-harvest management options for a key phytosanitary pest of South African export fruit.

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CHAPTER 3

BEHAVIOURAL ORIENTATION OF THE SHELTER-SEEKING GRAIN CHINCH BUG, *Macchiademus diplopterus* TO VISUAL TARGETS IN AN ARENA

3.1 INTRODUCTION

Like many organisms (insects and higher animals) which employ migration skills as a strategy to escape various environmental challenges, adult grain chinch bugs (GCB) *Macchiademus diplopterus* (Distant) (Hemiptera: Lygaeidae) are known to migrate in search of shelter when host plants, such as wheat and other grain crops are harvested in the summer season (Myburg & Kriegler 1967). For many migrating insect species, adverse conditions such as high temperatures and drought may come along as a result of changes in the local environment or due to the onset of a new season. The migrating insects may move long distances or simply move a few kilometres into new habitats to survive immediate ecological or seasonal changes. Some insects make use of complicated navigational techniques that may depend on natural maps and compasses and in some cases magnetic cues and polarised light (Mouritsen & Frost 2002; Stalleicken et al. 2005).

Once migration has begun, feeding and reproduction activities come to a halt or are suspended for a while depending on the species (Kennedy 1985). Migrating organisms also have the potential to choose where to settle and this behaviour is known as habitat selection (Huntingford 1984; Carde 2000; Merlin et al. 2012). The distribution of a species after migration is also limited by the behaviour of members choosing their habitats. This behaviour may be influenced by food or predator risk leading individuals into dense habitats (Hilden 1965; Verner 1975; Bechard 1982; Wywialowski 1987).

In insects, host profile or habitat acceptance during shelter selection depends on completing a series of multiple sensory processes (Atkins et al. 1987). These multiple sensory signals contribute towards modifying insect behaviour leading to the adoption of suitable body orientation positions (Rowell & Reichert 1985). The influence of learning and inherited choices in selecting habitats are not yet known in many insects (Rolstad et al. 2000). To a larger extent, environmental cues are usually adopted and used in decision making while landscape and terrain forms are likewise utilised in selecting sheltering sites. Visual and olfactory cues have been used by many foraging insect species in evaluating their surroundings with the additional use of taste sensory channels (Lanier 1983; Fawcett & Johnstone 2003). It is known that certain terrestrial insects such as the desert locust, *Schistocerca gregaria* and *Lymantria* caterpillars are capable of utilising visual information of tree trunk arrangement when orienting in their habitats (Vinson 1998). They achieve this through discriminating different plant types and forms that are synonymous with their host habitats (Vinson 1984; Rolstad et al. 2000; Olson et al. 2003).

Multiple laboratory experiments have used wood crickets *Nemobius sylvestris* to show that terrestrial visual signals aid in orientating in their natural environment using similar cues such as tree stem formations (Campan & Gautier 1975; Beugnon 1982). Similar experiments proved that the visual behaviour of *N. sylvestris* was a result of positive scototaxis, which involves the movement towards low reflection targets (Campan & Medioni 1963; Campan & Lacoste 1971). The spider, *Arctosa variana,* shows an escape reaction in its natural environment towards the direction of dark objects guided by the position of the sun (Papi & Tongiorgi 1963). It reacts to signals from terrestrial structures such as a horizon silhouetted against the sky assisting it to orientate towards river banks (Campan & Gautier 1975).

Foraging insects are known to use the most accurate, easy and readily available terrestrial structures for directions, an action which mainly relies on visual cues (Thorsteinson 1960). Insects are likely to use the most important indicators such as shape, colour, arrangement and magnitude of habitat in locating hosts for shelter or

food, despite having poor eye resolution (Honegger 1981; Michaud & Mackauer 1994; Hoffmeister et al. 1999; Fischer et al. 2003; Lobdell et al. 2005). Multimodal cues have been investigated in wood boring beetles that demonstrated strong visual preferences enabling them to distinguish between hosts and non-hosts (Campbell & Borden 2005).

Since GCBs start searching for sheltering sites before entering into aestivation, the aim of this study was to determine whether they orientate themselves towards certain visual profiles. This follows after Campan & Gautier (1975) who suggested that insects living in low contrast environments have the ability to orient using visual landmarks that they learn to use from the beginning of life. Orientation responses to these various targets and how they may induce positive visual reactions in adult GCBs is not yet known. Therefore, in this chapter I look at visual behavioural orientation responses of GCBs to gain an understanding of how this behaviour may play a role in influencing where to seek shelter during the aestivation season. The objective was to test their visual preferences towards four different shapes of different colours. This is crucial as it contributes to an insightful understanding of the GCB shelter-seeking behaviour. Understanding how GCBs select shelter sites may be used in creating visually attractive targets for development of effective traps. Effective traps would assist in the development of GCB monitoring and management practices, to assist in reducing fruit infestations and thereby diminishing the rejection of South African export fruit by importing countries.

3.2 MATERIALS AND METHODS

3.2.1 Insect collection

Aestivating adult GCBs were collected from sheltering sites under the bark of blue gum trees *Eucalyptus globulus* during the period January to March 2015, in areas surrounding Malmesbury (33.45ºS,18.73ºE) and Wellington (33.38ºS,18.59ºE) in the Western Cape, South Africa. Insects were brushed from bark and trees into ventilated plastic containers. Loose bark from blue gum trees were placed inside the collection containers to provide shelter for the insects. Insects were immediately transported to the laboratory and kept in the plastic containers at ambient temperature until use. Experiments were conducted within 7 days of collection.

3.2.2 Behavioural response to visual cues experiments

Behavioural response experiments were conducted in a test arena constructed of Perspex material in the form of a square measuring 50 cm x 50 cm x 30 cm (Fig. 3.1). It was covered on the outside with a sheet of white paper around all the four sides and the arena floor. This was done to limit the vision of the insects to targets inside the arena. The lid of the arena was designed to fit tightly to the arena to prevent the escape of insects. The centre point on the floor of the arena was marked as the insect release point.

Fig. 3.1. Test arena constructed of Perspex material with all four sides and floor covered with a sheet of white paper.

The behavioural response of adult GCBs to visual cues was tested in three successive experiments. The first experiment tested their response to black silhouettes of four different shapes; rectangle, square, triangle and circle. The second experiment tested responses to four different colours (black, red, green and yellow) on rectangular shaped silhouettes (preferred shape from the previous experiment). The final experiment tested responses to a vertical striped pattern in two different combined colours (preferred colours in the colour preference experiment).

All experiments were conducted inside a laboratory which was illuminated with fluorescent lights (2500 lux at height 3 m) resembling the natural sunlight. The lights provided an even coverage of the entire room and the experiments were conducted at room temperature (22-26 ºC) at relative humidity conditions of 65-75 %. Within the arena paper silhouettes were used as targets representing the different visual profiles/landmarks found in the natural environment. Targets were cut from 1 mm thick paper sheets to equal relative sizes according to area (cm^2). In the behavioural response to different shapes, rectangular, square, triangular and circular targets were cut from black paper and a shape was affixed to the centre of each inner wall in upright position, with the base of the shape touching the arena floor (Fig 3.2).

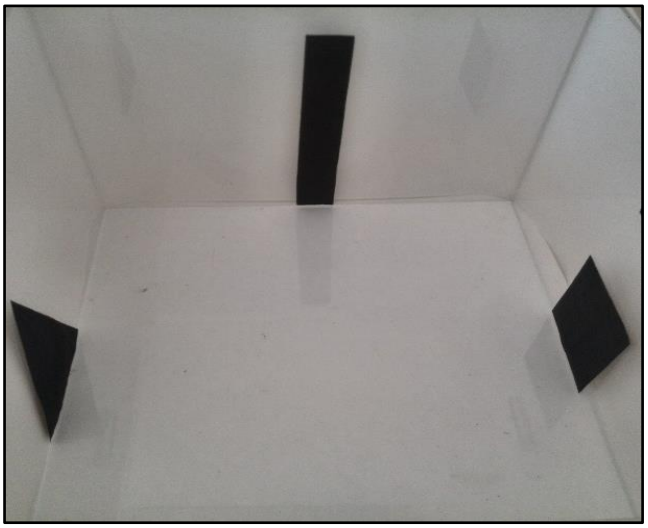

Fig. 3. 2. The test arena showing only three of the black shapes (circular shape not shown here) and how they were firmly affixed to the middle of walls inside the arena before insects were introduced.

For response to different colours, targets were cut from black, red, green and yellow paper and affixed to the inner walls (Fig 3.3).

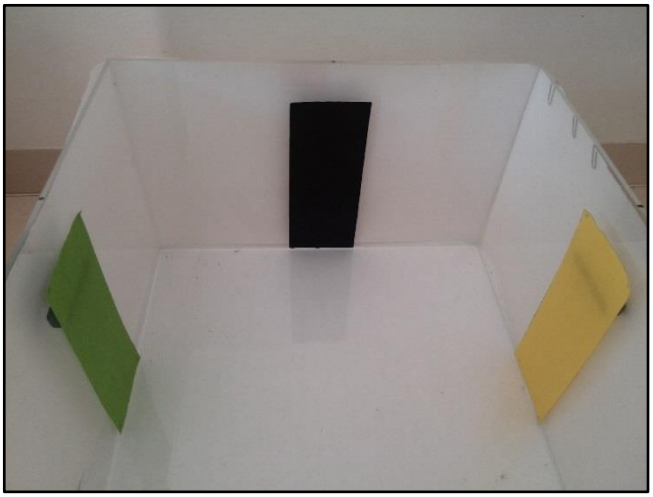

Fig. 3.3. The placement of vertical rectangle shapes of different colours on inner walls of arena showing only three of the four coloured shapes (red shape not shown here).

To test the response to vertical stripes of black and white, as well as red and white colour patterns, the two targets were affixed next to each other on one wall, leaving the other 3 walls of the arena blank (Fig 3.4).

Fig. 3.4. The placement of vertical stripes of black and white, red and white colour patterns and how they were affixed next to each other on one wall inside the arena.

Each run in a behavioural response experiment was carried out by releasing 20 insects into the centre of the arena. The lid was placed on the arena and the insects were exposed to targets within the arena for a period of 30 min. After each exposure period the position of the insects inside the arena was recorded. Insects that were at the targets or within a 5 cm radius of a target were recorded as being on-target. Insects that had moved from the centre of the arena, but were not at or close to a target, were recorded as being off-target, and insects that did not move from the centre were recorded as sedentary. Once insect positions were recorded, insects were removed and the arena was rotated at 90^0 in a clockwise direction to cancel any directional influence on further experiments, and the inner walls of the arena were cleaned using 70% ethanol. Insects already used were not retested to minimise the effects of previous experience. As a control run, no target was presented inside the arena and the position of insects after 30 min was recorded. Response to shape experiments were repeated 8 times (N = 160), response to colour 16 times (N = 320) and response

to vertical striped colour pattern 12 times (N = 240). Four control experiments were carried out using 80 insects for each response experiment.

3.2.3 Statistical Analysis

Statistical analysis of data was based on SAS's general linear models procedure (SAS Enterprise Guide 2014). Shapiro-Wilk test was used to test for normality of data for all experiments. In the case where the data were not normally distributed non-parametric data analysis was performed using Kruskal-Wallis test with multiple comparison tests for pairwise comparisons. Significant means were separated using the Bonferoni t-test at P = 0.05.

3.3 RESULTS

3.3.1 GCB responses to shapes

In the experiments testing the behavioural response of adult GCBs to four shapes in the arena, the rectangle had the highest number of insects visiting with 33.15%, followed by the square with 16.88%. A moderate 13.75% of the insects visited the triangle, while another 13.75% also moved from the centre but did not go to any particular shape and were recorded as off-target. The circle attracted 11.88% of the insects while 10.63% of the insects did not move from the centre of the arena and remained sedentary (Fig 3.5).

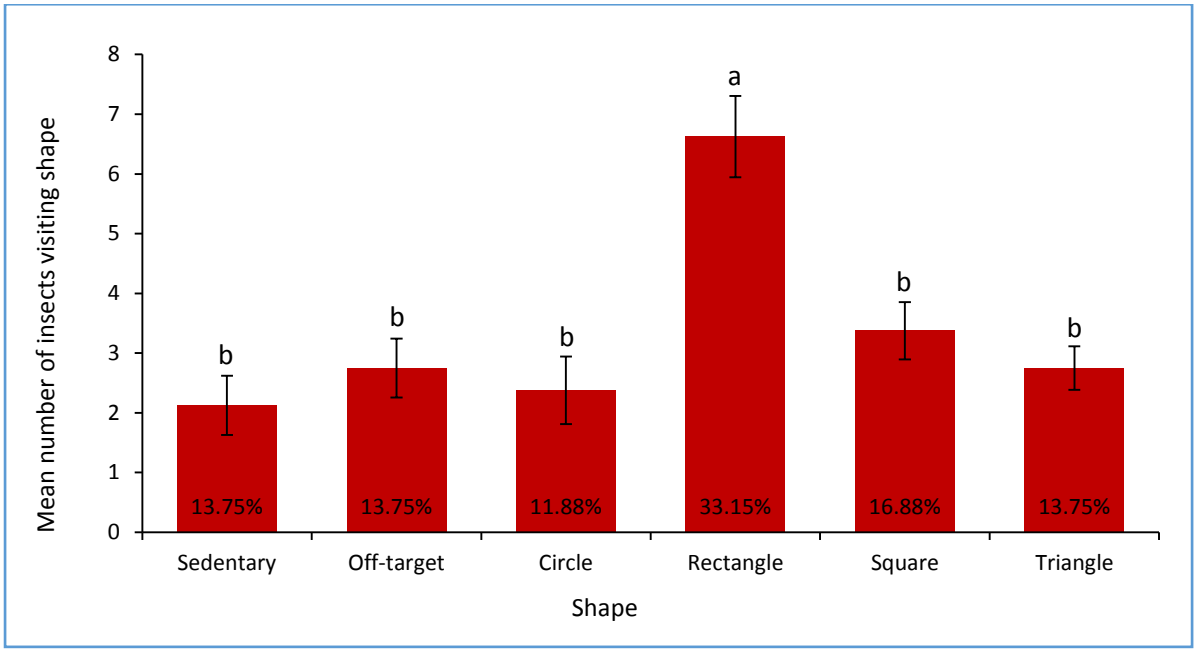

Fig. 3.5. Mean and percentage number of insects (±S.E) recorded as sedentary, off-target and on-target visiting the four shapes affixed to walls of arena in the GCB behavioural response to shape experiments. Means with the same letter are not significantly different (α = 0.05). N= 160.

Significantly more insects (P = 0.0001) visited the rectangle shape and settled there more than they did for the other three shapes. There was no significant difference (P > 0.05) between insects visiting the square, triangle and circle targets or off-target and sedentary insects.

3.3.2 GCB responses to colour

Using the preferred shape, as observed in the previous experiment, the influence of colour on the GCB responses was tested, and the number of insects visiting each coloured rectangle were recorded (Fig 3.6). The results indicated that 39.07% of the insects preferred orienting towards red rectangle targets while 34.69% chose black rectangles. Green rectangle targets had 10.94% of the visits, while 6.25% of the insects went off-target or remained sedentary in the middle of the arena. Yellow targets had the least number of insects visiting them with only 3.44% choosing this colour.

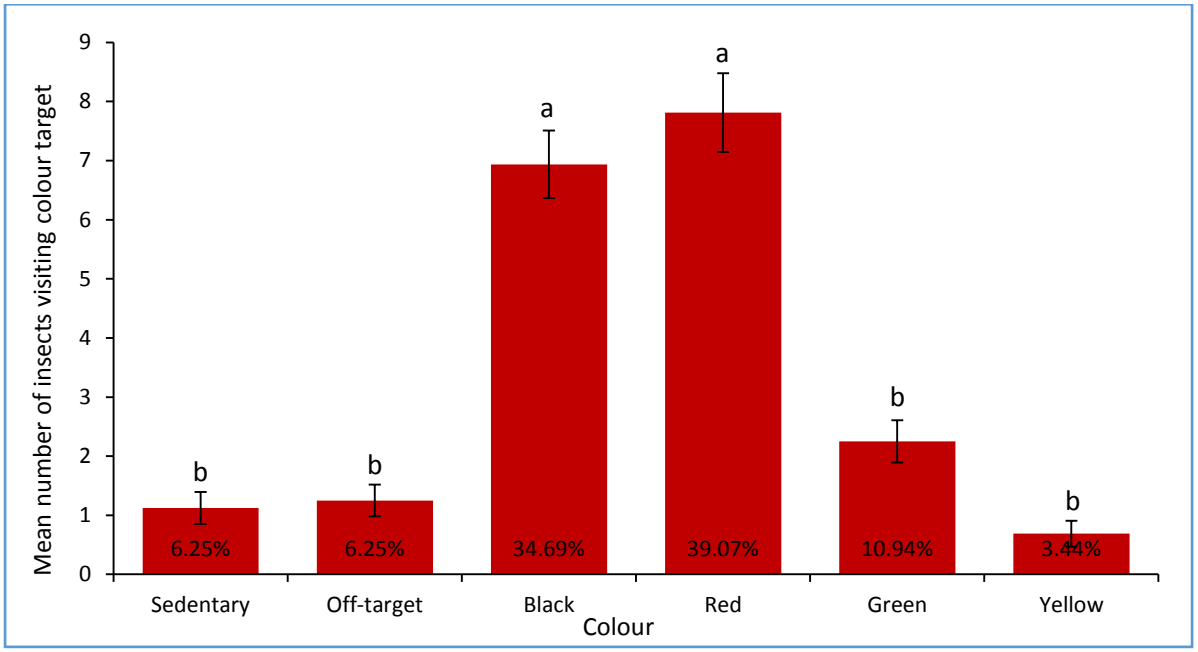

Fig. 3.6. Mean and percentage number of insects (±S.E) recorded as sedentary, off-target and on-target insects visiting the rectangular targets of four different colours in the GCB behavioural response to colour experiments. Means with the same letter are not significantly different (α = 0.05). N = 320.

Insect visits to black and red rectangle targets were not significantly different (P > 0.150) from each other, but both were significantly different (P = 0.0001) from the rest of the other coloured rectangle targets. The results show that GCBs oriented more towards the two dark contrasting colours, black and red, during these experiments.

3.3.3 GCB responses to striped colour patterns

Responses to striped patterns using the two preferred colours seen in the previous experiment, red and black, were tested using vertical stripes of black and white, and red and white and the mean number of insects visiting each pattern were recorded (Fig 3.7). The black and white pattern had the highest number of visits with 41.25 % compared to the red and white pattern with 36.25 % of the visits. Only 13.75% of the insects went off-target, while 8.75% remained sedentary in the centre of the arena.

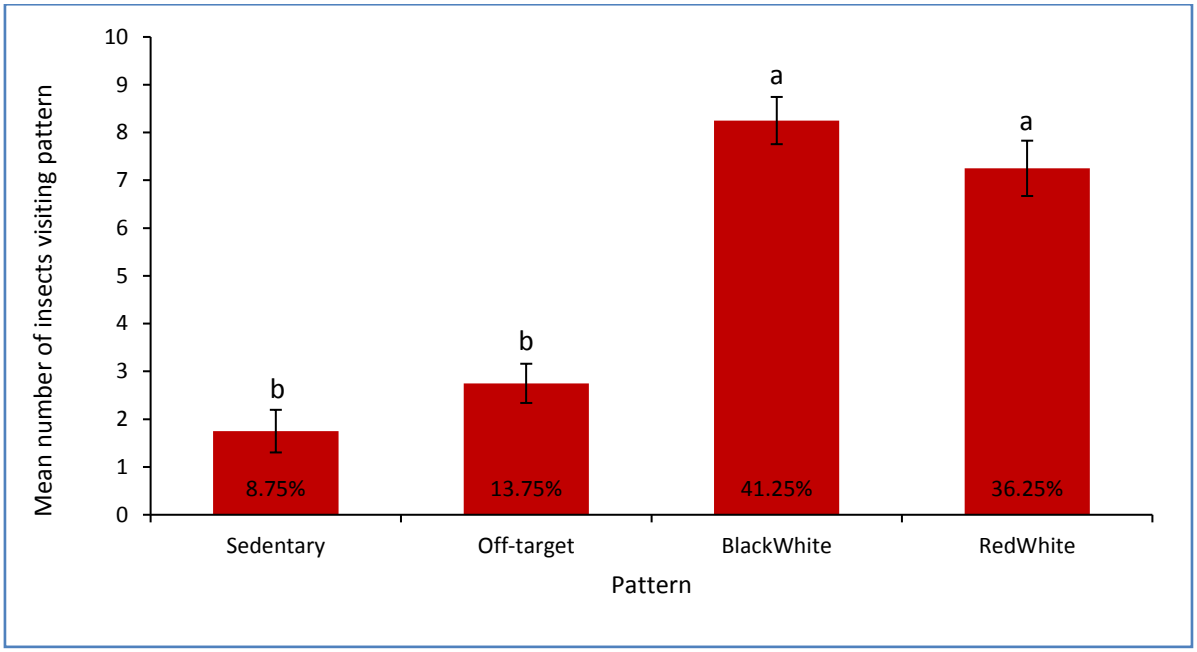

Fig. 3.7. Mean and percentage number insects (±S.E) recorded as sedentary, off-target and on-target insects visiting each striped colour pattern. Means with the same letter are not significantly different (α = 0.05). N = 240.

There was no significant difference (P > 0.153) between insects visiting the black and white pattern and the red and white pattern, but both striped patterns were significantly different (P = 0.0001) from insects that went off-target and those that remained sedentary. This indicates that GCBs were equally responsive to both black and white, as well as the red and white stripes during the experiments. This illustrates the possibility of a similar influence of the two dark contrasting colour patterns in inducing attraction reactions in GCBs when placed as targets in an arena.

Both the dark and upright striped rectangles tested in either of the two colour patterns, black and white and red and white, triggered an equally positive scototactic reaction in GCBs. Both colour patterns induced the insects to orient and move towards the dark upright striped shapes. Control experiments were carried out without targets presented inside the arena and the GCBs showed no particular direction preference in their movements. More than 90% of the insects stayed mostly in the middle of the arena floor away from the walls (results not shown). This indicated that bugs oriented towards certain directions only when there were targets in sight.

3.4 DISCUSSION

Aestivating GCB adults exhibit positive scototactic behaviour when presented with different visual cues. GCBs had a tendency to move towards vertical rectangular shaped targets more than square, triangle or circle targets, and bugs oriented more towards dark vertical surfaces by preferring to move towards black and red rectangle shapes, more than they did for green and yellow rectangle shapes. A similar level of attraction to both black and red, with or without vertical white stripes, is also indicative of positive scototaxis. Insects do not see red, but rather, are known to see colours more at the far left of the light spectrum, close to violet (Chapman 1998). The GCBs probably saw red as dark colour. Although the results from this study were not always overwhelming in the numbers of bugs observed as preferring one target over another, the differences were significant, indicating preference.

The present study is comparable to the visual fixation responses observed in the wood-cricket, *N. sylvestris*, whose scototactic orientation was described by Jeanrot et al. (1981). Their work measured the effectiveness of the different areas of the eye comparing the responses of insects to dark visual targets. They demonstrated that black stripes induced strong visual orientation in crickets placed on a horizontal plane facing upright targets. Additionally, Jeanrot et al (1981) observed that the ability to move heads freely in the wood-cricket enlarges their effective visual field by almost 20 ^{o} towards rectangular black visual targets. Campan & Gautier (1975) also showed that black and white bars induced *N. sylvestris* to orient towards vertical targets more than they responded to similar horizontal targets. The same is true for *M. diplopterus* which responded positively towards all dark vertical rectangle targets in this study. A possible explanation for target preference behaviour observed in both species, *M. diplopterus* and *N. sylvestris*, concerns the habitats they live in , and being surrounded by wood landmarks of dark tree trunks that are vertically oriented. Visual terrestrial cues provided by such landmarks have been revealed to be used by *N. sylvestris* to guide its orientation (Campan & Gautier 1975). The similarity in visual appearance of

landmarks between these two species' environments is reasonable enough to assume a similar orientation reaction to vertical objects.

In a study involving the field cricket, *Acheta domesticus*, scototactic responses were tested towards various shaped targets in an arena using a compensatory treadmill and it was seen that the crickets oriented towards horizontal dark targets (Atkins et al. 1987). However, vertical targets induced little positive orientation and this may also be related to its natural environment which is mainly composed of horizontal features such as rocks and cracks. The appearance of the environment influences the preference for targets (Atkins et al. 1987). Anderson (1989) stated that species from shady habitats usually orient towards familiar silhouettes that resemble areas with vegetation cover such as bushes and trees.

The level of visual responses observed in GCB and attraction to vertical dark objects, may be a reflection of commonality with their natural habitat when surrounded by a variety of other trees including blue gum trees. These vegetation structures provide vertical dark visual images from the position of GCBs in the wheat fields. These dark structures become their targets for seeking shelter sites when migrating from harvested wheat fields at the onset of aestivation. Some insect species such as the wandering spider, *Cupiennius salei* are able to make such strict direction choices due to the enhanced attractiveness of large vertical images that supply huge stimulus changes on the retina of the eye (Kaps & Schmid 1996). Vertical contours and inclined surfaces are known to trigger strong visual attraction signals that compel the insect to follow the direction of the visual stimulus (Lanier 1983; Lindgren 1983; Anderson 1989; Finch & Collier 2000; Campbell & Borden 2005).

Two species of bark beetles, *Dendroctonus psedotsugae* and *Ips paraconfusus* utilize visual cues with the aid of two photoreceptors that react to blue light at a maximum flux of (450 nm) and green light at a maximum flux of (520 nm) (Groberman & Borden 1982). These bark beetles are also known to orient their bodies toward suitable shelter by making use of vertical stem silhouettes (Lanier 1983; Finch & Collier 2000). The

mountain pine beetles, *D. ponderosae* (Schonherr 1977) and *Trypodendron lineatum*, are both attracted to traps of dark colours such as black, brown and red and not yellow and white (Dubbel et al. 1985). Stacked black funnel traps create vertical silhouettes that resemble host tree conifers and are used in trapping most coniferophagous beetles (Lindgren 1983; Campbell & Borden 2005).

The findings from this study are encouraging and present a platform for further work on visual attractants of various forms for the GCB. This increases our knowledge and understanding of its visual orientation behaviour and how this influences the selection of sheltering sites at aestivation. This provides a good starting point for the development of novel management strategies against the phytosanitary pest. Dark rectangular shaped traps placed on the wheat-to-fruit orchard interface may help to reduce fruit infestations in orchards. For the South African fruit producers, this contributes towards lowering the risk of suffering fruit consignment rejections in the export market.

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CHAPTER 4

GENERAL DISCUSSION

The grain chinch bug is a quarantine pest that infests export fruit and as a result, threatens to negatively impact international trade in fruit products from South Africa. This study is an addition to a limited number of studies looking at mitigating the GCB pest problem that confronts the export fruit industry. The objectives of the study were to develop methods for GCB trapping using pheromone lures and modified traps, as well as better understand orientation behavior during aestivation. This was conducted by testing a previously identified aggregation pheromone lure, as well as a newly formulated sex pheromone lure in a field trapping trial using modified traps that incorporate shelter (Chapter 2); and by testing the visual responses of GCBs to shapes of different colours in a localized visual field in an effort to better understand their visual orientation behavior for the development of suitably attractive traps (Chapter 3). This chapter interprets the findings from both chapter 2 and chapter 3 and also provides recommendations for future research.

The present study compared the aggregation pheromone and tested the sex pheromone as well, using modified traps incorporating sheltering components, but was unsuccessful in attracting significant numbers of GCBs in these traps. This may be due to the fact that pheromone volatile compounds become unstable when impregnated into rubber septa dispensers in field experiments. This might have reduced their efficacy in dispensing, as in the case of the stink bug, *Chlorochora sayi* (Millar et al. 2010). Weather agents such as wind speed, humidity and temperature influence the rate of lure release under field conditions and may lead to poor lure attraction.

The compounds hexanal, *(E)*-2-hexenal, *(E)*-2-hexenol and tridecane, identified as the constituents of both the aggregation pheromone (Okosun, 2012) and the sex pheromone, in the present study, were identified before as defensive secretions by

Oliver et al. (1996). *(E)*-2-octenal and *(E)*-2-octenol were also previously identified by Okosun (2012) as part of the aggregation pheromone. Some of the major chemicals identified by Oliver et al. (1996) which were tridecane, *(E)*-2-octenal, *(E)*-2-hexenal and hexanal have been reported to stimulate alarm and aggregation reactions in true bug species including the cabbage bug, *Eurydema rugose* and the southern green stink bug, *Nezara viridula* (Ishiwatari 1976; Lockwood & Story 1985). It is most likely that GCBs use the same compounds as aggregation, sex or defence pheromones, but in different concentrations and ratios for various roles according to season.

Attraction pheromones present in GCBs, may be short distance or contact pheromones. This can be supported by the evidence from laboratory bioassays conducted by Okosun (2012) that showed some attraction in both sexes by the female pheromone compounds hexanal, *(E)*-2-hexenal and tridecane. These compounds however, did not elicit attraction under field conditions in a field trial that followed the laboratory assessments. The main reason for this is more likely due to the presence *(E)*-2-hexenal and *(E)*-2-octenal in relative concentrations high enough to inhibit the attraction capability of other compounds such as nonanol and decanal. The present study contributed to our knowledge around the pheromone compounds in GCBs by identifying four more volatile compounds found in GCBs during the active season: nonanal, decanoic acid and icosane which were found in marginally higher proportions in females than in males, and decanal, which was substantially higher in females. These volatile compounds may in the future be used in formulating an attractive sex pheromone lure to increase our ability to optimize the trapping of GCBs.

Cuticular hydrocarbons such as tridecane may also be used in optimizing GCB trapping. Tridecane was found in the highest quantities in samples collected in this study, and all previous GCB chemical ecology work reported here. Such hydrocarbons are waxy compounds which provide protection from desiccation and function on the cuticle assisting species to discriminate each other (Kather & Martin 2012). Insects also use these hydrocarbons to identify mating partners and to know where they have been by recognizing their own scent (Everaerts et al. 2010). A good example is that of

the cricket, *Gryllodes sigillatus* that recognizes its former mating partners by the scent it leaves on them after mating (Weddle et al. 2012). In *Drosophila melanogaster* flies, hydrocarbons work as short distance or contact pheromones (Everaerts et al. 2010).

In addition to the use of scents, the importance of shelter in soliciting attraction responses in aestivating GCB is highly evident as reported by Addison (2004), Johnson & Addison (2008) and Okosun (2012). They all found high numbers of bugs caught in cardboard bands tied around tree trunks during GCB orchard abundance inspections. As such, the use of modified traps in the present study was intended to improve trap catches since the insects exhibit a strong shelter seeking behavior. Despite using the modified traps, no improved trap caches were noticed in baited traps. This could be as a result of the size, position, shape and colour of traps used in the field trial which may not have been attractive to the bugs.

GCBs exhibit a preference for dark vertical shapes when seeking shelter. This attraction towards dark vertical objects suggests that the development of traps of a suitable dark and vertical nature would be more efficient in trapping the bugs. The GCB has a tendency to move toward low reflecting areas before they go against gravity when they reach the target. This could be due to the perception of images under a simple nervous system which is highly responsible for vertical silhouette preference in many insect species seeking shelter (Lanier 1983; Muller 2010). Low reflecting objects are considered attractive due to the contrast perceived at the edges of a vertical silhouette (Smith 1993). Further investigations of this potential for attraction needs to be tested in the field. Only a few investigations have been carried out on visual discrimination for sheltering sites especially for true bug species (Campbell & Borden 2006).

This study has also shown the influence of other cues associated with the shelter seeking behaviour of the GCB during the aestivation period, such as moisture and temperature. The bugs might be attracted to areas that offer conducive moisture and low temperature levels, such as found under cardboard bands tied around trees.

Similar to what was found by Addison (2004), Johnson & Addison (2008) and Okosun (2012), more GCBs were sheltering under cardboard bands tied around tree stems in the present study.

Given the new knowledge on GCB chemical ecology and visual behavior, as well as understanding GCB bio-ecology, future research should focus on using cropping systems in combination with pheromone baited traps and physical barriers against the GCB in order to control it effectively. These management tactics have been used successfully before, against three green stink bugs of cotton, namely: *Euschistus servus*, *N. viridula* and *Chinavia hilaris*. The strategic placement of soybean trap crops, stink bug pheromone baited traps and synthetic physical barriers between peanut and cotton fields achieved a reduction of stink bug densities in cotton (Tillman et al. 2015). Hokkanen (1991) describes trap cropping as a strategy for managing dispersing insect pests by using an attractive plant to harbor the pest thereby reducing the chances of entry into crop fields. The GCBs are known to disperse into fruit orchards at the wheat to fruit orchard interface which offers an opportunity to evaluate the impact of trap crops and physical barriers at these interfaces preventing the entry of the bugs into fruit orchards.

For example, providing a dense stretch of wheat crop on the periphery of fruit orchards, may trap and delay GCBs from entering into orchards until fruit is removed from trees. Similarly, dark coloured vertical barriers placed at the periphery of wheat fields may help in trapping GCBs preventing them from reaching fruit orchards. These innovative vertical traps can be made from affordable materials such as paper or plastic. Vertical object discrimination is a beneficial evolutionary mechanism that offers an insect the opportunity to find a vertical more secure hiding place (Campbell & Borden 2005) and this behaviour can be manipulated in order to trap the pest. Combining both methods may improve the effectiveness of these solutions. If an attractive pheromone is formulated in the future, both trapping crops and physical barriers may be used in combination with the pheromone to achieve better results.

The findings from this study edifies the development of novel monitoring methods against the GCB. The gathered knowledge on the chemical and visual ecology of the pest is a step forward in the development of appropriate and effective traps in the future. Ultimately, this will help in reducing the risk of exporting GCB contaminated fruit to those markets importing fruit from South Africa.

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