CHEMICAL ECOLOGY AND ECO-PHYSIOLOGY OF THE GRAIN CHINCH BUG,

Macchiademus diplopterus (Distant) (HEMIPTERA: LYGAEIDAE: BLISSIDAE), A

PHYTOSANITARY PEST OF SOUTH AFRICAN EXPORT FRUIT

by

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ABSTRACT

The grain chinch bug, *Macchiademus diplopterus,* is an endemic pest of cultivated grain crops and wild grasses in the south-western Cape region of South Africa. In early summer when host plants dry out, adult grain chinch bugs aggregate in large numbers in shelter sites in surrounding areas and enter into aestivation. These shelter sites sometimes include the stalk or calyx ends of fruit, and shelter-seeking bugs can also contaminate export fruit cartons, consequently posing a phytosanitary/quarantine risk to importing countries. Presently, there are no feasible pre- or post-harvest control measures to manage this quarantine risk. The aggregating behaviour of grain chinch bugs suggests the involvement of pheromones. Therefore, investigating the chemical ecology of grain chinch bugs for potential use in control measures is the focus of the first research chapter of this study. Gas chromatography-mass spectrometry (GC-MS) was used to identify headspace volatiles collected from aggregating bugs. Olfactometer bioassays were conducted to assess the attractiveness of each gender to separate sexes, individual compounds and a mixture of the compounds as a formulated lure. The lure was tested in field trapping trials with delta and bucket traps. In the bioassays with the live insects the response of each gender to live females was greater than the responses of each gender to live males, suggesting that females may disseminate the pheromones more efficiently than males. The following eight volatile compounds were indentified from the GC-MS analysis: hexanal, (*E*)-2-hexenal, (*E*)-2-hexenol, (*E*)-2-hexenyl acetate, (*E*)-2-octenal, (*E*)-2-octenol, (*E*)-2-octenyl acetate and tridecane. In the bioassays with individual compounds, three of these eight compounds, hexanal, (*E*)-2-hexenal, and tridecane, elicited attraction of both females and males. The formulated lure was attractive to both males and females in the laboratory bioassay, but this attraction was not evident in the field. In the field, there was only one occasion when a significantly higher number of bugs were caught in baited traps compared to unbaited traps.

Trap catches were very low compared to the actual level of infestation in the field which was evident from corrugated cardboard bands tied around tree trunks which contained many sheltering bugs. The low trap catches seen in the field were partly due to competition between the synthetic pheromone lure and the natural pheromones emitted by aggregating live insects. Also, the characteristic shelter-seeking behaviour of grain chinch bugs influenced trap catches, as more bugs were found in places that provide shelter, like cardboard bands and walls of the delta traps. This behavior of aestivating bugs could be used to the advantage of trapping bugs by integrating sheltering sites into traps in future trials. Also, the lure needs to be improved for optimum efficiency in the field. The second research chapter also addresses the quarantine risk posed by grain chinch bugs, by investigating the thermal biology of bugs to ultimately facilitate the development of effective post-harvest treatments. Critical thermal minimum and maximum temperatures (CT_{min} and CT_{max}) of both active and aestivating bugs were subjected to critical thermal limits analysis. The CT_{min} and CT_{max} of aestivating bugs were not affected by gender ($p > 0.05$). There was a decrease in CT_{min} from the active period into aestivation for both males (2.8°C to 1.0°C ($\pm$ 0.1)) and females (2.1°C to 0.6°C ($\pm$ 0.1)). Also, for CT_{max} there was an increase in tolerance from the active period into the aestivation period for both males (49.9°C to 51.0°C ($\pm$ 0.1)) and females (49.9°C to 51.5°C ($\pm$ 0.1)). To determine the plasticity of grain chinch bug thermal tolerance, aestivating bugs at 27 weeks into aestivation, were acclimated at different temperatures and photoperiods [18°C (10L:14D) and 26°C (16L:8D)] for a period of seven days. Both low (18°C) and high (26°C) acclimation temperatures and photoperiods increased CT_{min} of aestivating grain chinch bugs at 14 weeks from 0.8°C to -1.2°C and -0.1°C ($\pm$ 0.1) respectively. However, CT_{max} was not altered by acclimation temperatures ($p > 0.82$). Field temperatures at collection sites were recorded to compare to grain chinch bugs thermal tolerance levels exhibited in the laboratory. These results, as well as the effects of acclimation treatments on the CT_{min} of bugs, have

implications for post-harvest treatments, and understanding the quarantine risk posed to importing countries. The information generated from this study can be used to further advance the development of both effective pre-harvest and post-harvest control measures to reduce grain chinch bug quarantine risk.

OPSOMMING

Die graanstinkluis, *Macchiademus diplopterus*, is 'n endemiese plaag van aangeplante graangewasse en wilde grasse in die Suidwes Kaap-provinsie van Suid-Afrika. In die vroeë somer wanneer gasheerplante uitdroog, soek groot getalle volwasse graanstinkluise skuiling in die omliggende gebiede en gaan in 'n somerrusperiode. Hierdie skuilplekke sluit soms die stam of kelk eindes van vrugte in en graanstinkluise kan ook uitvoer-vrugte kartonne kontamineer. Gevolglik word lande wat vrugte uit Suid-Afrika invoer, aan die fitosanitêre kwarantynrisiko van stinkluisbesmetting blootgestel. Tans is daar nie haalbare voor- of na-oes beheermaatreëls om hierdie kwarantyn risiko te bestuur nie. Die aggregasiegedrag van graanstinkluise dui op die betrokkenheid van 'n feromoon. 'n Ondersoek van die chemiese ekologie van die graanstinkluis vir moontlike gebruik in beheermaatreëls is die fokus van die eerste gedeelte van hierdie studie. Gaschromatografie-massaspektrometrie (GC-MS) is gebruik om die vlugtige organiese verbindings in die bodamp van die saamgetrosde stinkluise te identifiseer. Olfaktometriese biotoetse is uitgevoer om die aantreklikheid van die insekte vir die teenoorgestelde geslag te bepaal, asook van die individuele verbindings en 'n mengsel van die verbindings as 'n geformuleerde lokmiddel in lokvalle. Die lokmiddel is getoets in veldproewe met deltatipe en emmertipe lokvalle. In die olfaktometriese biotoetse met die lewende insekte is die reaksie van beide geslagte teenoor lewende wyfies groter as die reaksie van die geslagte teenoor mannetjies, wat daarop dui dat wyfies die feromoon meer doeltreffend as mannetjies versprei. Die volgende agt verbindings is geïdentifiseer met behulp van GC-MS-analise: heksanaal, (*E*)-2-heksenaal, (*E*)-2-heksenol, (*E*)-2-heksenielasetaat, (*E*)-2-oktenaal, (*E*)-2-oktenol, (*E*)-2-oktenielasetaat en tridekaan. In die biotoetse met individuele verbindings het drie van die agt verbindings, hexanal, (*E*)-2-hexenal, en tridecane, lokaktiwiteit vir beide geslagte getoon. Die geformuleerde lokmiddel was aantreklik vir beide geslagte in laboratorium toetse, maar soortgelyke lok is nie in die

veld gevind nie, waar daar net een keer 'n aansienlike groter getal graanstinkluise met lokmiddel gevang is in vergelyking met lokvalle sonder lokmiddel. Die getal graanstinkluise in lokvalle was baie laag in vergelyking met die werklike vlak van besmetting in die veld, wat duidelik geblyk het uit die getalle graanstinkluise wat skuiling gesoek het in die geriffelde karton bande wat om boomstamme vasgemaak was. Die lae lokvalvangste in die veld was deels te wyte aan die kompetisie tussen sintetiese feromoon en die natuurlike feromoon van saamgetrosde insekte. Die kenmerkende aggregasiegedrag van graanstinkluise het lokvalvangste beïnvloed, aangesien meer stinkluise gevind is in plekke wat skuiling bied, soos die kartonbande en die binnekant van die delta-lokvalle. Hierdie skuilings van graanstinkluise kan in toekomstige proewe uitgebuit word deur vir meer skuilplek in lokvalle voorsiening te maak. Die formulering en die aanbieding van die lokmiddle moet ook verbeter word vir 'n optimale doeltreffendheid in die veld. In die tweede hoofstuk word die kwarantynrisiko van die graanstinkluis aangespreek deur die ondersoek van die termiese biologie van stinkluise om uiteindelik die ontwikkeling van doeltreffende na-oes behandelings te fasiliteer. Kritiese termiese minimum en maksimum temperature (CT_{min} en CT_{max}) van beide aktiewe en rustende graanstinkluise is bepaal deur analise van die kritiese termiese beperkings van die insek. Die CT_{min} en CT_{max} van rustende graanstinkluise is nie geraak deur geslag nie ($p > 0.05$). Daar was 'n afname in CT_{min} van die aktiewe tydperk tot in rus, vir beide manlike (2.8°C tot 1.0°C (± 0.1)) en vroulike insekte (2.1°C tot 0.6°C (± 0.1)). Ook vir die CT_{max} was daar 'n verbetering in toleransie vanaf die aktiewe tydperk tot in die rusperiode vir beide manlike (49.9°C tot 51.0°C (± 0.1)) en vroulike insekte (49.9°C tot 51.5°C (± 0.1)). Om die aanpasbaarheid van die termiese toleransie van die graanstinkluis te bepaal, is graanstinkluise 27 weke na aanvang van die rusperiode geakklimatiseer by verskillende temperature en fotoperiodes [18°C (10L: 14D) en 26°C (16L: 8D)] vir 'n tydperk van sewe dae. Beide lae (18°C) en hoë (26°C) akklimatiseringstemperature en fotoperiodes

het onderskeidelik die CT_{min} van rustende graanstinkluise op 14 weke verhoog van 0.8°C tot -1.2°C en -0.1°C (± 0.1). Daar is egter geen effek op CT_{max} deur akklimasie temperature nie (p > 0.82). Veldtemperature is ook bepaal om te vergelyk met graanstinkluis termiese toleransie vlakke wat in die laboratorium bepaal is. Hierdie resultate, sowel as die gevolge van die akklimasie behandelings op die CT_{min} van graanstinkluise, het implikasies vir na-oes behandelings, en begrip van die kwarantyngevaar wat dit inhou vir vrugte-invoerlande. Die inligting wat uit hierdie studie voortvloei, kan gebruik word om die ontwikkeling van beide effektiewe voor-oes en na-oes beheermaatreëls te bevorder en om die kwarantynrisiko wat graanstinkluise inhou, te verminder.

DEDICATION

This dissertation is dedicated to God almighty, to my parents, Mr & Mrs Olatunji Ogidan and to my darling husband, Dr Kazeem Oare Okosun.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1. GENERAL INTRODUCTION

The grain chinch bug, *Macchiademus diplopterus* (Distant) (Heteroptera: Lygaeidae: Blissinae) is an indigenous pest of cultivated grain crops in the south-western Cape region of South Africa. During the aestivation phase of its life cycle grain chinch bugs aggregate in sheltering sites in the vicinity of host plants. The south-western Cape is an important grain and fruit-growing area in South Africa and consequently, fruit orchards near cultivated grain crops are often infested with aestivating grain chinch bugs. Since this pest is endemic to South Africa, overseas markets importing fruit from South Africa impose quarantine or phytosanitary restrictions on trade to prevent the introduction of a new pest. To mitigate the phytosanitary risk posed by this pest and maintain export markets, intervention needs to start at the orchard level. Currently there are no feasible pre-harvest or post-harvest treatments to reduce infestation of grain chinch bugs on deciduous fruits. Information on the basic biology of the grain chinch bug is needed to facilitate the development of such control practices. This thesis focuses on certain aspects of the chemical ecology and eco-physiology of the grain chinch bug, with the aim of future development of effective monitoring and post-harvest treatment tools for this important quarantine pest.

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1.1 Taxonomic classification, morphology and distribution in South Africa

Macchiademus diplopterus belongs in the order Heteroptera, a group informally and universally termed as 'bugs'. The evolutionary success of the order Heteroptera is evident in the diversity within the group (Schuh & Slater, 1995). This success is mainly attributed to the herbivorous members that are abundantly widespread across the world (Schaefer & Panizzi, 2000). The sucking and piercing method of feeding of herbivorous heteroptera enables them to penetrate plant tissue and escape many plant defences (Panizzi, 1997). Plant feeding bugs are important pests of many crop plants, as they cause localized injury to plant tissues, weaken plants by removing sap, and may also transmit plant pathogens (Schaefer & Panizzi, 2000).

Within the Heteroptera, *M. diplopterus* belongs to the family Lygaeidae. Lygaeids are primarily seed feeders and referred to as the 'seed bugs', however, the subfamily Blissinae are sap suckers that do not feed on seeds (Sweet, 1960; Slater, 1976). The Blissinae are probably the most economically important subfamily within the Lygaeidae, being specialized for feeding on monocotyledonous plants (Slater & Wilcox, 1973). They attack graminaceous plants (Poaceae), including economic grasses such as wheat, corn (maize), rice, sorghum, barley, rye, oats, and many types of millet, as well as grasses for pasture and hay forage for livestock and dairy production (Sweet, 2000). Thus, the Blissinae threaten the plants that produce not only the majority of the world's food, but also other economic products. The Blissinae have a worldwide distribution, but the status of the different pest species belonging to this subfamily varies considerably from area to area, which makes accidental introductions to new areas a constant threat (Sweet, 2000).

Species belonging to the genus *Macchiademus* were initially placed in the genus *Atramdemus*, because of similarity of external features (Slater, 1967), but more detailed morphological studies showed that five closely related species in the Cape region of South

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Africa formed an isolated generic unit from *Atramdemus* (Slater & Wilcox, 1973). The genus *Macchiademus* was thus established to receive these species, based on the uniqueness of the sperm reservoir of the male phallus. *Macchiademus* are restricted to the south-western Cape region of South Africa, and *M. diplopterus* is the most common and economically important of the five species in the genus. In a survey conducted in natural vegetation, *M. capensis, M. acuminatus, M. nigritus* and *M. angustus* were found in highland areas confined to particular host plants (Slater & Wilcox, 1973). These hosts included robust clump-growing perennial plant species such as *Juncus lomatophyllus* (Family Juncaceae)*,* as well as plant species from the family Poaceae such as *Ehrharta erecta, Pennisetum macrourum, Sporobulus capensis* and *Pentaschistus curvifolia,* but development on the latter three species was not confirmed (Slater & Wilcox, 1973). Most of the *M. diplopterus* specimens were found in lowland, relatively dry disturbed areas where grasses did not form dense clumps. Native host plants of *M. diplopterus* are *Ehrharta longifora*, *E. erecta*, *E. calycina*, *Pentaschistus thunbergii*, *P. macrourum, Triticum aestivum,* and *T. aveneae* species. Introduced weed grasses that are also hosts of *M. diplopterus* include, *Hordeum murinum*, *Avena fatua*, *A. sativa*, *Bromus catharticus*, *B. diandrus*, *Poa annua*, and *Lolium multiflorum* (Myburgh & Kriegler, 1967; Slater & Wilcox, 1973).

Macchiademus diplopterus are mostly macropterous (capable of flight) but a small percentage are brachypterous (incapable of flight), with the wings reaching the third and fourth abdominal tergum (Slater & Wilcox, 1973). The ability to fly is a contributing factor to *M. diplopterus* being the most economically important of the five species, since the other four *Macciademus* species are mostly brachypterous. Presence of brachyptery in *M. diplopterus* indicates a former restriction to a more permanent habitat prior to the introduction of new host plants (Slater & Wilcox, 1973). The dominance of macroptery is the consequence of strong selection for migration to and from new host plants (introduced weeds and cultivated

grain crops), and to aestivation sites (Slater & Wilcox, 1973). The production of cultivated grain crops, such as wheat, started in the 17th century in the Cape, thus there has been enough time for the development of migration patterns and host transfer to these new hosts (Sim, 1965).

The limited distribution of *M. diplopterus* seems to be related to a correlation between its life cycle and the winter rainfall area of the south-western Cape of South Africa (Slater & Wilcox, 1973). The survey carried out by Slater and Wilcox (1973) showed that infestations are most severe in the drier climate and in drier, warmer years. A recent survey conducted over a period of three years in the fruit-growing areas of the Western Cape showed that areas with significantly lower average monthly relative humidity and minimum temperatures had higher numbers of grain chinch bug than other areas (Johnson & Addison, 2008). Areas with the highest numbers of grain chinch bug infestations were Ceres, Porterville and Piketberg.

1.2 Biology and seasonal cycle

Adult grain chinch bugs emerge from aestivation in autumn and fly to host plants to feed and reproduce. During this active reproductive phase, adults mate and lay eggs in rows on the inside of the leaf sheaths on host plants. A single female may lay a total of 50 - 150 eggs. The elliptically shaped eggs are white to light yellow, gradually changing colour to orange just before hatching (Fig. 1) (Sim, 1965; Slater & Wilcox, 1973; Matthee, 1974). The duration of the egg stage is 20 - 30 days. The abdomen of the nymphs is banded in white and red; the anterior abdominal segments more white, the posterior more red, with a dark chocolate brown head, thorax and wing pads (Fig. 2). The pronotum is uniformly dark chocolate to black. The nymphs develop through five instars (ranging from 1.66 to 3.28 mm in length) during a period of approximately 6 weeks, to reach the adult stage. Adult grain chinch bugs reach 4 - 5 mm in length, are dark brown to black with silvery-grey wing membranes (Fig. 3).

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Distinctions between males and females are made by comparison between body markings at the end of the abdomen; also males tend to be smaller than females (Slater & Baranowski, 1978).

Fig. 1: Eggs laid in rows within leaf sheath.

Fig. 2: Grain chinch bug nymphs.

Fig. 3: Grain chinch bugs aggregating underneath loose bark of a *Eucalyptus* tree.

When host plants dry out or are harvested in summer, the new generation migrates to aestivation sites, where they remain quiescent until the following autumn (Sim, 1965; Slater & Wilcox, 1973; Matthee, 1974). There is one generation of grain chinch bugs in a year, since as the new generation develops, the adults of the original population die. During migration grain chinch bugs travel in large numbers and can fly long distances to sheltering sites. This migratory shelter-seeking behaviour enables them to congregate in large numbers in various sheltering sites which includes anywhere on nearby trees, particularly under the bark (e.g on *Eucalyptus* trees). Grain chinch bugs seeking shelter in fruit orchards are the most problematic, as they shelter within bunches of grapes, at the stalk and calyx ends of fruits such as apples (Fig. 4) and peaches or in the navels of oranges (Giliomee, 1959; Myburgh & Kriegler, 1967; Annecke & Moran, 1982). The presence of grain chinch bugs on fruit poses a phytosanitary threat to importing countries.

Fig. 4: Grain chinch bugs infesting calyx end of apple.

1.3 Phytosanitary or Quarantine status

Since grain chinch bugs shelter on fruit and there is a possibility of bugs occurring on packed fruit that is exported to overseas markets, *M. diplopterus* is classified as a key phytosanitary pest. Consequently, quarantine restrictions to prevent the spread of grain chinch bug are imposed by importing countries.

Initially, interception of live adults on fruits such as apricots, peaches, apples and pears from South Africa gave the misconception of grain chinch bug as a fruit feeder (Herring, 1973). It was later realised that the presence of bugs on export fruit is as a result of the migratory shelter-seeking behaviour of this pest (Slater & Wilcox, 1973). One of the earliest reports of grain chinch bug as a quarantine pest of fruit was from peaches and apricots exported to the United States of America (USA) (Myburgh & Kriegler, 1967). However, grain chinch bugs can infest any fruit type (Johnson & Addison, 2008). In certain cases more than 50% of table grapes presented for export were rejected due to contamination with grain chinch bugs, and in the past season (2010/11), the export market most affected by grain chinch bug contamination was pears packaged for the USA (F. Moller pers. comm.)

The invasion potential of *M. diplopterus* in countries like the USA is considered to be high, since infestations of *M. diplopterus* in South Africa are similar to those of the chinch bug complex found in North America. The infestations of *M. diplopterus* on wheat, barley and oats have often made the production of these agricultural products unprofitable in the drier grain-growing area of the Western Cape Province of South Africa (Matthee, 1974; Annecke & Moran, 1982). Sap is sucked out of the stems of the grasses and this impairs growth, resulting in stunted appearance and drying of leaves (Matthee, 1974). The plants often die before production of grain ears. In cases where ear production does occur, the ears are also attacked and the developing grains sucked out (Matthee, 1974). The North American

complex consists of three species, namely, *Blissus leucopterus leucopterus, B. leucopterus hirtus,* and *B. insularis,* all of which are serious pests of grain crops and grasses in North America (Herring, 1973; Sweet, 2000). The common chinch bug, *Blissus leucopterus leucopterus* Say is the most notorious of the three pest species (Sweet, 2000). It feeds on a wide variety of host grasses, but is particularly damaging to corn. The adults hibernate in protected sites on non-hosts which include some grasses and ground covers (Sweet, 2000). The hairy chinch bug, *Blissus leucopterus hirtus* Montandon is one of the major insect pests attacking lawns, golf courses and turfgrasses in the north-eastern United States. The Southern chinch bug, *Blissus insularis* Barber, is a pest of St. Augustine grass lawns, a turf and pasture grass grown throughout the southern United States. Control options for handling chinch bug outbreaks in the USA include application of insecticides, the removal of weeds which grow among the infested and dead grasses, and the planting of healthy grass once the chinch bug populations have moved out of the infested area (Sweet, 2000). The control of chinch bug outbreaks in Florida runs into millions of dollars annually. Therefore, another potential chinch bug pest entering the USA on imported fruit is strictly avoided.

In addition to the similarity between the grain chinch bug in South Africa and the North American chinch bug complex, there is also evidence of high cold tolerance levels of grain chinch bug. This further highlights the threat of introduction of *M. diplopterus* into new areas. Live grain chinch bugs found on peaches and nectarines exported to the USA survived normal pre-cooling and in-transit cold storage treatment (Myburgh & Kriegler, 1967). This cold tolerance behaviour suggests that grain chinch bugs may have the potential to establish populations in regions with colder prevailing temperatures than South Africa.

1.4 Management of the quarantine risks

Any country involved in international trade of agricultural products has to contend with quarantine insect pests that must be controlled or managed to ensure that markets for the export products are maintained. Pre- and post-harvest measures are therefore put in place for management of insect pests on these agricultural products.

Pre-harvest management of quarantine insects is incorporated into the general management of insect pests on a farm. Monitoring systems, chemical and biological control programmes and cultural practices all cumulatively make up an integrated pest management system which is implemented as a systems approach to pest management in the orchard. Post-harvest management of quarantine insects also incorporates some cultural practices but is focussed on the application of specific physical postharvest treatments applied to packed fruit. Knowledge of insect behaviour and physiology are imperative in the development and implementation of these pre- and post-harvest control measures.

In the orchard, manipulation of insect communication forms the basis for monitoring systems, as well as biological control measures such as mass trapping, attract-and-kill and mating disruption. A good understanding of insect chemical ecology and the use of chemical signals is important in the success of these control measures. Very little is known about the chemical ecology of *M. diplopterus*, but their aggregation behaviour suggests the presence of an aggregation pheromone, which may be useful in monitoring and trapping bugs.

Post-harvest treatments for *M. diplopterus*, other than fumigation with methyl bromide, do not exist. Thus, the potential of alternative treatments such as heat and cold treatments need to be investigated. The success of such treatments is based on a good understanding of the ecophysiology of the insects and their thermal tolerance levels. Ambient temperature affects physiological and biochemical processes in insect metabolism (Chown & Terblanche, 2007;

Bowler & Terblanche, 2008). Understanding insect response to fluctuating temperatures could aid in the development of temperature treatments as a postharvest control measure.

These two areas of research, chemical ecology and eco-physiology of *M. diplopterus*, form the basis of this dissertation. As part of this general introduction, I will now discuss a general background for each of these aspects of the study and focus on the specific research questions addressed in chapters 2 and 3.

1.4.1 Chemical Ecology

Chemical ecology is the study of the chemicals involved in the interactions of living organisms and is based on the production of signalling molecules known as semiochemicals. These semiochemicals are important in insect behavioural and olfactory communication for intra-specific and inter-specific relationships (Birch & Haynes, 1982; Aldrich, 1988). Semiochemicals mediate interactions between organisms; if this interaction is between different species, the compound is known as an allelochemical (e.g. kairomones, allomones or synomones), but if the interaction is between members of the same species, it is known as a pheromone (e.g. sex, alarm, or aggregation pheromones) (Howse, 1998). Kairomones give the receiver a selective advantage over the emitter (e.g. parasites or predators use the smell of their prey to locate it). Allomones provide a selective advantage to the emitter (e.g. defensive secretions from prey act as irritants that deter predators). Synomones are advantageous to both the emitter and receiver (e.g. attractions of pollinating insects to plants, or plants damaged by insect feeding emit volatiles that attract their predators). Pheromones facilitate different important behavioural activities in insects, such as migration, reproduction, and ultimately facilitate the maintenance of colonies (Aldrich et al., 1999). Chemical compounds that constitute semiochemicals include aldehydes, esters, alcohols, monoterpenoids, sesquiterpenoids and ketones (Moraes et al., 2008).

The importance of pheromones in insect chemical ecology

In Heteropterans, scent glands known as methathoracic scent glands, present in both the immature and adult stages, are used for the purpose of chemical communication. These glands produce the pheromones that serve in mate location, defense and aggregation (Aldrich, 1988).

The importance of pheromones in mating of true bugs cannot be over emphasized, because mating behaviour is triggered by sex pheromones which increase the probability of successful mating. The sex pheromones in Heteroptera are released by both sexes but mainly by male bugs. Release by females may also act as an attractant for predators to prey on the eggs, and in that case act as a kairomone (Aldrich, 1988; Demirel, 2007). It has been shown that most parasitoids use sex and defence pheromones of the host as kairomones for host finding (Moraes et al., 2008).

Alarm pheromones are also important in insect communication, as they cause dispersal of bugs from the source of the signal by increasing space between individuals. This benefits the group by reducing intra-specific competition and avoiding impending dangers (Birch & Haynes, 1982; Demirel, 2007). Compounds used as alarm pheromones may also be used as defence secretions to deter predators thereby acting as an allomone (Demirel, 2007; Moraes et al. 2008). The alarm pheromones are produced by both adults and nymphs and may be specific or non-specific to a particular life stage (Leal et al., 1994; Prudic et al., 2008). For example, the alarm pheromone may cause dispersal in nymphs, but not elicit any behaviour in adults; also adult secretions could cause dispersal in adult but not have behavioural effects on larval aggregation (Prudic et al., 2008).

Aggregation pheromones are important in Heteroptera as they induce a behavioural response in the organism, leading to an increase in their density in the vicinity of the pheromone source (Birch & Haynes, 1982). This increase in numbers is beneficial to insects as it provides protection against predators, facilitates overcoming host resistance (for feeding) and mate location (Aldrich, 1988; Demirel, 2007; Moraes et al., 2008). Aggregation pheromones do not necessarily trigger mating behaviour but may be involved in attraction for both larvae and adults (Millar, 2005). Aggregation pheromones are important in the migration and aestivation behaviour of Lygaeidae (Aldrich et al., 1999).

Applications of pheromones as a pest control tool

Aggregation and sex pheromones can be applied as pest control tools for either direct monitoring, mass trapping and attract-and-kill techniques, or for mating disruption (Aldrich, 1988; Moraes et al., 2008).

Monitoring: Pheromone-based monitoring is a major component of the integrated pest management strategy for early warning and detection of pests. Some important factors that ensure effective pest monitoring when using pheromone baited traps are the attractant source, its controlled release device, as well as trap placement. Only a small proportion of a population is sampled by pheromone-based monitoring, but the information retrieved from pheromone traps is used to set thresholds for timing of chemical treatments, timing of other sampling methods or for risk assessment (Jones, 1998; Witzgall et al., 2010). Monitoring enables accurate assessment of timing of pest emergence and the size of adult populations (Birch & Haynes, 1982). Some of the successful pheromone-based monitoring systems for pest species in South Africa are used for codling moth (*Cydia pomonella*) in apple and pear orchards (Jones, 1998; Riedl et al., 1998), false codling moth (*Thaumatotibia leucotreta*) in

citrus orchards (Grout et al., 1998) and mealybugs (*Planococcus ficus*) in vineyards (Walton & Pringle, 2004).

Mass trapping and attract-and-kill: Mass trapping uses lures for one or both sexes to attract insects to a source in which they are trapped, either in water or on an adhesive device, but not necessarily killed. Attract-and-kill differs from mass trapping because lured pests are either killed or sterilised by a sterilizing agent at the point source of the attractant, thereby effectively eliminating pests from the population. Mass trapping is considered to be an old method of pest control. Attract-and-kill has been used effectively for false codling moth, codling moth, potato tuber moth (*Phthorimaea operculella*), Mediterranean fruit fly (*Ceratitis capitata*), olive fly (*Bactrocera oleae*) and the cotton boll weevil (*Anthonomus grandis*), in South Africa, South America, USA and Switzerland (Stotter, 2009; Jones, 1998; Lösel et al., 2000; Witzgall et al., 2010).

Mating disruption: This method of control incorporates the use of synthetic sex pheromones to flood the treatment area with pheromone, thereby causing sexual confusion and preventing mating between males and females. This is based on the principle of using a large number of point sources of synthetic sex pheromones in the orchards, thereby reducing the ability of a male to locate a female for successful mating (Brunner & Knight, 1993; Jones, 1998). Mating disruption programmes have been developed for false codling moth and codling moth in South Africa (Grout et al., 1998; Pringle et al., 2003), and for pink bollworm (*Pectinophora gossypiella*) in the United States (Jones, 1998).

Previous work on the application of pheromones to control *M. diplopterus* in deciduous fruit orchards in South Africa was done using seven general stink bug (Family: Pentatomidae) pheromones in wing traps during the early aestivation period of grain chinch bugs (November and December) (Addison, 2004). General stink bug pheromones were not effective in

trapping grain chinch bugs, suggesting that this lygaeid bug probably has a species-specific pheromone that needs to be investigated and identified for use in field trapping grain chinch bugs. Hence, identification of the chemical compounds comprising the aggregation pheromone, and formulating a lure (the objectives of chapter 2) is an important initial first step to future development of a pre-harvest pheromone-based monitoring or mass trapping system for grain chinch bug in deciduous fruit orchards.

1.4.2 Ecophysiology of insects

Ecophysiology is the study of the physiology of organisms with respect to their adaptation to the environment. Physiological and biochemical processes in insect metabolism are affected by external factors, such as ambient temperature, and ectothermic organisms require mechanisms to tolerate changes in their thermal environment. Environmental temperature influences behaviour, activity, energetics, species abundance, distribution, habitat location, reproductive performance and survival of organisms (Angilletta et al., 2002; Chown & Nicolson, 2004; Jumbam et al., 2008). Although insects adjust behaviour to moderate the effects of environmental temperature fluctuations, response to these fluctuations results in knock down of insects, prolonged coma, irreversible trauma and finally death (Chown & Nicolson, 2004). Insight into the thermal biology of an insect has implications for the development of effective temperature treatments that can be used as postharvest treatments for quarantine pests.

Insect thermal biology and critical thermal limits (CTLs)

An organism's thermal tolerance defines the range of temperatures at which an organism can function optimally, or still recover from if pushed to its limits at either end of that range. There are a number of factors that influence insect thermal tolerance. These include the condition or state of the insect when exposed, the severity of the exposure and thermal history

(Lutterschmidt & Hutchison, 1997; Hoffmann et al., 2003). In some instances, insect thermal tolerance is also influenced by life stage or gender, environmental cues like changing light conditions (daylength) and daily temperature variations (Hoffmann et al., 2003; Chown & Terblanche, 2007; Bowler & Terblanche, 2008; Nyamukondiwa & Terblanche, 2009). The upper and lower limits of this thermal tolerance range are known as the critical thermal limits (CTLs) and temperatures beyond these limits are lethal to organisms (Chown & Nicolson, 2004). Critical thermal minima (CT_{min}) and maxima (CT_{max}) are measured by cooling or heating an animal from an initial temperature until there is physiological failure (Chown & Nicolson, 2004). The visual detection of CTLs includes behavioral traits such as loss of righting response, onset of spasms, knockdown, salivation, capsizing or heat paralysis and panting in ectotherms (Lutterschmidt & Hutchison, 1997; Lighton & Turner, 2004). An inability to escape from the adverse conditions ultimately results in death.

In determination of thermal tolerance of organisms, the ramping rate or rate of temperature change is an important factor that must be taken into account, because the duration of exposure is directly related to thermal tolerance (Lutterschmidt & Hutchison, 1997), and both rate of temperature change as well as start temperature affects CTLs (Terblanche et al., 2007). Cooling rates influence an insect's capacity to survive low temperature, while heating rates can affect an insect's ability to survive high temperature (Chown & Nicolson, 2004). An insect's flexibility to develop into any of several phenotypic states, depending on the environment, is known as phenotypic plasticity, and allows for an organism to adapt to, and survive in a changing environment (Seebacher, 2005; Deere & Chown, 2006). Duration of exposure to sublethal conditions may vary from minutes to hours or days to weeks which may cause acclimation responses in insects that are phenotypically plastic (Hoffmann et al., 2003).

Acclimations in insects

Acclimation is a form of phenotypic plasticity, in which long term exposure to sublethal thermal conditions results in physiological changes that enable an insect to counter the effects of the exposure and survive under extreme conditions which would otherwise be lethal (Hoffmann, 1995). Short term exposure to sublethal thermal conditions gives rise to a hardening response with reversible physiological changes, while an acclimation response consists of both reversible and irreversible physiological changes (Cossins & Bowler, 1987; Hoffmann et al., 2003; Nyamukondiwa & Terblanche, 2010). Insect response to acclimation and hardening can be different within species depending on traits involved such as their levels of thermal tolerance (Hoffmann et al., 2003; Marais et al., 2009). Physiological changes which occur by exposure to a particular temperature are aimed at survival upon exposure to more extreme conditions (Hoffmann, 1995; Hoffmann et al., 2003). Understanding the mechanisms underlying insect thermal biology and its ability to acclimate to fluctuating conditions is necessary to improve the post-harvest disinfestation techniques used for control of quarantine insect pests.

Post-harvest temperature treatments for phytosanitary pests

Quarantine restrictions are developed to protect a region's agricultural industry from the introduction of damaging insect pests which may be found on imported agricultural products (Mitcham et al., 2002). In order to gain access to export markets, approved post-harvest disinfestation treatments that control associated quarantine insect pests, are required. The current post-harvest treatments include fumigation, reduced atmospheric pressure, washing, high energy (such as irradiation, radio frequency or microwave), controlled atmospheres (low oxygen and high carbon dioxide) and temperature treatments (high or low), (Paull, 1994; Mitcham et al., 2002; Neven, 2003). The different heat treatments used for disinfestation include, high temperature forced air, hot water dips, drenches or sprays, vapour heat and hot

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air, while cold treatments are mainly carried out by cold storage treatment at packhouses or during transportation (Paull, 1994; Neven, 2003). Some of the benefits of such temperature treatments are that they are residue-free, have easy application and can also be used for disease control (Couey, 1989; Paull, 1994).

Insect sensitivity to temperature is due to its poikilothermic nature, consequently increasing or decreasing insect body temperature causes a simultaneous increase or decrease in metabolism and respiration up to a critical thermal limit (Neven, 2000). The response to heat treatment leads to irreversible shock, which may eventually cause death (Chown & Nicolson, 2004). An important factor in development of quarantine postharvest treatments is knowledge of the insects' physiological condition (i.e. weakness), or capitalising on the difference in physiological responses of the produce and its arthropod insect pest (Neven, 2003). In order to achieve effective post-harvest temperature treatments, a balance between produce tolerance and pest intolerance is required. However, a limiting factor of temperature treatments is that thermal tolerance of the crop is often less than that of its associated arthropods pests (Couey, 1989; Neven, 2000). Temperature treatments that kill infesting insects can damage the fruit by causing scalding, internal browning and decay, thereby rendering the fruit unmarketable (Lay-Yee & Rose, 1994). Nevertheless, a number of effective treatments have been developed and some are applied on a commercial scale. Post-harvest temperature treatments with hot-water is effective in eradicating all quarantine insect pests of red ginger flowers (Hara et al., 1996), and hot-water immersion of guava with subsequent cold storage eradicates all the immature stages of Caribbean fruit fly (*Anastrepha suspensa*) (Hallman, 1994). Hot-water immersion and vapour heat treatments of tropical fruit are effective against Queesnland fruit fly (*Bactocera tyroni*), Meditteranean fruit fly (*Ceratitis capitata*), and Cook Island fruit flies *Bactocera melanotus* and *B. xanthodes* infestations and do not damage the fruit (Heard et al., 1991; Armstrong et al., 1995; Waddell

et al., 1997). High temperature treatments are more suited to tropical fruits than temperate fruits, as tropical fruits are more tolerant of the heat applied to disinfest fruits of insect pests. Thus, heat treatments for temperate fruits are more difficult to develop. For example, post-harvest heat treatments of temperate fruits, against oriental fruit moth (*Grapholita molesta*) and codling moth (*Cydia pomonella*) infestations, caused fruit damage as the fruits were intolerant to the high temperatures, but the treatment was effective against the pests (Yokoyama & Miller, 1987; Yokoyama et al., 1991).

One of the other factors that could affect the use of temperature treatments for insect pests is pre-exposure to non-lethal or elevated temperatures. Pre-exposure of insects to thermal conditions enhances its thermal tolerance and affects their response to disinfestation treatments (Lester & Greenwood, 1997). For example, exposure to non-lethal thermal conditions prior to disinfestation treatments caused enhanced thermal tolerance of Queensland fruit fly to subsequent heat treatments (Waddell et al., 2000). Pre-treatment thermal conditioning during harvest and storage caused an increase in heat resistance in codling moth and light brown apple moth (Lester & Greenwood, 1997; Yin et al., 2006), which eventually compromised the effectiveness of subsequent thermal treatments. Also, thermal tolerance induction was caused by pre-exposure to hot-air before hot-water immersion treatment of mealybugs (Hara et al., 1997).

In addition to the effect of prior exposure to altered temperature conditions, the physiological state of insects also needs to be taken into consideration when applying temperature treatments. Insects in diapause, a state of reduced activity, respond differently to treatments compared to when not in diapause. Lay-Yee & Whiting (1996) reported that non-diapausing mites were less tolerant to post-harvest treatments compared to diapausing mites. This may be an important factor to consider when developing post-harvest temperature treatments for

grain chinch bugs, since infestation of fruit occurs during the aestivation phase of their life cycle, a period of reduced activity

The quarantine risk potential posed by *M. diplopterus* is great since there are no effective non-toxic, post-harvest treatments available. This creates a need to investigate all potential alternative treatments. Temperature treatments may be the easiest to apply, and thus, investigating an aspect of grain chinch bug physiology to determine its thermal tolerance (objectives of chapter 3) is an important first step. The insight to be gained will enhance the development of alternative and effective post-harvest temperature treatments for this quarantine pest.

1.5 Study objectives

My overall project aim is to improve basic knowledge of grain chinch bug physiology and ecology to ultimately improve management of grain chinch bug at both orchard level and in packhouses, and thereby help maintain fruit market accessibility. My individual objectives are:

1)   To investigate and identify the chemical compounds that make up *Macchiademus diplopterus* aggregation pheromone, and to evaluate a lure formulated from the identified compounds in a field trapping trial.

2)   To determine the critical thermal limits of active and aestivating grain chinch bugs, as well as the effect of acclimation on aestivating bugs.

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CHAPTER 2

AGGREGATION PHEROMONES OF *MACCHIADEMUS DIPLOPTERUS*, THE GRAIN CHINCH BUG, AND ITS POTENTIAL USE AS A LURE IN TRAPPING SYSTEMS

1. INTRODUCTION

The migratory shelter-seeking behaviour of grain chinch bug, *Macchiademus diplopterus* (Distant) causes many problems for producers intending to export fresh fruit produce to overseas markets. When host plants (wild grasses and cultivated grain crops e.g. wheat) dry out or are harvested, grain chinch bugs move in large numbers and seek sheltering sites in which to aestivate and avoid desiccation during adverse summer periods. This migration coincides with the ripening and harvesting periods of deciduous fruits in the Western Cape, South Africa, and as a consequence orchards which are in close proximity to wheat fields, become infested with grain chinch bugs. Seeking shelter in small crevices, grain chinch bugs can infest citrus, pome and stone fruit at the calyx and stalk ends, or shelter within bunches of grapes. The risk of contaminated fruit being packed for export to overseas markets has made the grain chinch bug a key phytosanitary or quarantine pest of South African export fruit. In recent years, over 55% of table grapes presented for export in one season were rejected due to the presence of grain chinch bugs (Johnson & Addison, 2008).

The risk of contamination by grain chinch bugs could be mitigated if producers could monitor for and control grain chinch bugs moving from host plants into orchards. At present, there are no effective tools for monitoring or control of this quarantine pest. A previous study tested the effect of colour in trapping grain chinch bugs, with five different coloured sticky traps,

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but none were found to be significantly effective in trapping bugs in comparison to the level of infestation observed in the orchards (Addison 2004). Since the shelter-seeking aggregating behaviour of grain chinch bugs suggests mediation by an aggregation pheromone, Addison (2004) also investigated the potential of seven different general stink bug pheromones in wing traps to trap grain chinch bugs. The results showed that none of the general stink bug pheromones attracted bugs, suggesting that *M. diplopterus* may have a species-specific pheromone which warrants further investigation, as it may be useful in developing a trapping system for this pest.

The aggregation pheromone is important in migration, colonisation of new hosts, aestivation and overwintering behaviour of the Heteroptera (true bugs) (Aldrich et al., 1999). The production of aggregation pheromone is by either one or both sexes and serves to attract other individuals of the group for the purpose of mating, feeding or avoiding desiccation and being preyed upon by predators or parasitoids (Aldrich, 1988; Demirel, 2007). Interference with this chemical communication and manipulation of pheromones enables control of insect pests through monitoring, attract and kill techniques, mating disruption, and mass trapping. The application of pheromones in monitoring and control is used in integrated pest management as alternatives to, or for reduced use of broad-spectrum chemical control (Jones, 1998). The information retrieved from pheromone traps is used to make informed decisions (e.g. determination of thresholds for timing of treatments), and for risk assessments, as well as accurate assessments of pest density (Birch & Haynes, 1982; Jones, 1998). Pheromone-based monitoring has been exploited for some other key quarantine insect pests in fruit orchards in the Western Cape, such as, codling moth (*Cydia pomonella*), false codling moth (*Thaumatotibia leucotreta*), vine mealybug (*Planococcus ficus*) and fruit flies (*Ceratitis* spp.) (Grout et al., 1998; Jones, 1998; Riedl et al., 1998; Pringle et al., 2003, Walton & Pringle, 2004). The polyphagous nature and high mobility of heteropterans complicates its control and

monitoring strategy (Millar et al., 2002), nevertheless, successful pheromone-based monitoring techniques have been developed for some heteropterans, such as *Euschistus* bugs and *Lygus* bugs (Cottrell, 2001; Leskey & Hogmire, 2005; Blackmer et al., 2008). Limited information is available on pheromone-based monitoring of Blissidae, especially chinch bug complexes. The present monitoring strategies applied for the North American chinch bug complex is labour intensive and is done mainly by direct scouting, vacuuming or floatation techniques on identified infested grasses (http://versicolor.ca/lawns/secM.html).

The aggregating behaviour of grain chinch bugs strongly suggests presence of an aggregation pheromone which, if synthetically reproducible, would be useful in monitoring and control of this quarantine pest. The aim of this study is two-fold; firstly, to investigate the chemical ecology of grain chinch bugs and identify the pheromone constituents used to mediate aggregation during the aestivation period; and secondly, to formulate a lure using these constituents and evaluate its efficacy in trapping grain chinch bugs in the field. This may then be used for future development of practical methods to monitor the time of dispersion of grain chinch bugs from wheat fields into fruit orchards, as well as for mass trapping.

2. MATERIALS AND METHODS

2.1 Collection of insects

Aestivating grain chinch bugs were collected from underneath the bark of bluegum (*Eucalyptus*) trees situated close to wheat fields, in Malmesbury, South Africa, during November-December 2008. The insects were collected at the onset of aestivation, as this is the period during which the migrating bugs exhibit aggregating behaviour. Insects were collected on sheets of paper and placed directly into sterilised 300-ml glass bottles. The bottles were closed with screw caps using aluminium foil seals to prevent absorption of the pheromone into the rubber seal in the lid. Females tend to be larger than males and the insects were sexed in the laboratory according to their relative sizes. In the laboratory the insects were used for the collection of the pheromone for qualitative and quantitative analyses, as well as in behavioural bioassays of the response of the bugs to live insects and to synthetic formulations of the pheromone.

2.2 Identification of the chemical constituents of the aggregation pheromone
2.2.1 Sample collection.

The volatile constituents of the pheromone released by aggregating aestivating grain chinch bugs were collected for gas chromatographic-mass spectrometric analysis (GC-MS) using a device described as a sample enrichment probe (SEP) (Burger et al., 2006; Burger et al., 2011). The volatile components of the pheromone were collected by exposing the sorptive phase of the SEP (56 mg of polydimethylsiloxane (PDMS) rubber) to the effluvium of 20 aggregating insects in screw-capped glass vials (140 mm x 20 mm) (Fig. 1) for periods of 4 h. The constituents of the secretions of male and female insects were collected separately.

Fig. 1: Glass vial with a sample enrichment probe (SEP). The insects aggregate on the PDMS sleeve of the SEP because it acts as disseminating medium and competes with the secreting insects when equilibrium is reached between the secreting insects and the PDMS sleeve.

2.2.2 Analytical methods

Electron impact mass spectra (EIMS) of the individual constituents of the aggregation pheromone were obtained at 70 eV on a Carlo Erba QMD 1000 GC-MS instrument consisting of a Carlo Erba 5300 gas chromatograph and a Trio 1 quadrupole mass spectrometer. The gas chromatograph was fitted with a split/splitless injector, which was operated at 220 °C, and a glass capillary column (40 m x 0.25 mm i.d., coated with 0.25 μm of PS-089-OH (DB-5 equivalent). The column was provided with an integrated retention gap of 2 m. Helium was used as carrier gas at a linear flow velocity of 28.6 cm/sec at 40 °C and the column oven was programmed at 4 °C/min from 40 °C to 220°C. The GC-MS interface and ion source were operated at 250 °C and 180 °C, respectively.

The collected volatile material was analysed by introducing the SEP into the injector of the GC-MS instrument where the volatiles were desorbed from the PDMS rubber and were swept into the capillary column by the carrier gas (He) to be separated on the stationary phase and ionized in the ion source of the MS. The SEP was left in the injector until completion of the analysis. Mass spectral data were acquired from m/z 40 to 250 at 0.9 s/scan with a 0.1 s interscan delay. The data were used to plot reconstructed total ion chromatograms (TICs) from which the mass spectra of the constituents of the pheromone were extracted. The constituents of the pheromone were tentatively identified by computerised comparison of their mass spectra with the available National Bureau of Standards library of mass spectra as well as by the interpretation of the resulting mass spectral data. The structures of the components of the pheromone were confirmed by retention time and mass spectral comparison with authentic synthetic compounds using GC-MS analysis. Except for (*E*)-2-octenyl acetate, the compounds required for retention time and mass spectral confirmation of the identities of the constituents of the pheromone were purchased from Sigma-Aldrich, Kempton Park, South Africa. (*E*)-2-octenyl acetate was synthesized by conventional esterification of (*E*)-2-octenol with acetic anhydride.

Quantitative data were obtained by integration of the peaks in the TICs using the Lab-Base software of the GC-MS instrument.

2.2.3 Formulation of a lure

Natural rubber strips (32 mm x 3 mm x 1 mm, 103 mg; Interstat, Waltons, Stellenbosch) were impregnated with a mixture of synthetic analogues by exposing the strips in a 2- ml glass vial to 1 µl of a mixture of the synthetic constituents made up according to the quantitative data shown in Table 1, until equilibrium was established; for at least 24 h. The composition of the

volatile material released by these impregnated strips was determined by exposing a SEP to the headspace gas of one of the bands in a glass vial (140 mm x 20 mm) capped with a Teflon-faced screw cap for 18 hours at 22 °C, as described above for experiments with the insects. The organic volatile compounds trapped on the SEP were analysed by GC-MS as described above.

2.3 Behavioural bioassays

2.3.1 Behavioural bioassay: response to live insects

To test the response of grain chinch bugs to either live male or female bugs, behavioural bioassays were performed at ambient temperature using a four-arm olfactometer (Fig. 2). The four-arm olfactometer consisted of three inter-connecting Perspex units forming a central chamber, with four glass tube attachments, similar to the olfactometer described by Pettersson (1970). The two outer units were circular discs (104 mm in diameter and 9 mm thick) which were secured to the top and bottom of the centre unit, forming an enclosed four-pointed star-shaped exposure chamber in the centre. Each of the four points ended in a 9 mm hole in the wall of the unit to which the glass tubes were attached. A 3 mm hole in the top disc served as an outlet for the air flowing through the chamber. The air stream through the olfactometer was generated by a suction pump drawing air through the four glass tubes, into the exposure chamber and out through the centre outlet in the top disc.
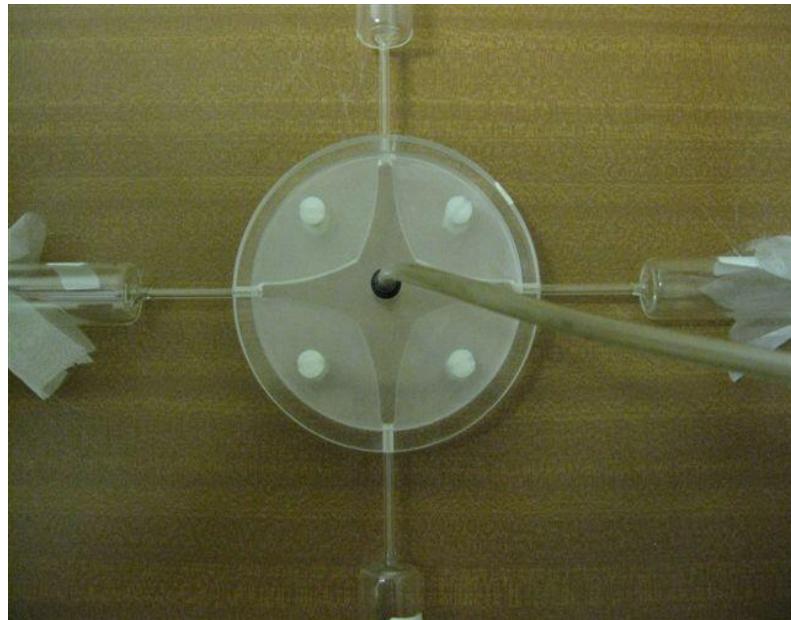
Fig. 2: Four-arm olfactometer used for laboratory bioassays.

Individual male and female test insects were separated from each other for about five hours before the commencement of bioassay. The protocol for the bioassay was based on that described by Verheggen et al. (2007). To assess the attractiveness of each gender to separate sexes using live insects, either ten male or female adult bugs were placed in one of the four test-arms as the odour source, while the other three arms were empty (controls). These single-choice tests were carried out to give an absolute measure of attraction of males relative to females exposed to the same odour source. Responses of 40 males and 40 females, tested in alternate batches of five, were observed for each of the odour sources (male or female). Test insects were released one at a time into the olfactometer through the centre hole in the top disc. The suction pump tube was attached to the outlet and switched on. The flow rate was maintained at 70 ml/min. Each test bug was observed for 3 minutes in the central chamber and allowed to choose the preferred odour source. If an insect entered one of the glass arms and remained there for 30 consecutive seconds, the test was terminated for that insect and the remaining time was awarded to that particular zone. After determining the response of five

insects the whole olfactometer was opened and cleaned with acetone, and the orientation of the set-up was rotated at 180° to check for positional effect.

2.3.2 Behavioural bioassay: response to identified compounds and formulated lure

To assess the response of each gender to the separate chemical compounds identified by GC-MS analysis, a mixture of the individual compounds and an optimised mixture used for the lure formulation, live insects were exposed to odours using the four-arm olfactometer. A pheromone-impregnated rubber strip was placed in one test arm and the other three test arms remained empty (controls). The responses of 40 males and 40 females to each individual compound and a mixture of these compounds were tested in December 2008 while the lure formulated for field trials was tested in December 2009, using the same protocol as described above for testing the response of insects to live males and females.

2.4 Field trapping trial

Five field sites were identified in areas with high numbers of grain chinch bugs, based on a recent survey of grain chinch bug distribution in the Western Cape (Johnson & Addison, 2008). These comprised of two pear orchards in the Prince Alfred Hamlet (PAH) area near Ceres, two nectarine orchards in the Kouebokkeveld (KBV) area near Ceres and a site with a row of bluegum trees adjacent to wheat and oat fields in Malmesbury. The selected orchards were also adjacent to wheat fields.

To test the response of grain chinch bugs to the formulated lure in the field, a pheromone dispenser was created using a 4-ml glass vial containing 100 µl of formulated aggregation

lure, covered with poly-ethylene film secured in place with cotton thread (Fig. 3). Two trap designs were tested, namely delta traps with sticky pads and bucket traps with vapour poison strips (Chempac (Pty) Ltd, Paarl, South Africa) (Fig. 4a and b). At each site traps were hung in the row of trees at the orchard boundary adjacent to the wheat field. In each row, traps with and without lures were positioned in a randomised order and hung approximately 10 m apart and at a height of 1.5 m. Three replications of the two trap types with and without lures were carried out in each row. Thus, there were a total of 12 traps at each site (3 baited delta traps, 3 unbaited delta traps, 3 baited bucket traps, and 3 unbaited bucket traps).

Fig. 3: Glass vial dispenser.

Corrugated cardboard bands provide convenient sheltering sites for grain chinch bugs and have been used in previous survey studies to monitor for the presence of grain chinch bugs (Addison, 2004; Johnson & Addison, 2008). In the present study corrugated cardboard bands were tied to the trunks of trees, in the same row, and at the same height as the traps, to help monitor chinch bug presence in test orchards (Fig. 4c). The traps and bands were examined fortnightly from December 2009 to February 2010, during four visits, and the number of grain chinch bugs caught in traps and sheltering in bands was determined.

Fig. 4: (a) Delta trap; (b) Bucket trap; (c) corrugated cardboard band with sheltering grain chinch bugs.

2.5 Statistical analysis

For behavioural bioassay trials, all experiments were checked for normality using the Shapiro-Wilks test. Non-parametric tests were used for analysis of those results that were not normally distributed. The responses of males and females were compared using a Mann-Whitney U test and T-test in Statistica 9 (Statsoft, USA). For the field trapping trial, the data was checked for normality using Shapiro-Wilks tests. Both trap and band catches were analysed using factorial ANOVA in Statistica 10 (Statsoft, USA) and Tukey-Kramer's post-hoc tests were used to identify statistically homogenous groups.

3. RESULTS

3.1 Identification of the chemical constituents of the aggregation pheromone

The eight volatile compounds identified from headspace samples of aggregating *M. diplopterus* and analysed by GC-MS are: hexanal, (*E*)-2-hexenal, (*E*)-2-hexenol, (*E*)-2-hexenyl acetate, (*E*)-2-octenal, (*E*)-2-octenol, (*E*)-2-octenyl acetate and tridecane. The chromatogram produced by GC-MS analysis used to identify these compounds is shown in Fig. 5. All of these compounds at the same ratios were identified from headspace samples collected from both male and female grain chinch bugs.
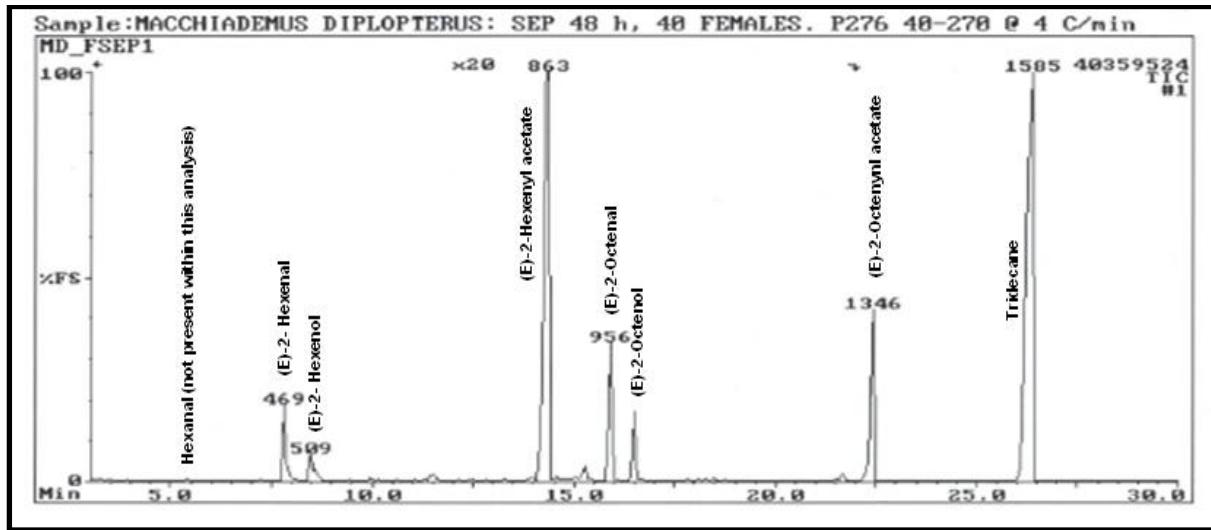

Fig. 5: Chromatogram of isolated chemical compounds from headspace sample of aggregating grain chinch bugs.

3.1.2 Formulation of a lure

The identified aggregation pheromone compounds were used to formulate a synthetic lure according to the quantitative analysis results shown in Table 1.

Table 1. Quantitative composition of the aggregation pheromone of *Macchiademus diplopterus* and the synthetic lure.

Constituents	Quantitative analyses of the natural secretion (Relative concentrations)[1]		Composition of synthetic lure[2]	Density of the constituents (g/cc)
	Males (n = 3)	Females (n = 3)		
Hexanal	3 – 4	2	4	0.815
2-Hexenal	2 – 3	1 – 2	2	0.846
2-Hexenol	0 – 2	0 – 1	1	0.849
2-Hexenyl acetate	2	3 – 4	2	0.898
2-Octenal	38 – 40	37 – 60	38	0.846
2-Octenol	1	1 – 2	1	0.843
2-Octenyl acetate	6	2 – 10	6	0.892
Tridecane	100	100	100	0.756

[1]Quantitative data were obtained by integrating the peaks of the respective constituents in the total ion chromatogram (TIC) of the natural secretion trapped on a sample enrichment probe at 22°C from 20 –50 males or females. The data were normalised with respect to tridecane = 100.
[2]The lure was made up by mixing volumes (µl) of the respective synthetic compounds in a glass vial.

3.2 Behavioural bioassays

3.2.1 Behavioural bioassay: response to live insects

In the bioassays using live insects in the test arm of the four-arm olfactometer, both male and female grain chinch bugs showed significant attraction to the test arm when it contained females (Fig. 6a). Thirty-three of the 40 males tested in the olfactometer chose the test arm with females over the empty control arms (p = 0.000106), and 32 out of 40 females tested chose the test arm with females rather than the empty controls (p = 0.001360). In contrast, when males were placed in the test arm, odours emanating from live males were not as attractive to either males or females (p > 0.05) (Fig. 6b). Only 11 out of the 40 test males exhibited some attraction to the test arm with males, and 8 of the 40 test females chose the test arm containing males.

3.2.2 Behavioural bioassay: response to identified compounds and formulated lure

Of the eight chemical compounds identified by GC-MS analysis, four were attractive to females, and three of these four were also attractive to males, when each gender's response to the individual compounds was tested. Hexanal, (*E*)-2-hexenal and tridecane elicited attraction of females (Fig. 7a) (p = 0.000705, p = 0.000014 and p = 0.000939 respectively), as well as males (Fig. 7b) (p = 0.019188, p = 0.000939 and p = 0.048580 respectively). Females also exhibited attraction to (*E*)-2-hexenyl acetate (p = 0.010082). The most attractive compound to females was hexanal, with 28 out of 40 females choosing the test arm over the empty control arms. Of the eight compounds (*E*)-2-hexenal attracted the most males, namely 25 out of 40. The least attractive compound, to both genders, was (*E*)-2-octenyl acetate, with 18 females and 17 males choosing the test arm. Both sexes showed an attractive response to the mixture of the chemical compounds. Of the 40 individuals of each sex, 27 females and 23 males chose the test arm.

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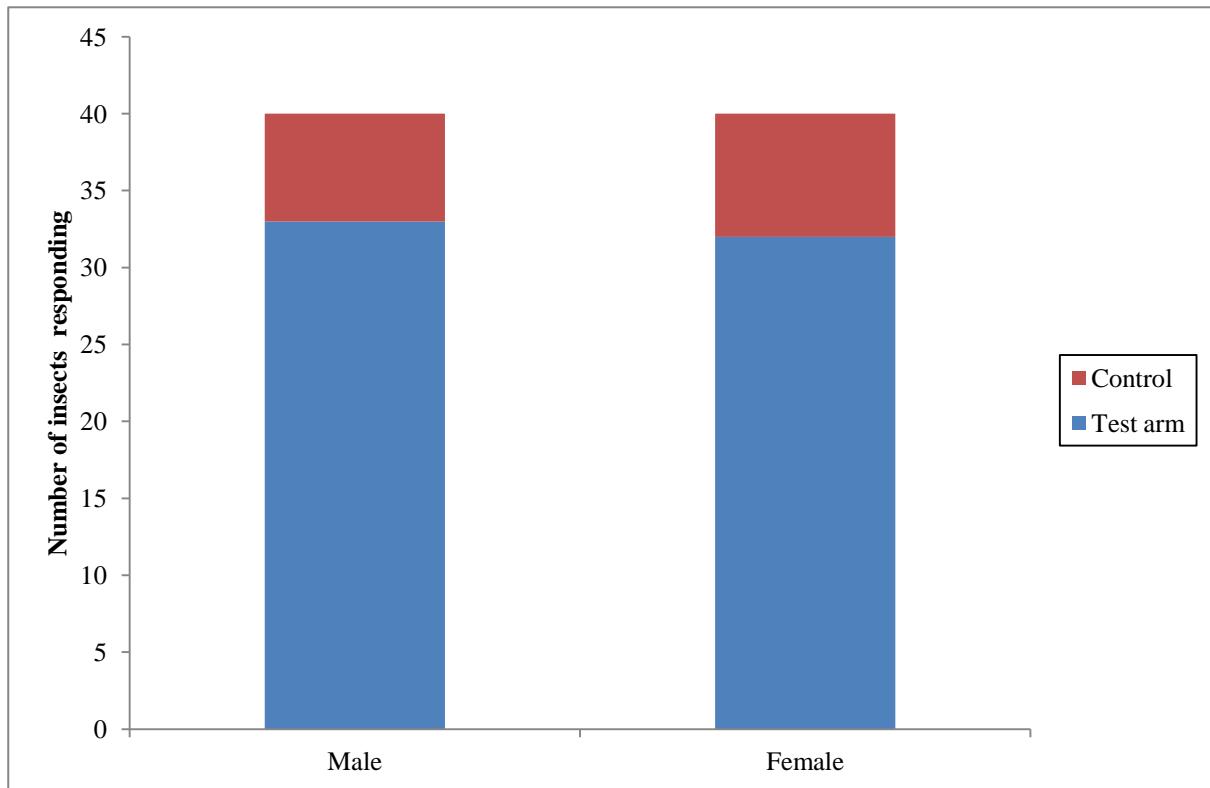
Fig. 6a: Behavioural bioassay responses of each gender to live females.
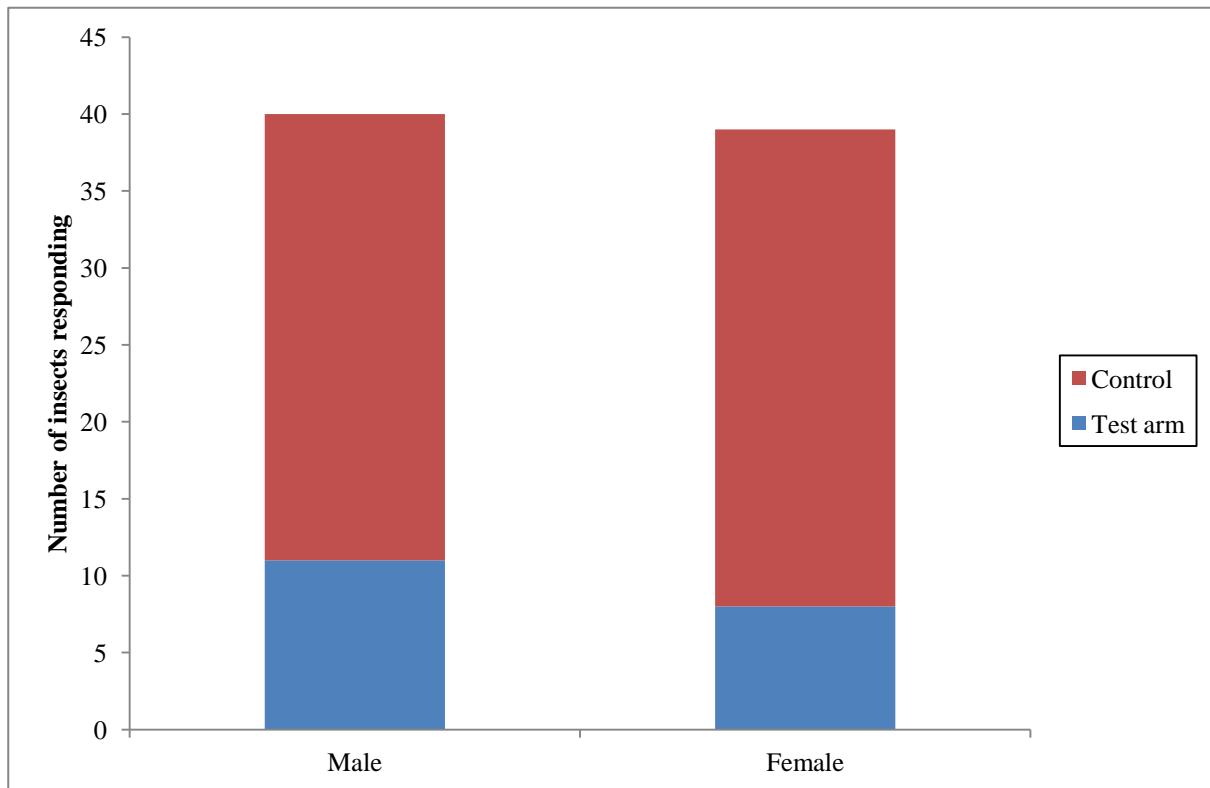
Fig. 6b: Behavioural bioassay responses of each gender to live males.
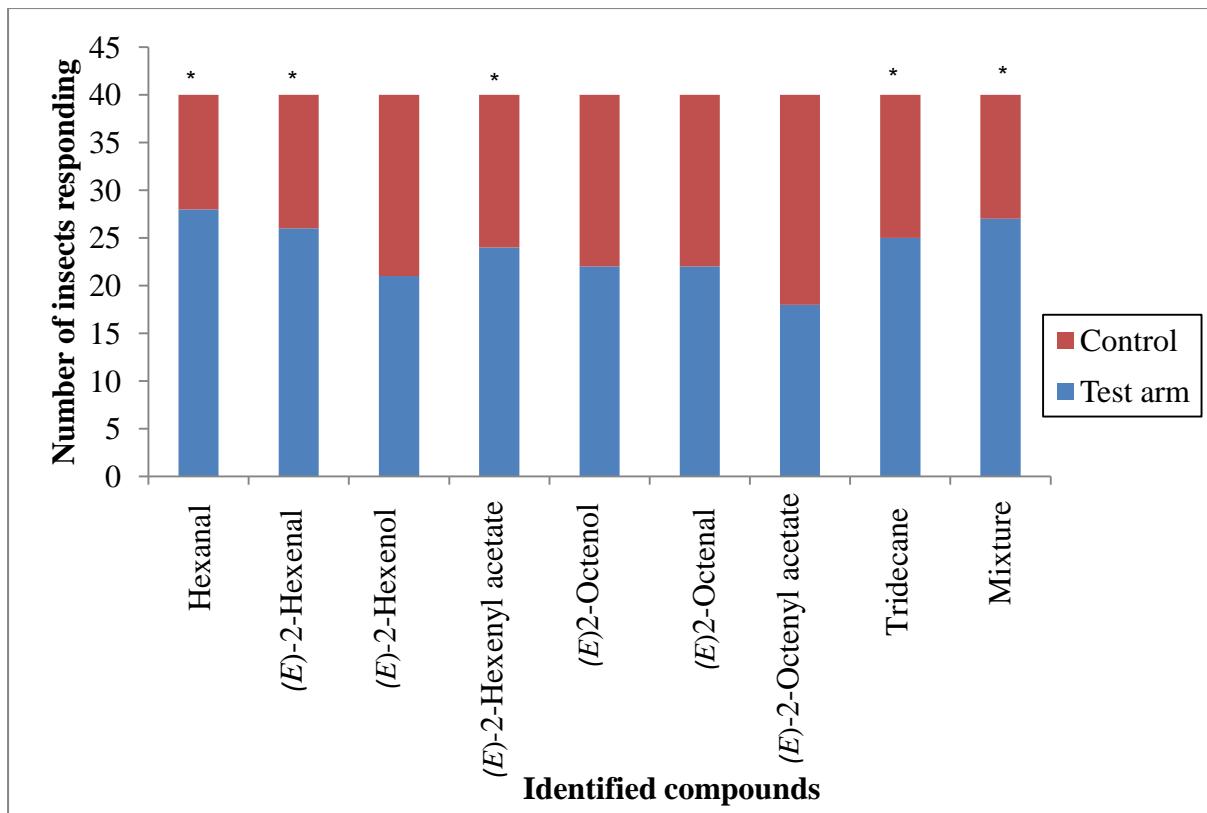
Fig. 7a: Behavioural bioassay responses of females to individual chemical compounds.
*denotes statistical significant difference
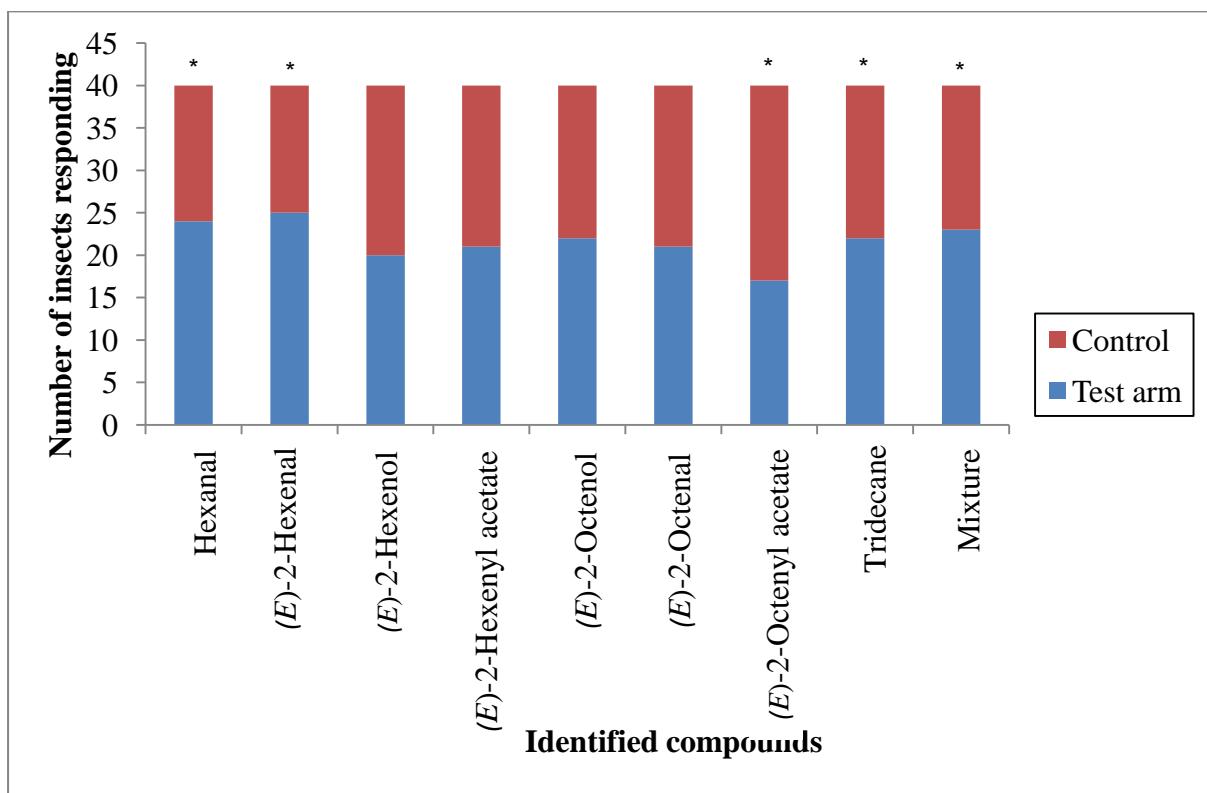
Fig. 7b: Behavioural bioassay responses of males to individual chemical compounds.
*denotes statistical significant difference

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The results from the laboratory bioassay with the lure formulated for field trials showed that the lure was attractive to both males and females (Fig. 8). Significantly more males (26) chose the test arm over the control arm (p = 0.000014) and 29 out of 40 females (p = 0.000939) moved into the test arm containing the lure.
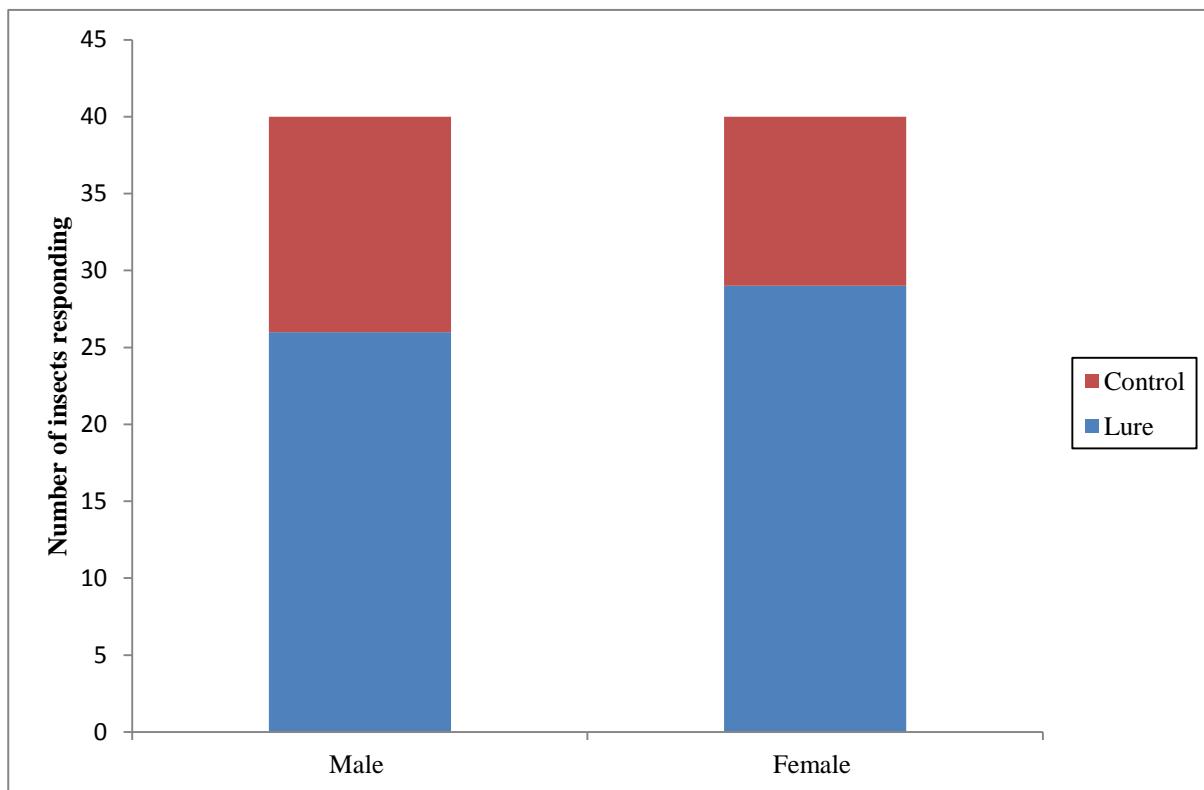
Fig. 8: Behavioural bioassay responses of each gender to the formulated aggregation pheromone lure.

3.3 Field trapping trial

Despite selecting sites in areas with a history of high grain chinch bug infestations, very low numbers of bugs were caught in the two pear orchard sites in Ceres (PAH) on the first visit and nothing was caught in traps or bands on subsequent visits, therefore, the two sites were excluded from the analysis. The remaining sites were the two nectarine orchards (Tandfontein A and B) in Ceres area (KBV) and the bluegum trees in Malmesbury area.

Across these sites, the two trap types and all collection visits, there was only one occasion when a significantly higher number of bugs was caught in baited traps compared to unbaited traps. This was the case for baited bucket traps on the first collection visit to Tandfontein B ($F_{(18, 96)}$ = 1.9547, p = 0.01987) (Fig. 9a). Across all sites and collection visits there was no significant difference in the number of bugs caught in the two different trap types ($F_{(3, 96)}$ = 2.2593, p = 0.08644). Also, there was no significant difference between different visits ($F_{(3, 96)}$ = 1.6108, p = 0.19194). By comparison of the sites, significantly higher numbers of bugs were trapped at Tandfontein A & B in Ceres area than in Malmesbury ($F_{(2, 96)}$ = 7.5753, p = 0.00088).

The trap catches, across all sites, visits and both trap types were significantly different from the band catches in all sites on different visits ($F_{(24, 228)}$ = 7.1524, p = 0.0000). Both delta and bucket traps (whether baited or not) caught significantly less bugs than corrugated cardboard bands ($F_{(4, 228)}$ = 72.362, p = 0.0000). The results showed that both delta and bucket catches were not representative of the level of infestation in the orchards when compared to corrugated cardboard band catches (Fig. 9b). Also there was a significant difference between the numbers of bugs caught in traps and cardboard bands on the first visit compared to other visits ($F_{(3, 228)}$ = 10.782, p = 0.00000). Cardboard band catches in Malmesbury were significantly higher than at the other two sites in Ceres ($F_{(2, 228)}$ = 14.312, p = 0.00000).
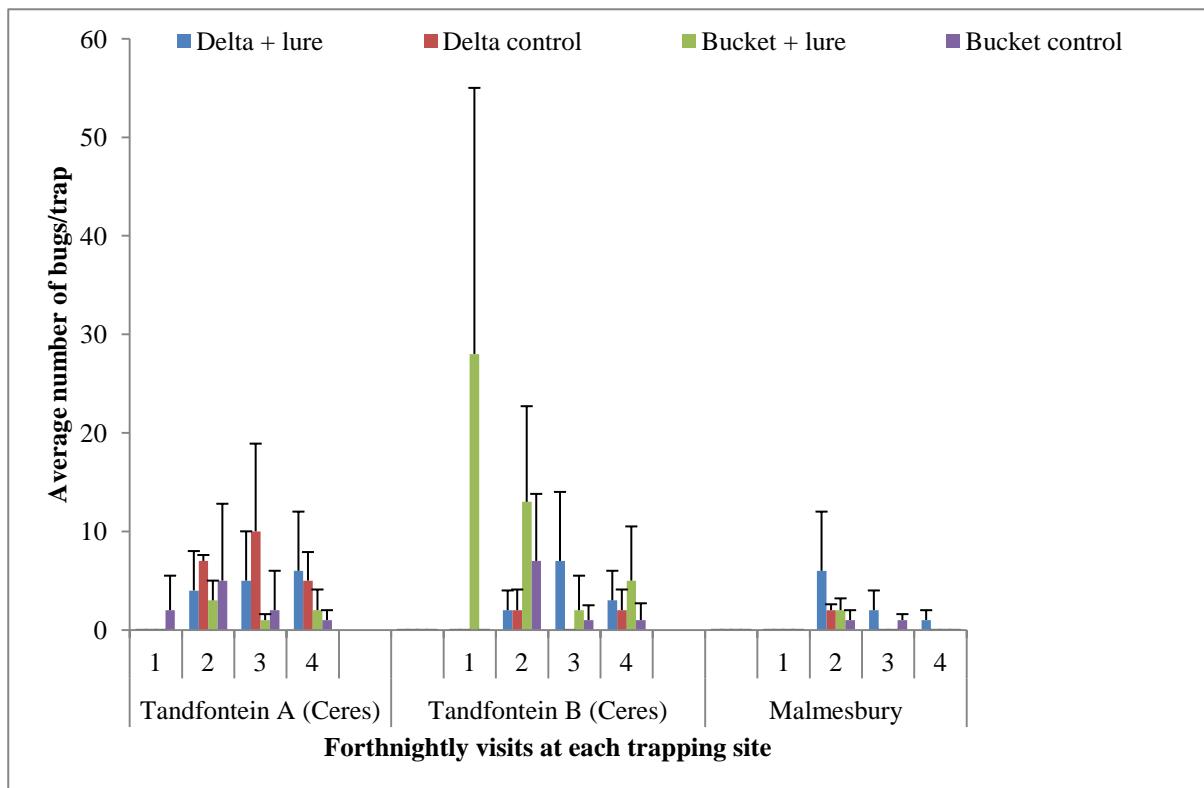
Fig. 9a: Average number of bugs caught per trap (+ S.D) at each visit at each site (December 2009- February 2010).
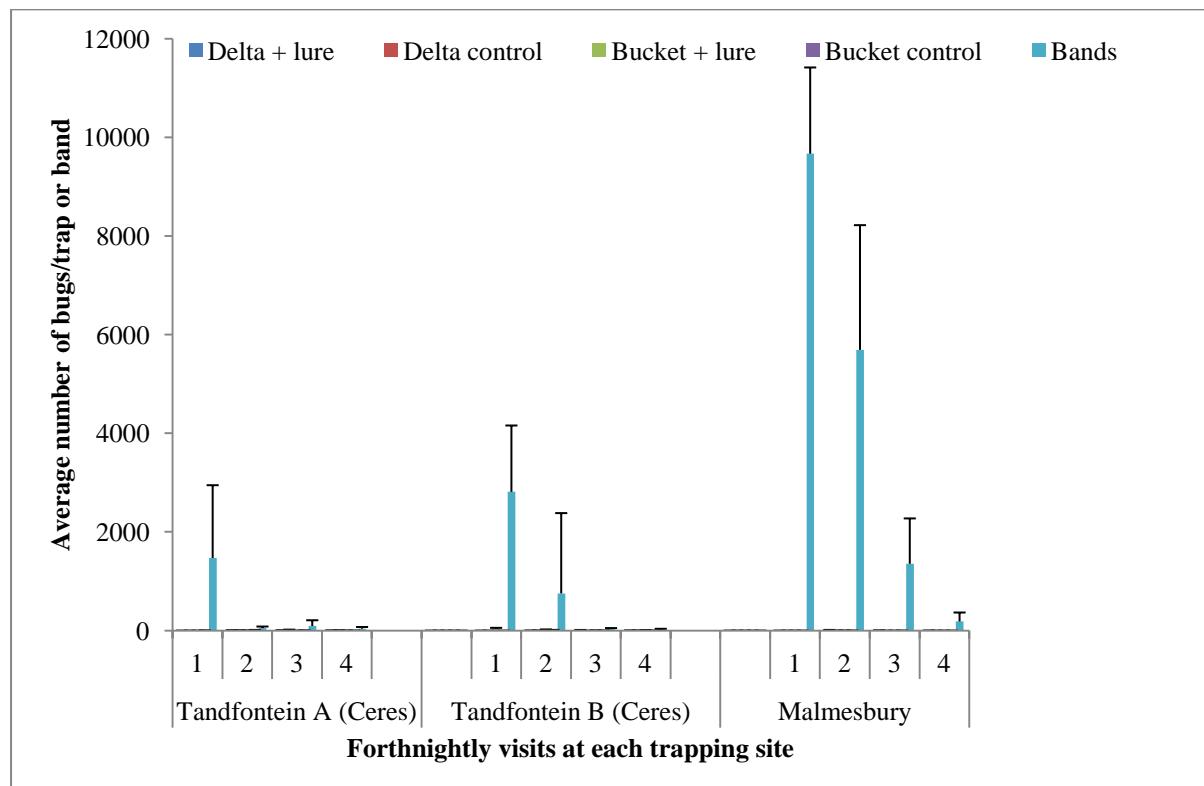
Fig. 9b: Average number of bugs caught per trap or band (+ S.D) at each visit at each site (December 2009 - February 2010).

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Towards the end of the trial it was observed that grain chinch bugs were also sheltering within the walls of the delta traps (Fig. 10). These walls are similar to the corrugated cardboard bands in that they create small spaces between two sheets. In 5 out of the 6 delta traps at the two sites in Ceres, higher numbers of bugs were found sheltering in the walls of delta traps with lures, compared to those without (Fig. 11).

Fig. 10: Bugs sheltering in walls of delta traps.
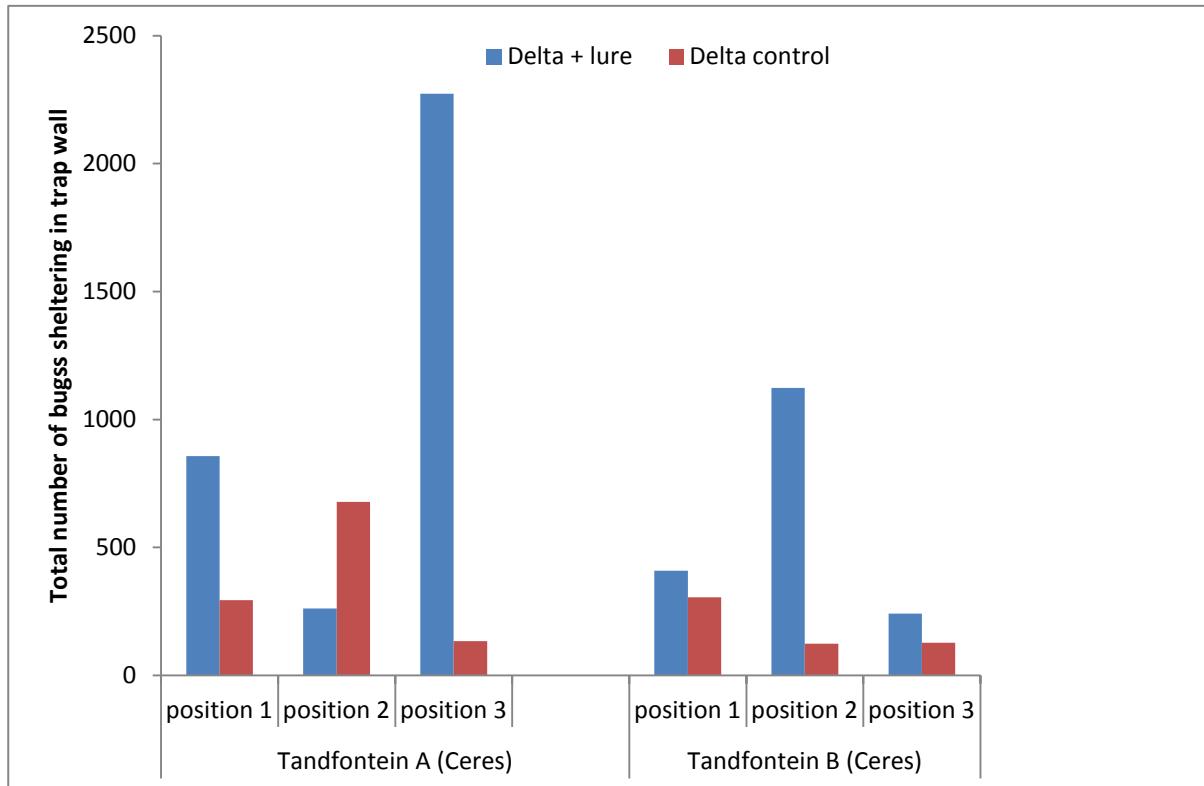
 Fig. 11: Total number of grain chinch bugs sheltering in the wall of delta traps (with and without lures) at the two field sites in Ceres.

4. DISCUSSION

Aggregation pheromone production in Heteropterans has been identified and recorded for well-researched families such as Pentatomidae, Miridae and Lygaeidae (Aller & Caldwell, 1979; Aldrich et al., 1999; Moraes et al., 2008). The aggregation pheromone in Heteropterans facilitates migration to a new source of food or to aestivation sites to escape from unfavourable weather conditions (Aldrich et al., 1999). The large aggregation formation during aestivation, mediated by aggregation pheromone, increases defense mechanisms, reduces desiccation and increases moisture to ensure survival through the aestivation phase (Aldrich, 1988; Bengtsson, 2008). Since aggregation pheromones are important in migration and aestivation behaviour of Heteropterans (Aldrich et al., 1999) and with the understanding that grain chinch bugs migrate to sheltering sites during early summer and form large aggregations through the aestivation period, the presence of an aggregation pheromone mediating this behaviour in this pest is highly likely.

Although the responses of grain chinch bugs to the chemical compounds produced in this study, in both the bioassays and the field trial, were not as strong as expected for an attracting pheromone, there are two observations that support the fact that grain chinch bugs do produce an aggregation pheromone. Firstly, during collection of the volatile chemical constituents being released from aestivating grain chinch bugs, using the sample enrichment probe, all the insects inside the vial aggregated on the probe as it became loaded with pheromone. Secondly, the laboratory bioassays with live insects in the test arms showed that the pheromone being released during aestivation was equally attractive to both males and females. This is indicative of an attractive response to an aggregation pheromone rather than a sex pheromone. Aggregation and alarm pheromones can be released from either one or both sexes, whereas sex pheromones are produced by only one gender to attract the other for

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mating (Oliver et al., 1996; Demirel, 2007). Although female grain chinch bugs did appear to disseminate the aggregation pheromone more efficiently than males, since females in the test arms attracted both sexes more than males in the test arms, the production of pheromone was not quantified. Qualitatively, however, there was no difference in the components of the pheromone produced by both sexes. The efficient dissemination of the pheromone by females may have influenced the positive response seen between males if the female pheromone had contaminated males prior to separation before commencement of the bioassay.

In the only other study on the chemical ecology of grain chinch bugs, Oliver et al. (1996) described the chemical compounds identified as constituents of a defensive secretion or alarm pheromone. This could be attributed to the methods used, since during sample collection, bugs were constantly agitated to produce volatiles that had a 'distinctive stinkbug odour'. Insects often use the same chemical compounds for aggregation and alarm pheromones (Raska, 2009). The behavioural response elicited is determined by the concentrations and ratios of constituents in the mixture (Demirel, 2007). Compounds such as tridecane, hexanal, (*E*)-2-hexenal and (*E*)-2-octenal, identified by Oliver et al. (1996), as well as in the present study, have been reported to be used by true bugs, such as the cabbage bug, *Eurydema rugosa* and the southern green stink bug, *Nezara viridula*, for both alarm and aggregation pheromones, but with different concentrations of the compounds in each (Ishiwatari, 1976; Lockwood & Story 1985). Another example of this dual use of a chemical compound has been reported for the bean bug, *Riptortus clavatus*, with (*E*)-2-hexenyl hexanoate as one of the components of the alarm pheromone (Leal & Kadosawa, 1992), as well as the aggregation pheromone (Yasuda et al., 2007). In the present study, the identified compounds are in fact those that elicit aggregation behaviour since insects were collected while aggregating during aestivation, and most importantly, insects were not disturbed during the headspace collection process. Furthermore, one of the compounds found in the defensive secretions of grain chinch

bugs, identified by Oliver et al. (1996), 4-oxo-(E)-2-hexenal, is known to be associated with defensive secretions of true bugs (Aldrich et al., 1991; Moreira & Millar, 2005; Prudic et al., 2008), and was not found in the present study.

Of the chemical compounds identified in this study, (E)-2-hexenol and (E)-2-octenol are two additional compounds to those previously identified by Olivier et al. (1996). These two compounds have also been isolated from other heteropterans, but their biological activity was not ascertained (Millar, 2005). In grain chinch bug these compounds may be only minor constituents of the aggregation pheromone, as they did not play a major role in attracting bugs.

The three individual volatile compounds that were the most attractive to both male and female grain chinch bugs, namely hexanal, (E)-2-hexenal and tridecane, may likely be the important constituents of grain chinch bug aggregation pheromone. These compounds have been reported to play a role in aggregation pheromone of true bugs (Ishiwatari, 1976; Millar, 2005; Moraes et al., 2008). In addition to mediating behaviour, the aliphatic hydrocarbon, tridecane, which is often found in high quantities in extracts of bugs, also serves as a solvent modulating the evaporation of other compounds (Aldrich, 1988). In this study, the relative quantity of tridecane was high, suggesting that it did serve as the carrier agent for the controlled evaporation of other compounds, but it was also attractive to both male and female grain chinch bugs, and thus also important in aggregation.

Of the eight compounds identified in this study the least attractive to both genders, (E)-2-octenyl acetate, has been reported as a constitutent of both aggregation and alarm pheromones in heteropterans (Ishiwatari, 1974; 1976). It is one of the two attractant components of the lygaeid bug, *Tropidothorax cruciger*, which was attractive to both sexes (Aldrich et al., 1997). Its attractive effect on male rice bug, *Leptocorisa chinensis* has also

been reported (Leal et al., 1996; Watanabe et al., 2009), while it is found as one of the components of the defensive and alarm pheromones of Neotropical stink bugs (Moraes et al., 2008).

Although attraction to the formulated lure was evident in the controlled conditions of the laboratory, in the field the lure is exposed to the changing external environment, and did not perform as well. Pheromone dose, component blend ratios, range of pheromone attraction or masking of the main pheromone components by other compounds in the mixture, and many other factors, reviewed in Millar et al. (2000), all contribute to the efficacy of lures. The volume of pheromone, i.e. pheromone dose, and correct combination of pheromone components, i.e. pheromone blend ratio, is essential for optimal trapping. Leskey & Hogmire (2005) reported that a higher number of *Euschistus* stink bugs were trapped in apple and peach orchards, when a higher volume of aggregation pheromone was used compared to a lower dosage. Millar et al. (2010) reported that a low concentration of aggregation pheromone was attractive to *Chlorochora* stink bugs, while high volumes elicited a repellent response in the field. Changes in pheromone blend ratio of the chemical constituents of an aggregation pheromone can either improve or reduce the efficacy of a lure. Optimisation of the blend ratio of components of larger grain borer, *Prostephanus truncatus* aggregation pheromone by increasing the amount of the major component improved trap catches by more than 20% (Hodges et al., 2004). On the other hand, increasing the amount of certain components in the blend ratio of Mexican rice borer, *Eoreuma loftini* aggregation pheromone significantly reduced the efficacy of the pheromone (Shaver et al., 1990). The poor efficacy of the lure used in the field in the present study could be attributed to the volume and blend of components used in this first trial. Additional work on the chemistry of grain chinch bug aggregation pheromone components is required to optimise its use in field trapping

The release rate of a dispenser is also an important factor affecting efficacy of lures. The rate of pheromone release depends on the volatility of the chemical components, as well as the material and structure of the pheromone dispenser. Different types of dispensers include rubber septa, poly-ethylene sachets, and plastic or glass vials. Rubber septa were found to be inefficient in dispensing the synthetic aggregation pheromone of *Chlorochora sayi* optimally (Millar et al., 2010). Authors attributed this to the low volatility of one of the important components of the pheromone blend and the slow rate at which it would diffuse through the rubber septum. In a study that compared the release rate of different dispensers for trapping the Mexican rice borer, *Eoreuma loftini*, a rubber septum dispenser was highly effective in the field, compared to polyvinyl chloride plastic rods and plastic vials (Shaver et al., 1990). A poly-ethylene sachet dispenser was found to be optimal in dispensing aggregation pheromone at a standard release rate for trapping strawberry weevil, *Anthonomus rubi* (Cross et al., 2006). In the present study, a glass vial covered with poly-ethylene film did not appear to give optimum release rate of pheromones for trapping grain chinch bugs in the field trials. Also, environmental conditions such as wind and high temperature during the trial might have contributed to the ineffective dispensing of the pheromones from the release devise. Therefore in future trials, other types of dispenser that will improve the release rate for trapping grain chinch bugs should be tested.

Synthetic pheromone lures in the field need to compete with the natural pheromones released by bugs in the field. In a study to test the response of the red palm weevil, *Rhynchophorus ferrugineus*, to synthetic aggregation pheromone in date palm plantations, weevils were more attracted to the natural pheromones released by large insect infestations (Abbas et al., 2006). Also, Athanassiou et al. (2006) reported the responses of two stored product pests, *Sitophilus oryzae* and *Tribolium confusum*, to traps baited with synthetic pheromone, food volatiles or live insects. Both species were more attracted to traps with live insects or food volatiles,

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compared to the synthetic pheromone. The large aggregations of grain chinch bugs in the cardboard bands, seen in the present study, also created a challenge for effective attraction of bugs to the lure. Live insects sheltering in the cardboard bands would continue to release aggregation pheromone, as opposed to trapped and killed insects in the delta and bucket traps. In addition, bugs trapped in the sticky pads in delta traps may have also released an alarm pheromone, which would have deterred other bugs from the vicinity of the traps. Furthermore, large numbers of grain chinch bugs were observed sheltering in the walls of delta traps which provided space suitable for sheltering. Bugs sheltering in the trap walls were alive and would also be releasing more pheromone to maintain the aggregation.

In the case of bugs sheltering in the walls of delta traps, the lure did appear to have some attracting effect since more bugs were found in the walls of baited delta traps than in unbaited traps. However, there is more evidence of trap catches being largely incidental, based on the number of bugs present in the field, since the average number of bugs sheltering in cardboard bands was, in some cases, thousands of times more than those found in the traps. This is similar to the results of a previous study which tested general stink bug pheromones to trap grain chinch bugs in the field. The results showed that numbers of pests caught in pheromone traps were very low at a time when grain chinch bug movement between fields and orchards was very high (Addison, 2004). The high number of grain chinch bugs found within cardboard bands here, and the bugs sheltering within the walls of delta traps, are indicative of the importance of a covered sheltering site for these bugs. This shelter-seeking behaviour should be used to the advantage of developing an effective trapping method. Incorporating sheltering sites into the trap design, together with an effective lure, would ensure an efficient trapping system for grain chinch bugs.

Improvement in the design of a trap can aid its efficacy. For example, Hogmire & Leskey (2006) used a modified general pyramid trap to trap stink bugs. Trap catches increased

fourfold and the improved catch was attributed to improved design which reduced escape of the attracted bugs. The modifications to the general pyramidal traps by Adachi et al. (2007) increased trap catches of fruit-piercing stink bugs *Plautia crossota stali* in the field. Furthermore, by modifying the two fixed components of general pyramidal traps to three easily detachable components, Adachi et al. (2007) recorded improved trap catches of fruit-piercing stink bugs *Plautia crossota stali* in the field. Adjustments to sticky pads in sticky stake traps increased trap catches of strawberry weevil *Anthonomus rubi,* in comparison to catches in funnel, delta and boll weevil traps (Cross et al., 2006). In the present study, to improve the efficacy of the traps, structural adjustments to accommodate the sheltering behaviour of the bugs might be useful to improve trap design and ultimately trap catch. In addition to trap design, trap colour can also play an important role in trapping efficacy. However, Addison (2004) reported no effect of colour on grain chinch bug trapping when brown, yellow, white, clear, black and red coloured sticky traps were tested. The trap catches were low and inconsistent, as bugs were not attracted to the colour but flew into the traps accidentally. The low trap catches in the yellow bucket and delta traps in the present study could not be due to colour, but are more likely a result of poor efficacy of the synthetic lure.

This study is an important first step in identification of the constituents of the grain chinch bug aggregation pheromone. The aggregation of bugs on the sample enrichment probe during the collection of headspace volatiles and attraction of both sexes to females in the behavioural bioassay indicates that the pheromone released elicits aggregation behaviour. Although the compounds identified elicited a low attracting response compared to what is expected of an attractant, the chemical compounds that are likely to be important in grain chinch bug aggregation were identified. The use of these compounds in a lure now needs to be optimised. The lure formulated from the mixture of identified compounds can be improved and effective trapping achieved if pheromone dose, pheromone blend ratios, as well as an appropriate

dispenser for optimum release rate could be improved. Moreover, the sheltering behaviour of grain chinch bugs, which contributed to the erratic nature of trap catches, could be used to improve trap catches by incorporating sheltering sites into the trap design. If all these shortcomings highlighted by this study could be overcome, intervention at orchard level with effective trapping and monitoring will help to reduce the phytosanitary risk posed by the presence of grain chinch bugs in fruit intended for export.

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CHAPTER 3

THERMAL TOLERANCE OF A QUARANTINE PEST, THE GRAIN CHINCH BUG, *MACCHIADEMUS DIPLOPTERUS* (DISTANT): IMPLICATIONS FOR POSTHARVEST CONTROL

1. INTRODUCTION

Temperature plays a critical role in determining ectotherm fitness and survival at various time-scales. As temperature increases or decreases from the optimal range, activity in an insect becomes limited, and may even result in death. However the lethality of a thermal exposure is dependent on a number of factors including rate of temperature change, photoperiod, humidity, nutritional status, and magnitude and duration of thermal exposure (Hoffmann et al., 2003; Terblanche et al., 2007; Liefting & Ellers, 2008).

Given the above factors, static and dynamic protocols can thus be used to assess insect thermal tolerance. Critical thermal limit (CTL) determination is one of the widely used methods in determining insect thermal tolerance (Lutterschmidt & Hutchison, 1997). Critical thermal maximum (CT_{max}) and minimum (CT_{min}) temperatures are the highest and lowest temperatures, respectively, that an organism can tolerate and retain normal functions. Beyond CT_{max} and CT_{min}, organisms become dysfunctional and may ultimately die (Chown & Nicolson, 2004). CTLs are measured by cooling or heating an animal at a controlled rate from an initial temperature to a point where there is physiological failure such as loss of righting response, onset of muscle spasms or knockdown (Lutterschmidt & Hutchison, 1997; Chown & Nicolson, 2004). However, insects facing a variety of thermal stressors can adjust their tolerance in order to continue performing optimally in unfavourable temperature

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conditions (Loeschcke & Hoffmann, 2002; Liefting & Ellers, 2008; Chidawanyika & Terblanche, 2010). This ability to adjust tolerance is termed phenotypic plasticity, and is defined as the ability of an organism to develop into any of several phenotypic states depending on the environment (Deere & Chown, 2006; reviewed in Chown & Terblanche, 2007). Phenotypic plasticity is important to a species' fitness since it allows for an organism to adapt and survive in a changing environment (Seebacher, 2005). A period of exposure to sub-lethal thermal conditions may be a short or long term exposure which gives rise to hardening and acclimation responses in phenotypically plastic organisms (Hoffmann et al., 2003). Acclimation responses of thermal tolerance in insects can occur in response to changes in temperature, photoperiod, humidity, or some combination of these (Hoffmann et al., 2003; Jumbam et al., 2008a). Such plasticity of temperature tolerance in insect pests may result in reduction of the effectiveness of thermally based post-harvest disinfestation treatments.

Post-harvest temperature treatments are applied against quarantine insect pests associated with fresh horticultural produce to mitigate the risk of spread through international trade (Hallman, 1994; Hara et al., 1996; Waddell et al., 1997). An effective post-harvest temperature treatment ensures that the treatment either increases or decreases produce temperature above or below the thermal tolerance of its associated insect pest. One of the limitations of temperature treatments for temperate fruit disinfestation is the inability of the fruits to withstand treatments without damage, which renders the fruit unmarketable. Also, pre-exposure to sub-lethal temperatures can affect insect response to subsequent temperature treatments. The sources of non-lethal temperatures could be from pre-harvest and packhouse temperatures before the actual disinfestation treatments. For instance, exposure to various elevated thermal conditions increases heat resistance of quarantine insect pests such as codling moth *Cydia pomonella* and light brown apple moth *Epiphyas postvittana* to

subsequent post-harvest temperature treatments (Hara et al., 1997; Lester & Greenwood, 1997; Yin et al., 2006).

Grain chinch bug, *Macchiademus diplopterus* (Distant), is an indigenous quarantine pest of export fruits from South Africa. Due to the risk of introduction of grain chinch bugs into new areas, quarantine restrictions are placed on fruits exported from South Africa. These require that approved disinfestation treatments be carried out before shipment, but presently there are no alternatives to fumigation with methyl bromide. The presence of live grain chinch bugs as contaminants in fruits has caused rejection of packed export fruit cartons (Myburgh & Kriegler, 1967; Johnson & Addison, 2008). Furthermore, Myburgh & Kriegler, (1967), anecdotally reported a cold tolerance trait in grain chinch bugs since insects survived cold storage during transit (-0.5°C to -1°C) and were found alive on export fruit at the destination port.

Since insect plasticity to temperature tolerance improves survival or activity in otherwise lethal environments, an understanding of *Macchiademus diplopterus* thermal tolerances would likely help in designing effective post-harvest protocols. Therefore, the aim of this study is to gain a better understanding of grain chinch bug thermal tolerance by determining the critical thermal limits of active and aestivating grain chinch bugs, as well as the effect of acclimation on aestivating bugs, for the development of effective post-harvest temperature treatments against this pest.

2. MATERIALS AND METHODS

2.1 Experimental insects

Test insects were collected in Malmesbury, Western Cape South Africa, during both the active and aestivating periods of the life cycle of *M. diplopterus*. Actively reproducing bugs were collected from July - September 2009 from wheat and oats fields. Aestivating bugs were collected from December 2009 - May 2010 from sheltering sites on *Eucalyptus* trees at different weeks into aestivation (2, 10 & 14 weeks for CTL determinations, and 27 weeks for acclimation treatments).

2.2 Critical thermal limits determination

Critical thermal limits were determined using a perspex organ pipe apparatus connected to a programmable water bath. The programmable water bath (Grant GP200-R4, Grant Investments, UK) was filled with a 1:1 mixture of propylene glycol and water to allow for subzero temperatures to be reached during the cool temperature phase. To record the chamber temperature, a type K thermocouple connected to a Fluke 54 series 2 (Fluke Cooperation, China) digital thermometer (accuracy $0.05°C$) was inserted into the control chamber (in organ pipes). Insects ($n = 10$) were loaded individually into a chamber in the organ pipe apparatus and the treatment was started. The assumption was that, for small insects ($<1g$), body temperature is always in equilibrium with environmental temperature; in this case, chamber temperature. Treatments started at 25ºC and ramped up or down, at 0.25ºC per minute following one of the protocols of Terblanche et al. (2007). The temperature at which each individual insect lost muscle function and was unable to respond to external stimuli by probing, was recorded as CT_{min} or CT_{max} for that individual. The experiment was conducted

on active and aestivating bugs at 2, 10 and 14 weeks into aestivation, with each gender separately and replicated 10 times (each insect serving as a replicate).

2.3 Effect of thermal and photoperiod acclimation on CTL of aestivating bugs

To determine whether a period of exposure to temperature variation affects the CT_{min} and CT_{max} of aestivating grain chinch bugs, insects were collected in the field 27 weeks into the aestivation period. These bugs were acclimated in an environment chamber at 18°C (10L: 14D) or 26°C (16L:8D), to reflect the average temperature and daylength for both active and aestivation periods, for a period of seven days. This period of seven days to acclimation temperatures has been shown to be representative of long-term response to altered temperatures (e.g. Terblanche et al., 2006; Nyamukondiwa & Terblanche, 2010). CT_{min} and CT_{max} experiments were conducted on 18°C and 26°C acclimated bugs, with each gender separately, as described above.

2.4 Field temperature data

To compare the thermal tolerance level of grain chinch bugs to what is experienced in the field where experimental insects were collected, the daily minimum and maximum temperatures from January to December 2010, in Malmesbury, South Africa, was obtained from the South African Weather Service.

2.5 Statistical analysis

Data were checked for normality using Shapiro-Wilks tests. The critical thermal limits and the effects of acclimation on both upper and lower thermal limits for both genders were analysed using factorial Analysis of Variance (ANOVA) in Statistica 8 (Statsoft, USA) followed by Tukey-Kramer's post- hoc test to identify homogeneous groups.

3. RESULTS

3.1 Critical thermal limits of active and aestivating bugs

The critical thermal minima for active and aestivating grain chinch bugs are shown in Fig. 1. There was a significant difference between the CT_{min} of males and females during the active period ($F_{(1, 152)} = 27.1$, $p < 0.001$). For both males and females there was a decrease in CT_{min} from the active period into aestivation, and the downward trend continued for weeks into the aestivation period ($F_{(3, 152)} = 82.0$, $p < 0.001$). The CT_{min} of active males decreased from 2.8°C to 1.4°C (± 0.1) at 2 weeks into aestivation and continued to decrease reaching 1.0°C (± 0.1) by 14 weeks into aestivation. The CT_{min} of active females decreased from 2.1°C to 1.2°C (± 0.1) at 2 weeks into aestivation, and continued to decrease reaching 0.6°C (± 0.1) by 14 weeks into aestivation.

The critical thermal maxima for active and aestivating grain chinch bugs are shown in Fig. 2. Unlike for CT_{min}, there was no difference between genders for CT_{max} during the active and different aestivation period collection time ($F_{(1, 152)} = 3.3$, $p= 0.072$). For both genders, CT_{max} increased for bugs collected from the active period into aestivation, and continued to increase for weeks into the aestivation period ($F_{(3, 152)} = 14.1$, $p < 0.001$). The CT_{max} of active males 49.9°C (± 0.1) was similar to that of males at 2 weeks into aestivation 50.0°C (± 0.1), but increased significantly to 51.0°C (± 0.1) by 14 weeks into aestivation. The CT_{max} of active females 49.9°C (± 0.1) was also similar to female bugs at 2 weeks into aestivation 50.1°C (± 0.1) and increased significantly to 51.5°C (± 0.1) by 14 weeks into aestivation.
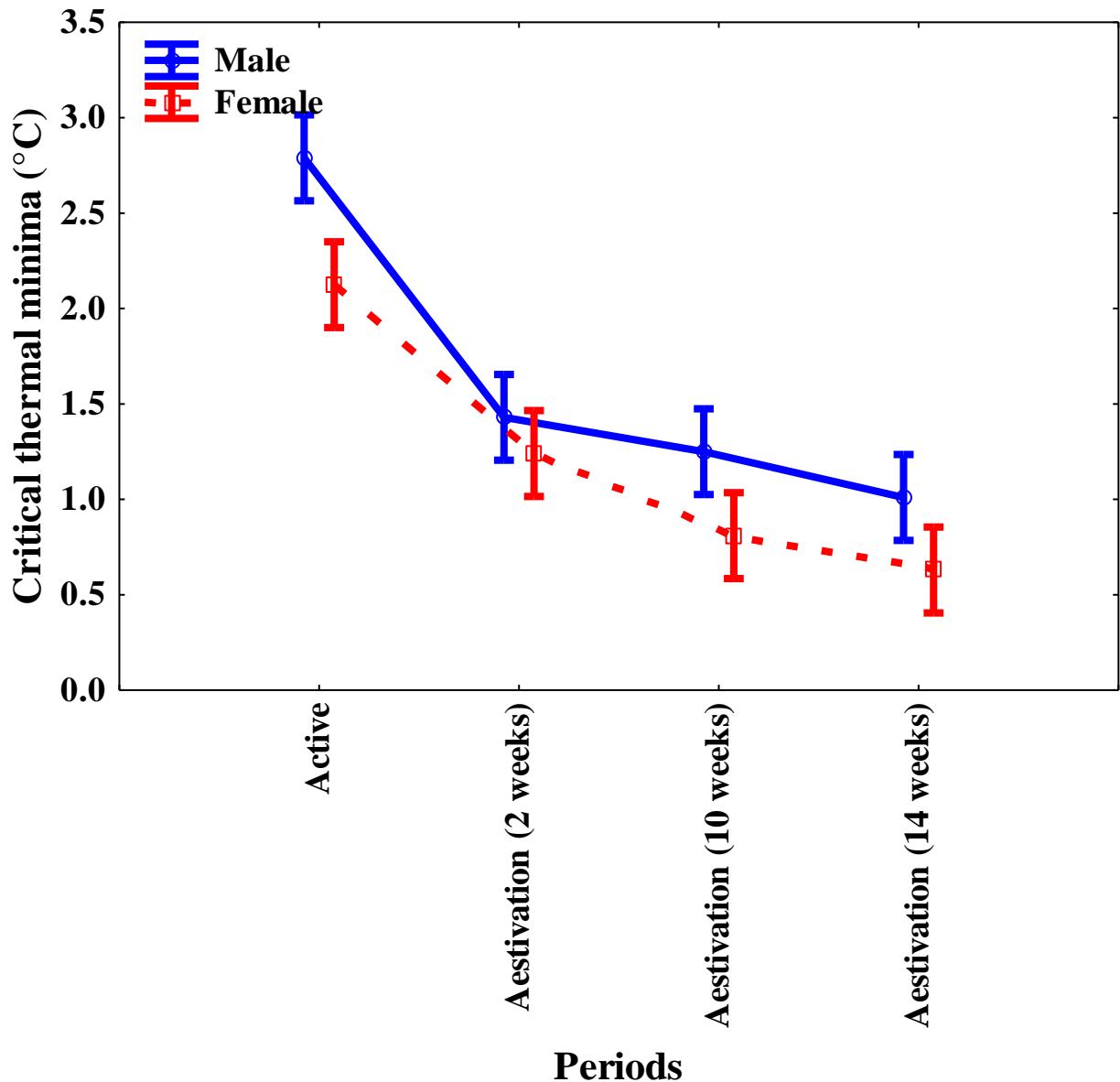
Fig 1. CT_{min} of active and aestivating male and female grain chinch bugs at different periods (mean ± 95% CL). n = 20 for each treatment.
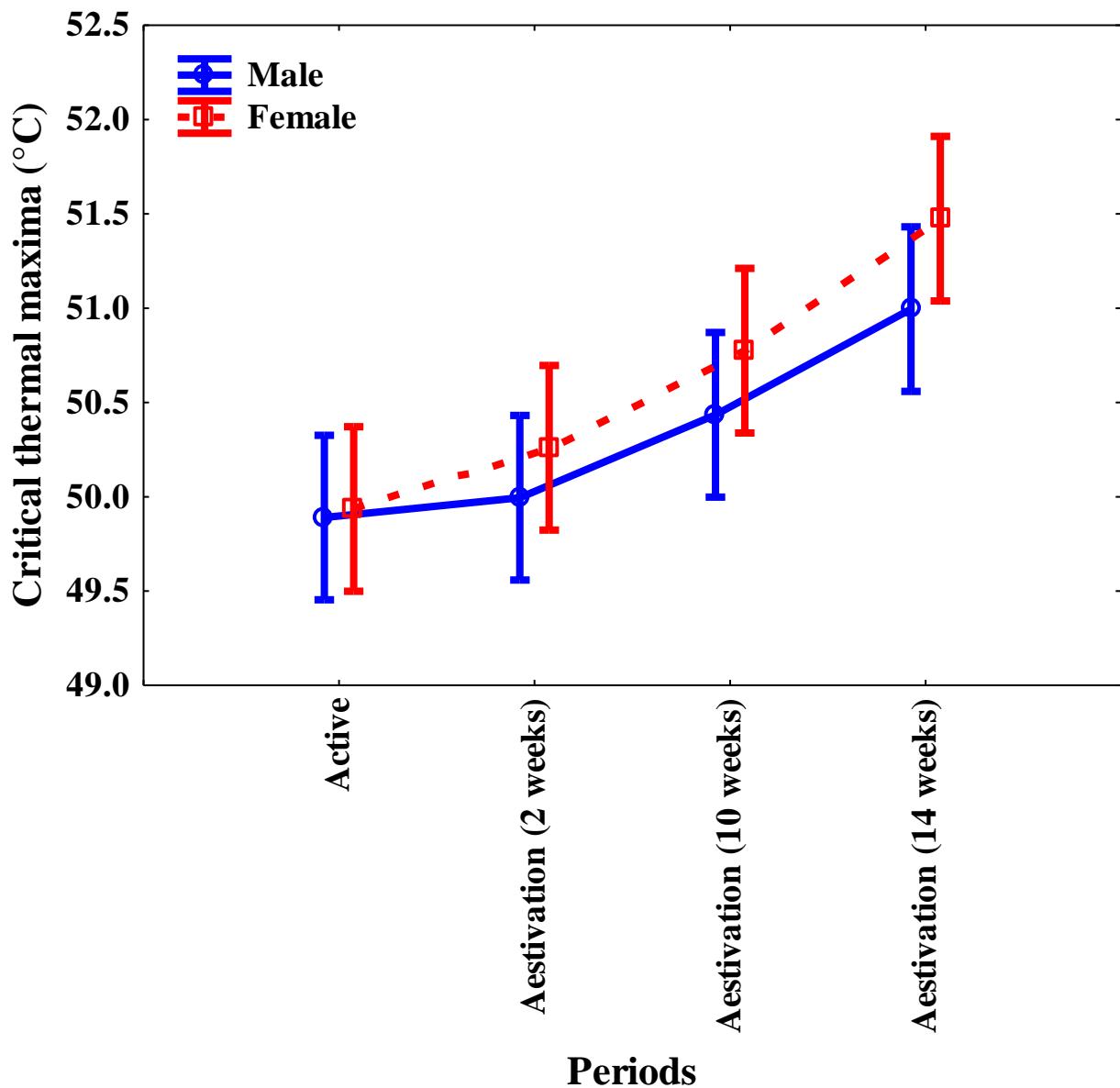
Fig 2. CT_{max} of active and aestivating male and female grain chinch bugs at different periods (mean ± 95% CL). n = 20 for each treatment.

3.2 Effect of thermal and photoperiod acclimation on CTL of aestivating bugs

The critical thermal minima of both male and female bugs at 14 weeks into aestivation and different acclimation treatments are shown in Fig. 3. At the low acclimation temperature of 18°C and short daylength photoperiod (10L:14D) for CT_{min}, there was no significant difference between male and female bugs ($p > 0.05$). In comparison to aestivating bugs at 14 weeks, which is the closest available date to the acclimated aestivating bugs at 27 weeks, CT_{min} reduced significantly ($p < 0.05$) from 1.0°C to -1.2 °C (± 0.1) in males and from 0.6°C to -1.2°C (± 0.1) in females. After acclimation at a high temperature of 26°C and long daylength (16L:8D), the CT_{min} of males (0.3°C ± 0.1) and females (-0.4°C ± 0.1) differed significantly. Also in comparison to aestivating bugs at 14 weeks, CT_{min} decreased for males from 1.0°C to 0.3°C (± 0.1) and for females from 0.6°C to -0.4°C (± 0.1). Generally, both low (18°C) and high (26°C) acclimation treatments significantly decreased CT_{min} of aestivating grain chinch bugs ($F_{(1, 76)} = 92.1$, $p < 0.001$), but low acclimation treatments had the greatest effect in lowering CT_{min} of bugs.

The critical thermal maxima of both male and female bugs at 14 weeks into aestivation and different acclimation treatments are shown in Fig. 4. At low acclimation temperature of 18°C and short daylength photoperiod (10L:14D), the CT_{max} of male and female bugs differed significantly ($p < 0.05$) by 0.4°C (± 0.1) from each other. In comparison to aestivating bugs at 14 weeks, there was no significant difference ($p > 0.05$) in CT_{max} of males. While in females, CT_{max} of acclimated aestivating bugs decreased to 51.1°C (± 0.1) at the low acclimation treatment. At the high acclimation temperature of 26°C and long daylength photoperiod (16L:8D), there was also a significant difference ($p < 0.05$) between males (51.0°C ± 0.09) and females (51.4°C ± 0.1) bugs. In comparison to aestivating bugs at 14 weeks, there was no significant difference ($p > 0.05$) in CT_{max}. Generally, acclimation treatments did not have any

significant effect ($F_{(1, 76)} = 0.05$, $p = 0.817$) on the CT_{max} of aestivating grain chinch bugs except for females at low acclimation treatments.
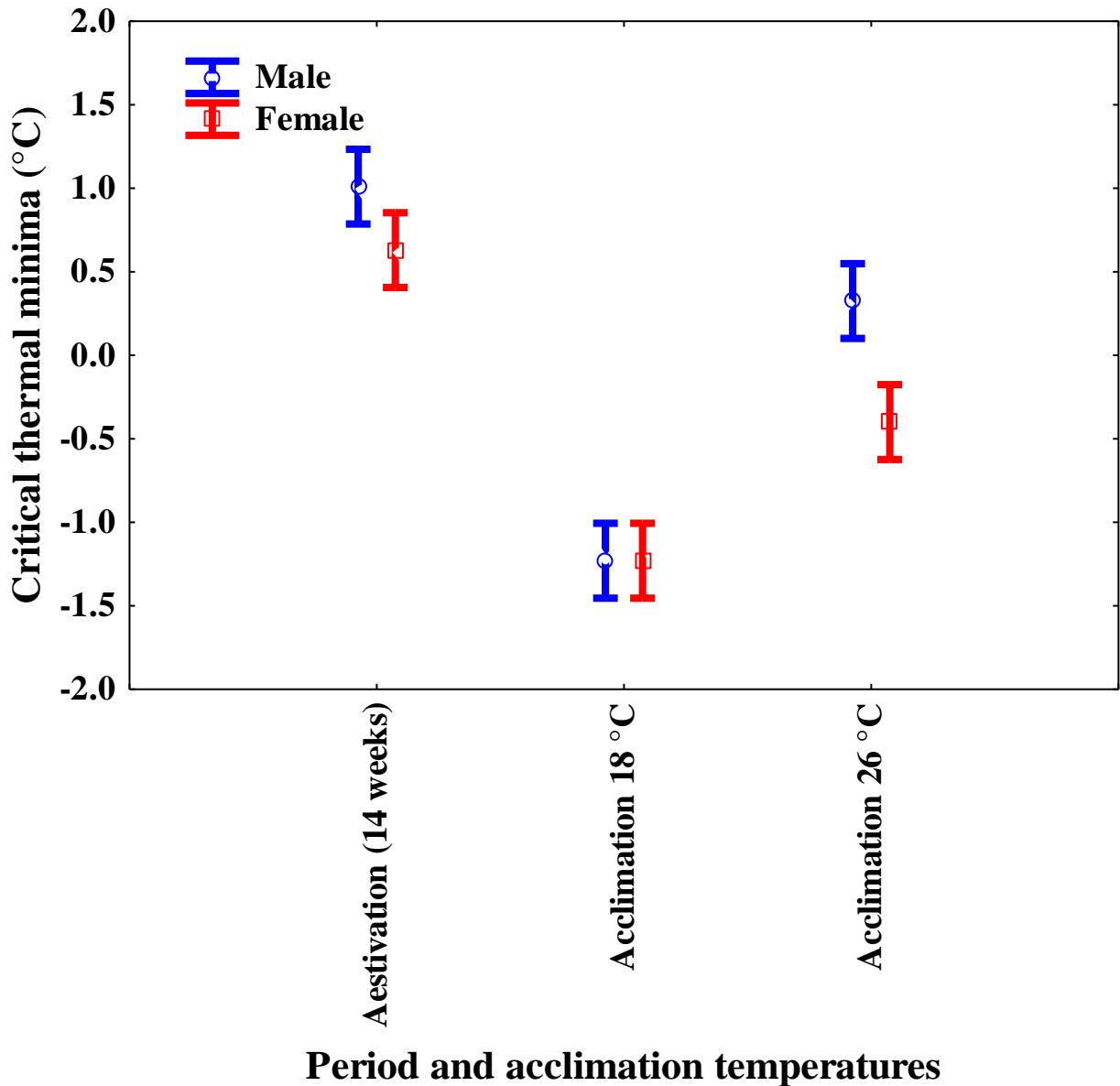
Fig 3. CT_{min} of both genders at aestivation (14 weeks) and aestivation (27 weeks) + acclimation temperatures (mean ± 95% CL). n = 20 for each treatment.
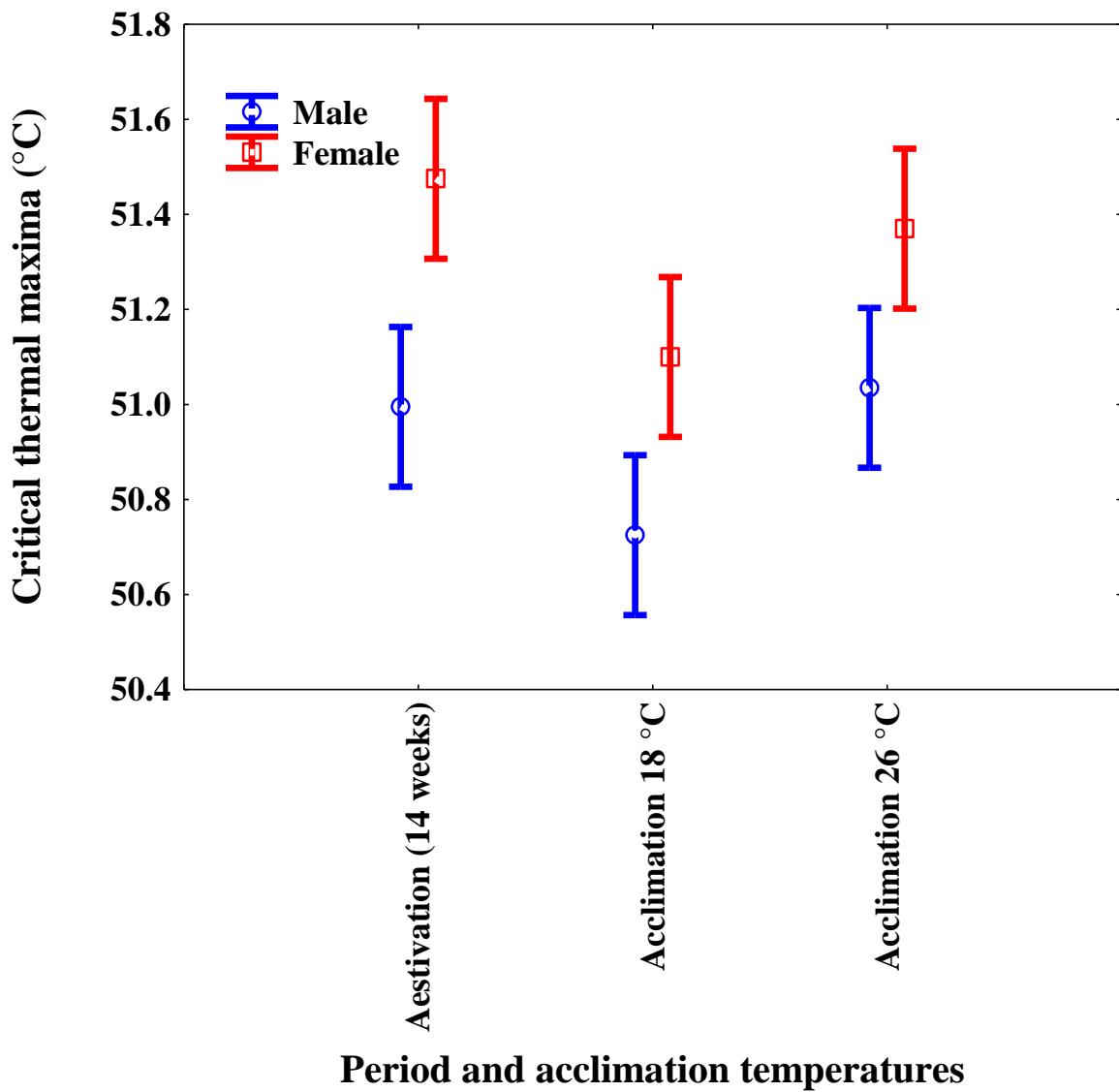
Fig 4. CT$_{max}$ of both genders at aestivation (14 weeks) and aestivation (27 weeks) + acclimation temperatures (mean ± 95% CL). n = 20 for each treatment.

3.3 The field temperature data

Temperature data from the field site where grain chinch bugs were collected for critical

thermal limit determination are shown in Fig 5.
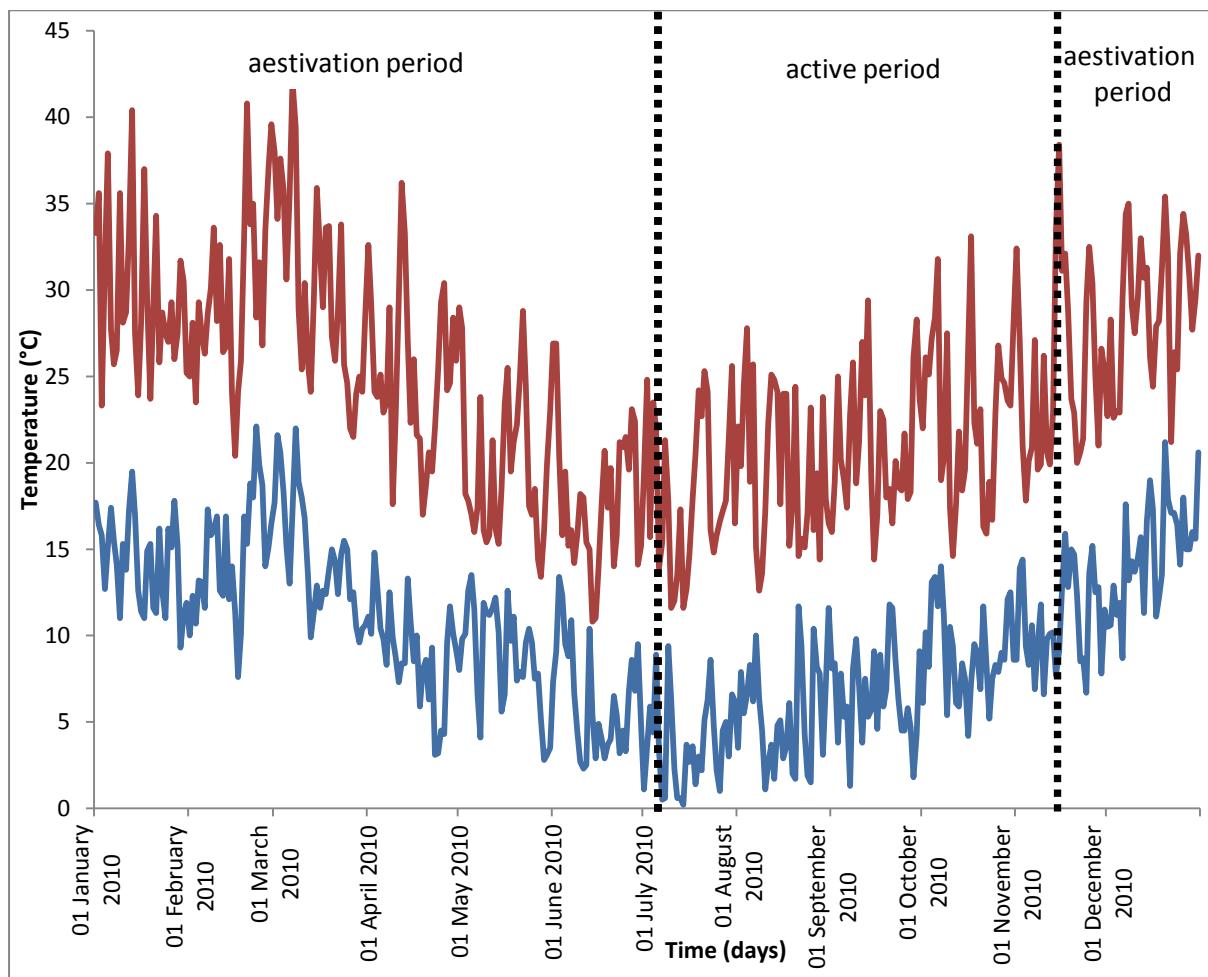
Fig. 5. Daily minimum and maximum temperature data for Malmesbury, South Africa, from

January to December 2010 (source: South African Weather Service). Dashed lines denote the

active and aestivation periods of grain chinch bug seasonal cycle.

The minimum temperature recorded during the winter which is the period when grain chinch

bugs are active was 1.8°C while the maximum temperature was 20.7°C. In summer, when

grain chinch bugs are in reduced activity and aestivating in sheltering positions, the minimum

and maximum temperatures experienced in the field were 12.6°C and 36.2°C, respectively.

4. DISCUSSION

Understanding the critical thermal limits of quarantine insect pests is valuable in assessing the potential of postharvest temperature treatments which can be applied to control these pests. The upper and lower thermal tolerances of grain chinch bugs increase as adult bugs move from actively reproducing on host plants, to becoming quiescent during the aestivation phase of their life cycle. Since it is the aestivating grain chinch bug that infests export fruit, the CT_{min} and CT_{max} values of aestivating, and not active bugs should be taken into account when considering potential postharvest temperature treatments. The increased thermal tolerance of aestivating bugs, seen here, does not hold promising prospects for the use of temperature treatments alone, to mitigate the quarantine risk posed by grain chinch bugs.

The lowest CT_{min} found in aestivating grain chinch bugs (0.6°C for females at 14 weeks) is similar to that found for some tropical and temperate insects such as temperate weevils, *Chirodica chalcoptera*, tsetseflies, *Glossina pallidipes* and Argentine ants, *Linepithema humile*, while the CT_{max} is higher than for those insects (Terblanche et al., 2005; 2008; Jumbam et al., 2008a). The highest temperature tolerance of aestivating grain chinch bugs (51.5°C for females at 14 weeks) is comparable to a thermophilic Central Australian ant, *Melophorus bagoti* which can tolerate temperatures as high as 53.0°C (Christian & Morton, 1992). Thus, a postharvest cold treatment alone would have to reach temperatures below 0.6°C for an extended period of time to result in mortality of grain chinch bugs, and in contrast, a heat treatment would need to reach temperatures above 51.5°C to be effective. Approved cold treatments for both tropical and temperate fruits against fruit flies ranges from 0.0 to 3.0°C, while treatments against temperate insects such as false codling moth is -0.6°C or below (USDA-APHIS 2011). Heat treatments are mainly applied on tropical fruits against fruitflies and temperatures in these treatments range from 43.3°C to 48.0°C (USDA-APHIS

2011). The feasibility of using heat treatments against grain chinch bugs may not be possible as fruit will not be able to withstand the heat that will be effective to cause mortality. While approved cold treatments for fruits against temperate insects which are below that of grain chinch bugs may be applicable for disinfestations treatments.

Pre-exposure of insects to a range of environmental temperatures or exposure to pre-treatment conditioning of crops to enhance the crop's thermal tolerance against damage, may induce thermal tolerance of associated insect pests as well (Joyce & Shorter, 1994; Lester & Greenwood, 1997). Such exposure to reduced or elevated, but non-lethal temperatures, can condition quarantine insect pests in such a way that subsequent lethal temperature treatments are less effective for disinfestation (Hara et al., 1997; Waddell et al., 2000). In the present study, both low and high temperature acclimation and varying photoperiod treatments showed that such exposures affect the CT_{min} of aestivating grain chinch bugs by increasing the level of cold tolerance. The cold tolerant nature of grain chinch bugs was first proposed in 1967, when live grain chinch bugs were intercepted on stone fruit exported to the USA from South Africa (Myburgh & Kriegler, 1967). In that study, pre-exposure of bugs to low temperatures through both rapid and gradual pre-cooling enabled grain chinch bugs to withstand subsequent cold treatment during storage. Moreover, rapidly cooled bugs were more tolerant to treatment as lower mortality was recorded compared to gradual pre-cooling treatment. The implications of the increase in cold tolerance of grain chinch bugs in that study, as well as the present one, is that bugs can be affected by prior exposure to sub-lethal temperatures, and can adjust their body temperature to resist actual cold disinfestation treatments. However, with regard to the effect of acclimation on CT_{max}, neither low nor high temperature acclimation and varying photoperiod treatments affected the CT_{max} of aestivating grain chinch bugs. This suggests that, irrespective of pre-treatment exposure, heat treatments alone, at temperatures at or above the CT_{max} of aestivating grain chinch bugs should result in

mortality, but it is important to note that grain chinch bugs have a very high CT_{max}. In the present study grain chinch bugs exhibited a CT_{max} as high as 51.5°C, and this may hinder the use of heat as disinfestation treatment for fruits that cannot withstand such temperatures.

Standalone temperature treatments are effective against certain quarantine insect pests as post-harvest disinfestation treatments (Hallman, 1994; Waddell et al., 1997), but combination treatments, such as controlled atmosphere temperature treatment system (CATTS), can improve efficacy by reducing the intensity of the treatment required and thereby also maintaining fruit quality (Neven & Mitcham, 1995; Chervin et al., 1997; Neven, 2008). Johnson and Neven (2011) used a controlled atmosphere waterbath system (CAWB) to simulate CATTS for testing the potential of such a treatment against grain chinch bugs. The maximum temperature reached during treatment was 46°C, and since this is more than 5°C below the CT_{max} of aestivating grain chinch bugs, it is not surprising that the treatment was not effective in achieving mortality. Even with the combined atmosphere stress of the controlled atmosphere, treatments were not efficacious. Johnson and Neven (2011) suggested that the physiological condition of reduced metabolism during aestivation could also have been a contributing factor to the ineffectiveness of the treatment.

The physiological state of insects, such as a reduced state of activity (diapause or aestivation), also affects its thermal tolerance and response to postharvest temperature treatments. Improved thermal tolerance in the diapausing state compared to the non-diapausing state has been shown in diapusing codling moth larvae (*Cydia pomonella*), boll weevils (*Anthonomus grandis grandis*) and Warehouse moth larvae (*Euphestia elutella*) (Slosser et al., 1996; Collins et al., 2006; Johnson, 2007). Consequently the control of diapausing insect pests on fruit generally requires longer treatment times compared to those for non-diapausing insects, and this extended time could be beyond the fruit's thermal tolerance limits (Lay-Yee & Whiting, 1996; Mitcham et al., 2003). In the present study, the state of physiological

arrestment (aestivation) may be a contributing factor to the differences seen in the tolerance limits of active and aestivating grain chinch bugs, as active insects were less cold and heat tolerant than aestivating bugs.

One of several factors contributing to variation in insect thermal tolerance has been attributed to gender, as reported for *Drosophilia melanogaster* (David et al., 1998; Folk et al., 2006). However, in some species, such as tseflies (*Glossina pallidipes),* Natal fruit fly (*Ceratitis rosa*), and false codling moth (*Thaumatotibia leucotreta*), gender does not affect thermal tolerance (Terblanche et al., 2007; Nyamukondiwa & Terblanche 2009; Stotter & Terblanche, 2009). In the present study, at different times of aestivation, there was no difference between male and female grain chinch bugs for both CT_{max} and CT_{min}. This could be due to the fact that during aestivation grain chinch bugs are quiescent (in a state of reduced activity) and are no longer actively feeding or reproducing, but need to shelter and survive the extreme summer conditions. Since the target phase for post-harvest disinfestation treatments is the aestivation phase, where no gender difference in both upper and lower thermal tolerance was observed, controlling for gender during future thermal tolerance studies is not required.

 The lowest temperature recorded in Malmesbury, Western Cape, South Africa where bugs were collected over the active period (winter) was 1.8°C, which was 0.3°C lower than the CT_{min} recorded in the laboratory assay (2.1°C) for active grain chinch bugs. The highest temperature recorded during the winter period in the field was 20.7°C, whereas active grain chinch bugs had a CT_{max} of 49.9°C in the laboratory assay. During the aestivation period (summer), the lowest and highest temperatures experienced in the wild were 12.6°C and 36.2°C, respectively. In the laboratory, grain chinch bugs were more cold (0.6°C) and heat (51.5°C) tolerant in comparison to what was experienced in the field. This suggests that grain chinch bugs have both lower and higher thermal tolerance activity than what is typically experience in the field, except in the case of the CT_{min} of active bugs. The CT_{min} of

aestivating grain chinch bugs in the present study were lowered by both heat and cold acclimation temperatures, but acclimation treatments did not increase CT_{max}. The results from this study are consistent with other reports on ectotherms which documented that at lower acclimation temperatures CT_{min} seems to decrease while there is no effect on CT_{max} (Klok & Chown, 2003; Terblanche et al., 2005; Jumbam et al., 2008b; Chown et al., 2009; Lachenicht et al., 2010). Physiological changes which occur by exposure to a particular temperature are aimed at survival and performance upon exposure to more extreme conditions (Hoffmann, 1995; Marais & Chown, 2008; Marais et al., 2009). The thermal tolerance levels of grain chinch bugs, which is different to temperatures recorded in the field, suggests a potential to survive in areas with climatic conditions different to those experienced in South Africa. Furthermore, the improvement of cold tolerance after acclimation treatment is of quarantine concern, as this indicates that grain chinch bugs have the ability to withstand colder temperatures through pre-exposure to decreasing temperature, and this might be a contributing factor to the invasion and establishment of bugs in colder temperatures in importing counties.

The results of this study clearly indicate the implications for the development of post-harvest disinfestations treatments and the risk of introduction of grain chinch bug into other regions. Thermally based treatments that will be effective against grain chinch bugs should note the high thermal tolerance level of these bugs. Also caution should be taken to guard against pre-exposure to sub-lethal temperatures which may induce a lower temperature tolerance, potentially reducing efficacy of low temperature disinfestation treatments. The risk of invasion and survival in a new area is substantiated by the fact that the CT_{mins} of aestivating grain chinch bugs were lower than the daily minimum temperatures experienced in the field in South Africa, also the CT_{max} estimates, were higher than the maximum temperatures recorded in the field. This suggests that bugs reaching export markets on packed fruit may

have the ability to survive in the colder climates of typical export destinations of South African fruit. To further understand the thermal biology of grain chinch bugs, it will also be valuable to determine the metabolic rate of aestivating insects, effects of ramping rates on thermal tolerance, recovery and survival rate after temperature treatment, as well as rapid cold hardening responses which may contribute to its survival on introduction to novel regions.

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CHAPTER 4

CONCLUDING COMMENTS

This study stemmed from the need to alleviate the problems posed by grain chinch bugs infesting export fruit and threatening international trade in agricultural products from South Africa. The specific objectives of the study were to firstly, identify the pheromone constituents used in aggregation by grain chinch bugs and formulate a lure for use in field trapping trials (Chapter 2); and secondly, to determine the thermal tolerance of both active and aestivating grain chinch bugs using critical thermal limits, in an effort to better understand their thermal biology, and its implications for the development of postharvest mitigation treatments (Chapter 3). This chapter summarises the findings in chapters 2 and 3, and provides recommendations for future studies towards developing effective pre- and post-harvest control tools for grain chinch bugs.

Pheromone-based monitoring as a pre-harvest measure helps to detect early infestation and reduce insect population in fruit orchards by trapping. All the pheromone compounds identified from aggregating grain chinch bugs in this study are known to be associated with migration and aestivation behaviour in heteropterans (Aldrich et al., 1999; Millar, 2005). It is not a coincidence that most of the compounds identified as components of the aggregation pheromone of grain chinch bugs were also identified as the bugs' defensive secretions or alarm pheromones by Oliver et al. (1996). This is most likely due to the fact that most insects use the same compounds as alarm and aggregation pheromones, but the concentrations and the ratios of the constituents is what determines the behavioural response it elicits (Lockwood & Story, 1985). Grain chinch bug aggregation pheromone constituents were identified in this

study, as bugs released into the vial using a sample enrichment probe to collect the volatiles, aggregated on the probe as it became loaded with pheromone.

In this study, the attempt to trap grain chinch bugs in the field using its own aggregation pheromone lure was not successful. This is partly due to a number of factors relating to the lure itself, such as pheromone dose, component blend ratios and release rate. In addition, the low efficacy and performance of the lure in the present study may also be due to competition between the synthetic pheromone and natural pheromones released by large infestations of bugs in the field. Also, alarm pheromone might have been released by trapped bugs in the baited traps, and this odour could have reduced attraction of bugs to the lure. Moreover, the importance of shelter for aestivating grain chinch bugs was evident as more bugs were found in places that provided shelter (e.g. cardboard bands and walls of delta traps) than places with the lure (e.g. on sticky pads in delta traps or inside bucket traps). This sheltering behaviour of grain chinch bugs contributed to the erratic nature of trap catches, and its importance needs to be considered and incorporated when developing traps for monitoring in future trials.

Thermally-based postharvest control treatments for grain chinch bugs are only feasible if the thermal limits of this species are obtainable in a mitigation treatment scenario. The eco-physiological aspects of this study indicate that bugs have both low CT_{min} (0.6°C) and high CT_{max} (51.5°C). Also, both low and high acclimation temperature treatments improved CT_{min}, but did not improve CT_{max}. These results suggest that cold treatment may not be feasible as a control tool because the low CT_{min}, and prior exposure to various environmental temperatures may cause bugs to adapt and ultimately survive cold temperature treatments. Heat treatments may be more attainable than cold treatments but it should be noted that grain chinch bugs have a high heat tolerance. Aestivating grain chinch bugs were more cold tolerant than actively reproducing and feeding bugs. This may be due to physiological arrestment of reduced activity in aestivating insects. The thermal tolerance levels of aestivating bugs, which

was lower than minimum temperatures and higher than maximum temperatures recorded in the infested area in South Africa, as well as improvement of cold tolerance by acclimation treatments, may enhance the ability of bugs to survive extreme weather conditions and adapt to a new environments.

Future research

This study represents an important first step in developing an aggregation pheromone lure for *Macchiademus diplopterus* trapping, as well as understanding the thermal biology of this key quarantine pest. A good pheromone-based monitoring system requires optimisation of the pheromone dose, component blend ratios, an appropriate and efficient dispenser and well-designed traps for capturing insect pests. Therefore additional work on the chemistry of the aggregation pheromone components of grain chinch bugs, identified in this study, should be investigated, as well as different lures and trap designs, so as to optimise trapping with a lure in the field.

In addition, this study has enhanced the knowledge about an aspect of grain chinch bug physiology which could be used in the furtherance of developing feasible non-chemical disinfestation treatments, as well as understanding the threat posed to importing countries. The thermal tolerance of aestivating grain chinch bugs suggests that bugs seems to be well cold and heat adapted, which could impact on post-harvest temperature treatments, as well as invasion potential. The prospect of using heat treatments against insect pests on temperate fruits may be diminished by its cost, and the length of time in achieving efficacious treatment, but combination treatments may reduce heat intensity and treatment time. These combination treatments may be feasible for grain chinch bugs in future, now that its thermal tolerance has been determined. Cold acclimation allows organisms to increase their cold

hardiness by reducing their metabolism. It will be of interest in future to determine the metabolic rate of aestivating grain chinch bugs, as both cold and high acclimation treatments enhanced cold tolerance.

Problems associated with developing a monitoring system for grain chinch bugs using its own pheromones in future have been alleviated a little, with the knowledge gained here regarding grain chinch bug chemical ecology. Also, to maintain current markets and expand to new areas, the insight gained into grain chinch bug thermal biology will help in developing effective mitigation treatments for this pest on export fruits in future.

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