Chemical composition of leaf essential oils of *Lantana camara* varieties in South Africa and their effect on the behavioural preference of *Falconia intermedia*.

Thesis submitted in fulfilment of the requirements for the degree of

MASTER OF SCIENCE

at

RHODES UNIVERSITY

by

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April 2016

Abstract

Lantana camara L. (Verbenaceae) is one the most problematic invaders in South Africa invading forest edges, sand dunes, and shorelines by forming impenetrable thickets. *Lantana camara* invasions degrade natural biodiversity, reduce the value of land and consequently it has been a target for biological control, over the last 50 years in South Africa. Studies that have reported on chemical profile of *Lantana camara* have been conducted around the world but not in South Africa. Hence, the first aim of the current study was to identify the chemical baseline of *L. camara* varieties in the Eastern Cape, South Africa. Recent studies have shown that feeding by one of the agents released against *L. camara*, *Falconia intermedia* (Distant) (Hemiptera: Miridae), induces anti-herbivory response through increased leaf toughness and trichome density. A preliminary study conducted also reported the production of volatile chemicals by one variety, Whitney Farm, due to feeding by the mirids. Therefore, the second aim was to determine the induced changes in chemical compounds of *L. camara* varieties after feeding by *F. intermedia*. A third aim was to determine the effect these chemical compounds have on the behaviour of *F. intermedia*.

To identify the chemical baseline of *L. camara* varieties, the essential oils of four *L. camara* varieties (East London, Port Alfred, Whitney Farm and Heather Glen) were analysed using gas chromatography mass spectrometry (GC-MS) and that resulted to the identification of 163 constitutive and 75 induced chemicals across the varieties tested. *Lantana camara* varieties showed different chemical classes but were highly dominated by terpenes. A great variation in the number of constitutive chemical compounds was found in all the varieties. There were 56 constitutive chemical compounds in the Whitney Farm variety, 41 in the East London variety, 36 in the Heather Glen variety and 30 in the Port Alfred variety. The Whitney Farm variety had the highest number (22) of unique constitutive chemicals identified when compared with other varieties. This indicates the chemical distinctiveness of the Whitney Farm variety from the other varieties. In the varieties tested, there were common chemical compounds identified in constitutive and induced (discussed below) states of the plants such as caryophyllene, hexane, naphthalene, copaene and α-caryophyllene. Besides naphthalene, the majority of chemical compounds in South African *L. camara* varieties were similar to compounds that have been identified across the world, suggesting that they are closely related. The expression of naphthalene in these varieties may be due to changes in the chemicals expressed over evolutionary time as predicted by the Novel Weapons Hypothesis.

Amongst the varieties, a great variation in chemical compounds and their concentrations was shown in the induced states of the plants. The concentration of constitutive caryophyllene ranged from (3.13 – 15.7) %, to (4.02 – 11.10) % after feeding. The concentration of constitutive hexane ranged from (6.13 – 71.19) %, to (33.3 – 75.8) % after feeding. The concentration of constitutive naphthalene ranged from (0.21 – 4.79) %, to (0.92 – 2.11) % after feeding. The concentration of constitutive copaene ranged from (0.57 – 1.57) %, to (1.20 – 2.72) %. Lastly, the concentration of constitutive α-caryophyllene ranged from (1.18 – 9.03) %, to (0.78 – 5.48) % after feeding. The changes in chemical concentrations in lantana varieties indicated that feeding by the mirid on *L. camara* varieties causes an induction by either reducing or increasing the chemical concentrations.

To determine the effect of the identified compounds on the behaviour of *F. intermedia* adults, olfactometer bioassays were conducted using a Y-tube technique. A significantly higher proportion of *F. intermedia* were attracted to undamaged leaves over damaged leaves and purified air. Undamaged leaves attracted 52 % of *F. intermedia* from the East London variety, 62.5 % from the Port Alfred variety, 56 % from the Whitney Farm variety, 58 % from the Lyndhurst variety and 54.5 % from the Heather Glen variety in dual choice trials versus damaged leaves. Furthermore, a significantly higher proportion of *F. intermedia* were attracted to damaged leaves over purified air. Damaged leaves attracted 67 % of *F. intermedia* from the East London variety, 67 % from the Port Alfred variety, 65.9 % from the Whitney Farm variety, 65.3 % from the Heather Glen variety and 64.5 % from the Lyndhurst variety.

Olfactometer bioassays were also conducted using purified standard compounds of four chemical compounds identified from essential oils, hexane was used as a positive control as it is reported to be an insect attractant in literature. Hexane was highly attractive to the mirids compared to three standard compounds caryophyllene, caryophyllene oxide and naphthalene at the rate of 80 %, 73 % and 80 %, respectively. The standard compounds tested against *F. intermedia* are major compounds contained by *L. camara* varieties and they have proven to have a repellent effect. This may indicate that after feeding by *F. intermedia*, the major compounds expressed by the plant varieties repel *F. intermedia* contributing to the invasiveness of this weed. The increased expression of hexane and caryophyllene after feeding may also indicate increased attraction to some insects, opening up the potential for third trophic level interactions in varieties where this is the case. This is the first study on the chemical composition of essential oils of *L. camara* in South Africa. Therefore, we recommend that

where appropriate chemical profile studies of the invasive alien plants should be considered during host specificity testing, and the vital role of chemical compounds on agent-weed interactions must be taken into consideration with other factors before and after the biological control agents are released.

Declaration

The following thesis has not been submitted to a university other than Rhodes University, Grahamstown, South Africa. The work presented here is that of the author.

Signed: ..... Date: .....

Dedicated to

my grandmother (mama), Mafusi Emmah Ngxande

24 February 1928 – 01 November 2014

Acknowledgements

First, I would like to express my gratitude to my supervisor Dr. Unathi Heshula and my co-supervisor Prof. Martin Hill for their continuous support, guidance and patience. I could not have done it without their thoughtful ideas and comments.

I thank Mrs Helen Holleman for proofreading my write-ups and helping me with English Grammar.

Gratitude is also due to the following people: Nkosinathi Kofi for his willingness to assist me in maintaining the plants and insects in Waainek Research Institute; my friends and colleagues; Kennedy Zimba, Samuel Motitsoe, Zolile Maseko, Lumka Mdodana and Sisanda Mvandaba for their assistance in my field trips; Zezethu Mnqetha and Ikponmoswa Egbon for watering my plants in my absence; Mr Vuyisile Dondashe at the Chemistry Department for supplying me with glassware; Pendrick Kotelo for assisting with laboratory equipment; Dr. Lenin Chari, Dr. Tatenda Dalu, Dr. Sydney Moyo and Thabisa Mdlangu for not closing their office door in times of desperation.

I thank the owners of the following farms, Lyndhurst Fam, Whitney Farm and Heather Glen Farm for allowing me to get plant cuttings in their farms. I also thank Jeanne van der Merwe and Babalwa Booi for their assistance in procuring the laboratory supplies.

I would also like to thank my family and friends for their prayers and support throughout my studies.

Finally, I would like to thank my loving husband, Bongolethu Koza and our beautiful daughter Kungawo Koza for their unconditional love, understanding and support they have given me during this time.

Most of all, I give thanks to my creator, God Almighty, for providing me with strength to finish my studies. “Through Christ, we are more than conquerors”.

I thank the Council Research Funding and Working for Water programme, an initiative of the Department of Environmental Affairs, South Africa for funding this study. Lastly, I thank Rhodes University for allowing me to use their facilities.

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CHAPTER 1
General Introduction

1.1 Problem statement

Lantana camara has been declared a weed in South Africa since 1954. To date, there have been 25 biological control agents released in an attempt to control the weed (Klein 2011). Nevertheless, lantana persists and invades new areas. Previous studies have suggested several factors that could contribute to the inadequate control of *L. camara.* These factors include climate incompatibility, release strategies, hybridization of the weed, predators and parasitism (Day and Neser 2000; Urban and Phenye 2005; Tourle 2010; Vardien 2012). A study by Heshula and Hill (2011) reported induced physical defensive traits of *L. camara* changing after feeding by one of the agents, *Falconia intermedia*, which affected the population of this mirid negatively. However, nothing is known about the role that herbivore induced chemical responses have on *F. intermedia* behaviour, and this is addressed in this thesis.

1.2 *Lantana camara*

Lantana camara Linn. (Verbenaceae), which is commonly known as lantana or wild sage, is one of the world's worst weeds (Holm *et al.* 1977) (Fig 1.1). *Lantana camara* is a tropical evergreen flowering shrub that originates from South and Central America (Stirton 1977; Ghisalberti 2000; Day *et al.* 2003). This plant can grow up to 10 m high as a vine with small flowers that vary from yellow to pink, red and orange (Fig 1.1 A) (Ghisalberti 2000; Day *et al.* 2003; Roy *et al.* 2004). Flowering of the plant takes place between August and March, however, if there is enough moisture and light, flowering can occur throughout the year. The stems are woody, square in cross section and when the plants are still young the stem is hairy with oval leaves that are about 2 – 10 cm long and 2 – 6 cm wide, covered in fine hairs. The leaves are bright green above and paler beneath, rough and with saw-like margins. They produce a distinctive odour when crushed. The fruit is round, berry-like and turns from a glossy green to purplish-black when fully matured (Fig 1.1 B) (Stirton 1977; Day *et al.* 2003). The seed production on a mature plant is about 12 000 seeds yearly (Langeland and Burks 1998). The clearance or burnt forest areas contributes to the spread of the weed as the seeds survive hot fires. Additionally, birds and rodents play a significant role in dispersing seeds. *Lantana camara* has a root system that is large and strong which enables it to coppice after fire and being cut (Priyanka and Joshi 2013).

Figure 1.1: The aerial parts of *Lantana camara* L. A = some of the different colours of lantana varieties, B = fruit

1.1.1 Invasiveness of lantana

Lantana camara mostly invades edges of the forest, sand dunes, beachfronts, plantations, and forests recovering from fires, forming impenetrable thickets (Cilliers 1983; Henderson 1995). *Lantana camara* invasions degrade the natural biodiversity, reduce the value of land and ingestion may lead to human and livestock poisoning (Wells and Stirton 1988; Henderson 1995; Day *et al.* 2003a). When the forest has been disturbed and spaces created, *L. camara* invades. It does not survive where the natural forests are thick, impenetrable and tall forming canopies (Richardson and van Wilgen 2004). It is also susceptible to low temperatures, brackish soils and low rainfall (Sharma *et al.* 2005; Baars *et al.* 2007; Vardien 2012). Varieties of the lantana were intentionally introduced from South and Central America via Europe for ornamental purposes into many countries of the world where they became invasive (Stirton 1977). South Africa is one of the countries that have recognized the invasiveness of this plant

and have thus declared it a weed by law: The Conservation of Agricultural Resources Act (Act no. 43 of 1983, amended in March 2001 section 15A) enforces landowners to control lantana invasion on their lands (Heystek 2006; Vardien 2012). In the National Environmental Management: Biodiversity Act regulations of South Africa, *L. camara* is placed in category 1b, stating that invasive species in this category may not be owned, imported into South Africa, grown, moved, or sold. Category 1b species are major invaders that may need government assistance to remove. The weed invades subtropical, warm and wet temperate provinces of South Africa: Limpopo, Mpumalanga, North West, Western Cape Eastern Cape and Gauteng (Fig 1.2). The weed was first recorded in 1858 in Western Cape (Cape Town) then dispersed along the coast to KwaZulu Natal in 1883 (Stirton 1977). Recent surveys done on *L. camara* invasion of land in South Africa have shown to have increased from 560 000 ha to more than 2 million ha (Kotze *et al*. 2010; Urban *et al*. 2011; Bhagwat *et al*. 2012).

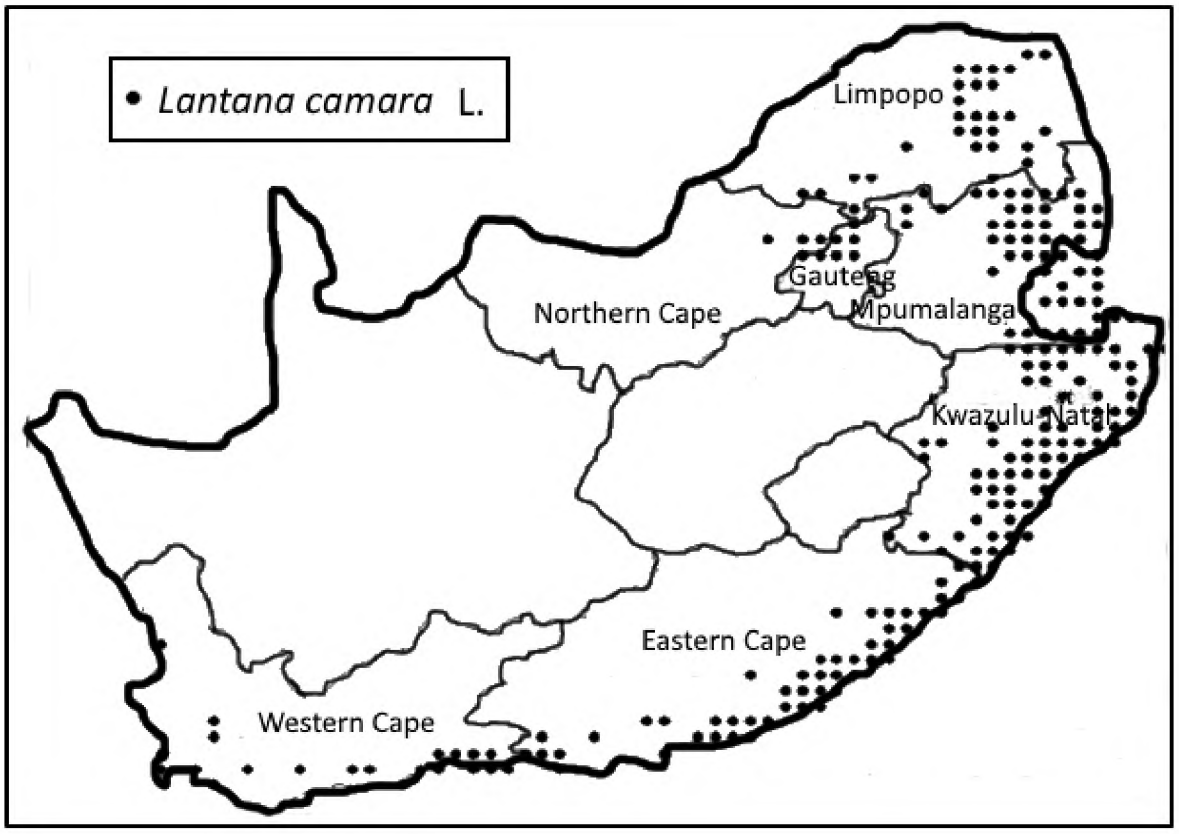

Figure 1.2: Geographical distribution of *Lantana camara* in South African Provinces (Henderson 2001)

1.1.2 Control methods

Three methods of controlling *L. camara* have been implemented: chemical, mechanical and biological (Cilliers and Neser 1991; Grobler *et al*. 2000; Marais *et al*. 2004). However, using

an integration of control methods gives the best results (Urban 2010; Vardien *et al.* 2012). There are aspects that need to be considered before selecting which method to use for the control of lantana: the geographic location, the size and density of the invasion (see below) (Vardien *et al.* 2012).

Herbicides are effective but they are expensive and may be harmful to natural vegetation. Costs of herbicidal use to control 100 % density coverage by *L. camara* were estimated in 2004 at around R572/h (Marais *et al.* 2004). In some areas, the costs of chemical control are sometimes equal to or even more than the land *L. camara* invaded (Grobler *et al.* 2000). Even though this method of treatment is effective, it is only a temporary solution (Cilliers and Neser 1991). Follow-up treatment is necessary to achieve good results. The size of plant and area it is on is also critical. The smaller the plant, the easier it is to control. There are 11 registered chemicals that are used for the control of lantana in South Africa (Euston-Brown *et al.* 2007), some of those are: glyphosate used for spraying the foliage, imazapyr for treatment of stem's base, picloram for painting cut stump and tebuthiuron, foliar spray is more effective than other treatments. Imazapyr is less expensive than the other two, picloram and glyphosate; therefore it most preferably that glyphosate is used for follow-up (Erasmus and Clayton 1992). Follow-up treatment on saplings and coppice growth by chemical is also effective after mechanical and fire control (Heystek 2006).

Chopping, sawing or felling of plants is one form of mechanical control. It is labour-intensive and costly and is suitable for medium-sized infestations as it requires extensive follow-up due to seedling recruitment. Follow-up must be done for both mechanical and chemical control (Heystek 2006; Vardien 2012). Marais *et al.* (2004) reported estimated costs of mechanical control for initial clearing of about R1000/ha and R600/ha for follow-up in 2002/03. Areas susceptible to soil erosion should not be cleared using the mechanical control as the manual labour could cause more degradation in vegetation (Grobler *et al.* 2000).

Biological control is one of the methods used in South Africa for control of invasive plants using their natural enemies and it offers a long-term and an environmentally friendly management tool. The biocontrol agents are brought from the region of origin of the invasive plant to where it is problematic with expectations that the rate of invasion will be reduced (Zalucki *et al.* 2007; De Lange and Van Wilgen 2010). The agents that are released for the biocontrol of an alien plant are host-specific causing the impact on targeted species only; hence,

it is preferable to other control methods (Van Wilgen *et al.* 2004). Biocontrol is recommended because it is self-sustaining, when agents are released, they disperse to other infested areas. The results might take longer to show, as agents need to establish first, and when they do, effective control becomes evident. Agents kill the plants slowly, not leaving an open space for other invasive plants to take over but allowing indigenous plants to grow back because control is over a long period of time (Binelli *et al.* 2001).

Like any other method of control, biological control has its disadvantages that hinder the programme, and this is evident in the biocontrol of *L. camara* in South Africa. Biocontrol agents have been released over several decades however, this invasive weed continues to invade. Biological control agents are faced with many challenges and some of those are the lantana varieties that are able to cross-fertilize producing new hybrids, climate incompatibility of the original country with countries where agents are released and release strategies (Sands and Harley 1980; Neser and Cilliers 1989; Day and Neser 2000). Further, past research has discovered that plants have the ability to defend themselves against herbivore damage (Heshula and Hill 2011).

Biological control of *L. camara* was initiated in South Africa in 1961/62 and by 1995, 18 biocontrol agents had been released (Baars *et al.* 2003; Vardien 2012). Since 1995, seven agents were released to make a total of 25 including the native moth, *Hypena laceratalis* Walker (Lepidoptera: Noctuidae). Out of that 25, 12 established with only seven agents showing some level of control of *L. camara* (Table 1.1), of which *Falconia intermedia* is one of the most recent and understudied species.

Natural enemy	**Feeding guild**	**Agent research status**	**Damage inflicted**	**Key references**
Aceria lantanae Cook (Acarina: Eriophyidae)	Flower galler	Released 2007, established	Extensive	Urban *et al.* 2001, 2011a
Calycomyza lantanae Frick (Diptera: Agromyzidae)	Leaf miner	Released 1982, established	Moderate	Baars and Neser 1999
Falconia intermedia Distant (Hemiptera: Miridae)	Leaf sucker	Released 1999, established	Moderate, localized	Baars *et al.* 2003

Hypena laceratalis Walker (Lepidoptera: Noctuidae)	Leaf feeder	Not released, native	Moderate	Baars 2003
Octotoma scabripennis Guèrin-Mèneville (Coleoptera: Chrysomelidae)	Leaf miner	Released 1971 – 1975, established	Considerable	Cilliers 1987
Ophiomyia camarae Spencer (Diptera: Agromyzidae)	Leaf miner	Released 2001, established	Considerable	Simelane 2002; Simelane and Phenye 2005
Teleonemia scrupulosa Stål (Hemiptera: Tingidae)	Leaf and flower sucker	Released 1972, established	Considerable	Baars and Neser 1999
Uroplata girardi Pic (Coleoptera: Chrysomelidae)	Leaf miner	released 1974 and 1983, established	Considerable, coastal	Baars and Neser 1999

Table 1.1: The most effective biological control agent species released in South Africa for *L. camara* (Klein 2011)

1.1.3 Biology of *Falconia intermedia*

Falconia intermedia is a leaf-sucking bug that feeds on *L. camara*. The fully-grown mirid is about 2 – 4 mm in length with dark-brown body, transparent wings and pale legs (Fig 1.3). The adult lays eggs on the underside of the leaf. Eggs hatch in about 10 – 14 days into green nymphs. Under laboratory conditions, nymphs develop into fully matured adults in approximately 15 – 20 days and the adult lives for about two months (Baars *et al.*; 2007; Heshula 2009). The adults and nymphs feed on leaves causing chlorosis on the upper surface and dark frass spots on the underside. Warm, humid climates are mostly preferred by *F. intermedia* as their population size increases under those conditions. *Falconia intermedia* is originally from Mexico, Florida and the Caribbean (Palmer and Pullen 1995; 1998). This agent was released in 1999 throughout the sub-tropical regions in South Africa. In each instance, mirids of about 5000 – 100 000 per site were released in subtropical regions (Heystek 2006). These large numbers of agents released built up a large population of *F. intermedia* that caused defoliation of *L. camara*. However, in winter, lantana drops its leaves and this results in high mortality of mirid populations. And so, when *L. camara* leaves grew back, mirids were absent (Heystek and Olckers 2003).

Figure 1.3: Adult of *Falconia intermedia* with damaged leaves of *Lantana camara*.

In the Eastern Cape, the lantana mirid was released in 2001 at five sites: coastal (East London, Whitney Farm and Port Alfred), and inland (Lyndhurst and Heather Glen) (Fig 1.4).

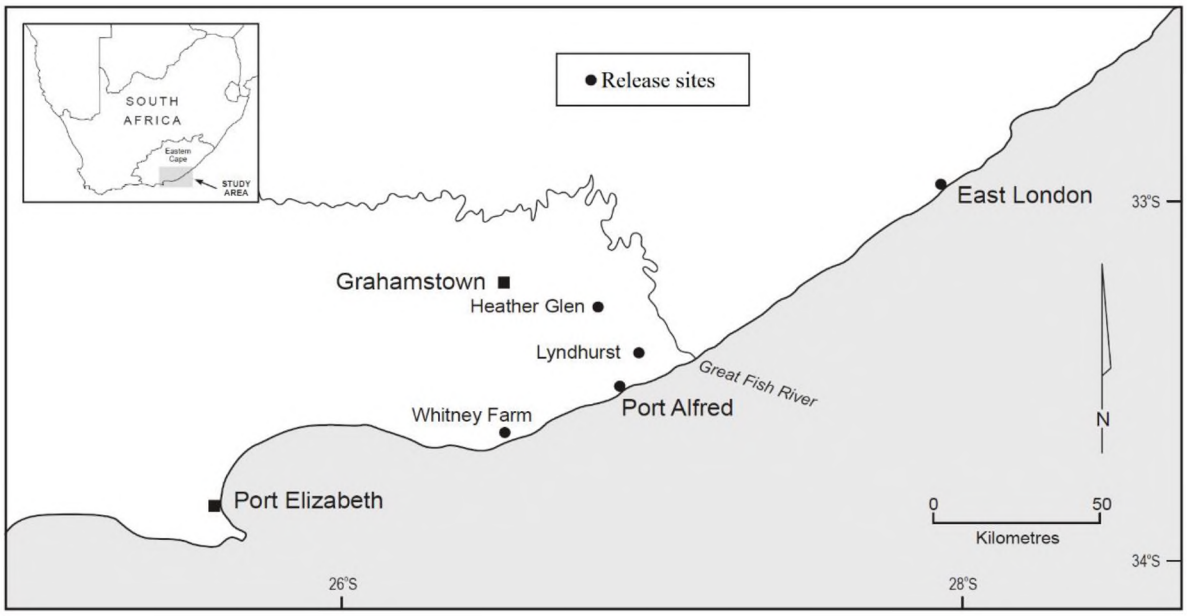

Figure 1.4: Sites where *Falconia intermedia* was released on *Lantana camara* varieties in the Eastern Cape (Heshula 2009)

1.2 Factors influencing the effectiveness of biological control of *Lantana camara*

Several factors have contributed to the lack of establishment and inadequate control of *L. camara* by biological control agents. One of the factors is exposure to parasitism and predation by natural enemies (Urban and Phenye 2005; Tourle 2010). When natural enemies attack agents, their population numbers decrease resulting in unsuccessful control of lantana. There are instances where these actions have been measured, including *Uroplata girardi*, parasitized by unknown eulophid species, *Ophiomyia camarae* negatively affected by African parasitoids

(Urban *et al.* 2011), a few generalist predator species attacking *Teleonemia scrupulosa* (Cilliers 1987a) and *F. intermedia* attacked by ants (Heystek and Olckers 2003; Tourle 2010).

Climate plays an important role in the success of biocontrol agents of weeds. *Lantana camara* has been able to adapt to a wide range of climates in South Africa (Heystek 2006). It is expected that agents will establish and perform well if invasive plants invade areas where climate conditions are similar with that of their region of origin (Day and Neser 2000). *Octotoma scabripennis* prefers climates that are humid covered with tall tree canopies, whereas, *T. scrupulosa* performs well in open, sunny habitats (Cilliers and Neser 1991; Day and Neser 2000). *Lantana camara* invasions are distributed in wide-ranging climatic conditions; from the Western Cape Province with winter rainfall; Mpumalanga and Kwa-Zulu Natal, where winter is moderate and has summer rainfall. On the other hand, inland in the Eastern Cape Province, winter is cold and windy. *Lantana camara* defoliates where it is cold and dry, which negatively impacts agent species that depend solely on leaves (Baars and Heystek 2003; Heshula 2009). A study conducted by Heshula (2005) revealed that of the five sites where *F. intermedia* was released in the Eastern Cape, the agent managed to establish at only two, East London and Whitney Farm. Recently, *F. intermedia* feeding damage was confirmed at third release site in Port Alfred (personal observation). Climatic conditions in these areas are compatible for lantana growth, and here the lantana has leaves through the year (Day and Neser 2000). On the other hand, at Heather Glen and Lyndhurst (the other two release sites) the mirid did not establish due to the colder winters (Heshula 2005). Under laboratory conditions the mirid established on all varieties.

Lantana camara has the ability to hybridize forming many plant varieties (Day and Neser 2000; Vardien 2012). Over 50 varieties of *L. camara* are documented in South Africa and have created a complex taxonomic problem (Wells and Stirton 1988; Munir 1996). Lantana varieties share similar morphological traits: colour of mature flowers, size of leaves, spines in stems and hairiness of leaves. However, although these varieties may share similar traits, it does not mean they are the same (Day and Neser 2000; Tourle 2010). This matter has not only presented challenges for taxonomists but also for biocontrol. The lantana varieties from which the agent was collected (in the country of origin) might be different from the varieties on which the agent was released on in South Africa. This is important for some agents and not others, for example, *Uroplata girardi* Pic was collected in Brazil on *L. tiliaefolia*, in South Africa and Australia it

has been one of the effective agents in control of all varieties of *L. camara* (Swarbick *et al.* 1995).

Release strategy is another crucial factor affecting agent success. Inaccuracy in methods used when agents are released can affect their establishment; the numbers should be enough to build up a population that will be effective in control; e.g. *U. lantanae* and *Eutreta. xanthochaeta* Aldrich numbers released in South Arica were below the minimum threshold (Cilliers and Neser 1991). The abundance of predators such as ants in releasing sites could threaten the build-up of agent population as they may feed on eggs, e.g. the hemipteran agent, *Prosopidopsylla flava* Burckhardt (Hemiptera: Psyllidae) and *Alagoasa parana* Samuelson (Coleoptera: Chrysomelidae) (Day and Neser 2000).
Understanding the interaction between plants and herbivores is crucial, especially in the context of controlling the invasive plants using biological control. Thus, an overview of the current theories proposed for the plant defence against insect herbivory is presented below.

1.3 Evolutionary history of plants and insects

470 million years ago, during the Ordovicial period, the first plant species (Embryophytes) appeared on land (Wodniok *et al.* 2011; Pires and Dolan 2012; Bowman 2013). Insect species colonized the earth approximately 412 million years ago in the Early Devonian period (McGavin 2001; Engel and Grimaldi 2004). Insects and plants have co-existed for about 350 to 410 million years (Labandeira 2007; Mithöfer *et al.* 2009; Wu and Baldwin 2010). Evidence of feeding by herbivores and the presence of spines for defence against damage by herbivores appears on fossilised plants in the Devonian (Labandeira 1998). This interaction is the driver behind co-evolution and the immense diversity between plants and insects.

1.3.1 Co-evolution of plants and insects

Insects are the most abundant group on earth, with about 500 000 species feeding on plants (McGavin 2001; Wu and Baldwin 2010). In response to the high number of insect herbivores consuming plants, plants co-evolved defences against these attackers. When plants developed defences to overcome their enemies, the enemies also co-evolved traits to defend themselves and increase their efficacy. The co-evolution between these two living organisms is "reciprocal cyclic", sometimes referred to as the "arms race" (Leimu *et al.* 2012; Turley *et al.* 2013); whereby every time the plant species evolves a trait to survive, the insect species feeding on it also evolves to adapt to that new state (Leimu *et al.* 2012). The co-evolved traits of plants can

be expressed morphologically, chemically or both. For example, pine species have developed toxic resin terpenoids in response to feeding by the sawfly, *Diprion pini* Linnaeus (Hymenoptera: Diprionidae). The sawfly, in turn, developed the ability to sequester the resin terpenoids in its foregut, and even use them to its advantage: releasing the volatile resin terpenoids to repel its predators (Mumm and Hilker 2006). The tobacco plant, *Nicotiana tabacum* Linn induces levels of nicotine as a counter attack; but the earworm caterpillar, *Helicoverpa zea* Boddie (Lepidoptera: Noctuidae), uses glucose on its saliva to suppress the nicotine levels (Utsumi 2011).

Plants are phenotypically plastic and this enables them to change their physical structure and functionality due to pressures exerted by the environment. Those pressures may be drastic weather conditions, herbivores or intraspecific competition (Agrawal 2001; Sultan 2003; Nicotra *et al.* 2010). Plants are also able to induce volatile organic chemicals after attack by herbivores with differences in the quality and quantity of major compounds. Damage to the plant can increase or reduce a particular compound, depending on the type of herbivore damage (Bruce *et al.* 2005).

1.4 Plant response to herbivory

Interactions between plants and phytophagous insects can be positive or negative to either of the organisms involved. Plants provide ants with food and shelter and in turn, ants protect plants against herbivore attack. Numerous insect species disperse plant seeds and assist in pollination (Gruber *et al.* 2009; Kessler and Heil 2011; Trowbridge and Stoy 2013). Insect orders that play a significant role in pollination are Hymenoptera, Lepidoptera, Coleoptera, and Diptera (Herrera 1996, Waser *et al.* 1996). These instances show the mutual relationships plants and herbivorous insects share. On the contrary, the majority of interactions between plants and herbivores have a negative impact on plants as insects attack plants for food, use plants as oviposition sites and shelter. The sessile plants have in turn evolved a number of different strategies for survival against biotic and abiotic factors. These include tolerance against herbivore damage, constitutive and induced defences (Howe and Schaller 2008; Miresmailli and Isman 2014).

1.4.1 Constitutive mechanism of defence

Constitutive defence is the type of defence that exists in plants before the attack by herbivores occur (Chen 2008; Wu and Baldwin 2010). This defence mechanism constitutes physical and

chemical defences. Physical defence is made of structural traits that hinder herbivores from moving on the plant surface, *inter alia*, leaf trichomes, occasionally containing poisons or irritants on the leaves, sharp prickles, spines, thorns, sticky-toxic resin and tough leaves (Franceschi *et al.* 2005; Schoonhoven *et al.* 2005; Mumm and Hilker 2006; Chen 2008; Howe and Schaller 2008; Speight *et al.* 2008; Mithöfer and Boland 2012; Trowbridge and Stoy 2013; Miresmailli and Isman 2014). Chemical defence on the other hand involves toxins found in structures such as glandular trichomes, laticifers and resin ducts which is expressed after the herbivore or pathogen attack (Duke *et al.* 2000; Dussourd and Hoyle 2000; Hallahan 2000). Trichomes produce defensive compounds which can be toxic and are released onto the surface of the plant, for example, silica, resins, lignin and wax (Mumm and Hilker 2006; Howe and Schaller 2008). Silica released into the spaces between cells (extracellular spaces) forms stone-like phytoliths that increase wear on insect mouthparts (Hanley *et al.* 2007). Resin is a sticky substance produced by *Pinus contorta* Douglas for defence and healing against herbivore damage. In pine species, resin is used to deter pine beetles, *Dendroctonus ponderosae* Hopkins (Coleoptera: Curculionidae) from laying eggs (Mumm and Hilker 2006). Lignin plays an important role in plant development and defence against pathogens and insect damage. It fills up the spaces in the cell walls between cellulose, hemicellulose and pectin components by making the plant stems stronger (Riederer and Schreiber 2001; Jung *et al.* 2006).

Plant chemicals are classified into two categories: primary metabolites and secondary metabolites. All plant cells produce primary metabolites and they are involved in the growth and reproduction processes of plants. Primary metabolites comprise amino acids, lipids, sugars, proteins and nucleic acids (Freeman and Beattie 2008; Vince and Zoltán 2011). There are two main functions that secondary metabolites have in plants: defence and attraction. Secondary metabolites defend plants from attack by herbivores, viruses, bacteria, fungi and against competition with other neighbouring plants for light, nutrients and space. They are synthesized in three pathways: jasmonate acid pathway, salicyclic acid pathway and ethylene pathway. When a plant is triggered by herbivory, salicyclic acid and jasmonate acid are activated and start defending a plant against damage. Salicyclic acid is synthesized when a plant is attacked by phloem-feeding whiteflies and aphids, while jasmonate acid and ethylene, are activated when plant is damaged by chewing herbivores and pathogens killing living tissues (Walling 2000; Moran and Thompson 2001; Jones and Dangl 2006; Kempema *et al.*, 2007; Van der Does *et al.* 2013). Chemical defence is made of a collection of compounds that are reserved in the tissues of plant and can be toxic to herbivores (Franceschi *et al.* 2005; Mumm and Hilker

2006; Chen 2008). This defence is mainly from secondary compounds that are found in plant species: phenolics, terpenoids and nitrogen containing compounds: alkaloids, glucosinolates and cyanogenic glycosides (Freeman and Beattie 2008; Vince and Zoltán 2011).

Phenolics are one of the largest groups of secondary metabolites that are derived from pentose phosphate, malonic acid and shikimic acid pathways (Balasundram *et al.* 2006; Freeman and Beattie 2008). These metabolite compounds are important in plants in different ways to support mechanical and chemical processes. Phenolics defend the plant against herbivores and pathogens that attack; moreover, they positively influence the growth and reproduction of plants. Pollinators and seed dispersal insects are attracted by phenolics because of colour and odour they provide to plants. Plants produce these metabolites with other kinds of compounds for protection. Those compounds include flavonoids, tannins, lignin, anthocyanins and furanocoumarins (Balasundram *et al.* 2006; Freeman and Beattie 2008; Vince and Zoltán 2011).

Alkaloids are some of the largest chemical compounds produced by the plants, derived from amino acids and large number of bitter-tasting, nitrogenous compounds (Freeman and Beattie 2008; Da Silva and Batalha 2011). They are mostly found in roots, leaves, seeds and fruits (Freeman and Beattie 2008). Alkaloids include cocaine, caffeine, nicotine, theobromine and morphine. Caffeine is toxic to insects and fungi and it is found in coffee, *Coffea arabica* L., tea *Camellia sinensis* L. and cocoa *Theobroma cacao* L. When the plants contain high concentrations of caffeine, neighbouring plant seeds are prevented from growing. This behaviour is called allelopathy, the inhibition of plant growth caused by the plants containing substances such as caffeine competing for resources (Freeman and Beattie 2008). Jain *et al.* (1989) reported similar behaviour by *L. camara* that have phenolic compounds inhibiting the growth of duckweed. This noxious weed also deters the growth of some agricultural crops such as corn (*Zea mays* L.), wheat (*Triticum aestivum* L.) and soybean (*Glycine max* L.) (Mersie and Singh 1987).

1.4.2 Induced mechanism

Herbivore damage may induce defence mechanisms following feeding and the defence mechanisms induced can be physical or chemical. The defence mechanisms directly affect herbivores by repelling or deterring them; and indirectly affect them by attracting their natural enemies (predators and parasitoids) through the release of volatile organic compounds (Karban

and Agrawal 2002; Gatehouse 2002; Mumm and Hilker 2006; Chen 2008; Mithöfer *et al.* 2009; Glinwood *et al.* 2011). Induced volatile chemical compounds, sometimes known as herbivore induced plant volatile (HIPVs) vary in quantity and quality depending on the type of damage. Induced defences are different from constitutive defences in that they are only released by plants after attack by herbivores (Mumm and Hilker 2006; Banchio *et al.* 2007; Chen 2008; Freeman and Beattie 2008; Vince and Zoltán 2011; Redondo-Gómez 2013). Leaf toughness is employed by plants as one of the important physical defences induced after the attack of herbivores and other environmental stresses. Toughness of the leaf has been shown to be induced in new growth of *L. camara* varieties (Heshula 2011). Additionally, the reduced nutritional quality in new leaves and increase of secondary compounds is evident in *Schima superba* Gardner and Champ (Theaceae) (Liu *et al.* 2010). Howe and Schaller (2008) stated that sometimes it is challenging to distinguish if defence traits should be regarded as induced physical, chemical or both for defence. This is because leaf toughness is often considered a physical defensive trait, and after herbivore attack, chemicals (phenolics) are emitted to deter herbivores from feeding (Schoonhoven *et al.* 2005). The toughness of the leaf however has a chemical basis as it consists of augmentation in cell wall constituents such as cellulose and lignin (Lucas *et al.* 2000). Tough leaves affect the mouthparts of piercing-sucking insects when attacking the plant tissues (Chapman 1995; Schoonhoven *et al.* 2005). Trichomes provide both physical and chemical protection against herbivore insects. They are hair-like structures on the surface of the plant, can be unicellular or multicellular and vary in shape from being spiral, straight, branched or unbranched. They also have different sizes, number of cells and chemical constituents (Werker 2000; Tian *et al.* 2012). Glandular trichomes discharge toxic substances including terpenoids, alkaloids and glucosinolates. The main role of glandular trichome is prohibiting or deterring herbivores from feeding. In potato and tomato species, glandular trichomes emit oils that prevent aphids from feeding (Freeman and Beattie 2008). Additionally, the trichome density is increased as induced responses, which results in less damage by herbivores (Dalin *et al.* 2008). This plant structure protects it not only from herbivores but also from drought and heat (Huttunen *et al.* 2010; Karioti *et al.* 2011).

Chemical defence is another mechanism that plants induce following herbivore attack. After the attack, chemical compounds are induced as volatiles into the air. Plants have an outer thin layer on the leaves that is made up of wax, which controls the emission of volatile chemicals that may serve to discourage or inhibit the herbivorous insects from feeding on plants (Riederer and Schreiber 2001; Jung *et al.* 2006). Volatiles induced function in two ways: as direct

defences and indirect defences. Direct defences deter herbivores from causing further damage to the plant (Pare and Tumlinson 1996; De Moraes *et al.* 1998). Indirect defences are used by plants to indirectly affect herbivorous insects by attracting their natural enemies. Volatiles emitted by damaged *Nicotiana attenuata* Torr. ex S. Watson are indirectly used by *Geocoris pallens* Fallén (Hemiptera: Geocoridae) a predator of mirid bug, to locate its prey *Tupiocoris notatus* Distant (Hemiptera: Miridae) (Kessler and Heil 2011).

Terpenoids are some of the chemical compounds commonly used by plants during induced chemical defence (Langenheim 1994; Takabayashi *et al.* 1994). They are also called isoprenoids and are one of the largest groups of compounds produced by all plant species. These compounds have more than 40 000 different molecules and they provide plant growth and protection from insects, fungus, bacteria and abiotic stresses (Aharoni *et al.* 2005; Vince and Zoltán 2011). The simplest terpenoid structure contains hydrocarbon isoprene (C_5H_8). The leaves release it as a volatile emission induced by the damage or abiotic pressures (Takabayashi *et al.* 1994). Terpenoids are commercially and ecologically important. They contribute commercially for the scent, colour and flavour in foods and cosmetics. Ecologically, they are toxic to insects and plants are protected from pathogen attacks (Aharoni *et al.* 2005).

Terpenoids are synthesized into two metabolites: primary and secondary, with primary metabolites mainly focusing on plant growth, whereas secondary metabolites are for defence mechanism (Vince and Zoltán 2011; Ormeño and Fernandez 2012). Diterpenes (gibberellins) and triterpenes (brassinosteroids) are important plant hormones that control the plant growth. The other majority of terpenoids are secondary metabolites (Freeman and Beattie 2008; Vince and Zoltán 2011; Mithöfer and Boland 2012). Terpenoids have volatile and non-volatile compounds but both protect the plant from biotic and abiotic stresses (Mumm and Hilker 2006; Mithöfer and Boland 2012). The volatile terpenoids are also used by plants to communicate with neighbouring plants, to alert others of danger. They also attract the predators and parasitoids of insect herbivores and these natural enemies are able to perceive the cues from infested plants (Bezemer and Van Dam 2005; Mumm and Hilker 2006).

Some plants may only use a single type of induced defence; either physical or chemical, while others may integrate these defences (War *et al.* 2012). Producing induced defences for protection can be costly to the plant. This is caused by high metabolic energy required for

producing these defences. However, those plants that use both are forced due to a large number of herbivores attacking them (Mumm and Hilker 2006).

1.5 Role of volatile chemicals on plant-host location by phytophagous

Chemical ecology is the study of chemicals that are used among intraspecific and interspecific interactions of organisms (Wortman-Wunder and Vivanco 2011). Insects have developed the ability of using chemical signals for foraging, locating host-plants and ovipositing sites, and for finding mates. When herbivorous insects are searching for host plants, they use visual, gustatory and olfactory receptors (Schoonhoven *et al.* 2005; Couty *et al.* 2006; Tasin *et al.* 2011). Previous studies have shown that herbivorous insects perceive the host plants even from a distance of about 100 metres using olfactory receptors (Bruce *et al.* 2005; Schoonhoven *et al.* 2005; Couty *et al.* 2006; Tasin *et al.* 2011). Insects only use visual cues when they are nearer the host plant, as visual cues will assist in recognising the plant by its colour and shape (Couty *et al.* 2006). Furthermore, the gustatory receptors are for the final step in locating the host plant by tasting the non-volatile and volatile compounds (Tasin *et al.* 2011; Städler 2002). Female adults mostly are the ones that locate host plant, as they need to lay eggs on suitable host plants (Bruce *et al.* 2005; Tasin *et al.* 2011). This is significant as the larvae are not mobile and are thus unable to make the choice for themselves. The chemicals used in this intra- and interspecific communication are known as semiochemicals (or infochemicals) (Bailey *et al.* 2006; Karlsson 2011). Semiochemicals are perceived by insects using olfactory receptors situated mostly on their antennae and mouthparts (McGavin 2001, Dahanukar *et al.* 2005).Semiochemicals are subdivided into two clusters: allelochemicals that are involved in interspecific communications and pheromones involved in intraspecific communication. Allelochemicals consist of allomones, kairomones and synomones. For an example: the wasp, *Mischocyttarus drewseni* Saussure (Hymenoptera: Vespidae) uses allomones to repel ants by secreting them on the stem of host plant (Guerrero 2004). Kairomones (plant volatiles) are mostly used by parasitoids to locate their host, e.g. a larval parasitoid, *Agathis bishopi* Nixon (Hymenoptera: Braconidae) on false codling moth *Thaumatotibia leucotreta* Meyrick (Lepidoptera: Tortricidae) (Zimba *et al.* 2015). Adult females are also able to locate the host plant for laying eggs using kairomones, e.g. in *Manduca sexta* Linnaeus (Lepidoptera: Sphingidae) (Waldrop *et al.* 1986; Ramaswamy 1988). However, synomones are favourable to both organisms involved.

Organisms of the same species are also able to communicate amongst each other using pheromones. Pheromones, as well are divided into different groups, alarm, aggregation and sex pheromones. Females use sex pheromones to attract males for mating, e.g. female adults of *Manduca sexta* emit sex pheromones to attract males for mating (Tumlinson *et al.* 1989) and these pheromones are used commercially to control pests. On the other hand, aggregation pheromones are used by males to attract females for mating, such behaviour is seen in Coleoptera, Hemiptera, Orthoptera and Homoptera (Reddy and Guerrero 2004). Whereas, when there is a predator attacking insects, alarm pheromone is used to alert the other insects to prime, e.g. in aphids (Hemiptera: Aphididae) (Verheggen *et al.* 2010; Vandermoten *et al.* 2012).

Volatiles are also used for communication among plants in the neighbouring area and in the parts of the same plant alerting others of the herbivore attack. In a case of the plant being attacked in the leaves, signal is sent throughout the parts of the plant (Glinwood *et al.* 2011). Terpenoid level is increased in foliage of *Gossypium herbaceum* L. (Malvales: Malvaceae) when its roots is attacked by *Agriotes lineatus* L. (Bezemer *et al.* 2004). Chemical constituents released after attack are used as host-finding cues by predators and parasitoids. *Microplitis mediator* Haliday (Hymenoptera: Braconidae) is a parasitoid of *Mamestra brassicae* (Linnaeus) (Lepidoptera: Noctuidae) which is a pest of cabbage, *Brassica oleracea* L. var. *capitata* L. (Brassicaceae) in Europe. This parasitoid perceives an integrated volatile blend from damaged plant and its host (Belz *et al.* 2012). Parasitoids are known to have a 'reliability-detectability problem', whereby cues emitted by the host are usually in low concentrations for parasitoids to locate them. Parasitoids have evolved methods of detecting not only cues from its host but cues from plant infested by its hosts (Belz *et al.* 2012). If the plant is attacked by non-host, parasitoids have the ability to tell difference in the emissions. Differences in volatile compound emissions depend on the concentration of each compound in the blend, and type of feeding guild also has an impact on volatile blend (Mumm and Hilker 2006; Girling *et al.* 2011). *Plutella xylostella* Linnaeus (Lepidoptera: Plutellidae) is a problematic pest of *Brassica oleracea* L. (Brassicales: Brassicaceae), its natural enemy *Cotesia vestalis* Haliday (Hymenoptera: Braconidae) can be used as a biocontrol agent, since it is attracted by volatiles emitted after host damage (Girling *et al.* 2011). These are the cases whereby volatiles are perceived in the third trophic level. However, before parasitoids and predators attack their host or prey, the phytophages need to locate their host-plants for feeding and egg oviposition. They

achieve this by using kairomones that play a significant role in host plant location (Meiners *et al*. 2005; Pitts *et al*. 2014).

1.6 Motivation for the study

Plants are the primary providers of food for insect herbivores; however, they exhibit damage when herbivores feed or oviposit eggs. In order for plants to survive this damage, they fight back by inducing defence responses. Induced responses vary depending on the type of plant species and herbivore feeding on them. *Lantana camara* varieties invading in the Eastern Cape share structural similarities, yet, they respond differently when attacked by the mirid, *F. intermedia* (Heshula 2009).

Figure 1.5: *Lantana camara* varieties from five sites in the Eastern Cape. Flower colour is among factors used to distinguish one variety.

The Port Alfred variety has a similar colour of flower to the Lyndhurst variety, which is dark pink. Whereas the other three varieties: East London, Whitney Farm and Heather Glen share the same colour of the flower, light pink (Heshula 2009; Heshula and Hill 2011). The flower colouring of these varieties is significant in setting them apart as the other parts of the plant are similar. Although these varieties share the same flower colour (Fig. 1.5), their response to the feeding by *F. intermedia* varies. Heshula (2009) discovered that the Lyndhurst, East London and Port Alfred varieties responded by increasing leaf toughness and trichome density. Toughness of the leaf is one way of deterring herbivores from feeding on the plant and has been considered the "most effective defence" mechanism (Garibaldi *et al*. 2011; Heshula and Hill 2011). Increased density of trichomes hinders or minimises the insect from attaching or moving from the plant surface (Björkman and Ahrné 2005). In contrast, the other two varieties (Heather Glen and Whitney Farm) did not show any induced physical traits for defence, instead

they seem to tolerate the feeding by the mirid. Furthermore, establishment of *Falconia intermedia* was sustained by these two varieties. A previous study revealed that there might be induced volatile chemicals emitted by these two varieties after feeding by *F. intermedia* (Heshula 2009; Heshula and Hill 2014). Emission of volatile chemical compounds by *L. camara* could have a negative effect on the mirid, such as, natural enemies of *F. intermedia* using these emissions to locate prey or host, or the volatiles repelling the agent from feeding or ovipositing.

1.7 Aims of the study

It has been over 50 years since *L. camara* has been targeted for control using biological control agents in South Africa. In the process, 25 agents have been released for the control of this alien invasive plant with variable results. Out of those 25 agents, only five leaf feeders established and caused notable damage to the weed. However, the damage exerted on *L. camara* by these agents is not sufficient to prevent it from invading new areas. The study conducted by Heshula and Hill (2011) reported that feeding by one of the recent agents released, *F. intermedia*, induced an increase in physical responses by varieties of this weed at sites around the Eastern Cape Province. The effectiveness of *F. intermedia* was reduced subsequent to the induced physical responses. All five varieties in the Eastern Cape showed an augmentation in leaf toughness. Three varieties (Lyndhurst, Port Alfred and East London) showed an increase in trichome density in new leaves. In a subsequent study, induced chemical responses were tested on two *L. camara* varieties or localities (Whitney Farm and East London) and the chemical responses were evident only in Whitney Farm variety (Heshula and Hill 2014). A 2.5-fold increase in the rate of emission of the volatile compounds sesquiterpene, beta-caryophyllene, was reported after *F. intermedia* feeding. According to research done, beta-caryophyllene is known for attracting natural enemies of herbivores (Rasmann *et al.* 2005; Köllner *et al.* 2008). It is however unknown whether the induced volatiles from *L. camara* varieties affect any repellence or attraction on *F. intermedia*. Thus, the overall aim of the study was to first identify the volatile profiles of these five varieties and investigate whether these chemical compounds were induced after *F. intermedia* feeding. Subsequently, I also set out to understand the impact these induced volatiles have on mirid behaviour. The following specific objectives were addressed:

1. Identification of the volatile leaf chemical compounds produced by the leaves of undamaged and damaged *L. camara* varieties. The identification of chemical

compound profiles gave an insight of whether the chemical profiles of these varieties varied with herbivory (Chapter 2).

2. Determination of the incidence of any induced volatile chemical responses subsequent to *F. intermedia* feeding from all five varieties in the Eastern Cape Province. This was to investigate if chemical responses had increased their emission intensity after feeding by *F. intermedia* (Chapter 2).
3. Using olfactometry techniques, major induced volatile chemical compounds identified were tested to determine their effect on the behaviour of *F. intermedia*. This was done to provide an understanding of whether these induced chemical compounds were attractants or repellents to *F. intermedia* populations (Chapter 3).
4. The implications of these research findings are discussed in the final chapter and highlighting critical factors that will require further research (Chapter 4).

Chapter 2

The chemical composition of *Lantana camara* essential oils and the *Falconia intermedia* feeding induced changes to their quality and quantity

2.1 Introduction

As in other angiosperms, *L. camara* produces essential oils with volatile components that play a significant role in plant functionality. Reports from previous studies have revealed that chemical compounds in the essential oils of *L. camara* comprised terpenes and terpenoids (Seth *et al.* 2012; Sousa *et al.* 2012). These studies have focused on the pharmacological activity of *L. camara* as the plant has been shown to have antibacterial and antifungal properties (Sousa *et al.* 2010; Medeiros *et al.* 2012; Seth *et al.* 2012; Sousa *et al.* 2012; Jawonisi and Adoga 2013). These studies identified a number of volatile compounds in *L. camara* worldwide with some common ones being germacrene-D, β-caryophyllene, β-elemene and α-copaene (Khan *et al.* 2002; Zoubiri and Baalioumer 2012; Passos *et al.* 2012). Essential oils of *L. camara,* and closely related Verbenaceae such as *Lippia* spp., have already been studied in other African countries such as Nigeria and Ghana (Folashade and Omoregie 2012), and it was found that the most abundant chemical compounds identified were similar (Pascual *et al.* 2001; Oliveira *et al.* 2006; Montanari *et al.* 2011; Gomide *et al.* 2013). Except for some preliminary work done by Heshula and Hill (2014) focusing on headspace volatile emissions, no similar work has been conducted in South Africa on the composition of constitutive chemical compounds in *L. camara* essential oils.

Terpenoids and terpenes are the main constitutive and feeding induced volatile chemical compounds identified in plant species. For example, feeding by, *Manduca quinquemaculata* Haworth on *Nicotiana attenuata* Torr. ex Wats (Solanaceae), induced the production of volatile compounds including, three terpenoids; trans-β-ocimene, cis-α-bergamotene and trans-β-farnasene, that strongly deter oviposition (Kessler and Baldwin 2001; Dudareva *et al.* 2004). Another tobacco species, *N. tabacum*, produces similar volatile compounds, including β-caryophyllene and α-humelene, following *Heliothis virescens* (Lepidoptera: Noctuidae) feeding, which is active at night (De Moraes *et al.* 2001).

Previous work conducted on *L. camara* has shown that there are a number of factors that contribute to the insufficient biological control of the weed by its agents, including the role of

herbivore-induced responses (Heshula and Hill 2011). As discussed in the previous chapter, induced plant responses against herbivory include increased emission of essential oils, also known as volatile oils, which are the fragrant essence of plants and can be used in plant defence (Kumari *et al.* 2014). In interspecific interactions where plants are targeted by herbivores they are used chiefly to deter herbivores from further feeding by repellence, as well as to attract natural enemies (predators and parasitoids) (De Moraes *et al.* 2001; Kessler and Baldwin 2001; Holopainen and Blande 2012; Dudareva *et al.* 2013; Kumari *et al.* 2014). Studies on numerous plant species indicate that responses to abiotic and biotic factors (e.g. plant growth stage, climate, soil properties, herbivore feeding type, elicitors in oral secretion of insect herbivores) changes the quality and quantity of volatile chemicals in essential oils (Turlings *et al.* 1993; Mattiacci *et al.* 1995; Banchio *et al.* 2007; Figueiredo *et al.* 2008; Msaada *et al.* 2009; Nurzyńska-Wierdak *et al.* 2012; Abdelmajeed *et al.* 2013). For example, concentrations of chemical compounds in essential oils of *Coriandrum sativum* L. Apiaceae are affected by plant growth stage and climate conditions. While linalool increases through eight maturing stages (Lawrence 1993; Msaada *et al.* 2009), adverse climatic conditions (cold) reduce linalool concentrations (Msaada *et al.* 2009). An increase in terpenoids and indole by *Zea mays* var Delprima was also reported in response to wet and dry soil respectively, while insufficient irrigation resulted in an increase in terpenoids of *Phaseolus lunatus* L. Fabaceae (Takabayashi *et al.* 1990; Gouinguené and Turlings 2002). Not only do abiotic factors have this effect but biotic factors have also been shown to have this impact on concentrations of induced chemical compounds. The feeding by pea leafminer, *Liriomyza huidobrensis* (Blanchard) (Diptera: Agromyzidae) on *Minthostachys mollis* (Kunth) Lamiaceae causes an antagonistic interaction between two major monoterpenes (menthone and pulegone). When one is reduced, the other is increased (Valladares *et al.* 2002; Banchio *et al.* 2007). Furthermore, an increase of terpenoids (E-β-ocimene) and heliocide is seen in the young leaves of *Gossypium hirsutum* L. Malvaceae after feeding by caterpillar, *Spodoptera littoralis* Boisd (Lepidoptera: Noctuidae) on 4-week-old leaves (Opitz *et al.* 2008).

Heshula and Hill (2011, 2014) highlighted induced responses by *L. camara* in response to feeding by one of its sap-sucking biological control agents, the mirid, *F. intermedia*. Feeding by *F. intermedia* on varieties from East London, Port Alfred and Lyndhurst induced morphological defences while an increase in the expression of some volatile chemicals (Beta-caryophyllene) was recorded in plants collected at Whitney Farm (Heshula and Hill 2014). Apart from this preliminary study by Heshula and Hill (2014), thus far there has been no other

report describing either the constitutive and induced chemicals of *L. camara* varieties, nor detailing the role of volatile chemical components in the biological control of the South African varieties of *L. camara*. Hence, the current study aimed to fill the gap in knowledge related to the identification of baseline and induced volatile chemicals emitted by *L. camara*. The specific aims for this chapter were to identify the constitutive chemical compounds of essential oils of *L. camara* varieties in the Eastern Cape Province, and then to assess induced chemical compounds of *L. camara* varieties by comparing the changes in quality and quantity of these essential volatile oils due to herbivory by *F. intermedia*.

2.2 Materials and Methods

2.2.1 Plant Material

In the Eastern Cape, there are five sites where *F. intermedia* was released to control *L. camara*: three coastal sites: East London (32 ° 58 ' 20 " S 27 ° 57 ' 24 " E), Port Alfred (33 ° 36 ' 16 " S 26 ° 52 ' 16 " E) and Whitney Farm (33 ° 40 ' 43 " S 26 ° 35 ' 49 " E); and two inland sites: Heather Glen Farm (33 ° 19 ' 28 " S 26 ° 47 ' 31 " E) and Lyndhurst Farm (33 ° 27 ' 11 " S 26 ° 53 ' 10 " E) (Heshula 2005; Heshula 2009) (Fig. 1.4). Forty lantana cuttings were taken from the plants at each of these sites. The tip of each cutting was dipped into water then into root growth hormone (Dip and Grow®, Fleuron (Pty) Ltd). The cuttings were planted in a pot filled with planting soil and transported to the greenhouse in Waainek Research Laboratory at Rhodes University. Cuttings were watered daily and fertilised bi-weekly using Seagro® until leaves started to sprout and roots to grow, after which they were transplanted into individual pots. Plants were continuously watered daily and fertilised bi-weekly. When the plants were fully matured, four plants from each variety were caged with *F. intermedia* feeding continuously. The remaining plants were kept free of insect feeding by spraying them with insecticide (Malathion DP Insecticide). The damaged plants (plants that the insects have fed upon) were used as treatments and undamaged plants were used as controls.

2.2.2 Insects

The individuals of *Falconia intermedia* was collected from the East London site. Branches with *F. intermedia* were cut and transported using pillowcases and reared in cages with four healthy plants on each in the greenhouse in Waainek Research Laboratory at Rhodes University. In the cages, 3 – 4 adult mirids per leaf were released to feed continuously for 21 days. After 21 days, mirids were removed from the damaged plants to new healthy plants to

maintain the culture of mirids. The leaves were then removed from treatment (damaged leaves) and control (insect-free) plants to run the extractions.

2.2.3 Essential oil extraction

Essential oils were extracted from plant leaves of *Lantana camara* varieties, 450 – 500 g of fresh plant leaves were immersed in distilled water in a round bottom flask. The contents of the round bottom flask were heated to 70 degrees Celsius by means of heating mantle. In the condensation process, water and oil separate and then the oil is collected (Seth *et al.* 2012, Ranjitha and Vikiyalakshimi 2014). Essential oils were extracted from treatment (damaged leaves) and control (insect-free) plants of all varieties. Chemical compounds were identified from essential oils of insect-free leaves and insect damaged leaves to compare the variations in the concentrations and their retention time. After five hours of heating had elapsed, an extra hour and a half was allowed for cooling down. Afterwards, essential oil was collected from the hydrosol. Excess water in essential oil was then dried up by anhydrous sodium sulphate (Na_2SO_4) and the oils stored in refrigerator at 4 ^oC until analysed.

2.2.4 Volatile chemical compounds analysis of essential oils

Gas chromatography mass spectrometry (GC-MS) analyses of volatile chemical compound was carried out by InnoVenton Analytical at Nelson Mandela Metropolitan University in Port Elizabeth. Agilent 6890N GC, 5973 MS Detector was used with Hewlett-Packard 5 medium polarity column (30 m X 0.25 mm i.d., 0.25 µm film thickness). Helium was used as a carrier gas at a constant flow rate of 1 ml/min. The oven was initially programmed at 45 °C, increased at 15 °C/min to 200 °C, and then ramped up from 200 °C to 240 °C at 15 °C/min for 20 minutes. The chemical compounds were identified by comparing identical retention time and chromatogram peaks using the NIST10/HPPEST/WILEY275 mass spectral libraries.

2.3 Results

2.3.1 Constitutive chemical profiles of *Lantana camara* varieties

Constitutive chemical compounds of four varieties (East London, Port Alfred, Whitney Farm and Heather Glen) were identified. The identified compounds belonged to different classes of chemicals. Sesquiterpenes were the dominant class followed by monoterpenes. Other miscellaneous chemical classes were also represented; including aliphatic aldehydes, aliphatic alcohols, alkanes, benzenoids and cycloalkanes (Table 2.2). In the constitutive state of the

plants, the Whitney Farm variety had the highest number (56) of compounds, the East London variety had 41 compounds, the Heather Glen variety had 36 compounds and the Port Alfred variety had the least number (30) of compounds identified (Table 2.1). The Whitney Farm variety had 22 unique constitutive compounds, two sesquiterpenes, one aliphatic aldehyde and alkane each, and some unknown compounds. The Heather Glen variety had four unique constitutive compounds, one sesquiterpenes and three unknown compounds. The Port Alfred variety had six unique constitutive compounds consisting of one benzenoid, two sesquiterpenes and three unknown compounds. The East London variety had three unique constitutive compounds that belonged to sesquiterpenes. The East London variety was characterised by hexane and methyl cyclopentane that had the highest concentrations of 71.19 % and 8.62 % respectively. The Port Alfred variety was characterised by 1,6-cyclodecadiene and caryophyllene with the highest concentrations of 18.22 % and 15.7 %, respectively. The Whitney Farm variety was characterised by caryophyllene and α-muurolene with the highest concentrations of 15.06 % and 7.5 % respectively. The Heather Glen was characterised by caryophyllene and 5-hepten-3-one, 2-(5-ethenyltetrahydro-5-methyl-2-furanyl)-6-methyl-, [2S-[2.alpha.(R*),5.alpha.]] with the highest concentrations of 15.56 % and 20.44 % respectively.

Table 2.1 Number of compounds identified on each treatment of *Lantana camara* varieties

Variety	Treatment	Number of compounds	Number of unique compounds
East London	Undamaged	41	3
	Damaged	39	2
Port Alfred	Undamaged	30	6
	Damaged	19	2
Whitney Farm	Undamaged	56	22
	Damaged	17	0
Heather Glen	Undamaged	36	4

2.3.2 Comparisons of chemical concentrations in undamaged and damaged leaves of the four *Lantana camara* varieties

Notable changes - increases and decreases - were observed in both the concentrations of various chemical compounds and the number of compounds identified across *L. camara* varieties after *F. intermedia* damage. The East London variety comprised 41 compounds from undamaged leaves and 39 compounds in damaged leaves. The concentrations of chemical compounds found before and after feeding, were reduced in: hexane from 71.19 % to 33.3 %, methyl

cyclopentane from 8.62 % to 3.77 %, 3-hexen-1-ol, (Z) from 4.38 % to 3.17 % and 1,6,10-dodecatrien-3-ol,3,7,11,trimethyl-[S-(Z)] from 5.44 % to 0.40 % (Table 2.2). Increases of concentrations were seen in compounds such as copaene from 0.64 % to 2.72 %, caryophyllene from 3.13 % to 7.59 %, 5-hepten-3-one, 2-(5-ethenyltetrahydro-5-ethenyltetrahydro-5-methyl from 1.11 % to 11.03 %, naphthalene from 0.21 % to 1.62 % and cycloisolongifolene from 0.17 % to 1.37 % (Table 2.2).

The Port Alfred variety had 30 compounds identified from undamaged leaves versus 19 compounds in damaged leaves. Reductions in chemical concentrations in this variety were seen in: naphthalene from 4.79 % to 0.92 %, caryophyllene from 15.7 % to 4.03 %, di-epi-α-cedrene from 9.61 % to 1.47 % and α-caryophyllene from 9.03 % to 1.89 % (Table 2.2). In contrast, other concentrations of several chemicals found in essential oil collected were increased after herbivore damage: pentane, 3-methyl from 0.15 % to 2.56 %, hexane from 6.13 % to 75.8 %, methyl cyclopentane from 0.32 % to 5.47 %, 1H-cyclopropa[a]naphthalene from 0.44 % to 0.57 % and 1,3-cyclohexadiene,5-(1,5-dimethyl) from 0.83 % to 2.28 % (Table 2.2).

The Whitney Farm variety had the highest number of compounds (56) identified from undamaged leaves and 17 compounds from the damaged leaves. Reductions in concentrations of chemicals in the essential oils collected were in copaene from 1.2 % to 0.7 %, 1H-cyclopropa[a]naphthalene from 2.64 % to 1.19 %, caryophyllene from 15.06 % to 11.1 %, bicyclo[4.4.0]dec-1-ene from 7.32 % to 1.44 %, and α-caryophyllene from 7.01 % to 5.48 %. Increases of concentrations of chemicals in the essential oil collected were in: pentane, 3-methyl from 0.5 % to 2.07 %, hexane from 6.95 % to 51.93 %, methyl cyclopentane from 0.92 % to 4.19 %, 1,6-cyclodecadiene, 1-methyl-5-methylene-8-(1-methylethyl)-[S(E,E)] from 7.32 % to 7.45 %, isoledene from 0.45 % to 1.88 %, naphthalene from 0.87 % to 2.11 %, α-cubebene from 0.19 % to 0.54 %.

The Heather Glen variety had 36 compounds identified from undamaged leaves. The chemicals in the essential oil of this varieties had these concentrations: copaene 0.57 %, α-cubebene 0.57 %, 1H-cyclopropa[a]naphthalene 1.32 %, caryophyllene 15.56 %, α-caryophyllene 6.39 %, 5-hepten-3-one, 2-(5-ethenyltetrahydro-5-ethenyltetrahydro-5-methyl 20.44 %, 1,6-cyclodecadiene 2.87 %, 1,3-cyclohexadiene,5-(1,5-dimethyl) 2.76 %, caryophyllene oxide 2.17 %, 1,6,10-dodecatrien-3-ol,3,7,11,trimethyl-,[S(Z)] 3.05 %, naphthalene 0.72 %.

2.3.3 Comparisons of common constitutive and induced chemical compounds in four varieties tested

There were chemical compounds eluted that were common to all *L. camara* varieties tested and they were expressed in constitutive and induced states of the plant. Four of these chemical compounds, copaene, caryophyllene, α-caryophyllene and naphthalene were found in all these varieties tested. Constitutive copaene peak in the East London variety was generated at 13.532 minutes with the concentration of 0.64 %. It was induced to 2.72 % concentration with its peak generated at 14.736 minutes after feeding by *F. intermedia*. In the Port Alfred variety, constitutive copaene peak eluted at 13.481 minutes with the concentration of 1.57 %. After the feeding by *F. intermedia*, copaene peak emitted at 13.499 minutes with the concentration of 1.2 %. In the Whitney Farm variety, the peak of constitutive copaene eluted at 13.499 minutes with the concentration of 1.2 %. After feeding by *F. intermedia*, copaene eluted at 13.479 minutes with the concentration of 0.7 %. In the Heather Glen variety, constitutive copaene eluted at 13.532 minutes with the concentration of 0.57 %. Constitutive caryophyllene peak was eluted at 13.926 minutes with the concentration of 3.13 % in the East London variety. In the Port Alfred variety, constitutive caryophyllene peak was generated at 13.931 minutes with the highest concentration of 15.7 % compared with other varieties. At 13.996 minutes, constitutive caryophyllene in the Whitney Farm variety was generated with 15.06 % concentration. Lastly, in the Heather Glen variety at 14.013 minutes constitutive caryophyllene eluted with the concentration of 15.56 %. After feeding by *F. intermedia*, induced caryophyllene was generated at 13.923 minutes with the concentration of 7.59 % in the East London variety. In the Port Alfred variety, caryophyllene concentration dropped to 4.02 %. Same response in the Whitney Farm was noted at 13.912 minutes, where caryophyllene was generated at 11.1 %. Constitutive α-caryophyllene peak eluted at 14.345 minutes with the concentration of 1.18 % in the East London variety and after feeding, it was reduced to 0.78 % at 14.244 minutes. In the Port Alfred variety, constitutive α-caryophyllene peak eluted at 14.241 minutes at the concentration of 9.03 %. The feeding by the mirid significantly dropped the concentration of α-caryophyllene to 1.89 % at 14.225 minutes. In the Whitney Farm variety, the peak of α-caryophyllene eluted at 14.290 minutes with the concentration of 7.01 %. At 14.228 minutes, α-caryophyllene peak eluted at the concentration of 5.48 %.In the Heather Glen variety, α- caryophyllene peak eluted at 14.350 minutes with the concentration of 6.39 %. Constitutive naphthalene peak in the East London variety was generated at 14.254 minutes with a concentration of 0.21 %; after feeding by *F. intermedia*, it was elevated to 1.62 % concentration and peak was generated at 15.790 minutes. In the Port Alfred variety, constitutive

naphthalene peak was generated at 14.851 minutes with a concentration of 4.79 %. Response to *F. intermedia* feeding dropped the concentration of naphthalene to 0.92 % and its peak was eluted at 14.706 minutes. In the Whitney Farm variety, constitutive naphthalene peak was generated at 14.873 minutes with a concentration of 0.87 % and induced to 2.11 % with a peak area at 14.715 minutes. The Heather Glen variety constitutive naphthalene peak was generated at 15.901 with a concentration of 0.72 %.

These following chemical compounds found in three of the varieties tested were hexane, cyclopentane-methyl and α-cubebene. In the East London variety, constitutive α-cubebene peak eluted at 13.193 minutes with the concentration of 0.12 %. The feeding by the mirid induced the concentration of α-cubebene to 0.44 % at 13.490 minutes. In the Port Alfred variety, constitutive α-cubebene was generated at 13.183 minutes with 0.34 % concentration and was not picked up on an induced state. At 13.194 minutes, the Whitney Farm variety peak of constitutiveα-cubebene was eluted with the concentration of 0.54 % and dropped to 5.48 % at 14.228 minutes. In the Heather Glen variety, the peak of α-cubebene was generated at 13.532 minutes with the concentration of 0.57 %. In the East London variety, constitutive hexane peak was identified at 1.811 minutes with a highly concentration of 71.19 % and was highly reduced to 33.3 % at 1.830 minutes after feeding by *F. intermedia*. In the Port Alfred variety, constitutive hexane was 6.13 % concentrated and its peak was generated at 1.811 minutes. A highly increase in hexane peak was generated at 1.805 minutes with 75.8 % concentration after feeding. In the Whitney Farm variety, constitutive hexane peak was eluted at 1.839 minutes with the concentration of 6.95 %. After feeding by *F. intermedia*, hexane peak was generated at 1.814 minutes and its concentration was highly elevated to 51.93 %. The East London variety had constitutive cyclopentane-methyl peak generated at 1.993 minutes with the concentration of 8.62 % and was reduced to 5.77 % after feeding with its peak eluted at 2.017 minutes. In the Port Alfred variety, constitutive cyclopentane-methyl concentration was 0.32 % generated at 2.003 minutes. It was then induced to 5.47 % with its peak eluted at 1.998 minutes. The Whitney Farm variety had its peak eluted at 2.021 minutes for constitutive cyclopentane-methyl with the concentration of 0.92 %. After feeding by *F. intermedia*, the peak was eluted at 2.006 minutes with the concentration induced to 4.19 %.

There were chemical compounds found in two of the varieties tested were copaene, caryophyllene oxide, 1,6-cyclodecadiene, (+)-epi-bicyclosesquiphellandrene, bicyclo[4.4.0]dec-1-ene, 2-isopropyl-5-methyl-9-methylene and 1H-

cyclopropa[a]naphthalene. The concentration of constitutive 1H-cyclopropana[a]naphthalene in the Port Alfred variety was 0.44 % and its peak was generated at 13.575 minutes. It was induced to 5.77 % after feeding by *F. intermedia* with the peak eluting at 13.578 minutes. In the Whitney Farm variety, the peak of constitutive 1H-cyclopropa[a]naphthalene was eluted at 13.606 minutes with the concentration of 2.64 %. *Falconia intermedia* feeding reduced the concentration to 1.19 % with its peak eluted at 13.650 minutes. The peak of constitutive 1,6-cyclodecadiene eluted at 14.499 minutes in the Whitney Farm variety with the concentration of 7.32 %. After feeding by the mirid, the concentration went up to 7.45 % at 14.442 minutes. In the Heather Glen variety, constitutive 1,6-cyclodecadiene eluted at 14.575 minutes with the concentration of 2.87 %. In the East London variety, constitutive (+)-epi-bicyclosesquiphellandrene peak eluted at 14.001 minutes with the concentration of 0.29 %. It was then induced to 3.37 % at 13.995 minutes after the feeding by the mirid. In the Whitney Farm variety, constitutive (+)-epi-bicyclosesquiphellandrene eluted with the concentration of 7.32 % at 14.499 minutes. The feeding reduced the concentration of (+)-epi-bicyclosesquiphellandrene to 1.44 % at 13.987 minutes. In the East London variety, constitutive bicyclo[4.4.0]dec-1-ene, 2-isopropyl-5-methyl-9-methylene peak was eluted at 14.001 minutes with the concentration of 0.29 %. The feeding by the mirid induced the bicyclo[4.4.0]dec-1-ene, 2-isopropyl-5-methyl-9-methylene peak at 13.995 minutes with 3.73 %. In the Port Alfred variety, constitutive bicyclo[4.4.0]dec-1-ene, 2-isopropyl-5-methyl-9-methylene peak was eluted at 14.499 with 7.32 % of concentration. It was then reduced at 13.987 minutes to 1.44 % after feeding by mirid. In the East London variety, constitutive caryophyllene oxide peak eluted at 16.757 minutes with the concentration of 0.89 %. The feeding induced caryophyllene oxide at 15.330 minutes to a concentration of 1.09 %.

Lastly, there were chemical compounds identified in only one variety: isoledene, di-epi-α-cedrene, isolongifolene,8,9-dehydro- and nerolidol. In the East London variety, constitutive isoledene peak eluted at 14.568 minutes with the concentration of 1.09 %. The feeding reduced isoledene at 15.260 minutes to a concentration of 0.54 %. In the Whitney Farm variety, constitutive isoledene peak eluted at 14.178 minutes with the concentration of 7.01 %. The peak was then reduced to 1.88 % at 14.554 minutes. The constitutive di-epi-α-cedrene in the Port Alfred variety was eluted at 14.605 minutes with the concentration of 9.61 %. It was highly reduced to 1.47 % after feeding by *F. intermedia* with the peak eluting at 14.594 minutes. The East London variety had constitutive isolongifolene,8,9-dehydro- peak generated at 15.268 minutes with the concentration of 0.17 % and was induced to 1.37 % after feeding with its peak

eluted at 15.923 minutes. In the East London variety, constitutive nerolidol peak eluted at 15.217 minutes with the concentration of 5.44 % and it was reduced after the feeding to the concentration of 0.4 % at 14.078 minutes.

Table 2.2 The concentrations of chemical compounds in the essential oils of leaves of undamaged and damaged *Lantana camara* varieties identified by GC-MS. Notes: T = treatment, EL = East London, PA = Port Alfred, WF = Whitney Farm, HG = Heather Glen, RT = retention time, Con. = concentration, U = undamaged, D = damaged, CG = Chemical Group, S = sesquiterpene, M = monoterpene, UC = unidentified compound, B = benzenoid, D = diterpene, AA_1 = Aliphatic aldehyde, AA_2 = aliphatic alcohol, AK = aliphatic ketone, AALK = aliphatic alkane ALC = alcohol, CA = cycloalkane, IT = irregular terpene, ALK = alkane, AHC = aromatic hydrocarbon

Compound	CG	EL Und		EL Dam		PA Und		PA Dam		WF Und		WF Dam		HG Und	
		RT	Con %	RT	Con %	RT	Con %	RT	Con %	RT	Con %	RT	Con %	RT	Con %
Pentane, 2-methyl	ALK			1.69	0.23					1.694	0.06				
Pentane, 3-methyl	ALK			1.755	2.00	1.736	0.15	1.736	2.56	1.759	0.50	1.739	2.07		
Hexane	ALK	1.811	71.19	1.830	33.30	1.811	6.13	1.805	75.81	1.839	6.95	1.814	51.93		
Methyl cyclopentane	CA	1.993	8.62	2.017	3.77	2.003	0.32	1.998	5.47	2.021	0.92	2.006	4.19		
Cyclohexane	CA	2.271	0.34	2.295	0.14										
2-Hexenal, (E)-	AA_1									6.342	0.03				
3-Hexen-1-ol, (Z)-	AA_1	6.400	4.38	6.419	3.17					6.417	0.28				
1-Hexanol										6.760	0.03				
Ethanol, 2-butoxy										7.503	0.55				
Bicyclo[3.1.0]hexane, 4-methyl-1-(1-methylethyl)-										7.888	0.04			8.290	4.26
1R-α-Pinene	M	7.472	0.13	8.023	0.30					8.027	0.45			7.477	1.10
Camphene	M	7.819	0.07	8.328	0.17					8.332	0.16			7.819	0.57
Bicyclo[3.1.0] hex-2-ene, 4-methyl-1-(1-methylethyl)	UC			8.729	2.53					8.985	0.11				
β-Pinene	M	8.365	0.11	8.810	0.28					8.813	0.34			8.370	0.82
1-Octen-3-ol		8.871	0.16							8.872	0.08			8.427	0.28
Bicyclo[4.1.0] hept-3-ene, 3,7,7-trimethyl	M			9.291	0.59	13.070	1.59			9.295	0.42				
Benzene, 1-methyl-3-(1-methylethyl)	B			9.537	0.09					9.541	0.05			9.194	0.27

Table 2.2 (Continues…) The concentrations of chemical compounds in the essential oils of leaves of undamaged and damaged *Lantana camara* varieties identified by GC-MS. Notes: T = treatment, EL = East London, PA = Port Alfred, WF = Whitney Farm, HG=Heather Glen, RT=retention time, Con. = concentration, U = undamaged, D = damaged, CG = Chemical Group, S = sesquiterpene, M = monoterpene, UC = unidentified compound, B = benzenoid, D = diterpene, AA_1 = Aliphatic aldehyde, AA_2 = aliphatic alcohol, AK = aliphatic ketone, AALK = aliphatic alkane ALC = alcohol, CA = cycloalkane, IT = irregular terpene, ALK = alkane, AHC = aromatic hydrocarbon

Compound	CG	EL Und		EL Dam		PA Und		PA Dam		WF Und		WF Dam		HG Und	
		RT	Con %	RT	Con %	RT	Con %	RT	Con %	RT	Con %	RT	Con %	RT	Con %
β-Myrcene	M	8.590	0.06											8.590	0.33
3-Carene	M	8.911	0.20	10.537	0.21					9.295	0.42			8.916	1.40
β-Phellandrene	M			8.986	0.17										
D-Limonene	M	9.258	0.11											9.264	0.67
Bicyclo[4.1.0]hept-2-ene, 3,7,7-trimethyl										9.418	0.03				
Eucalyptol	M	9.647	0.39	9.655	1.84					9.658	1.12			9.328	1.22
1,3,6-Octatriene, 3,7-dimethyl-, (Z)	M	9.921	0.08	9.820	0.19					9.819	0.32			9.526	0.35
1,4-Cyclohexadiene, 1-methyl-4-(1-methylethyl)	UC	9.708	0.05	10.190	0.24					10.001	0.08			9.713	0.15
1,6-Octadien-3-ol, 3,7-dimethyl		10.312	0.10											10.312	0.15
Cyclohexene, 1-methyl-4-(methylethylidene)										10.359	0.08				
Bicyclo[2.2.1] heptan-2-one, 1,7,7-trimethyl-, (1R)	UC	11.166	0.49	11.168	0.96					11.167	0.41			10.996	0.72
3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)		11.530	0.18	11.532	0.24									11.392	0.40
1,3,7-Octatriene, 3,7-dimethyl		9.520	0.07					10.541	0.55						
3-Cyclohexene-1-methanol, .alpha., .alpha. 4-trimethyl-	UC	11.569	0.09	11.687	0.25			11.691	0.05						
Bicyclo[3.1.1] hept-3-en-2-one, 4,6,6-trimethyl-, (1S)	UC	11.840	0.11	11.837	0.16			11.333	0.02						
(+)-4-Carene	M							10.359	0.08						
Borneol	M	11.307	0.11					11.461	0.13						

Table 2.2 (Continues…) The concentrations of chemical compounds in the essential oils of leaves of undamaged and damaged *Lantana camara* varieties identified by GC-MS. Notes: T = treatment, EL = East London, PA = Port Alfred, WF = Whitney Farm, HG = Heather Glen, RT = retention time, Con. = concentration, U = undamaged, D = damaged, CG = Chemical Group, S = sesquiterpene, M = monoterpene, UC = unidentified compound, B = benzenoid, D = diterpene, AA_1 = Aliphatic aldehyde, AA_2 = aliphatic alcohol, AK = aliphatic ketone, AALK = aliphatic alkane ALC = alcohol, CA = cycloalkane, IT = irregular terpene, ALK = alkane, AHC = aromatic hydrocarbon

Compound	CG	EL Und		EL Dam		PA Und		PA Dam		WF Und		WF Dam		HG Und	
		RT	Con %	RT	Con %	RT	Con %	RT	Con %	RT	Con %	RT	Con %	RT	Con %
6-Octen-1-ol, 3,7-dimethyl-	IT							11.946	0.74						
Bornyl acetate										12.590	0.02				
1,5,5-Trimethyl-6-methylene-cyclohexene										12.959	0.04				
Phenol, 2-methoxy-3-(2-propenyl)						13.251	0.11								
α-Muurolene	S									14.617	7.50				
Copaene	S	13.532	0.64	14.736	2.72	13.481	1.57	13.499	1.20	13.499	1.20	13.479	0.70	13.532	0.57
α-Cubebene	S	13.193	0.12	13.490	0.44	13.182	0.34			13.194	0.19	15.790	0.54	13.532	0.57
1H-Cyclopropa[a]naphthalene, 1a,2,3,5,6,7,7a,7b-octahydro-1,1,7,7a-tetramethyl-,[1a.α.,7.α.,7a.α.,7b.α)]	AHC					13.585	3.18	13.578	0.57	13.606	2.64	13.581	1.19	13.644	1.32
1,3-Cyclohexadiene, 5-(1,5-dimethyl-4-hexenyl)-2-methyl-, [S-(R*, S*)]-						13.663	0.83							13.730	0.40
1H-Benzocycloheptene, 1,2,4a,5,6,7,8-hexahydro	B					13.781	0.19								
Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene														13.842	0.54
1H-Cyclopental[1,3]cyclopropa[1,2]benzene, octahydro-7-methyl-3-methylene-4-(1-methylethyl)-, [3aS-(3a.α.,3b.β.,4.β.,7.α.,7aS	B							13.984	0.49					14.083	1.70
γ-Himachalene	S									13.798	0.32				
Tricyclo[5.4.0.0 (2,8)] undec-9-ene, 2,6,6,9-tetramethyl	UC			13.784	0.15									14.880	3.47
Caryophyllene	S	13.926	3.13	13.923	7.59	13.931	15.70	13.909	4.02	13.996	15.06	13.912	11.10	14.013	15.56

Table 2.2 (Continues…) The concentrations of chemical compounds in the essential oils of leaves of undamaged and damaged *Lantana camara* varieties identified by GC-MS. Notes: T = treatment, EL = East London, PA = Port Alfred, WF = Whitney Farm, HG = Heather Glen, RT = retention time, Con. = concentration, U = undamaged, D = damaged, CG = Chemical Group, S = sesquiterpene, M = monoterpene, UC = unidentified compound, B = benzenoid, D = diterpene, AA_1 = Aliphatic aldehyde, AA_2 = aliphatic alcohol, AK = aliphatic ketone, AALK = aliphatic alkane ALC = alcohol, CA = cycloalkane, IT = irregular terpene, ALK = alkane, AHC = aromatic hydrocarbon

Compound	CG	EL Und		EL Dam		PA Und		PA Dam		WF Und		WF Dam		HG Und	
		RT	Con %	RT	Con %	RT	Con %	RT	Con %	RT	Con %	RT	Con %	RT	Con %
Bicyclo[4.4.0]dec-1-ene, 2-isopropyl-5-methyl-9-methylene	UC	14.001	0.29	14.003	0.90					14.499	7.32	13.987	1.44		
Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene-,[S-(R*,S*)]-						14.070	1.21								
1,6,10-Dodecatriene, 7,11-dimethyl-3-methylene-, (Z)	S			14.078	0.40									14.174	0.41
Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-	B	14.375	0.83	14.372	4.52									14.500	8.00
(+)-Epi-bicyclosesquiphellandrene	S					13.995	3.37								
α-Caryophyllene	S	14.345	1.54	14.244	0.78	14.241	9.03	14.225	1.89	14.290	7.01	14.228	5.48	14.350	6.39
1H-3a, 7-Methanoazulene, 2,3,4,7,8,8a-hexahydro-3,6,8,8, tetramethyl-,[3R-(3.α.,3a.β.,7.β.,8a.α.)]	UC			14.607	3.25	16.102	0.61	14.337	2.44	14.397	5.11	14.597	1.68		
5-Hepten-3-one, 2-(5-ethenyltetrahydro-5-methyl-2-furanyl)-6-methyl-, [2S-[2.alpha.(R*),5.alpha.]]	AK	15.177	1.11	15.185	11.03									15.382	20.44
Epizonarene	UC			15.260	0.54			14.064	0.51			14.554	1.88		
Aromadendrene	S	14.338	0.34												
Di-epi-α-cedrene (I)	S					14.359	11.11					14.335	5.67		
1,6-Cyclodecadiene, 1-methyl-5-methylene-8-(1-methylethyl)-[S-(E,E)]-	UC	14.455	0.57			14.460	18.22			14.499	7.32	14.442	7.45	14.575	2.87
Cyclohexene, 6-ethenyl-6-methyl-1-(1-methylethyl)-3-(1-methylethylidene)-(S)-	UC					15.054	0.40			14.617	7.50				
1,5-Heptadiene, 2,5-dimethyl-3-methylene														14.708	6.91

Table 2.2 (Continues…) The concentrations of chemical compounds in the essential oils of leaves of undamaged and damaged *Lantana camara* varieties identified by GC-MS. Notes: T = treatment, EL = East London, PA = Port Alfred, WF = Whitney Farm, HG = Heather Glen, RT = retention time, Con. = concentration, U = undamaged, D = damaged, CG = Chemical Group, S = sesquiterpene, M = monoterpene, UC = unidentified compound, B = benzenoid, D = diterpene, AA_1 = Aliphatic aldehyde, AA_2 = aliphatic alcohol, AK = aliphatic ketone, AALK = aliphatic alkane ALC = alcohol, CA = cycloalkane, IT = irregular terpene, ALK = alkane, AHC = aromatic hydrocarbon

Compound	CG	EL Und		EL Dam		PA Und		PA Dam		WF Und		WF Dam		HG Und	
		RT	Con %	RT	Con%	RT	Con %	RT	Con %	RT	Con %	RT	Con %	RT	Con %
1,3-Cyclohexadiene, 5-(1,5-dimethyl)	UC					13.663	0.83	14.471	2.28	13.071	0.83			14.612	2.76
1,4-Methanoazulene, decahydro-4,8,8-trimethylene						14.974	0.80								
10s, 11s-Himachala-3(12), 4-diene										15.002	0.94				
Caryophyllene oxide	S	16.757	0.89	15.330	1.09	15.316	0.76					15.314	0.37	15.532	2.17
Nerolidol/1,6,10-Dodecatrien-3-ol,3,7,11,trimethyl-,[S-(Z)]-	S	15.217	5.44	14.078	0.40	20.895	1.74			14.109	1.01			15.184	3.05
1H-Cycloprop[e]azulene, decahydro, decahydro-1,1,7-trimethyl-4-methylene-,[1aR-(1a.α.,4a.β.7.α.,7a.β.,7b.α)]		15.429	0.16			15.947	0.47			15.440	0.95				
Isoledene	S	14.568	1.09	15.260	0.54	14.549	0.62			14.178	0.45	14.554	1.88		
β-Humulene	S					15.412	0.33								
γ-Elemene	S	15.308	1.08	15.118	1.74	15.118	1.74					15.111	0.54		
Di-epi- α-cedrene	S					14.605	9.61	14.594	1.47						
β-Guaiene	S			15.608	1.53										
Azulene, 1,2,3,3a,4,5,6,7-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1R(1.alpha.,3a.β.,4.α.,7.β)]	UC	15.671	0.87							15.077	0.64			15.644	0.62
(Z,Z)-α-Fernesene	S	15.719	1.10												
3-Cyclohexen-1-carboxaldehyde, 3,4-dimethyl														15.789	0.67
α-Cardinol	S													16.174	0.29
Naphthalene	AHC	14.254	0.21	15.790	1.62	14.851	4.79	14.706	0.92	14.873	0.87	14.715	2.11	15.901	0.72

Table 2.2 (Continues…) The concentrations of chemical compounds in the essential oils of leaves of undamaged and damaged *Lantana camara* varieties identified by GC-MS. Notes: T = treatment, EL = East London, PA = Port Alfred, WF = Whitney Farm, HG = Heather Glen, RT = retention time, Con. = concentration, U = undamaged, D = damaged, CG = Chemical Group, S = sesquiterpene, M = monoterpene, UC = unidentified compound, B = benzenoid, D = diterpene, AA_1 = Aliphatic aldehyde, AA_2 = aliphatic alcohol, AK = aliphatic ketone, AALK = aliphatic alkane ALC = alcohol, CA = cycloalkane, IT = irregular terpene, ALK = alkane, AHC = aromatic hydrocarbon

Compound	CG	EL Und		EL Dam		PA Und		PA Dam		WF Und		WF Dam		HG Und	
		RT	Con %	RT	Con %	RT	Con %	RT	Con %	RT	Con %	RT	Con %	RT	Con %
Isoaromadendrene epoxide										15.349	2.23				
Guaia-3, 9-diene										15.628	1.46				
Cycloisolongifolene, 8,9-dehydro	UC	15.268	0.17	15.923	1.37					15.943	3.42				
β-Panasinsene	S	15.429	0.16												
2-Pentadecanone,6,10,14-trimethyl										17.724	0.05				
9,12,15-Octadecatrienoic acid										18.527	0.08				
1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl	UC					20.895	0.11			14.109	1.01				
n-Hexadecanoic acid										19.671	0.07				
Phytol	D	22.737	1.60			22.210	0.82								
1,6,10,14-Hexadecatetrene-3-ol,3,7,11,15-tetramethyl										20.939	0.25				
Hexadecane	ALK									22.089	0.04				
9,12,15-Octadecatrien-1-ol, (Z,Z,Z)										24.191	0.02				
Octacosane	AALK									24.528	0.02				
Heptacosane	AALK									33.429	0.13				
Octadecane	AALK									43.141	0.13				

2.4 Discussion

Among the South African *L. camara* varieties tested, constitutive compounds in undamaged leaves identified differed in the number of compounds each variety constituted. The total number of compounds identified in the Port Alfred variety was 30, in the East London variety 41, in the Heather Glen variety 36 and in the Whitney Farm variety 56. It was found that the compounds in essential oils were dominated by terpenes (monoterpenes and sesquiterpenes). Our findings are comparable with other studies conducted in relation to the number of compounds and classes of chemicals identified. Khan *et al.* (2002) and Saikia and Sohoo (2011) conducted research on *L. camara* in India and these studies identified 71 and 41 of chemical compounds, respectively, the majority of which were terpenes. In Madagascar and Cameroon, Ngassoum *et al.* (1999) reported 70 and 59 chemical compounds respectively identified on lantana varieties, while Mollenbeck *et al.* (1997) identified 19 chemical compounds in Madagascar. Furthermore, this similarity in the majority of compounds identified was also maintained in various other studies on native and invasive *Lantana* species (Montanari *et al.* 2011; Filho *et al.* 2012; Zoubiri and Baaliouamer 2012). Within the South African varieties tested, not only did the Whitney Farm variety have the highest number of compounds, but it also had the highest number of unique compounds (22) identified compared to other varieties. This means that the Whitney Farm variety is chemically distinct from other varieties tested, and that chemical variability could be used with other factors to separate *L. camara* varieties. It could also suggest that different chemical interactions could be expected from plants of this variety that will not be seen in the other three sites.

There was a trend shown in the total number of compounds identified before and after *F. intermedia* feeding across three varieties where this was tested. The total number of compounds identified after feeding was reduced in each of the varieties, indicating an induced response. Such reduction in chemical compounds after feeding was reported in *Brassica oleracea* var. sabauda (Conti *et a*l. 2008), and *Baccharis spicata* (Asteraceae) and *Schinus polygamus* (Anacardiaceae) (Damasceno *et al.* 2010). This herbivory induced reduction has however never been reported for any Verbenaceae plant, including *L. camara*. The expression of chemical compounds after induction varied depending on the variety fed on. The expressions of most terpenes were elevated whereas others were reduced. Constitutive defence are expressed more than induced defence that are costly, as they require more energy for enzymatic processes than constitutive defences (Karban and Myers 1989; Baldwin 1998; Opitz *et al.* 2008). This may

explain why there were more compounds in the constitutive rather than feeding induced state of the varieties tested.

Even though that was the case, some compounds (hexane, copaene, caryophyllene, naphthalene and α-caryophyllene) were present in relatively high concentrations in constitutive and induced states of the plants in each of the varieties. Caryophyllene has been identified in the chemical profiles of *L. camara* varieties from many studies. In addition, the research on different plant species identified caryophyllene as one of the major compounds in plants; Noge and Becerra (2009) in five *Bursera* species, in infested citrus fruit (Kendra *et al.* 2011; Van der Walt 2012) and in essential oils of *Commiphora leptophloeos* (Da Silva *et al.* 2015). No studies conducted on essential oils of *L. camara* varieties before have identified naphthalene, making this the first study to make this finding. However, studies of *Juniperus* spp. (Cupressaceae) and *Eriotheca longitubulosa* (Bombacaceae) both identified naphthalene in the chemical components of their essential oils (Adams 1998; McFarlane *et al.* 2003). Naphthalene is commercially used as insecticides and insect repellents (Bolton and Eaton 1968; Chen *et al.*, 1998), and it may be possible that the lantana varieties tested use this compound in a similar manner. Hexane was also not identified as one of the chemical constituents of *L. camara* varieties, but it was one of the major compounds in our results. Tatsuka *et al.* (1990) and Baraldi *et al.* (1999) identified hexane to be constituted by fruit flowers. It will be interesting whether future studies on the same or other varieties would discover the same chemicals. If so, these newly identified compounds may be indicating the chemical changes in *L. camara* varieties over time.

The feeding by *F. intermedia* changed the quality of chemical compounds comprised by lantana varieties tested. The concentrations of chemical compounds were either increased or reduced following the herbivore feeding. In the East London variety, an increase of the most number of compounds was observed in the following compounds: 1R-α-pinene; camphene; β-pinene; 3-carene; eucalyptol; 1,3,6-octatriene,3,7-dimethyl-(z); 3-cyclohexene-1-methanol, .alpha., .alpha. 4-trimethyl-; and caryophyllene oxide, nerolidol and isoledene concentrations were reduced. In the Whitney Farm variety, reduction was seen in isoledene. Although these chemicals were not unique across the varieties, they were only expressed after the feeding in the East London and Whitney Farm varieties. These compounds may not be the major compounds identified, but may have significant impact to the quality of chemical blend in the varieties. With these findings, the East London variety showed to be responding differently to feeding by *F. intermedia*.

The chemical compounds identified in constitutive and induced states of the lantana varieties are among the compounds previously identified in other plant species. Feeding by gypsy moth (*Lymantria dispar* L.) on *Quercus ilex* L. resulted in an increase of monoterpenes, homoterpenes and sesquiterpenes. Chemical compounds such as β-caryophyllene, germacrene and linalool were highly induced on infested holm leaves. Some traces of other compounds (α-humulene, δ-cadinene, β-bourbolene) were not detectable on uninfested leaves, but were highly expressed on infested leaves (Staudt and Lhoutellier 2007). Loughrin *et al.* (1995) reported the high emission of terpenes such as ocimene by lima beans and corn leaves when infested by herbivores. The variation in quality and quantity of chemical compounds identified from essential oils of *L. camara* varieties might have effect on the behaviour of *F. intermedia*. Hence, it is crucial to look at the role these chemical compounds identified have on the behaviour of *F. intermedia*.

Chapter 3

The effect of induced defence responses by *Lantana camara* on the behaviour of *Falconia intermedia*

3.1 Introduction

Foraging, oviposition and sheltering activities by phytophagous insects on host-plants can induce plants to emit either volatile or non-volatile chemicals for defence. There are more than 1700 volatile chemicals released by different parts (roots, flowers, leaves and fruit) from more than 90 plant families (Knudsen and Gershenzon 1993; 2006; Dudareva *et al.* 2006; Maffei *et al.* 2007). The survival and productivity of phytophagous insects depend on their ability to differentiate host from non-host-plants. This is achieved by perceiving and detecting specific volatile blends emitted by the host-plants (Bruce *et al.* 2005; Pickett and Glinwood 2008; Binyameen 2013). Phytophagous insects use three types of receptors (visual, gustatory and olfactory) in locating host-plants, food, oviposition sites and finding mates. Chemoreceptors play a significant role in detecting and perceiving the chemicals at a long and short distances emitted by plants. Chemicals are usually the first to be used by insects to detect volatiles from host-plants (Shorey 1973; Hansson and Stensmyr 2011; Webster 2012). As such, the study of pre- and post-feeding olfactory responses is important in understanding the interactions between plants and herbivores.

The emission of volatile chemical compounds by host-plants may attract or repel the herbivores after feeding and possibly attract the herbivore's natural enemies. In general, polyphagous insects (generalist) have a wide range of volatile chemicals that they have to overcome in order to feed, whereas the monophagous/oligophagous (specialist species such as *F. intermedia*) have a narrower range of volatile chemical combinations to overcome (Fürstenberg-Hägg *et al.* 2013). Specialists such as the buckeye caterpillar (*Junonia coenia*) (Adler *et al.* 1995), the tobacco hornworm (*Manduca sexta*) (Harvey *et al.* 2007) and the monarch caterpillar (*Danaus plexippus*) (Zalucki *et al.* 2001) are negatively affected by plant defences. Biological control agents are specialists, and when the levels of plant defence are higher, it may be a challenge for them to control alien invasive plants that increase the expression of the chemicals for defence (Callaway and Ridenour 2004).

Different combinations of induced volatile chemicals in plants have different effects on phytophagous insects. These induced volatile chemicals have been proven individually important in a volatile chemical blends. Even though plant species may belong to the same genus, sometimes the behavioural response of the phytophagous insects to the volatiles emitted by these plants may differ because of the difference in concentrations of the induced volatile chemicals emitted. For example, the volatile chemical compounds of four species of genus *Tagetes* L. (Asteraceae) demonstrated this. Two insect species, *Ceratitis capitata* Wiedemann (Diptera: Tephritidae) and *Triatoma infestans* Klug (Hemiptera: Reduviidae), are affected differently by chemical compounds in the essential oil of these plants. *Tagetes rupestris* and *T. terniflora* release similar volatile chemical compounds that differ in their concentrations. Olfactometer results showed that the chemical compounds from both plant species moderately repelled *T. infestans*, whereas *T. rupestris* was more strongly repelled. By contrast, *Tagetes minuta* was attractive only to males of *C. capitata*, while *T. terniflora* was attractive to both male and female (Lopez *et al.* 2011). Bernasconi *et al.* (1998) showed that corn leaf aphids, *Rhopalosiphum maidis* Fitch (Rhynchota, Sternorrhycha: Aphididae), the winged and wingless were both repelled by (E)-β-farnesene induced in *Z. mays*.

The ability of the plant to withstand damage by phytophagous insects depends on the synthesis of induced volatile chemical compounds to deter or hinder the insects. During the emission of volatiles in a chemical bouquet, some compounds may work synergistically with one another. For example, in grain foods, red flour beetle, *Tribolium castaneum* (Coleoptera: Tenebrionidae), induces the pure terpenes β-caryophyllene and α-pinene. When both are present these two compounds have a synergistic relationship, which inhibits the development of larvae and causes severe toxicity in both adults and larvae (Chaubey 2012). While feeding-induced volatile chemicals are detrimental to phytophagous insects, some may be beneficial. The bioassays conducted by Dickens (2002) showed the attraction of *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae) (Colorado potato beetle) larvae to three out of six volatile chemical compounds emitted by damaged potato. The adults of Colorado potato beetle were attracted to the plants whether their larvae or beet armyworm species had fed on the leaves (Bolter *et al.* 1997). The Colorado potato beetle larval behaviour of not discriminating between undamaged and damaged leaves works in its favour, as it has a guaranteed source of food (Dickens 2002). To investigate plant responses to damage or predation by insects, it is essential that all the volatiles are identified and their effects assessed individually and in combinations.

The use of specific phytophagous insects as biocontrol agents on invasive plants in South Africa has been growing over the past century (Annecke and Moran 1978; Van Sittert 2002; Moran *et al.* 2013). Although the biocontrol method has been considerably successful, the defence mechanisms that some target weeds have been able to elicit has hindered its efficiency. Over the past five decades, 25 biological control agents have been released on the invasive plant *L. camara*, but results have been variable (Urban *et al.* 2011). In Chapter 2, feeding by *F. intermedia* affected chemical compounds produced by *L. camara* varieties. Concentrations of some chemical compounds increased while others decreased. These variations may lead to inconsistent behaviour by *F. intermedia*, depending on the variety each mirid feeds on. Some varieties may tolerate feeding by the mirid, whereas some may repel or be detrimental to the insect.

The hypothesis tested here is that volatile chemical compounds emitted by *L. camara* varieties play a significant role in the behaviour of *F. intermedia*. Therefore, there is a need to understand the responses of the plant to feeding by the biological agents. Further, the response of the agent *F. intermedia* to volatiles of *L. camara* varieties is not known. Specifically, the attractiveness or repellence of *F. intermedia* to undamaged and damaged leaves of *L. camara* varieties and the effect of individual authentic standard compounds to the behaviour of *F. intermedia* was tested.

3.2 Materials and Methods

3.2.1 Insects

The rearing of the *F. intermedia* culture was described in Chapter 2. The culture was reared and maintained on caged *L. camara* varieties placed in greenhouses at Waainek Research Laboratory, Rhodes University. Plants were watered daily and fertilized bi-weekly to maintain the insect population. Other insects such as ants, spiders and mealybugs found in cages were removed to avoid predation and competition with *F. intermedia* for resources.

3.2.2 Plant varieties tested

Plants used in olfactometer bioassays were collected from five varieties of *L. camara*, namely from East London, Port Alfred, Whitney Farm, Lyndhurst and Heather Glen.

3.2.2.1 Undamaged and damaged leaves of *Lantana camara* varieties

In each cage, (Fig 3.1 A) four pot plants of each variety of *L. camara* were exposed to *F. intermedia* for continuous feeding until the leaf surface was yellow due to chlorosis. On each leaf, 3 – 4 adult mirids were released to reach the damage rating of 80 – 100 %. Heshula (2005) defined the damage intensity per leaf area fed on by the mirid. After 21 days of continuous feeding, five to six leaves from undamaged and damaged plants (Fig 3.1 B) were collected for olfactometer trials. *Falconia intermedia* were removed from the collected leaves. Leaves from both treatments were collected from the varieties five minutes prior to bioassays.

Figure 3.1 Damaged plants: A = Caged plants from all varieties, B = Chlorotic leaf surface.

3.2.3 Y-tube olfactometer set-up

A glass Y-tube (Belz *et al*. 2012) was used to conduct the olfactometer bioassays (Fig 3.2). Y-tube cylindrical arms were 150 mm long with the diameter of 250 mm (Fig 3.2 H). Air was transported by two air pumps (Fig 3.2 A) through Teflon tubes (10 mm in diameter) (Fig 3.2 D) to two conical flasks containing active charcoal (Fig 3.2 B). The activated charcoal trapped the impurities in the pumped air, after which the air was passed to two conical flasks containing distilled water (Fig 3.2 C) (500 ml) to humidify the air. Humidified air was then passed to two airflow meters (Fig 3.2 E) that controlled the airflow at 500 ml/min to the last two conical flasks used as odour sources (Fig 3.2 F). Odours were then passed to the Y-tube by Teflon tubes. The entry points of the Y-tube choice-arms were closed by gauze mesh to prevent the insects from escaping or getting into the Teflon tubes. A white Perspex box (Fig 3.2 G) was used to enclose the Y-tube to prevent the insects from receiving visual information. On top of the Perspex box, a glass pane with two holes 110 mm apart was placed to hold the tubes in position. The Y-tube had three small markings on it: one at the base-arm set as a starting line (Fig 3.2 J) and two on the choice-arms (Fig 3.2 I) at 50 mm each from the arms' intersect. Odour contamination of the system was prevented by first washing all the tubes, conical flasks,

gauze mesh and Y-tube with detergent, rinsing them with distilled water, then spraying them with 70 % ethanol before drying them in the oven at 120 °C for 2 hours after every five insects tested.

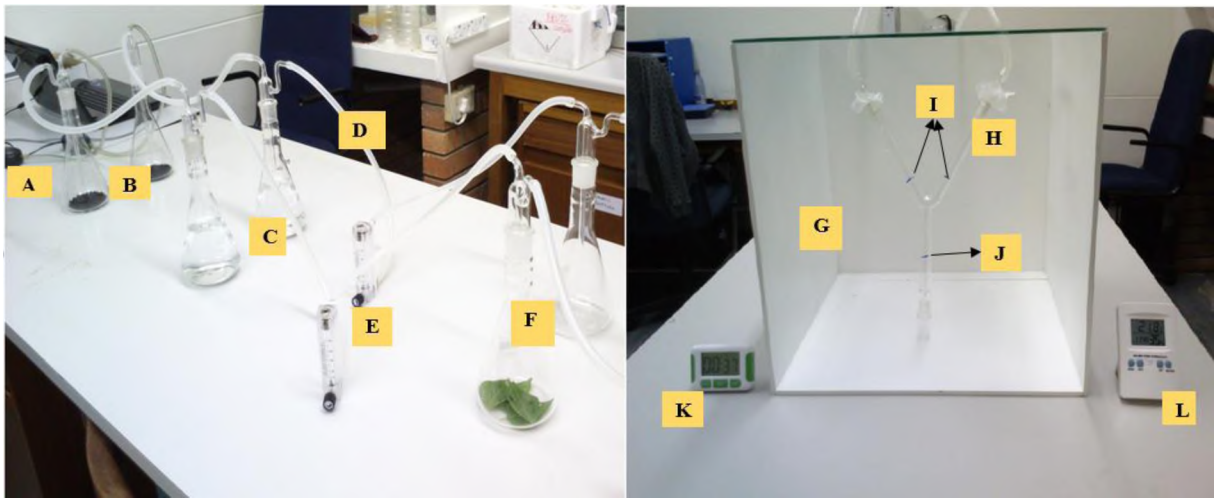

Figure 3.2 The olfactometry set-up. A = Air pumps; B = Activated charcoal filter; C = Distilled water; D = Teflon Tubes; E = Flow meters; F = Odour containers; G = Perspex box enclosing the Y-tube; H = Y-tube; I = Choice lines; J = Starting line; K = Stopwatch; L = Hygrometer.

3.2.4 Odour sources tested

Two sets of trials were conducted: first, to test the attractiveness of *F. intermedia* to undamaged and damaged leaves of *L. camara* varieties. Secondly, the responses of *F. intermedia* to individual standard chemical compounds identified from *L. camara* essential oils (chapter 2) were tested.

3.2.4.1 Individual authentic standard compounds

The major chemical compounds (in chapter 2) identified from *L. camara* varieties were selected for the olfactometry bioassays with *F. intermedia*. Due to the unavailability of other major chemical compounds identified, only five were used to conduct the experiments. The five individual authentic standard compounds were procured from Spellbound Laboratory Solutions were: Caryophyllene ≥ 98 % (Merck), Caryophyllene Oxide ≥ 98 % (Merck), Hexane ≥ 97 % (Merck), Naphthalene and Phytol ≥ 99 % (Merck). The concentrations of chemical compounds identified in lantana varieties varied (Table 2.2) and because of that, a standard dilution was made. A chemical solution was prepared by diluting pure compounds with a volume of hexane to make up a volume of 5 µg/ml. Hexane is used as solvent in many studies, (Lekganyane *et al*. 2012; Bhargava *et al*. 2013; Mariajancyrani *et al*. 2014; Zimba *et al*. 2015). Furthermore, hexane is also reported to be an attractant to various insect herbivores (Tatsuka

et al. 1990; Baraldi *et al.* 1999). For that reason, and because of preliminary observations confirming this for *F. intermedia*, we used it in our bioassays as a positive control. A dose of prepared chemical solution was applied to the filter paper (Qual, 150 mm diameter, grade 3-HW, Munktell, Sweden) at a volume of 0.1 µg/ml and left for one minute for solvent evaporation under the fume hood. Similarly, a volume (0.1 µg/ml) of hexane was also applied to the filter as a control. Each filter paper was placed into a different conical flask to serve as odour sources in olfactometer Y-tube bioassays.

3.2.5 Olfactometer bioassays

The conditions of the room where the olfactometer bioassays were conducted were controlled, with temperature of 22 ± 3 °C and relative humidity of 40–60 %. The intensity of two fluorescent tubes used in the room was 5 lux. The bioassays were conducted between 9:00 and 17:00. Due to insects' poor response in Y-tube horizontal orientation (Desouhant *et al.* 2005; Belda and Riudavets 2012; Zimba *et al.* 2015), many replicates with known compounds were performed to optimise the set-up prior the bioassays. Prior to bioassays, five individuals of *F. intermedia* were collected from caged plants using a vial with aspirator and kept in the dark for 30 minutes to stabilize. Olfactometer trials were conducted on seven treatments: undamaged leaves against damaged leaves, undamaged and damaged of all five varieties against the air (control), four individual chemical standards against hexane (control). Leaf material was changed after five insects to avoid biasing the results. A maximum of 10 minutes was allowed to stabilise the airflow in the system. Thirty individuals of *F. intermedia* were used for each of the chemical compound trials. Twenty to forty individual insects were used for each of the undamaged leaves, damaged leaves and air bioassays to make a total of 334 individuals run. An individual insect was then placed at the entrance of the Y-tube base-arm allowing it to move into the tube. Once the insect passed the starting line, it was given 15 minutes to make a choice and when that time elapsed, an insect was removed using an aspirator. When the time set for each individual had elapsed without the insect making choice, it was recorded as no-choice.

3.2.6 Statistical analyses

Statistical analyses were conducted using R-statistical package version 3.2.0. The olfactometer bioassays for choice test were analysed using Kruskal-Wallis chi-square tests (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$) to determine the statistical difference between undamaged and damaged leaves, undamaged and air (control), damaged and air (control), chemical compounds and

hexane (control). Asterisks denote the significant differences in the analysis. The insects that did not make a choice were discarded and not included in the analysis.

3.3 Results

3.3.1 The attractiveness of *Falconia intermedia* to undamaged or damaged leaves

Olfactometer experiments were conducted on five *L. camara* varieties for undamaged and damaged leaves, undamaged leaves and air (control), damaged leaves and air (control). *Falconia intermedia* individuals showed a highly significant preference for undamaged leaves when tested against damaged leaves for all varieties, indicated by the proportion of insects orientating towards the source smell in the assay (Fig 3.3 A). In the East London variety, 52 % of the total number of insects tested orientated towards the undamaged versus 48% that moved towards the damaged odour source (X^2 = 17.978, df = 1, $p < 0.001$). In the Whitney Farm variety, 56 % of total number of insects orientated towards the undamaged versus 44 % that moved towards the damaged odour source (X^2 = 22.265, df = 1, $p < 0.001$). In the Lyndhurst variety, 58 % of total number of insects orientated towards the undamaged versus 42 % that moved towards the damaged odour source (X^2 = 22.189, $p < 0.001$). In the Heather Glen variety, 54.5 % of total number of insects orientated towards the undamaged versus 45.5 % that moved towards the damaged odour source (X^2 = 21.717, df = 1, $p < 0.001$). Lastly, in the Port Alfred variety, 62.5 % of total number of insects orientated towards the undamaged versus 37.5 % that moved to the damaged odour source (X^2 = 21.985, df = 1, $p > 0.001$). The majority of *F. intermedia* walked up the y-tube within a few minutes after being released at the base of the Y-tube to the arm with the undamaged leaves and landed on the gauze mesh that prevented them from escaping to the Teflon tubes. Highly significant preference of *F. intermedia* to undamaged leaves against purified air was shown by all varieties (Fig 3.3 B), in the East London variety, 68.2 % of the total number of insects orientated towards the undamaged versus 31.8 % that moved towards the purified air (X^2 = 22.265, df = 1, $p < 0.001$). In the Port Alfred variety, 66.8 % total number of insects orientated towards the undamaged versus 33.2 % that moved towards the purified air (X^2 = 22.179, df = 1, $p < 0.001$). In the Whitney Farm variety, 66.2 % total number of insects orientated towards the undamaged versus 33.8 % that moved towards the purified air (X^2 = 22.392, df = 1, $p < 0.001$). In the Heather Glen variety, 70 % total number of insects orientated towards the undamaged versus 30 % that moved towards the purified air (X^2 = 22.199, df = 1, $p = 0.001$) and in the Lyndhurst variety, 66 % total number of insects orientated towards the undamaged versus 34 % that moved towards the purified air (X^2 = 22.104, df = 1, $p < 0.001$). Similar observations were made on damaged leaves against

purified air (control), where high preference to damaged leaves by *F. intermedia* was seen (Fig 3.3 C). In the East London variety 67 % total number of insects orientated towards the damaged versus 33 % that moved towards the purified air ($X^2 = 22.346$, df = 1, $p < 0.001$). In the Port Alfred variety, 67 % total number of insects orientated towards the damaged versus 33 % that moved towards the purified air ($X^2 = 22.159$, df = 1, $p < 0.001$). In the Whitney Farm variety, 65.9 % total number of insects orientated towards the damaged versus 34.1 % that moved towards the purified air ($X^2 = 22.189$, df = 1, $p < 0.001$). In the Heather Glen variety, 65.3 % total number of insects orientated towards the damaged versus 34.7 % that moved towards the purified air ($X^2 = 22.119$, df = 1, $p < 0.001$) and in the Lyndhurst variety, 64.5 % total number of insects orientated towards the damaged versus 35.5 % that moved towards the purified air ($X^2 = 21.999$, df = 1, $p = 0.001$).

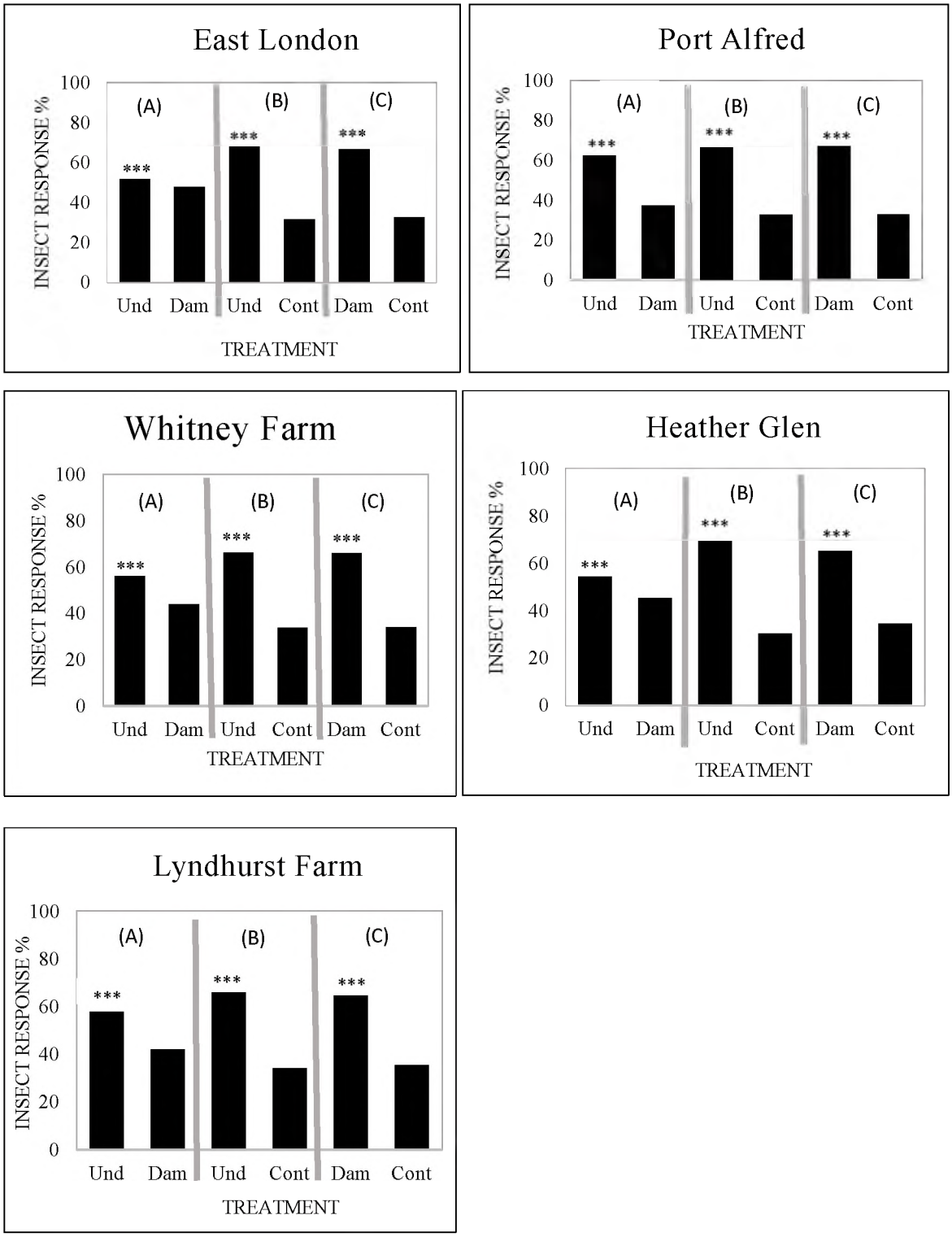

Figure 3.3 The *Falconia intermedia* responses in Y-tube bioassays. Comparisons were made between (A) undamaged leaves vs damaged leaves, (B) undamaged leaves vs control (air), and (C) damaged leaves vs control (air) of *Lantana camara* varieties. The Kruskal-Wallis chi-square (X^2) test was performed for each treatment to show the significant difference (Number of insects per bioassay = 20 - 40, * indicates $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, non-significant (n.s) > 0.05).

3.3.2 Response of *Falconia intermedia* to individual chemical compounds

The olfactory response of *F. intermedia* was tested against four individual chemical compounds identified in essential oils of *L. camara* varieties (chapter 2). The two-way interaction was highly significant, with 80 % of *F. intermedia* choosing hexane (control) over caryophyllene ($X^2 = 13.33$, df = 1, $p = 0.0003$). Caryophyllene showed a repellent effect on *F. intermedia* (Fig 3.4 a). In fact, this repellent reaction by *F. intermedia* was also observed for caryophyllene oxide and naphthalene. Seventy three percent of *F. intermedia* individuals chose hexane (control) over caryophyllene oxide ($X^2 = 6.533$, df = 1, $p = 0.010$). Caryophyllene oxide had a significant repellence on *F. intermedia* individuals (Fig 3.4 b). Eighty percent of *F. intermedia* individuals were attracted to hexane (control) over naphthalene ($X^2 = 10.8$, df = 1, $p = 0.001$), (Fig 3.4 c). On the other hand, the effect of phytol to *F. intermedia* individuals was not significant, with 40 % of insect individuals choosing hexane (control) and 60 % attracted to phytol ($X^2 = 1.2$, df = 1, $p = 1$) (Fig 3.4.d).

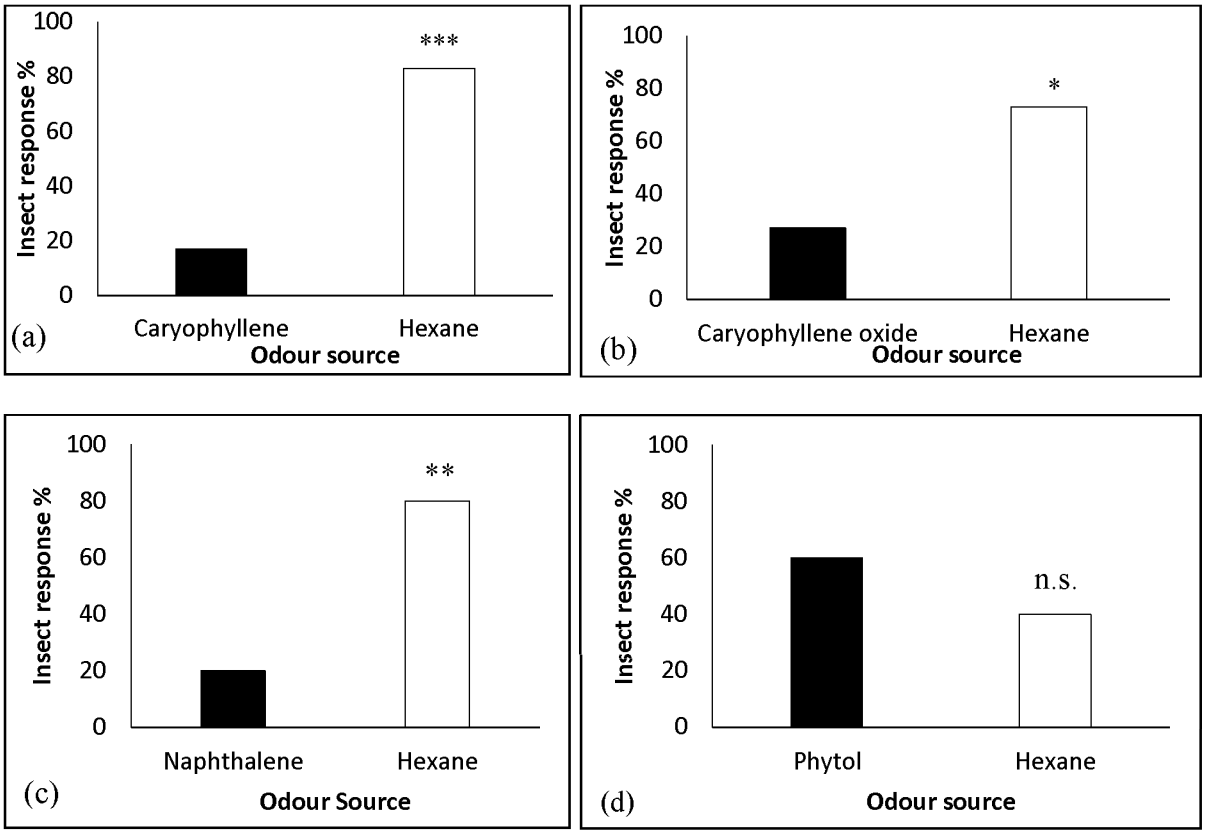

Figure 3.4 The insect responses in Y-tube bioassays. *Falconia intermedia* were exposed to four chemical compounds versus hexane (control). The chi-square ($X^{2)}$ test was performed for each treatment to show the significant difference (Number of insects = 30, * indicates $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, non-significant (n.s) > 0.05).

3.4 Discussion

As anticipated, volatile chemical compounds emitted by *L. camara* varieties play a significant role in attracting the vast majority of *F. intermedia* to undamaged leaves on tested varieties. In the olfactometer bioassays conducted across all varieties, *F. intermedia* individuals were highly attracted to undamaged leaves over damaged leaves and purified air. Additionally, damaged leaves were preferred by the mirids over the purified air. This shows that the hierarchy of preference by mirid individuals is undamaged plants first and then damaged plants. The high preference of damaged leaves by *F. intermedia* over purified air suggests that *F. intermedia* without any source of food or shelter would rather opt for the damaged leaves. Although not tested for significance, there were also further indications of preference for undamaged leaves from separate trials whereby in some varieties a higher proportion of individuals orientated towards undamaged as opposed to damaged leaves' sources in assays versus air (e.g. Heather Glen, Lyndhurst Farm (Fig 3.3 B & C)). According to Meiners *et al.* (2005), phytophagous insects prefer healthy and undamaged plants to damaged and/or infested plants and this was attributed to the higher quality nutrition acquired. Bernasconi *et al.* (1998) also supported this, where corn leaf aphids *Rhopalosiphum maidis* were only reported to feed and survive on undamaged maize seedlings compared to plants exposed to caterpillar regurgitate. Another mechanism that could explain this preference is the insect repellence from, or attraction to plants via volatile chemicals emitted. Dicke (1986), reported volatiles released by infested Lima plants to have significant role in *Tetranychus urticae* Koch (Trombidiformes: Tetranychidaei) dispersal and feeding behaviour, where they fed only on undamaged plants. In another study, the females of *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) were reported to be deterred by volatile chemicals emitted by damaged bean leaves (*Phaseolus vulgaris* L. cv. Borlotto Fabaceae) (Egger *et al.* 2016). In fact, in a previous study conducted, caryophyllene was reported to be increasingly emitted following the feeding by *F. intermedia* in the Whitney Farm variety (Heshula and Hill 2014). Caryophyllene is known to have a repellent effect in some insect herbivores, suggesting that it played a role in the repellence of *F. intermedia* individuals (discussed below). It is quite clear that in the majority of cases damaged or infested plants are chemically and physiologically less attractive to phytophagous insects.

In further observations made in Y-tube olfactometer bioassays of individual chemical compounds *F. intermedia* showed a highly negative response to three tested chemical compounds caryophyllene, naphthalene and caryophyllene oxide. The sesquiterpenes,

caryophyllene and caryophyllene oxide are reported as one of the major compounds that negatively affect insect species in different plant communities. The negative effect of these two chemical compounds has been reported previously on other insect species. In specialist insects, Langenheim *et al.* (1980) have reported deterrent effects of caryophyllene. In Brassica species, caryophyllene showed inhibitory properties towards the movement of *Phyllotreta cruciferae* (Goeze) (Coleoptera: Chrysomelidae) (Gruber *et al.* 2009). Zimba *et al.* (2015) also showed that caryophyllene in citrus fruit had a repellent effect on the parasitoid *Agathis bishopi* (Nixon) (Lepidoptera: Braconidae). A leafcutter ant, *Atta cephalotes* L. (Hymenoptera: Formicidae) induces a strong deterrent effects of caryophyllene oxide from *Hymenaea* species (Langenheim 1994). The majority of studies state clearly the impact these two chemical compounds have on insect species. The responses of the mirids to chemicals tested in this study correlates with previous studies that demonstrated negative response by herbivory insects. Therefore, our findings could give us an indication of how these sesquiterpenes affect the behaviour of *F. intermedia*.

Naphthalene on the other hand, has never been identified before as one of the chemicals contained by any *L. camara* variety. Nevertheless, Adams (1998) and McFarlane *et al.* (2003) reported naphthalene in essential oils of *Juniperus* spp. and *E. longitubulosa* respectively in their studies. Naphthalene is well known as commercially used insect repellent (Bolton and Euton 1968; Daisy *et al.* 2002; Galera *et al.* 2004; Debboun *et al.* 2006). Therefore, our findings on the effect of naphthalene to *F. intermedia* were not surprising as they showed significant repellence of the mirids. More studies are needed to ascertain whether the presence of naphthalene in the tested varieties suggests co-evolution of new compounds, or if they were experimental artefacts in the GC analysis used. Hexane on the other hand is reported as an attractant in previous studies (Tatsuka *et al.* 1990; Baraldi *et al.* 1999), thus it was used as a positive control in our bioassays. In chapter 2, it was shown as one of the major compounds in *L. camara* varieties before and after feeding by *F. intermedia*. In all but one of the dual choice tests conducted, hexane proved to be highly attractive to mirid individuals.

Even though a few major chemical compounds were tested for their effect on *F. intermedia* behaviour, the findings of the few that were tested give an indication of the impact these chemical might have on the mirids. The present study established that caryophyllene, α caryophyllene and naphthalene plays a critical role in plant defensive mechanism and ecologically as repellents to this biological control agent. In turn, hexane was shown to act as

an attractant to the mirid. Therefore, more studies are needed for better practice of biological control of alien plants species. In future studies, testing the effect of other major herbivore induced chemical compounds by *L. camara* varieties in multichoice olfactometry by the mirids should also be considered. Also field-based studies as highlighted will be significant to try to understand how and when plants' induced responses affect biological agents' establishment on lantana.

CHAPTER 4
General Discussion

4.1 Introduction

In 2001, *Falconia intermedia*, was released in the Eastern Cape in an effort to control the varieties of the highly invasive weed, *Lantana camara*. High numbers of *F. intermedia* were seen establishing in lantana varieties and causing major defoliation to the stands. Soon after this, a major reduction in population numbers was seen (Baars 2000a; Heshula 2005; Heystek 2006). A number of factors were commonly reported to be contributing to the reduction of agent populations in general. These include incompatible climate conditions (Heystek 2006), release strategies (Cilliers and Neser 1991), hybridisation (Day and Neser 2000; Vardien 2012), and natural enemies (Urban and Phenye 2005; Tourle 2010). A subsequent post-release evaluation study of *F. intermedia* revealed that the weed induces physical responses for defence following herbivore feeding (Heshula and Hill 2011). In another short study, one of the *L. camara* varieties (Whitney Farm) tested was shown to induce chemical responses in the form of herbivore induced plant volatiles after feeding by *F. intermedia* (Heshula and Hill 2014). As this was the only study on the chemical interaction of this alien plant, this gap in knowledge on the chemical profiles and interactions affecting the behaviour of *F. intermedia* prompted this study. The overall aim of this study was to identify the constitutive and induced chemical compounds due to mirid feeding and further how induced chemical compounds affect the behaviour of *F. intermedia*. In particular, I set out to:

a) Identify constitutive chemical compounds of essential oils of *L. camara* varieties in the Eastern Cape Province.
b) Assess the induced chemical compounds of *L. camara* varieties, by comparing the changes in quality and quantity of these volatile oils due to feeding by *F. intermedia*.
c) Determine the effect of induced volatile chemical compounds on the behaviour of *F. intermedia* and provide an understanding of whether these induced chemical compounds were attractants or repellents to *F. intermedia*.

The discussion below is based on the above aims, and seeks to reflect on the extent to which they were achieved.

Primarily, this study is believed to be the first of its kind to be conducted in South Africa, where the chemical baseline from essential oils of *L. camara* varieties is determined. Studies on *L.*

camara chemical constituents in other countries have been conducted, but focus was on potential pharmacological uses of the plant. Gas chromatography mass spectrometry analysis of essential oils of four (East London, Port Alfred, Whitney Farm and Heather Glen) *L. camara* varieties showed variations in constitutive chemical compounds identified on undamaged leaves (Chapter 2). The chemical variation was expressed across the varieties in quantity and quality of chemical constituents. The Whitney Farm variety had the highest number of chemical compounds (56), followed by the East London variety (41), the Heather Glen variety (36) and Port Alfred variety with the lowest chemical compounds (30) identified in the essential oils of undamaged leaves. The Whitney Farm variety had the greatest number of unique compounds when compared to other varieties tested. This indicates the chemical distinctiveness of the Whitney Farm variety, and may indicate that this variety may have chemical interactions with different ecological consequences compared to other varieties. There is already some evidence of this from headspace volatile chemical studies that Whitney Farm *L. camara* responds to *F. intermedia* feeding differently than plants from East London variety by increasing by almost 3 times emission of the defensive chemical caryophyllene (= beta caryophyllene) (Heshula and Hill 2014). In addition to commonly used morphological traits (e.g. flower colour, spininess, leaf morphology), chemical profile may be a useful additional method in distinguishing *L. camara* varieties apart. For this to happen however, more studies on other lantana varieties invading South African provinces are necessary. Even though there were chemical variations across the varieties tested, the majority of chemicals identified are common in lantana varieties across the world. This suggests that even if lantana varieties in South Africa are not the same as other varieties in the world they are closely related. Even though there is evidence of natural hybridisation by varieties of *L. camara*, most varieties were distributed worldwide from hybrids made by European horticulturists, and therefore such genetic and chemical commonality is not surprising. The South African varieties tested are characterised by different chemical classes such as terpenes, aromatic hydrocarbons, benzenoids, aliphatic aldehydes, aliphatic ketones, and aliphatic alcohols. The majority of chemical compounds produced by the *L. camara* varieties tested here belonged to terpenes, and this has been reported by the researchers conducted studies on the chemical composition of plants from Verbenaceae family (Oliveira *et al.* 2006; Montanari *et al.* 2011; Seth *et al.* 2012; Sousa *et al.* 2012; Gomide *et al.* 2013). This study may therefore also be helpful for the ethnopharmacological and essential oils industry in South Africa, as it might provide some insight and leads into possible essential oils exploration.

Amongst the compounds that were identified and can be used to characterise South African varieties, caryophyllene has proven to be one of the major compounds found across all varieties tested. Caryophyllene is one of the common compounds identified to be produced by Verbenaceae species in different countries. Interestingly, for the first time an aromatic hydrocarbon, naphthalene was identified as one of lantana chemical constituents. This chemical compound was never identified to be a component of the chemical profile of any lantana variety worldwide. This could suggest that new chemical compounds are expressed by these varieties over time. The novel weapons hypothesis (NWH) predicts that in the non-native country, invasive plants reallocate resources to chemicals for defence against competition with native plants and insect herbivores (both generalist and in this case specialist which are released for invasive weed for control). According to NWH, chemical compounds are expressed in higher concentrations or new compounds are reallocated (Callaway and Ridenour 2004; Ridenour *et al.* 2008). It will therefore be interesting if future chemical studies of *L. camara* varieties continue to uncover this chemical compound to ascertain and to prove that it was not experimental artefact in the experimental method.

Lantana camara varieties responded to the damage of *F. intermedia* by expressing chemical compounds in various concentrations. The variation was not only recorded in the chemical concentrations but in the number compounds expressed following the herbivore damage. The East London variety had the most compounds (39) identified in the induced state, followed by the Port Alfred variety with 19 compounds and surprisingly the Whitney Farm variety had the lowest number (17) of compounds. Three monoterpenes were elevated in damaged leaves due to feeding by the mirid, (1R-α-pinene, camphene and β-pinene) in the East London variety. Markedly different compounds were expressed in the Port Alfred variety, 4-carene and borneol. It is unlikely that these compounds were newly expressed in an induced state of the plant, as they were not identified in constitutive state of the plant. It is more likely that they were expressed but in minute trace quantities that were not identifiable by the GC-MS. Caryophyllene was expressed in higher concentrations in damaged leaves following the feeding. In the East London variety, concentration of caryophyllene was elevated to 7.59 %, but in the damaged leaves of both the Port Alfred and Whitney Farm variety, reductions in concentrations of caryophyllene (4.02 % and 11.1 %) were recorded. Interestingly an increase in caryophyllene was recorded post-feeding in Whitney Farm in a previous study (Heshula and Hill 2014). The differences in the trends observed may however be due to the different methods used in that and the current study. Heshula and Hill (2014) looked at headspace volatile

sampling with real time Solid Phase Micro Extraction (SPME) analysis, while the current study used hydrodistillation as a technique to extract essential oils.

The terpenes, monoterpenes and sesquiterpenes are regarded as feeding deterrents and toxins to herbivores feeding on plants (Karban and Baldwin 1999; Köllner *et al.* 2008; Opitz *et al.* 2008). Some of these terpenes are novelty induced following the feeding by herbivores (Callaway and Ridenour 2004, Callaway *et al.* 2008). Caryophyllene, caryophyllene oxide and naphthalene elicited a repellent effect on *F. intermedia* individuals when tested against these compounds. The results of the bioassay are expected due to the reported effect these chemical compounds have on insect herbivory. Caryophyllene in essential oils of *Pienna angolensis* and *P. quandrifolia* L. (Verbenaceae) repelled *Sitotroga cerealella* L. (Lepidoptera: Gelechiidae) (Adjalian *et al.* 2015). Bougherra *et al.* (2015) also showed the repellent effect of caryophyllene on *Sitophilius zeamais* Motsch. (Coleoptera: Dryophthoridae). Though some chemical compounds, monoterpenes in particular, were not tested against *F. intermedia* individuals, their effect on herbivores is well known. For example, myrcene and α-pinene kills pests (*Rhyzopertha dominica* F.) of stored-products (Ogendo *et al.* 2010). The monoterpene, camphene has insecticidal effects on *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) (Yildirim *et al.* 2013). The impact of these terpenes on insect herbivores is quite clear, in that they are elevated for defence. This supports the outcomes of Y-tube bioassays, when *F. intermedia* individuals were tested against undamaged and damaged leaves (Chapter 2). There were a statistically significant higher number of *F. intermedia* individuals preferring the undamaged leaves. The outcomes of the bioassays conducted gives an indication of what may be occurring in the field. When *F. intermedia* damages the leaves of *L. camara* varieties chemicals are released that clearly show a repellent and negative effect on the behaviour of *F. intermedia*.

According to Heystek and Olckers (2003), *F. intermedia* does not control all varieties of *L. camara* effectively. Furthermore, Heshula and Hill (2011) reported on induced physical responses following the feeding by *F. intermedia* on *L. camara* varieties were recorded. In another study, an increase in the volatile emissions of beta caryophyllene (caryophyllene) was reported in the Whitney Farm variety after *F. intermedia* feeding (Heshula and Hill 2014). These limitations may have contributed to the insufficient control of invasive *L. camara* varieties by *F. intermedia*. The past findings are now supported by the results we have obtained from the current study, whereby *L. camara* varieties were chemically distinct from each other.

The chemical distinction of each variety may suggest various effect on *F. intermedia* individuals and may extend the limitations to the control of the weed, *L. camara*. Some alien invasive plants, such as *Centaurea maculosa* Lam. (Asteraceae) (Ridenour *et al.* 2008) use induced responses in the non-native country for their survival (Inderjit *et al.* 2006). From the results of this study, NWH could explain *L. camara* varieties chemical defensive responses against biological control agent, *F. intermedia*. Changes in the constitutive chemical compounds identified in undamaged leaves of *L. camara* varieties were seen following damage by *F. intermedia*. Some chemical compounds concentrations were increased and some were reduced in damaged leaves. The number of compounds was reduced after *F. intermedia* feeding across the varieties.

4.2 Conclusion

It is evident that *L. camara* varieties tested show a great variation of chemical compounds in their essential oils and that showed the distinction in varieties. Furthermore, the herbivore defence chemicals vary greatly depending on varieties and this is the reason some varieties repel secondary feeding and others not. It was clear that when *F. intermedia* was given a choice in Y-tube bioassays, that damaged leaves had a repellent effect on the mirid. This finding was supported by bioassays looking at various chemical compounds where caryophyllene, α-caryophyllene and naphthalene were repellents. Undamaged leaves were attractive to mirids, as was hexane and phytol. Therefore, the general conclusion of chemical compounds induced following the feeding by the mirid is that some have a repellent effect on *F. intermedia*; hence, they may contribute to the weed's continued growth and invasion of new areas. Accordingly, the biological control programme for *L. camara* varieties in South Africa will continue encountering different factors that are complex. The effects of induced volatile chemicals pointed out on this study are in addition to other factors that were previously studied on why *F. intermedia* are not succeeding to control of *L. camara* (Cilliers and Neser 1991; Day and Neser 2000; Urban and Phenye 2005; Heystek 2006; Tourle 2010; Heshula and Hill 2011; Vardien 2012; Heshula and Hill 2014). Therefore, we recommend that, chemical profile studies of the invasive alien plants should be part of the host specificity testing, and the vital role of chemical compounds on agent-weed interactions must be taken into consideration with other factors before the biological agents are released.

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