INDUCED PLANT RESPONSES OF DIFFERENT *LANTANA CAMARA* L. (VERBENACEAE) VARIETIES TO HERBIVORY BY *FALCONIA INTERMEDIA* (DISTANT) (HEMIPTERA: MIRIDAE)

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Abstract

A highly variable invasive shrub, *Lantana camara* L. (Verbenaceae), has been notoriously difficult to control thus far despite a well established biological control programme in South Africa. A promising leaf-feeding biological control agent, *Falconia intermedia* (Distant) (Hemiptera: Miridae), released to control this invasive plant eventually crashed at three out of five sites in the Eastern Cape Province. In the Mpumalanga Province, after initially colonising and building up high numbers on the *L. camara* stands the agent populations crashed. Several reasons for these population crashes have been suggested, but induced plant defences have not been investigated. Although plants face the challenge of herbivory by various organisms while remaining immobile, some plants may possess the ability to induce physical and/or chemical defensive responses following feeding and thus prevent further plant tissue damage and loss.

Laboratory trials were conducted to determine the existence, nature and effect of physical and chemical feeding-induced responses of *L. camara* on the performance of the leaf-feeding biological control agent, *F. intermedia*. *Lantana camara* plants used in the study were obtained from five localities in the Eastern Cape Province, South Africa, while the insect culture was established from field populations. Plants from all varieties on which *F. intermedia* was released significantly increased the toughness of their leaves compared to control treatment plants. In addition, plants from three localities: Lyndhurst Farm, East London and Port Alfred, significantly increased trichome density after prolonged feeding by *F. intermedia*. On the three varieties showing increases in these two factors (i.e. leaf toughness and trichome density), oviposition, survival and feeding damage by the mirid agent was significantly lower on previously damaged plants. A significant negative correlation between trichome density and population numbers was found ($R^2 = 0.52$, $p < 0.0003$), suggesting that an increase in trichome density strongly contributes to a reduction in *F. intermedia*'s growth. The growth and reproduction of the resistant plants was not significantly impacted by *F. intermedia* feeding. The defensive responses were found to be plant systemic and rapidly induced as they were elicited

1

and expressed throughout the plant in both damaged and undamaged leaves within five weeks after insect release.

Leaf toughness and trichome density were not significantly increased after feeding on plants from Whitney Farm and Heather Glen Farm. On the contrary, mirid individuals performed significantly better on plants from Whitney Farm and Heather Glen Farm than on plants of other varieties, indicating their susceptibility and suitability to the agent and the lack of induced resistance against the agent. Plants from all localities besides East London showed some level of tolerance and overcompensated for feeding damage by increasing plant growth and reproductive factors on plants fed upon. This was however only significant in two variables of the more susceptible localities, Whitney Farm and Heather Glen Farm. This increase in plant fitness did however indicate an induced defence response by these plants to feeding, a response designed to lessen the effects of agent feeding.

Headspace volatile analysis was used to investigate any volatile chemical responses by *L. camara* due to *F. intermedia* feeding at two of the five localities chosen: East London and Whitney Farm. There was no significant difference in headspace volatiles emitted by leaves of plants from the East London insect infested and control treatment plants. On the Whitney Farm damaged plants however there was a 2.5 fold increase in the emission intensity of one of the three main compounds, later identified as Beta-caryophyllene. Three major chemical constituents which were found to be common to leaf volatiles of the two varieties were identified through gas chromatography-mass spectrometry (GC-MS) from the damaged and undamaged leaves of these two varieties. The methods used in collecting leaf volatiles were shown to be significant in the strength of chromatogram peaks. Using general authentication methods and purified standards, one of these was identified as the sesquiterpene, Beta-caryophyllene ($C_{15}H_{24}$). This compound is one of the major constituents found in isolations of *L. camara* varieties worldwide. This is the first such work done on a variety of *L. camara* in South Africa, and hopefully the beginning of more in-depth studies of the volatile organic chemicals from the numerous naturalised varieties of *L. camara*.

2

It is suggested that the sum of these responses may play a role bigger than is currently understood in this plant-insect relationship. It is also argued that feeding induced plant defences may play an important role in attempts to control alien plants using insect agents.

Table of Contents

5

List of Tables

8

List of Figures

10

11

Acknowledgements

CHAPTER 1

GENERAL INTRODUCTION

1.1   Plant defences against herbivores

Plants and insects share one of the most important ecological relationships in nature. The Plant Kingdom consists of the highest biomass among living organisms, while quantitatively insects are believed to have the highest number of species worldwide. Having co-evolved for over 350 million years, the extent of the interaction between these two taxa permeates all levels of the organisms, from population genetics to basic biochemistry. Interaction with insects may benefit the host plant, as in the case of pollination and seed dispersal. However insects are also major herbivores. An estimated 400 000 species feed on different niches of about 300 000 known vascular plant species (Schoonhoven *et al.* 2005). Herbivory by insects has been postulated as an important driving force in the terrestrial diversification of plants (Ehrlich and Raven 1964). Insect herbivores may remove anything from, on average 15%, to almost 100%, during outbreak years, of the above-ground biomass of terrestrial plants (Cyr and Pace 1993). Being sessile, plants seem defenceless against the injurious feeding activity of a variety of herbivores. Advances in biochemical and plant ecological research have however shown this to be untrue. To minimise and reduce the impact of the removed plant tissue, plants have evolved a variety of mechanisms including avoidance, resistance and tolerance to insect herbivory. Such characteristics that confer some fitness advantage to the plant expressing them are generally known as plant defences. Plant defences against herbivore attack may be expressed at all times (constitutive) or only when a plant has sustained a certain level of attack by herbivores (induced).

Plants may avoid or escape herbivore attack by expressing traits that reduce the likelihood of being found by herbivores (Boege and Marquis 2005). This strategy stems from the fact that plants aggregated in numbers are easier to locate than plants which are spatially isolated. Therefore, plants growing in isolation reduce their apparency and are less likely to be found by

13

herbivores. Even though it is unlikely that rarity is an adaptation to insect herbivory, it might lead to the desirable consequence of decreased herbivory (Schoonhoven *et al.* 2005). Timing of the development of vulnerable plant tissue, such as leaves, fruits, and seeds, may also be an adaptation by plants to negatively affect the distribution and abundance of insect herbivores. This asynchrony in the development of plant tissue and the insect herbivore life-cycle results in the insects being presented with lower plant quality (Wint 1983; Hunter *et al.* 1997).

Plants may further resist and reduce herbivore efficiency by varying their physical and chemical characteristics. This may be achieved by means of direct and/or indirect defences once a plant has been located by herbivores. Resistance is defined from the perspective of the herbivore as any plant genetic trait that results in a decrease in the performance of the herbivore on its plant host (antibiosis), or that impact on the preference of the herbivore during host plant selection (antixenosis) (Karban and Baldwin 1997; Schaller 2008). A number of physical defensive characteristics are effective in reducing insect herbivory. Glandular and non-glandular trichomes, or leaf hairs, reduce feeding by forming a barrier that makes it difficult for insects to attach or insert their mouthparts into plant leaves and stems. Glandular trichomes further reduce insect herbivory by releasing substances that may repel herbivores from feeding (antixenosis), be noxious or immobilise herbivores on the leaf by being sticky (Howe and Schaller 2008).

Increased leaf toughness is another plant trait that is considered to be a good indicator of resistance as it is widely reported to be inversely related to insect herbivore activity. Other traits that may act as anti-herbivore defences include waxy leaves, variations in leaf nutrients (e.g. N, C, H), and the release of resin flows (Eigenbrode and Espelie 1995; Lombardero *et al.* 2006). Although distinct from chemical defences, physical defensive characteristics are final expressions of the same biochemical processes generating chemical defensive traits (Schoonhoven *et al.* 2005). Whether a plant trait manifests physically or chemically, it is potentially a resistance factor if it negatively affects host plant selection, oviposition or feeding of an insect herbivore (Howe and Schaller 2008).

14

Plants also resist herbivores by accumulating toxic biochemical compounds as chemical defences. Primary metabolites may act as defensive chemicals by presenting herbivores with extremely low, high or unbalanced levels of nutrients, thereby tampering with their nutritional requirements (Simpson and Raubenheimer 2001). Defensive functions within the plant are usually facilitated by secondary metabolites. Unlike primary metabolites that are essential for life functions in every organism, secondary metabolites are not directly involved in the growth, development or reproduction of an organism, but they do contribute to the fitness of the organism by influencing ecological interactions (Vickery and Vickery 1981). Primary and secondary metabolites are produced by different metabolic pathways, with molecules necessary for secondary metabolism provided by primary metabolites. The secondary metabolite pathways are specific to plant family and genus and the specific constituents in a certain species have been used to help in systematic determination and chemotaxonomy (Mann 1987).

Secondary metabolites, which are broadly classed into terpenoids, flavonoids and phenyl propanoids (Croteau et al. 2000), have also been referred to as natural products due to their role as sources of dyes, polymers, fibres, glues, oils, waxes, flavouring agents, perfumes and drugs (Bassman 2004). Most of the compounds isolated from the weed Lantana camara L., the subject of this study, are terpenoids, along with alkaloids, steroids and essential oils. Terpenoids, synthesised from 5-C isoprene units, are the largest and most structurally diverse secondary metabolites found in plants. They comprise about 25 000 compounds with a variety of ecological functions (Bassman 2004). Many of these compounds are used as defensive chemicals by plants and deter feeding and oviposition by herbivores.

Terpenes may also be volatile and readily evaporate in air. These volatile oils, also known as essential oils, are hydrophobic and usually contain an essential aromatic compound. Plants are the primary producers of volatile oils, and the volatiles are generally obtained by distillation. Other methods such as cold-pressing and solvent extraction are also used to obtain the volatile oils. Essential oils are important commercially and are used in medicine as stimulants, correctives and carminatives. They are also used for food flavouring, fragrances, massage oils

15

and aromatherapy. Essential oil extracts and fractions are used to repel insects and other arthropods that are pests of humans, livestock and pets (Thomas and Schumann 1993). From an ecological point of view, volatiles secreted from aerial parts of plants play an important role in attracting pollinators. More recently, volatile-mediated plant herbivore relationships leading to the attraction of herbivore natural enemies have been reported (Dicke 1994). Other plants within the emission range of the emitter plant have been shown to cue in to the volatiles induced in response to herbivore damage (Arimura *et al.* 2001; Kessler *et al.* 2006; Kost and Heil 2006). The receiver plants in turn may use this chemical information to prime themselves against possible attack. The emission and use of these compounds when under attack is thus complex and manifold in the different plant groups and each plant must be studied independently.

Once damaged, plants become activated to respond to herbivory. Plants may increasingly express traits that reduce the negative impacts of the damage encountered (e.g. compensatory growth, increased photosynthesis, resource reallocation) (Stowe *et al.* 2000). The ability of plants to re-grow and reproduce after being damaged is known as tolerance. The extent to which a plant is able to show tolerance is known as compensation (Strauss and Agrawal 1999). In this regard, three levels of compensation are recognised; complete compensation (fitness in damaged equals fitness in undamaged plants), over-compensation (fitness in damaged more than fitness in undamaged) and under-compensation (fitness in damaged less than in undamaged plants). Plants increase their tolerance by increasing photosynthetic rates, increasing relative growth rates, increasing branching or tillering, and re-allocating root carbon reserves for above-ground reproduction and growth. Just as a combination of physical and chemical defensive measures may be used by plants, a combination of methods may be evident to their benefit (e.g. resistance and reducing negative impacts) (Howe and Schaller 2008).

The toxic compounds produced and the production mechanisms by both induced and constitutive defences may be the same in a given plant species (van Dam *et al.* 2001). In the induced state however, a particular compound may be up-regulated or increased in expression. Induced plant responses to herbivory may be direct or indirect. The plant- and herbivore-specific responses

16

may be immediately induced against herbivores causing immediate negative impact (rapid induced resistance) (Haukioja 1990), or the induced effect may affect the rates of herbivory in the following season (delayed induced resistance), or later in the same season (intermediate delayed induced resistance) (Agrawal 1998; Boege 2004; Stevens and Lindroth 2005). However the response to early season feeding may also have the effect of increasing the susceptibility of the plant to herbivores (Lempa *et al.* 2004). This may be achieved by the lowering of plant quality (Karban and Baldwin 1997).

Plant responses to herbivore attack and damage that directly affect the herbivore are known as direct induced responses. When induced, direct plant defences may be effective by decreasing plant quality, thus reducing feeding on the plant (Baldwin 1988, Stout *et al.* 1998; Berenbaum and Zangerl 1999). Inducible physical barriers to feeding such as thorns, spines and waxes found on the foliage of the plants are effective in this regard. Physical strength and toughness of leaves, induced or constitutive, are reported in many studies to negatively affect herbivory and insect development negatively (Agrawal and Spiller 2004; Wheeler 2001; 2006). Younger leaves and plants with softer leaves, and higher leaf moisture are preferred to older and harder leaves. Similarly, rapidly induced increases in the density of leaf trichomes is a barrier to herbivory and results in decrease in feeding and herbivore performance (Pullin and Gilbert 1989; Mauricio and Rausher 1997; Dalin and Bjorkman 2003). The effect of leaf nitrogen is more herbivore specific. Some herbivores prefer leaves with high leaf nitrogen (Wheeler and Center 1996; Prudic *et al.* 2005), while others performed significantly better on plants with a low nitrogen content (Cornelissen and Fernandez 2001).

Indirect induced defences on the other hand are responses characterised by the emission of volatile organic chemicals. The chemical information plays an important role in inter- and intraspecific communication. Volatiles emitted by damaged plants have been reported to attract natural enemies of the herbivores from the third trophic level. By predation and parasitism of the herbivores these natural enemies may reduce the damage to the plants (Tumlinson *et al.* 1993; De Moraes *et al.* 1998; Dicke and Vet 1999). The volatiles are also important in mediating

17

intraspecific communication. Undamaged plants may interpret the chemical signals from damaged plants and in turn prime themselves physically or chemically for defence (Agrawal 2005; Arimura *et al.* 2001; Dicke *et al.* 2003). Besides herbivore feeding, insect oviposition has also been shown to induce defensive responses from plants (Hilker and Meiners 2002). Whether they are expressed constitutively or induced, plant defences are physiologically costly and plants need to respond to a particular threat appropriately (Baldwin and Preston 1999; Hoballah *et al.* 2004).

1.2 An introduction to the problem plant: *Lantana camara*

Lantana camara L. *sensu lato* (Verbenaceae), hereafter referred to as lantana is a thicket-forming shrub. It is thought to have arisen through deliberate horticultural and natural hybridisation, selection and somatic mutation from a number of similar, and probably closely related, central and South American species (Swarbrick *et al.* 1995). This area is regarded as its generic epicentre, and from here the plant was introduced to the rest of the world as an ornamental plant via Europe (Stirton 1977). It is normally 2-4 m tall, but is capable of becoming a climber reaching up to 10 m in height. It grows well in regions receiving consistent rainfall, but it tolerates drought very well through defoliation. Flowering occurs throughout the year in areas with good light and moisture conditions, with peaks in wetter months of the year (Swarbrick *et al.* 1995). In cooler or drier conditions, flowering occurs in the warmer and wetter summer months, due to frost and drought damage (Graaff 1986).

1.2.1 Spread and impact

Lantana has become naturalised in more than 50 countries and is declared a weed in these areas. It was first introduced into South Africa via Cape Town in 1858, and KwaZulu-Natal in 1883 (Stirton 1977) and was declared a weed in 1954. Since introduction, it has naturalised and spread to Limpopo, Gauteng, Mpumalanga, KwaZulu-Natal provinces, and the coastal regions of the Eastern Cape and the Western Cape provinces (Fig 1.1). Versfeld *et al.* (1998) reported that lantana had invaded a total area of 2 235 395 ha in South Africa and 2 297 ha in the Eastern Cape. According to Robertson *et al.*'s (2003) prioritisation system for the management of alien

18

species in South Africa, *L. camara* received the highest prioritisation score based on criteria such as its invasiveness, spatial characteristics, potential impact, potential for control and the conflict of interests with the economic and informal sectors that arises from controlling the weed. The weed invades forests, riverbanks, creek banks, fence lines, grassland, and is common in open and semi-open plant communities such as road verges, grasslands and woodlands. It aggressively pioneers invasion of disturbed ecosystems, tending to crowd out indigenous flora and suppress natural vegetation, thus reducing the carrying capacity of veldt (Gentle and Duggin 1997).
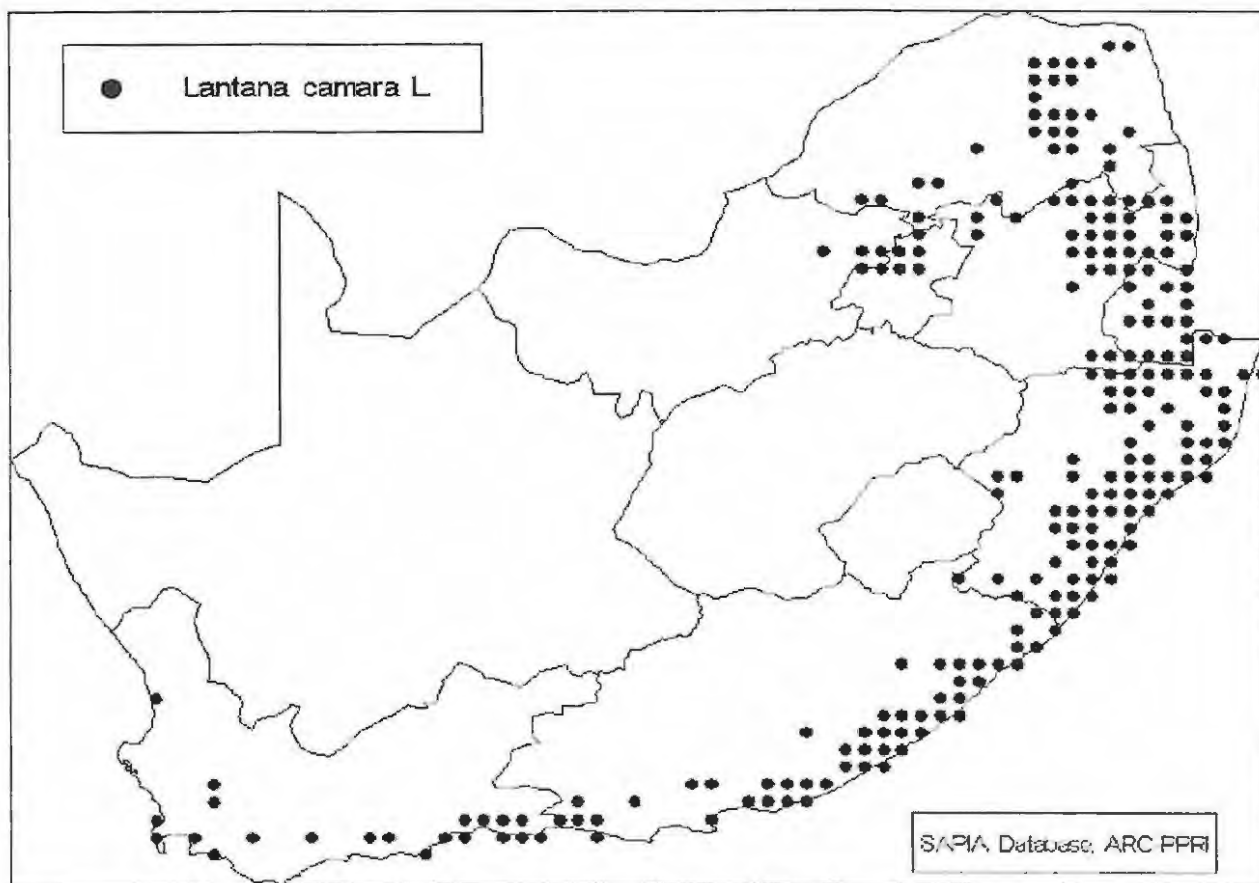
Figure 1.1 Distribution of *Lantana camara* in South Africa based on quarter square degrees in which the plant was found (Henderson 2001).

19

Through allelopathic suppression of indigenous plant species, lantana invasions also interrupt regeneration processes (Gentle and Duggin 1997) and reduce the biodiversity of natural ecosystems in Australia. Lantana leaves, stems and fruit of some varieties contain a number of chemicals toxic to animals, especially two cyclic triterpenes lantadene A and B (Sharma *et al* 1992) that primarily affect ungulates (Everist 1981). The most common symptoms of lantana poisoning in sheep, cattle, and horses are photosensitization, acute and chronic damage to the liver, kidneys and gut, paralysis of the gall bladder, internal haemorrhage and sometimes death within 1-6 days (Fourie *et al.* 1987). The expected annual impact of cattle mortalities in South Africa was estimated to be in excess of US$429 293 (at an exchange rate of R3.96 to US$1 in 1996) (Kellerman *et al.* 1996). As lantana usually invades fields and pastures where livestock feed, this exposes the animals to these potentially damaging chemicals and increases the chances of livestock feeding on them. Green lantana fruits also contain toxins and have caused sickness and death in children (Morton 1994). A number of studies have also reported larvicidal and insecticidal activity of lantana extracts against some organisms (Grainge *et al.* 1984; Saxena *et al.* 1992; Bouda *et al.* 2001).

1.2.2   Variation within species

The genus *Lantana* L. (Verbenaceae) is native to Central and South America, with a few species occurring in Africa and Asia (Munir 1996). There are four distinct groups recognized within this genus: *Lantana* sections *Calliorheas, Sarcolipia, Rhytocamara* and *Camara*. The *Lantana* section *Camara* is further divided into 3 complexes based on *L. urticifolia, L. hirsuta,* and *L. camara* (Day *et al.* 2003) (Fig. 1.2). The latter complex includes the primary weedy complex referred to as *L. camara* L. *sensu lato*. Lantana is part of a synthetic complex that consists of several morphologically similar *Lantana* species (Day and Neser 2000). Incomplete records collected by Stirton (1977) suggest that this complex is a hybrid from wild *Lantana* L. sect *Lantana* species sourced from Mexico, Brazil and throughout the West Indies. A long process of man-mediated horticultural hybridisation and selection then followed resulting in about 650 selected and named cultivars (Stirton 1977). Unlike the diploid natural wild *Lantana* taxa, the complex is polyploidy with triploid, tetraploid, pentaploid and hexaploid forms reported (Stirton 1977; Sanders 2006). Due to hybrid vigour speciation is likely, leading to new varieties of *L.*

20

camara. Parental *Lantana* in the native ranges are morphologically, physiologically, or chemically dissimilar to the *L. camara* varieties in the invaded ranges (Smith and Smith 1982).

L. micrantha

L. bahamensis

L. leonardiocum

L. urticoides

L triplinervia

L. urticifolia urticifolia

L. urticifolia zanonii   *L. urticifolia* complex

L. urticifolia "hispida" ined.

L. urticifolia "tiliifolia" ined.

L. urticifolia moldenkei

L. urticifolia "moritziana" ined.

L. urticifolia portoricensis

L. hirsuta

L. insularis
                                  L. hirsuta complex   *Lantana sect. Camara*
L. armata

L. viscosa

L. camara

L. depressa

L. hodgei   *L. camara* complex

L. cujabensis

L. kingii

L. ovatifolia

Figure 1.2 Three complexes of *Lantana* section *Camara* and their species (Day *et al.* 2003).

21

Hybridisation in the field (Spies and Stirton 1982 a; b; c) giving rise to different varieties, has further increased the genetic variability of lantana under field conditions, making this complex a challenging target weed for control. There is reported to be up to 50 different varieties of *L. camara* in South Africa (Spies and Stirton 1982 a; b; Graaff 1986), with about 17 varieties naturalised in the Kwazulu-Natal province alone (Stirton and Erasmus 1990). The varieties are generally differentiated by means of morphological characteristics such as spininess of shoots; size, toughness and hairiness of leaves; and spininess of the main stem. The different varieties of lantana differ also in growth form and vigour, chemistry and toxicity to livestock, susceptibility to attack by biological control agent, and cytology and fertility (Radunz 1971; Spies and Stirton 1977; Smith and Smith 1982). The lantana varieties are differentiated using distinctive morphological characteristics, including the variation in shoots, flower colour, leaf, stem and growth characteristics (Smith and Smith 1982; Stirton and Erasmus, 1990). A national survey of biological control agents on *L. camara* revealed that the most common flower and throat colour combinations found among lantana varieties were pink-orange, pink–pink, Light pink –pink, Dark pink –orange, Dark pink –red, Light pink-orange, Pink-yellow, Dark pink – pink, Red –red, Orange –orange, Dark pink –yellow, Orange-red and White-white, respectively (Baars and Heystek 2003; Heystek 2006). The most common varieties in South Africa have been reported to be the 017 Orange, 150 Orange, White pink 029, Light pink 009 and Dark pink 010 (Heystek 2006; Table 1.1). Despite these designations however, there still remains a need for proper molecular studies on all South African varieties, as spininess and flower colour vary widely and are thus unreliable as indicators of the different varieties (Scott *et al.* 1997; Sanders 2006).

1.2.3   Control

Three main methods for controlling lantana are employed: chemical, mechanical, and biological. Chemical control using registered herbicides is effective but very costly. Chemicals registered for use in South Africa include glyphosate, imazapyr, picloram and tebuthiuron. These herbicides may be used in controlling lantana as foliar spray, cut stump treatment, and coppice growth applications (Grobler *et al.* 2000). The cost of clearing a high density cover (75%-100%) of *L. camara* in South Africa using herbicides was estimated to be R572 per hectare (Marais *et al.* 2004). The effective use of this method of control, however, is limited by the fact that lantana

22

Table 1.1 Morphological features of the most common varieties of *Lantana camara* found in South Africa

L. camara variety	Leaves	Stem	Mature flower and throat colour
WP 029	Leaves with few hair	Medium hairiness, medium thorniness, green when new, shrubby	Light pink flower, yellow pink throat
Orange 017	Leaves medium hairiness	Very hairy, new stems medium thorniness, old with few thorns, prostrate	Orange-red flower, orange throat
Orange 150	Leaves with few hair	Medium hairiness, new stems very thorny, old medium thorniness, shrubby	Orange flower, orange throat
Light pink 009	Leaves with few hair	Medium hairiness, new stems medium thorniness, old with few thorns, shrubby	Light pink flower, orange throat
Dark pink 010	Leaves few hairs	Very hairy, very thorny, shrubby	Dark pink flower, orange throat

stands are usually impenetrable, thorny, and in the middle of desired vegetation. Non-target effects on native vegetation may thus occur due to use of herbicides.

Mechanical control by means of chopping and sawing, although very effective, is labour-intensive and expensive. Clearing a *L. camara* high density cover was estimated to cost R1000 per hectare using mechanical methods such as felling (Marais *et al.* 2004). There are also additional costs incurred in keeping cleared areas weed-free, which were estimated to be R600 per hectare (Marais *et al.* 2004). This control measure produces only temporary relief as seedlings and coppice growth from stems and roots rapidly re-infest cleared areas (Cilliers and Neser 1991). Controlled low-to-moderate intensity fires appear to reduce invasion by lantana and can be an effective preventative management strategy (Gentle and Duggin 1997). Like the use of

mechanical and herbicide control options however, use of fire also has the potential to negatively affect natural areas and vegetation in the vicinity.

Another control option for weedy invasive plants is biological control using various insect natural enemies and pathogens. Specialised natural enemies are imported from the weed's native range in an attempt to reinstate their limiting effect on the abundance and spread of the weed. This process involves four broad steps: identification of an introduced plant as weedy, surveys for natural enemies in the native ranges of the weed, laboratory testing to determine the host specificity of chosen natural enemies, release and post-release evaluation of the natural enemy. This is still one of the best options in the quest to control lantana. Biological control provides a long-term and cost-effective means of controlling weeds, as agents may be self-sustaining following establishment. Despite being considered as marginally successful, the biological control programme of lantana in South Africa was reported to have a 22 fold return on economic investment based on the rate of spread of the weed, the potentially invadable area, the impact of the biocontrol agents on the weed populations and spread, the cost of biocontrol research and implementation, and quantified benefits of preventing further invasion to water use and land value loss (Van Wilgen *et al.* 2004). Without the released biological control agents the total invaded area by *L. camara* was calculated and predicted to be 360 km^2 more than was determined in 2000 (Van Wilgen *et al.* 2004). Unlike herbicidal application, biological control is environmentally safe and agents are specific to the target weed. Agents are slow acting and thus inhibit the formation of large empty areas where other invaders can establish.

Biological control of alien plants does have disadvantages. The importation, host-specificity testing and eventual release of an insect agent is time-consuming and expensive. Van Wilgen *et al.* (2004) determined that the cost of biological control research since the inception of lantana research programmes until 2000 was in excess of R17.3 million. It takes on average 3-5 years of research to release a natural agent (Broughton 2000), and a variable amount of time for the insect population to establish and increase. In addition, no weed has ever been eradicated by a biological control insect, leading to questions on the effectiveness of controlling weeds in this

manner. However, the aim of biological control is not eradication, rather to reduce the target weed population to below an ecologically or economically determined threshold.

It is also difficult to predict the establishment and performance of released biological control agents on the different varieties of *L. camara*. Several agents are reported to exhibit varietal preference in studies conducted (Haseler 1966; Harley 1973; Cilliers and Neser 1991; Broughton 1999; Urban and Simelane 1999; Day and Neser 2000). The morphological and physiological differences among the different *L. camara* cultivars can be substantial to the point where each cultivar could be considered a different species (Smith and Smith 1982; Cilliers and Neser 1991). Furthermore, the cultivars are reported to display differences in vulnerability to insect herbivores, with most agents able to develop on just a few varieties (Radunz 1971; Taylor 1989, Broughton 2000; Day *et al.* 2003).

1.3   Biological control of *Lantana camara* in South Africa

A biological control programme targeting *L. camara* was initiated in South Africa in 1961/62. Five natural enemies were deliberately introduced initially: two leaf chewing moths: *Hypena laceratalis* Walker (Lepidoptera: Noctuidae) and *Neogalia sunia* (Guenèe) (Lepidoptera: Noctuidae), the leaf tying moth *Salbia haemorrhoidalis* Guenèe (Lepidoptera: Pyralidae); the fruit-mining fly *Ophiomyia lantanae* (Froggatt) (Diptera: Agromyzidae), and the leaf-feeding lace bug *Teleonemia scrupulosa* Stål (Hemiptera: Tingidae) (Table 1.2). Two of the released insect species, *H. laceratalis* and *O. lantanae* (Table 1.2) were already established in South Africa before the initiation of the lantana programme. *Hypena laceratalis* is indigenous to Africa and has been found to be synonymous with *H. jussalis* Walker, while *O. lantanae* was misidentified as *O. rhodesiensis* (Spencer), the confusion arising from misidentification by Oosthuizen (1964) during a pre-release survey. *Hypena laceratalis* larvae are active in late summer and autumn and damage seedlings and new lantana growth considerably (Baars and Neser 1999). The overall impact of *H. laceratalis* is somewhat limited however due to larval parasitism by several indigenous parasitoid species. Populations therefore never grow to levels where plants are significantly damaged.

25

Ophiomyia lantanae is widespread and abundant but has little impact on seed viability. It is under some level of parasitism throughout it range, but this does not seem to affect the high level of fly infestation in the field (Baars and Heystek 2003). Parasitoid species belonging to four families were found on samples of *O. lantanae*-infested fruits and reared to adulthood in the laboratory (Cilliers 1987a, Baars and Heystek 2003). Even after three releases in 1962, 1968 and 1983, the noctuid moth, *N. sunia*, failed to establish. *Salbia haemorrhoidalis* Guenée established after release but its potential for control is greatly reduced due to parasitism by a native parasitoid (Oosthuizen 1964, Baars and Neser 1999). The fifth species released, *T. scrupulosa*, is widely established and abundant after a number of releases from various countries. The insect is considered the most damaging of all the agents released on lantana. It causes periodic defoliation of *L. camara*, and a decrease in seed production. The tingid populations are at their peak in midsummer and consequently cause most of the damage during this time (Cilliers 1987b). It is not clear whether the release of the genetically variable *T. scrupulosa* populations imported from different parts of the globe has contributed to the success and efficacy of the insect in South Africa (Cilliers and Neser 1991).

Among the more successful biological control agents released on lantana were two hispine beetles, *Octotoma scabripennis* Guèrin-Mèneville (Coleoptera: Chrysomelidae) and *Uroplata girardi* Pic (Coleoptera: Chrysomelidae), and the leaf-mining fly *Calycomyza lantanae* (Frick) (Diptera: Agromyzidae). Two leaf-mining beetles, *Octotoma scabripennis* and *Uroplata girardi*, were released in the 1970s in the subtropical areas of the country. Both have established, with *U. girardi* reaching high populations in coastal KwaZulu-Natal. On the other hand, *O. scabripennis* is abundant at inland sites, where populations peak intermittently and in only a few areas (Baars 2002). At their population peaks, both species cause defoliation of *L. camara* stands (Cilliers 1987b). The leaf-mining fly, *C. lantanae* (Frick), is widely established in the distribution range of *L. camara* (Table 1.2). The fly causes characteristic blotch mines on leaves of actively growing plants, seedlings and coppice growth. The impact of *C. lantanae* populations is limited by heavy larval parasitism (Baars 2002). The herringbone leaf-mining fly, *Ophiomyia camarae*

26

Spencer (Diptera: Agromyzidae), is highly damaging at higher initial densities and reduced above ground biomass of lantana significantly (Simelane 2002, Simelane and Phenye 2004). Releases were made in KwaZulu-Natal Province in 2001 and the agent has dispersed north into Mozambique, Zimbabwe, Tanzania, and south along the South African coastline (Simelane, pers. comm.).

1.4   Factors influencing success of biological control agents

Despite the release of a large number of agents the success of biological control of lantana in South Africa has been limited. The failure to establish, and lack of effective control of biological control agents has mainly been attributed to a variety of factors including the genetic diversity of lantana in the native and naturalised ranges, effect of *L. camara* varieties on agents, incompatible

27

Table 1.2 Present status of agents released on *Lantana camara* in South Africa (from Baars and Neser 1999).

Order/Family	Natural enemy	Origin	Main releases	Mode of attack	Status	Damage inflicted
Hemiptera						
Tingidae	*Teleonemia scrupulosa* Stål	Mexico via Hawaii via Australia via Mauritius Florida, USA	1961 1971 1984 1989	Flower and leaf sucker	Widely established in large numbers across entire lantana range, severe seasonal and sporadic damage	Complete defoliation and abortion of flowers
	Teleonemia elata Drake	Brazil via Australia	1972	Sap sucker	Not established	-
	Leptobrysa decora Drake	Colombia and Peru	1972	Leaf sucker	Not established	-
Miridae	*Falconia intermedia* (Distant)	Jamaica	1999	Leaf sucker	Established in warm moist distribution range of lantana with population fluctuations	Extensive defoliation at high density
Coleoptera						
Cerambycidae	*Plagiohammus spinipennis* (Thomson)	Mexico via Hawaii via Australia	1973	Stem borer	Not established	-
Chrysomelidae	*Alagoasa parana* Samuelson	Brazil	1985	Leaf chewer	Not established	-

28

Order/Family	Natural enemy	Origin	Main releases	Mode of attack	Status	Damage inflicted
Coleoptera	*Octotoma scabripennis* Guèrin –Mèneville	Mexico via Hawaii via Australia	1971 1974	Leaf miner	Established in warm moist eastern range of lantana, localised abundance inland	Extensive defoliation localised
Chrysomelidae	*Octotoma championi* Baly	Costa Rica via Australia	1978 1995	Leaf miner	Persisted in low numbers after last release, unconfirmed establishment	Unknown
	Uroplata girardi Pic	Paraguay via Hawaii via Australia	1974 1983	Leaf miner	Abundant in KZN region, present in low numbers in warm moist inland range	Extensive coastal defoliation
	Uroplata lantanae Buzzi and Winder	Brazil via Australia	1984	Leaf miner	Not established	-
	Uroplata fulvopustulata Baly	Costa Rica via Australia	1978	Leaf miner	Not established	-
Diptera						
Agromyzidae	*Calycomyza lantanae* (Frick)	Trinidad via Australia Florida, USA	1982 1989	Leaf miner	Widely established in low numbers. Heavily parasitized	Unknown
	Ophiomyia camarae Spencer	Florida, USA	2001	Leaf miner	Established and dispersed North and South along coast	Unknown

Order/Family	Natural enemy	Origin	Main releases	Mode of attack	Status	Damage inflicted
Agromyzidae	*Ophiomyia lantanae* (Froggatt)	Mexico	1961	Fruit miner	Widely established and abundant	Low impact, some parasitism
Tephritidae	*Eutreta xanthochaeta* Aldrich	Mexico via Hawaii	1983	Stem galler	Not established	-
Lepidoptera						
Noctuidae	*Neogalia sunia* (Guenée)	USA	1962	Leaf chewer	Not established	-
	Hypena laceratalis Walker	Kenya, Zimbabwe	1962	Leaf chewer	Widely established and abundant. Larvae often parasitized	Considerable damage to seedlings and new growth
	Autoplusia illustrata Guenée	Colombia via Hawaii	1984	Leaf chewer	Not established	-
Pyralidae	*Salbia haemorrhoidalis* Guenée	Cuba via Hawaii	1962	Leaf-tying and feeding	Widely established in low numbers, more abundant coastally, larval parasitism	Some impact at high densities on the coast
Pterophoridae	*Lantanophaga pusillidactyla* (Walker)	Mexico	1984	Flower, fruit and seed chewer	Widely established in low numbers, possibly parasitized	Unknown

Order/Family	Natural enemy	Origin	Main releases	Mode of attack	Status	Damage inflicted
Lepidoptera						
Tortricidae	*Epinotia lantana* (Busck)	Hawaii	1984	Flower-peduncle and shoot-tip borer	Widely established in low numbers	Unknown
Gracillaridae	*Aristea onychote* Meyrick	Unknown	Unknown	Leaf miner	Widely established in low numbers, heavily parasitized	Unknown

climatic conditions in the native versus introduced ranges of *L. camara*, the employment of improper release methods, and predation and parasitism of natural enemies (Baars and Neser 1999, Broughton 2000, Day and Neser 2000, Day and Zalucki 2009).

The weedy complex *L. camara* does not exist in any areas as a native plant species, and it was previously unclear which plant species natural enemies for biological control should be collected on in exploratory surveys. For the most part, potential natural enemies were sourced from closely related plants in the genus (Day and Urban 2004). This is different to many biological control programmes where the potential agents are sourced from the same species in the country of origin, thus making for better agent-weed suitability (Day and Zalucki 2009). It has been suggested that this is a reason for lack of adequate biological control as each plant species has an associated suite of natural enemies (Day and Neser 2000; Day *et al*. 2003). Collection of natural enemies on closely related species thus increases the chances of better establishment and performance. Classical biological control efforts of genetically variable entities such as *L. camara* are considered more difficult to achieve (Sheppard 1992).

Recent studies have indicated that most effort in surveys should be put into exploring natural enemies from *Lantana urticifolia* Miller plants. DNA analyses have shown that the Australian *L. camara* is most closely related to *L. urticifolia* among Central and South American *Lantana* species. Using only morphological characteristics a South African lantana sample was identified as a cross between *L. camara* and *L. urticifolia* (Sanders pers. comm., in Day and Urban 2004). Indeed a retrospective analysis of biological control agents has revealed that most agents that have established in South Africa and Australia are from agents collected on *L. urticifolia* and *tiliifolia* (considered a subspecies of *L. urticifolia*) (Sanders pers. comm., cited by Day and Neser 2000).

The variability within *L. camara* in South Africa has been proposed as the second major factor for the limited success of lantana. Varieties display differences in susceptibility to insect herbivores, with most insects performing and developing best on only a few specific varieties. Varieties are generally classified by means of morphological differences such as

32

size and hairiness of leaves, spines found on shoots and the colour of the matured flowers in the inflorescence. The differences between some varieties can be substantial, to the point where each variety can be considered a different species (Cilliers and Neser 1991). Several agents are reported to exhibit varietal preference in studies conducted mainly in the laboratory (Haseler 1966; Harley 1973; Cilliers and Neser 1991; Broughton 1999; Urban and Simelane 1999; Day and Neser 2000). While possibly having some effect on establishment, recently conducted field surveys suggest that the impact of varietal preference in terms of performance may have been overestimated. In field surveys conducted in Australia and South Africa agents' abundance and impact were reported similar on sites with lantana stands of mixed varieties (Baars and Heystek 2003; Day *et al.* 2003).

The diverse climatic conditions in the distribution range of *L. camara* in South Africa have been cited as another reason for the failure of agents on lantana (Cilliers and Neser 1991; Day and Neser 2000). Not only has the weed infested the warm and subtropical coastal regions of the country, such as KwaZulu-Natal, but it is also found throughout the highveld region (altitude > 1500m) (Henderson 2001) with dry and frosty winters. The plant is capable of surviving adverse conditions, such as drought and very cold winter temperatures, by defoliating. Consequently leaf-feeding natural enemies introduced from tropical regions to temperate climes have a small chance of establishing (Sands and Harley 1980). Extensive defoliation during winter may leave leaf-feeding insects with neither food nor shelter, leading to widespread starvation, increased mortality and non-establishment. Extremely cold winter temperatures become a barrier for the natural enemies introduced from warm tropical regions and may lead to widespread mortalities in their populations. Lack of over-wintering mechanisms, such as egg, pupal or adult diapause by introduced insects, may also contribute to non-establishment of agents released. Insect populations either are wiped out completely, or are greatly reduced during these harsh South African winters.

Cilliers and Neser (1991) also proposed other factors that prevent establishment of natural enemies after release. These are (a) herbicidal and mechanical destruction of sites before the insects have had a chance to establish and disperse (e.g. *Octotoma championi* Baly (Coleoptera: Chrysomelidae), *Uroplata lantanae* Buzzi and Winder (Coleoptera:

33

Chrysomelidae)); (b) predation of their eggs, especially by ants (e.g. *Alagoasa parana* Samuelson (Coleoptera: Chrysomelidae)); (c) unsuitable microhabitats (e.g. *A. parana*), and (d) the numbers released being below a minimum threshold for populations to survive (e.g. *U. lantanae, Eutreta xanthochaeta* Aldrich (Diptera: Tephritidae), *Teleonemia elata* Drake (Hemiptera: Tingidae), *Lantanophaga pusillidactyla* (Walker) (Lepidoptera: Pterophoridae). According to Broughton (2000), releases of a low number of individuals in an introduction attempt accounted for 23.6% of all cases of failure in lantana programmes worldwide.

1.5 Recent evaluations and status of imported biological control agents

The biological control programme against *Lantana camara* (Verbenaceae) in South Africa has thus far seen 22 deliberate releases of insect biological control agents (Table 1.2; Julien and Griffith 1998; Zalucki *et al.* 2007). However, only 13 of these agents have established, and three of these are considered the most effective in the country (Baars 2002). There has been renewed emphasis not only on increasing pressure on the most attacked niche of lantana, the leaves, but also to source additional biological control agent species that damage other niches of the plant. To this end a number of mostly Central American potential agents have been imported into South African quarantines recently. After host specificity screening, the candidate biological control agents had varying degrees of suitability (Table 1.3).

Adults and larvae of the trimorphic flea beetle *Alagoasa extrema* Jacoby (Chrysomelidae: Alticinae) were observed to be feeding on leaves of *Lantana* species in surveys conducted in the subtropical and tropical areas of Mexico. This agent showed some potential with larvae reducing above-ground biomass and common *L. camara* varieties tested supporting a viable number of individuals over a few generations (Williams 2005; 2006). When tested against non-target indigenous plants however, *A. extrema* was shown to be oligophagous and therefore deemed unsuitable for further testing and release in South Africa (Williams and Duckett 2005). Another agent from Mexico attacking a different niche of the plant, the root-feeding *Longitarsus* sp. (Coleoptera: Chrysomelidae: Alticinae), showed promising results in tests (Simelane 2005). The beetle has a narrow feeding range, preferred *L. camara* strongly, has long-lived adults, and larvae which are highly damaging to the roots of *L. camara*, and is yet to be released.

A stem-galling fly collected in Mexico sourced via Hawaii, which failed to establish in a 1983 release, *E. xanthochaeta* (Cilliers and Neser 1991), was imported again and subjected to further host specificity tests. Such tests had not been conducted on it at the first release with results from tests conducted in Hawaii and Australia considered sufficient for releases in South Africa. The agent proved unsuitable for release following the latest host specificity tests conducted at the Plant Protection Research Institute (PPRI) (Mabuda 2005). *Eutreta xanthochaeta* developed and showed equal preference for *L. camara* and non-target native *Lippia* species. Tests conducted on a leafhopper, *Barela parvisaccata* Young (Hemiptera: Cicadellidae), produced similar results as above (Phenye and Simelane 2005).

Table 1.3 Host specificity (HS) status of recently imported *Lantana camara* enemies.

Order/ Family	Natural enemy	Origin	Year imported	Type of damage	Status
Coleoptera					
Brentidae	*Coelocephalapion camarae* Kissinger	Mexico	1997	Larvae petiole galler	To be released
Chrysomelidae	*Alagoasa extrema* Jacoby	Mexico	2000	Leaf feeder	Unsuitable*
	Longitarsus sp.	Mexico	2000	Root feeder	To be released
Hemiptera					
Cicadellidae	*Barela parvisaccata* Young	Mexico	2000	Sap sucker	Unsuitable*
Homoptera					
Membracidae	*Aconophora compressa* Walker	Mexico via Australia	1995	Stem sucking	Unsuitable*
Lepidoptera					
Geometridae	*Leptostales ignifera* (Warren)	USA and Mexico	2005	Leaf feeder	To be released

* Host specificity tests showed agent to be polyphagous / oligophagous on indigenous species

The petiole-galling weevil, *Coelocephalapion camarae* Kissinger (Coleoptera: Brentidae), proved more successful and promising in tests conducted in quarantine conditions. Adults and nymphs were highly injurious to *L. camara* leading to leaf desiccation and a reduction in plant biomass (Baars *et al.* 2007). Permission to release was obtained in 2007/08, but the agent is yet to be released due to a crash of the culture (L. Madire - PPRI, pers. comm.). The highly damaging stem-sucker, *Aconophora compressa* Walker (Homoptera: Membracidae), was also imported into South Africa for quarantine testing. The reproductive performance of the adults of this species and preference from multi-choice trials was equal or higher on a native and an ornamental plant than on lantana (Heystek and Baars 2005). It was therefore unsuitable as an agent and was duly rejected.

1.6   Biology of *Falconia intermedia*

The leaf sucking bug, *Falconia intermedia* (Distant) (Hemiptera: Miridae), was among the biological control agents recently collected to augment control of *L. camara* in South Africa and is the topic of this thesis. Two separate populations were collected, one from Jamaica in 1994 and another from Guatemala in 1997. The mirid is endemic to Mexico, Central America and the Caribbean Islands and its adults and nymphs are highly active and mobile, especially when disturbed (Palmer and Pullen 1998). The adult is about 2 mm long and 0.9 mm wide, with a dark brown body and pale legs. The adults have a 3 – 5 day pre-oviposition period in which mating takes place repeatedly (Baars 2002). The translucent pale green eggs are laid adjacent to the veins in isolation or in small groups on the under-surface of leaves and are covered in frass. The eggs take 10 – 14 days to hatch under laboratory conditions (Baars *et al.* 2003). The emergent nymphs are green and take 15 – 20 days to go through five instars to adulthood. The life span of the insect is about two months and it may have up to nine generations in a year (Baars 2002).

The adults and nymphs possess a piercing and sucking rostrum which is used to feed on the intercellular tissue of lantana leaves. Feeding mainly occurs on the underside of the leaf, where midday temperatures are cool and the mirid can remain hidden from predators. Slight to moderate feeding causes white chlorotic specks visible on the upper leaf surface, possibly impeding the photosynthetic capability of the leaf. Severe feeding damage causes entire

36

shrubs to appear silvery white and leaves to abscise. This agent was shown to have a sufficiently narrow host range under host specificity trials, with *L. camara* varieties being the most preferred among plants tested (Baars *et al.* 2003). There was significantly higher oviposition on lantana than on any other species in multi-choice trials. Permission for release was granted by the regulating authorities in 1999. The first releases were made in April 1999 at experimental sites along in KwaZulu-Natal, Mpumalanga and Limpopo Provinces (Heystek, pers. comm.). Further releases were made at five sites in December 2001 in the Eastern Cape Province.

1.7 Motivation

In an effort to intensify the feeding pressure exerted by biological control agents, *F. intermedia* was released on *L. camara* infestations found in South Africa. In a number of release sites in the Mpumalanga Province the leaf sucking bug established well and built up its populations to large numbers. After initial releases of the mirid, populations built up and caused severe damage to the lantana stands, resulting in defoliation and leaves turning silvery-white. However, the large populations of the agent declined despite the fact that some bushes in the stands were undamaged. In other field releases in the Eastern Cape Province, *F. intermedia* established at two coastal sites with the white-pink variety of lantana and failed to establish at three inland sites (Heshula 2005). Among the three inland sites with no establishment, one had the same variety of lantana as the coastal sites while two had a dark-pink variety. In laboratory no-choice trials, no significant differences in *F. intermedia* oviposition preference were observed among the five Eastern Cape varieties of *L. camara* (Heshula 2005). However there were differences in performance as the adult mirids performed better on white-pink varieties from one coastal and one inland site. The lack of sustained damage has been ascribed to adverse climatic conditions and lantana variety incompatibility in the above cases (Heystek pers. comm.; Heshula 2005).

The above reports are consistent with other reports from reviews that have cited *L. camara* varietal incompatibility as being important in limiting the effectiveness of biological control agents in South Africa (Day and Neser 2000; Zalucki *et al.* 2007). The range of *L. camara* varietal differences covers both the physical and chemical characteristics of the plants. It is

therefore possible that a variety of physical and chemical plant quality traits may act as plant defences to discourage herbivore feeding activity. Even more intriguing is the possibility that *L. camara* plants have the ability to mount defensive responses against *F. intermedia* and other agents. The events surrounding the Mpumalanga release mentioned above are consistent with such a response scenario, as a variety suitable at initial release became unsuitable after heavy feeding. Such defensive responses have been reported in many other ecological studies as affecting herbivores. Despite this and the above reports, there is a scarcity of published studies exploring the mechanisms by which *L. camara* varietal responses might limit the effectiveness of biological control agents.

1.8   Aims

Biological control evaluations have reported on many factors that may be responsible for non-establishment of agents on *L. camara*. However, there is no published work on the effect that physical characteristics and phytochemicals may have on the biological control agents of *L. camara*. This is despite the well-known fact that it is well known that plants mount chemical resistance against herbivores when attacked. This project sets out to investigate the possible role of induced defences of selected *L. camara* varieties primarily on the performance and survival of *F. intermedia* (Figure 1.3).

In previous field releases in subtropical parts of South Africa *F. intermedia* established successfully and built up impressive populations. Consequently whole lantana stands were heavily damaged by the mirid. This was however not maintained as populations decreased to negligible numbers. The present study seeks to investigate reasons for this decline by examining the effect of previous plant damage on subsequent feeding by natural enemies (Chapter 2). Previous damage has been reported to negatively affect future feeding in a number of studies (Agrawal 1998; Boege 2004; Stevens and Lindroth 2005). This thesis attempts to determine whether previous damage weakens or strengthens the plant in relation to the biocontrol agent.

Plants have an arsenal of defences that they may employ against herbivory. Some of the most important are slight variations in plant quality due to induction by herbivores. Plant quality characteristics refer to secondary metabolites of a plant, as well as to the physical characteristics of the plants (e.g. leaf characteristics). No studies have yet examined how these other factors affect the performance of biological control agents on lantana. Chapter three of this study deals with the effect of leaf toughness, leaf trichome density, leaf nutrients and leaf moisture content on the performance of the biological control agent *F. intermedia* was investigated (Chapter 3).

Damage by many plants is known to elicit a chemical response in the form of volatile organic chemicals. In laboratory and field conditions these induced volatiles are found to play an important role in intra- and interspecific communication. Though ubiquitous, such responses may not be immediately employed by all plants. Advanced chromatographic techniques (High Performance Liquid Chromatography/Gas Chromatography–Mass Spectrometry) were used to determine the presence of volatile defensive responses by *L. camara* varieties induced by *F. intermedia* (Chapter 4). The major components of two main varieties in the Eastern Cape were identified. Studies involving the isolation and elucidation of constitutive chemicals from leaves of *L. camara* have been conducted elsewhere in the world, but only two such reports has been published on South African lantana thus far (Barton *et al.* 1954; 1956), indicating lantana sample variation of the constitutive triterpenoid content. The effect of feeding by biological control agents on *L. camara* has not yet been explored. It is hoped that the work done will further resolve factors affecting the success of this agent. More broadly, it will also increase our understanding of the insect-plant interaction and how these may have affected the success of the biological control programme against *L. camara*.
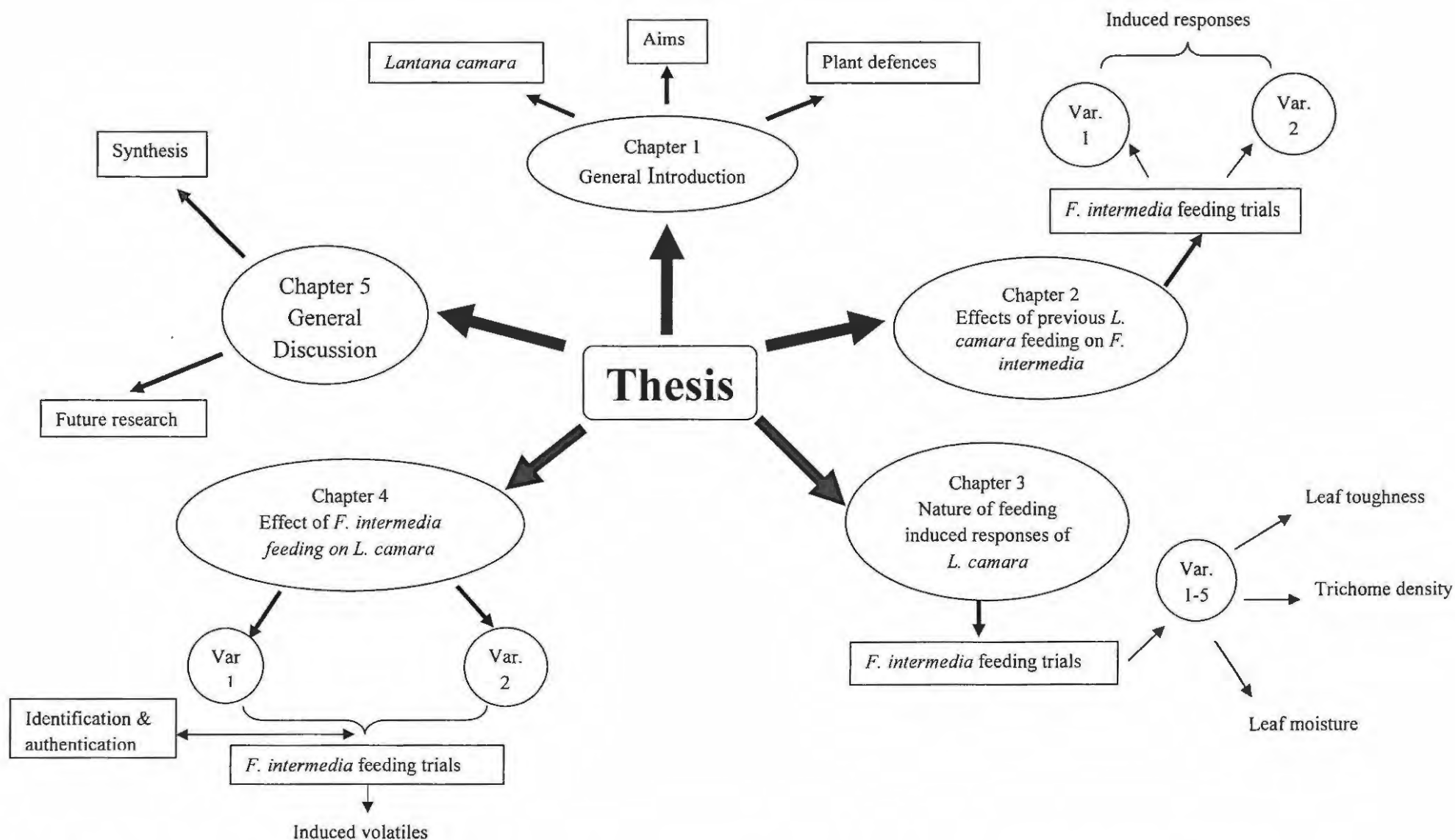
Figure 1.3 Outline of the current study to investigate *Falconia intermedia* induced physical and chemical responses of *Lantana camara*

CHAPTER 2

Effect of previous *Falconia intermedia* feeding on *Lantana camara* on subsequent feeding activity

2.1   Introduction

An ever increasing number of ecological studies have revealed that plants mount varying levels of responses to herbivory (Karban and Baldwin 1997; Gatehouse 2002). Evidence from research has shown that besides possessing constitutive defences, plants are able to resist or minimise negative effects to their fitness by means of various responses following damage or stress. Herbivore damage triggers responses which consist of various plant modifications. These involve changes in physical and/or chemical plant quality factors (Karban and Baldwin 1997). The changes induced may have the effect of increasing plant resistance to herbivores, thereby decreasing herbivore performance. Alternatively the responses may be an induced defence, reducing the costs of damage to the fitness of the plant, or increasing tolerance to herbivory, while not necessarily decreasing herbivore efficacy. Thirdly, responses may act to increase the suitability of the plant to subsequent feeding by herbivores. This is known as induced susceptibility.

It is evident from the literature that induced responses are widespread, with direct and indirect resistance having been recorded in over 100 plant species (Karban and Baldwin 1997). The timing of the responses is also an important determinant in the effectiveness of a response. Changes in plants may occur within hours of damage (Traw and Dawson 2002), days or weeks (Baur *et al.* 1991) or may be expressed in the new season (Dalin and Bjorkman 2003). The type of responses vary greatly and thus generalisation and prediction is impossible, especially for a plant-insect relationship such as *L. camara* varieties and *F intermedia* on which responses have not previously been studied. In addition, leaf feeding insects such as *F. intermedia* do not remove as much tissue as leaf chewing insects, and thus responses to feeding by insects with this feeding mode are different. Indeed the biochemical pathways activated by leaf chewing insects are different from the pathways activated by phloem-feeding insects (Walling 2000).

41

In the context of biological control of weeds, the presence and significance of such responses in the success of biological control agents has not been adequately studied. This is despite the fact that once agents are released on problem plants it is expected that they will sustain and increase populations while dispersing onto conspecifics, thus placing sustained pressure on the population. A response from the plant species that shifts the plant-herbivore interaction either pro-plant or pro-herbivore holds a great deal of significance for the success of biological control.

In this chapter feeding trials were conducted to test whether previous feeding induces a response by *L. camara* varieties. The hypotheses tested were the following:

a) Feeding by *F. intermedia* result in a defensive response by *L. camara* varieties expressed by a decrease in subsequent *F. intermedia* performance.

b) The induced effect is expressed rapidly and is effective within a short period of time.

c) The induced responses result in greater plant fitness expressed as an increase in plant growth and reproduction.

2.2   Materials and Methods

2.2.1 *Falconia intermedia* culture

A culture of *F. intermedia* was acquired from a *L. camara* stand with an infestation of the mirid found at a site near Nahoon Beach, East London, Eastern Cape (grid reference: 27°57'24"E 32°58'20"S). Whole lantana branches with adults and nymphs on the leaves were transported to the glasshouse facility at the Zoology and Entomology Department, Rhodes University. The culture was maintained in cages on potted East London variety of *L. camara*, described in Table 2.1. The plants were watered and fertilised regularly with a liquid fertiliser (Seagro®, Premier Fishing SA (Pty) Ltd).

42
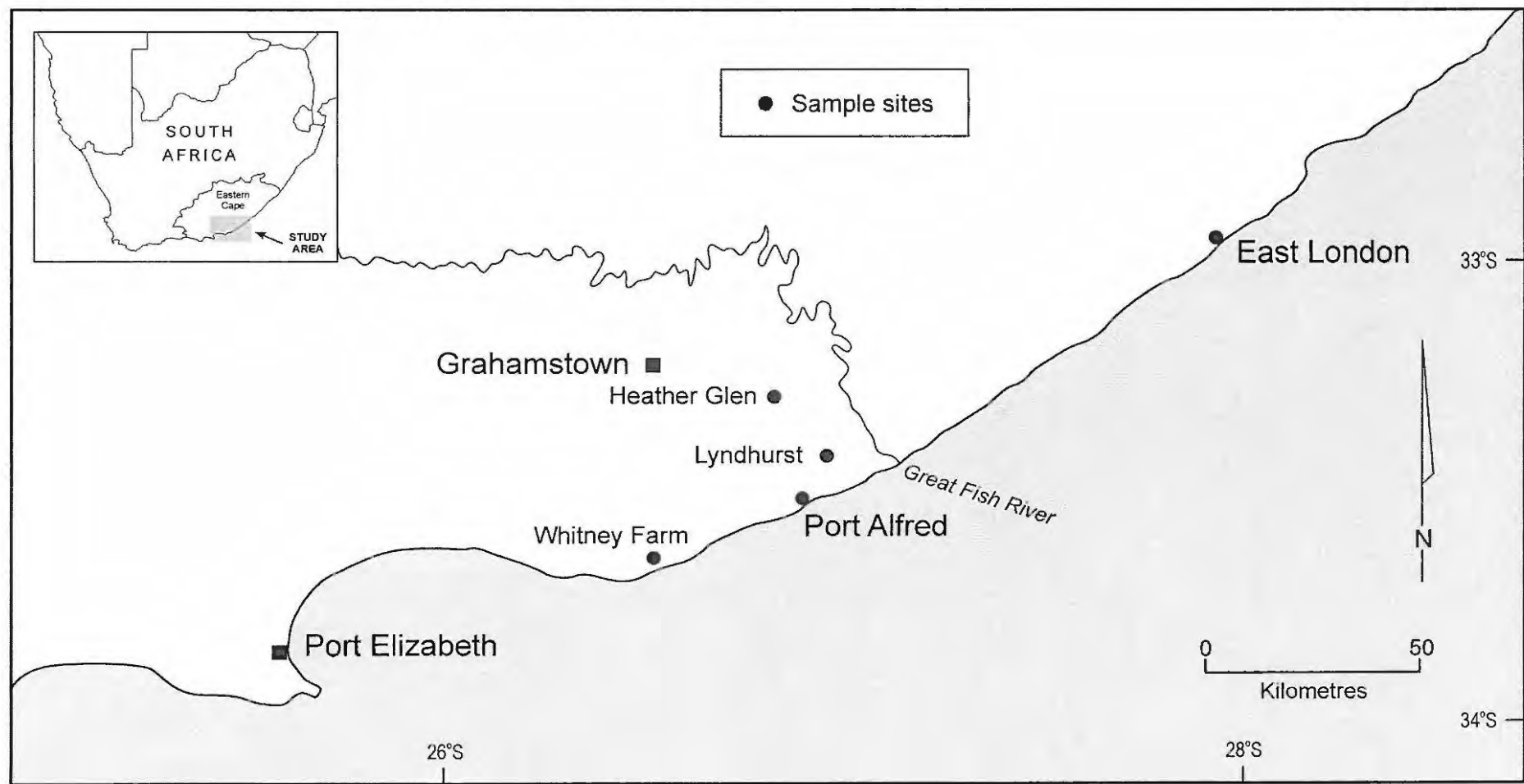
Figure 2.1 *Lantana camara* sites in the Eastern Cape from where cuttings were made.

2.2.2 Plant material preparation

Two varieties of *L. camara*, acquired from Whitney Farm and Lyndhurst Farm sites respectively and described in Table 2.1, were used in the bioassays. Field releases of *F. intermedia* made in 2001 at these two sites resulted in no establishment on Lyndhurst Farm lantana, and establishment and good performance on Whitney Farm lantana (Heshula 2005). Due to consistent spraying by the farm owners, the dispersal of the agent at Whitney Farm is limited to only a few stands. The distinguishing morphological features used to differentiate these varieties are described in Table 2.1.

Fifty shoots of about 15cm long with no *F. intermedia* damage and minimal damage by other herbivores were cut from *L. camara* bushes on each site. All the leaves except the apical leaf pair were removed and the shoots were wrapped in moist paper for transportation to the nursery. The cuttings were dipped into a root growth hormone (Dip and Grow®, Fleuron (Pty) Ltd) and planted in river sand to encourage root growth. Cuttings were allowed to develop roots for 4 – 6 weeks. Cuttings were subsequently transplanted into individual pots in a standard potting mixture and allowed to grow. All plants were fertilised fortnightly with a liquid fertiliser with a N: P: K formulation of 1:1:1 (Seagro®) and watered as necessary before and during the experiments.

All plants were kept insect-free by the application of a contact insecticide, Chlorpirifos®, until 10 days before the commencement of the experiments. Both the induction and post induction experiments were conducted in a green house tunnel facility equipped with fans. Ambient temperatures ranged between 14 °C at night and 38°C in the daytime. The temperature measurements did not take into account the effect of the microclimate around the plants and on the top and bottom surfaces of the leaf. The temperature around a leaf may be more than 10 °C below the ambient temperature (Schoonhoven *et al.* 2005). A midday cold water spray over the cages and the floor of the tunnel was applied on hot days to increase humidity and cool down the ambient temperature around the cages in the tunnel in an attempt to minimise heat induced stress on plants and insects.

2.2.3 Induction experiment

Ten plants from the two original localities (Whitney Farm and Lyndhurst Farm) were chosen for the induction experiment. Five of these plants were induced by *F. intermedia* adult feeding. The infested plants were confined in cages. A mean number of 1 adult per 4 lantana leaves (0.25 adults per leaf), rounded off to the nearest one, and were released on the five test treatment plants labelled as Test (T). The adult per leaf pair density was used successfully in studies previously conducted and would allow for comparative analysis of the results between the trials (Heshula 2005).

Falconia intermedia were allowed to feed on whole plants for 21 days. Five other plants were chosen as controls with no manipulation or mirids and were labelled as control (C). Control plants were also confined in cages to control for the effect of the cages. Control and test plants were all watered once daily and fertilised weekly with a liquid fertiliser with a N: P: K formulation of 1:1:1 (Seagro®).

2.2.4 Post-induction experiment

Following the induction experiment described above, the *F. intermedia* individuals (adults and nymphs) were removed from the test (T) treatment plants after 21 days. After removal a contact insecticide, Chlorpirifos®, was applied to prevent any herbivory on the plants of the two treatments above. A period of 10 days was allowed to pass due to the persistence period of the insecticide. Eggs were removed by means of a small brush throughout this period. The induced plants of the test treatment (T) were now referred to as test induced (Ti) treatment plants. Similarly, the plants from the induction experiments previously referred to as control (C) were renamed induced control (Ci) plants (see Fig. 2.2). A mean number of 1 adult per 4 lantana leaves (0.25 adults per leaf), rounded off to the nearest one, were released on the five induced control (Ci) and five test induced (Ti) treatment plants per variety. Five plants per variety with no mirid individuals were also included as post induction trial controls (Cp) in this section of the experiment (see Fig. 2.2). Plants in all three treatments were placed into cages. Watering and fertilising regimes were the same as for the induction experiment.

2.2.5 Plant and insect parameters measured

For both the induction and post-induction experiments the same *L. camara* and *F. intermedia* parameters were measured in the same way. A sample of 10 leaves per plant per treatment was taken from different branches of each plant to give a sample of 50 leaves for the five plants of each treatment. From this a mean for each of the five plants of a treatment was generated. From this sample the number of eggs, number of adults and nymphs were counted at the end of the induction. The ten leaves were scored for *F. intermedia* feeding damage to give an indication of the extent of the agent's feeding activity. Overall damage score was determined as a product of the area of leaf damaged (0-4) and the intensity of the feeding damage (0-3). Leaf area damage ratings ranged from 0% (=0), 1-25% (=1), 26-50% (=2), 51-80% (=3), and >81% (=4), while damage intensity scoring ranged from no damage (0), few and uneven speckles in affected area (=1), uniform white speckling in affected area (=2), and white speckling merging that appear white/yellow in affected area (=3).

To avoid recording the accumulation of damage made during the induction experiment as damage in the post induction experiment, leaves damaged from the test treatment (T) plants during the induction experiment were marked. This was achieved by means of a small permanent ink mark (dot) on the stem close to the leaf petiole and not on the leaf itself. The marked leaves were not selected as part of the random sample at the end of the post induction experiment, and thus allowed us to directly compare pre- and post-induction parameters of the treatments and the systemic response of lantana to damage. The leaves chosen were previously undamaged, and therefore any response to feeding could be determined based on the level of feeding of the random sample leaves. Most individuals were expected to feed on undamaged leaves.

Plant parameters were measured at the beginning and end of the experiments (day 0 and 21 for induction; day 31 and 52 for post-induction trial). Number of leaves was counted at the beginning and end of the trial and relative growth rate determined. Relative growth rate (RGR) was determined by using the formula;

$$RGR = ln(\text{final}) - ln(\text{initial}) / \text{number of weeks}$$

Relative growth rate describes the growth rate of a final value relative to its initial value over a given period of time, in this case weeks. Reproductive parameters of *L. camara*, the number of flowers (inflorescences) and seeds (infructescences) were measured and compared between treatments. Length of the longest continuous branch (leading shoot), and the number of side branches emanating from the main branch were measured and the percentage growth rate determined. The relative growth rate in height of the plant from the base of the stem to its highest point (plant height) was also measured.

2.2.6   Statistical Analyses

Data were tested for normality using normal probability plots and Shapiro-Wilk test and were generally acceptably normal with homogenous treatment variances. Analyses of the insect-infested treatments were done by means of paired T-tests (dependent measures t-test). Plant reproductive and relative growth rate data for plant parameters were non-normal and were subjected to a non-parametric Kruskal-Wallis ANOVA, and medians were separated by multiple comparison tests. Endpoint data were analysed at the 5% significance level on the Statistica 8.1® (StatSoft, Inc. 2006).
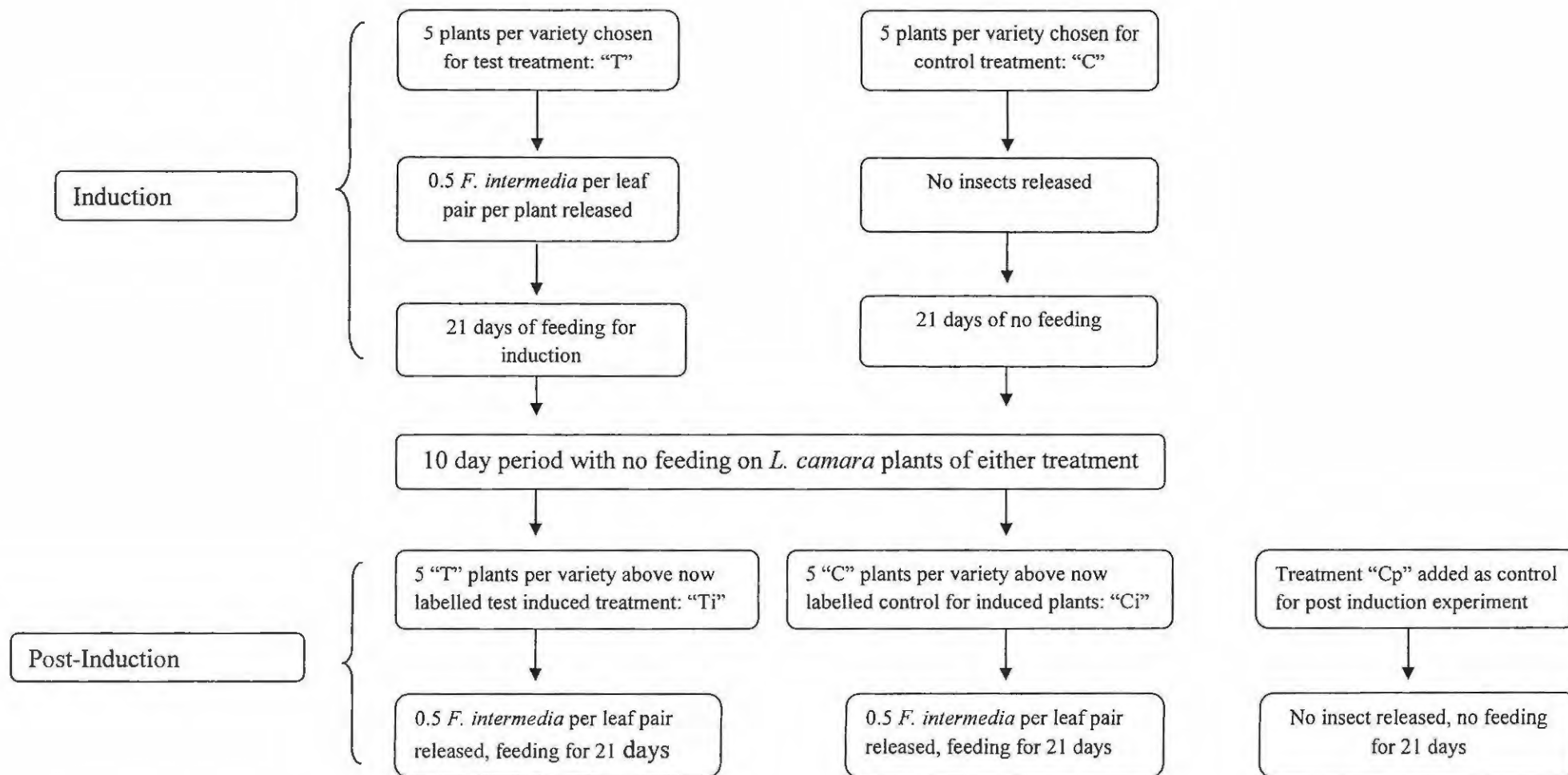
Figure 2.2 Diagrammatic representation of the induction and post-induction experimental design for each of the two varieties tested, Lyndhurst and Whitney Farms.

Table 2.1 Distinguishing characteristics and the original locations of *Lantana camara* varieties used during laboratory trials.

L. camara source site	Distinguishing morphological features	Flower and throat colour	Grid reference
Whitney Farm	Leaves large, broad, dark and medium hairiness; shoots spiny, main stem spiny	Light pink flower, orange throat	33°40'43"S 26°35'49"E
Lyndhurst Farm	Leaves small, tough and few hairs; shoots hairy and spiny; main stem with few spines	Dark pink flower, yellow throat	33°27'11"S 26°53'10"E

2.3 Results

2.3.1 *Falconia intermedia* performance, growth and reproduction

A trend in the mirid performance, growth and reproduction emerged between the induction and post induction treatments within each variety. The trend observed was different between the two varieties tested. Among the three insect-inoculated treatments, plants from Whitney Farm had no significant affect on any of the measured performance, growth and reproductive parameters of *F. intermedia*. On the other hand, *F. intermedia* impact, reproduction and growth on Lyndhurst Farm induced (Ti) plants was in many instances significantly lower than on the un-induced (T) and control induced treatment plants (Ci).
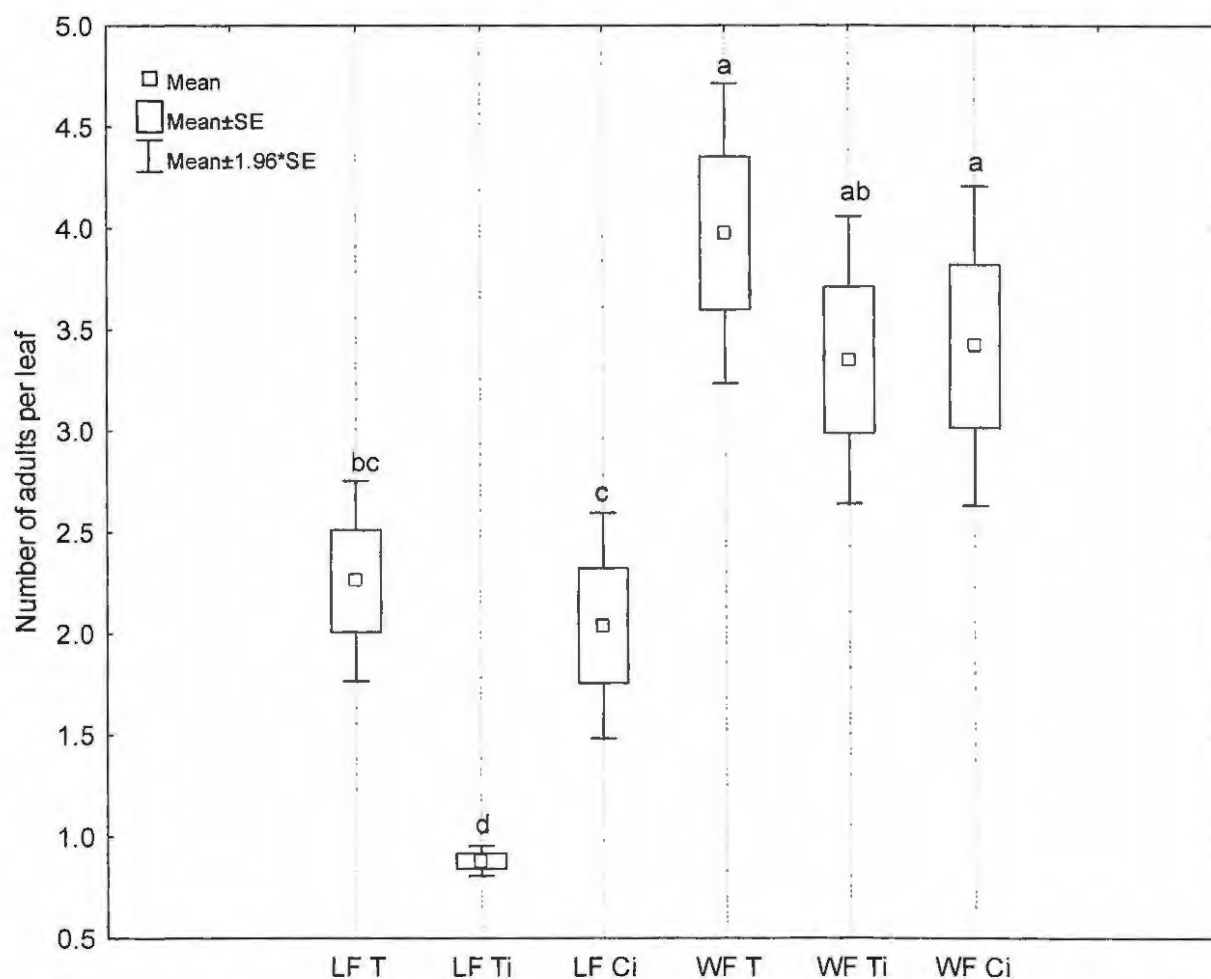
Figure 2.3 Box and whisker plots of *Falconia intermedia* number of adults on two *Lantana camara* varieties (Lyndhurst Farm – LF, Whitney Farm – WF) subjected to three treatments (T – un-induced, Ti – induced, Ci – control induced). Plots with the same letters are not significantly different (p > 0.05, paired T - tests).

The number of adults and nymphs found was significantly higher on Whitney Farm plants than on Lyndhurst Farm plants (ANOVA; $F_{(2, 27)} = 18.784$, $p = 0.00001$) (Figure 2.3 & 2.4). Furthermore the rate of growth of adults per weeks was significantly higher on the Whitney Farm variety ($F_{(1, 18)} = 26.844$, $p = 0.00006$) (Figure 2.4). The rate of growth of *F. intermedia* adults per week on the Lyndhurst Farm induced (Ti) treatment plants was significantly lower than those of all other treatments (Figure 2.4). The number of adults and nymphs at Whitney Farm was greater than Lyndhurst Farm by a factor of 2.07 and 1.91, respectively. The number of adults found on the induced treatment (Ti) plants of Lyndhurst Farm was significantly lower than the un-induced treatment (T; $t = -5.238$, $df = 4$, $p = 0.006$) and the induced control treatment (Ci; $t = -4.138$, $p = 0.0140$) (Fig. 2.5). Similarly the number of *F. intermedia* nymphs was significantly lower on the Ti treatment than the un-induced treatment (T; $t = -2.877$, $df = 4$, $p = 0.045$) on plants from Lyndhurst Farm (Figure 2.5).
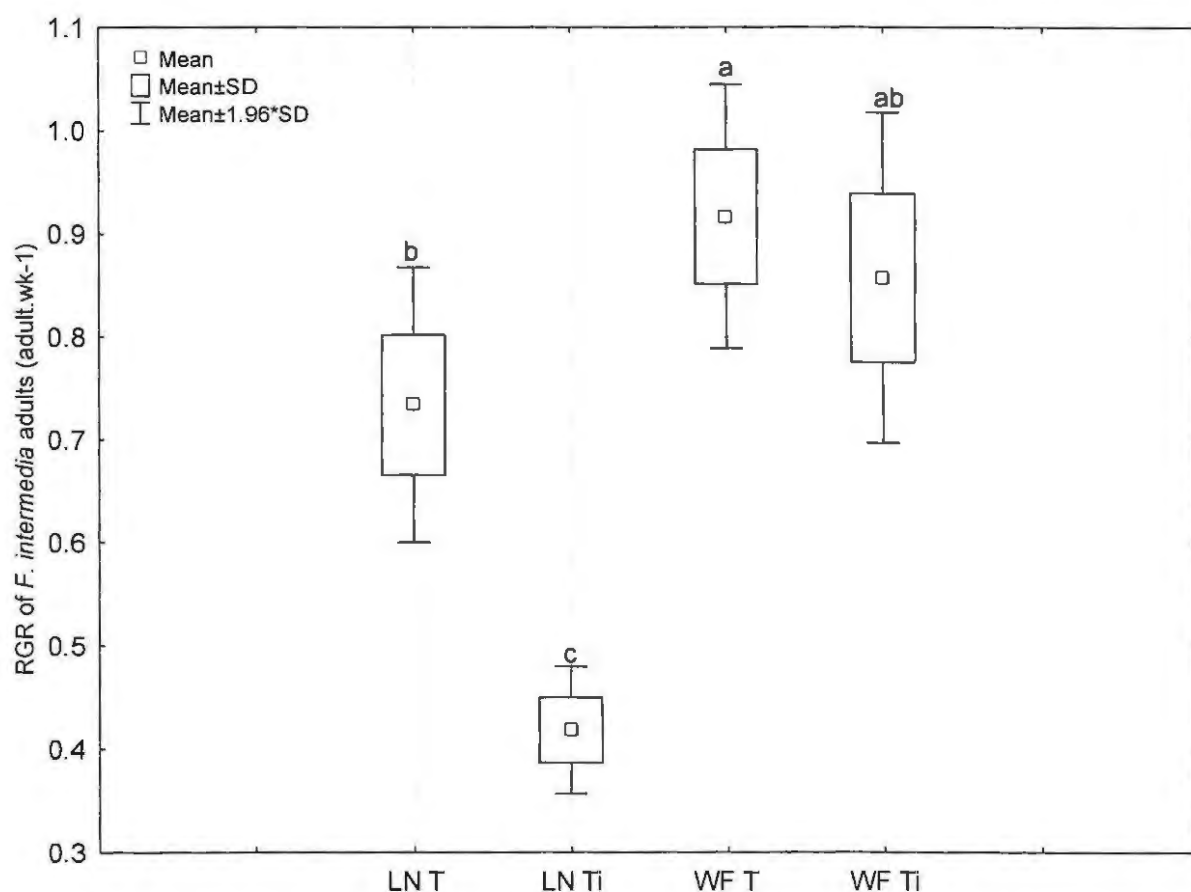
Figure 2.4 Box and whisker plots of relative growth rate (RGR) of *Falconia intermedia* adults per week on two *Lantana camara* varieties (Lyndhurst Farm – LF, Whitney Farm – WF) subjected to three treatments (T – un-induced, Ti – induced). Plots with the same letters are not significantly different ($p > 0.05$, paired T - tests).

51

Unlike the number of adults, the numbers of nymphs on the Ti and Ci treatments of Lyndhurst Farm were not significantly different (t = -2.497, df = 4, p = 0.067). Not only were the numbers of adults and nymphs higher on the Whitney Farm that Lyndhurst Farm plants overall, there were no statistically significant effect due to the different insect infested treatments of Whitney Farm.
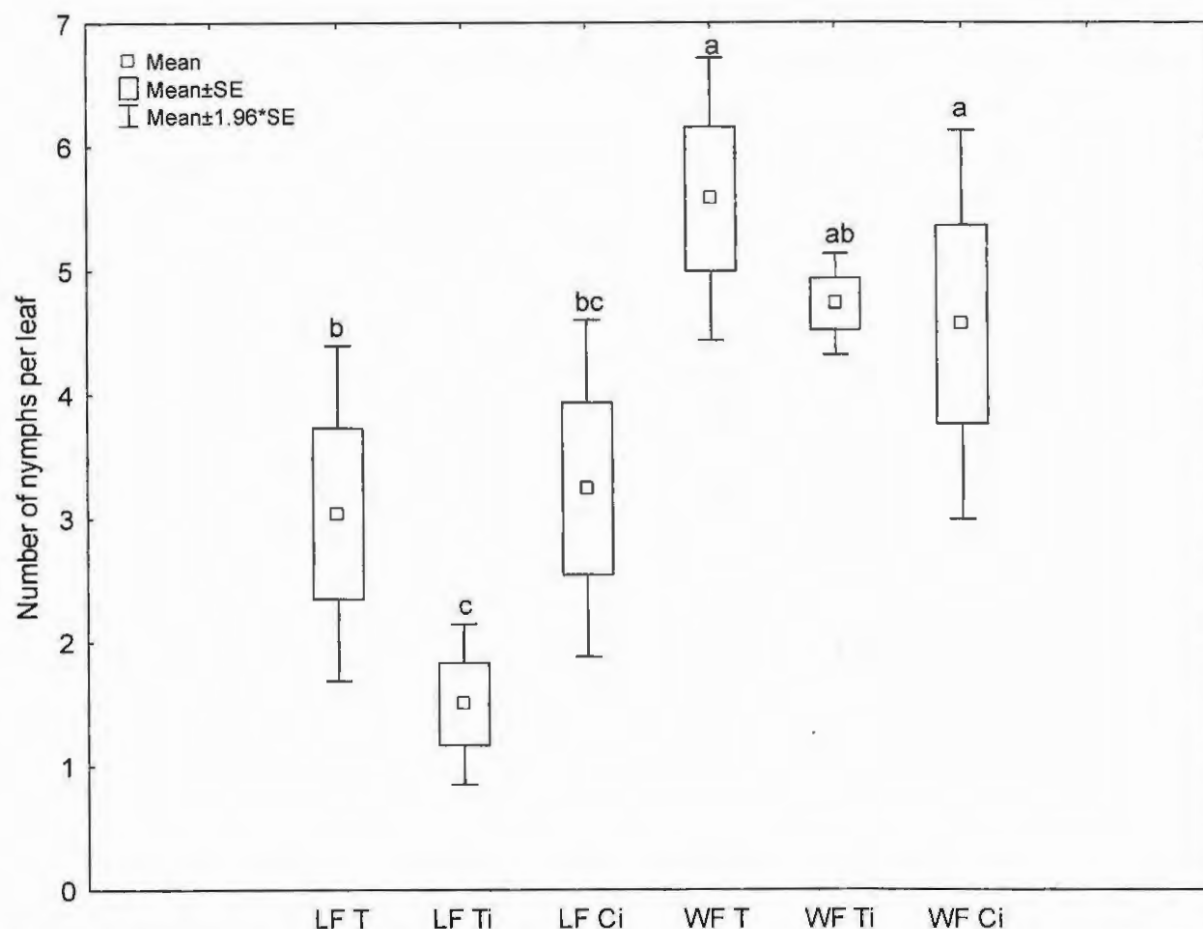
Figure 2.5 Box and whisker plots of *Falconia intermedia* number of nymphs on two *Lantana camara* varieties (Lyndhurst Farm –LF, Whitney Farm – WF) subjected to three treatments (T – un-induced, Ti – induced, Ci – control induced). Plots with the same letter are not significantly different (p > 0.05, paired T - tests).

The number of eggs laid by *F. intermedia* on the Whitney Farm plants was significantly higher than the number of eggs laid on the Lyndhurst Farm lantana (ANOVA; $F_{(1, 28)}$ = 55.722, p = 0.00000) (Figure 2.6). The different treatments once again had no significant effect on the number of eggs laid by *F. intermedia* females on Whitney Farm plants, resulting

in no statistically significant differences among the Whitney Farm treatments (Figure 2.6). A different result emerged among the Lyndhurst Farm treatments, where the plants of the induced treatment (Ti) had significantly fewer eggs laid on them than plants of the un-induced (T; t = -8.4462, df = 4, p = 0.001) and the Ci treatment plants (t = -5.1434, df = 4, p = 0.007).
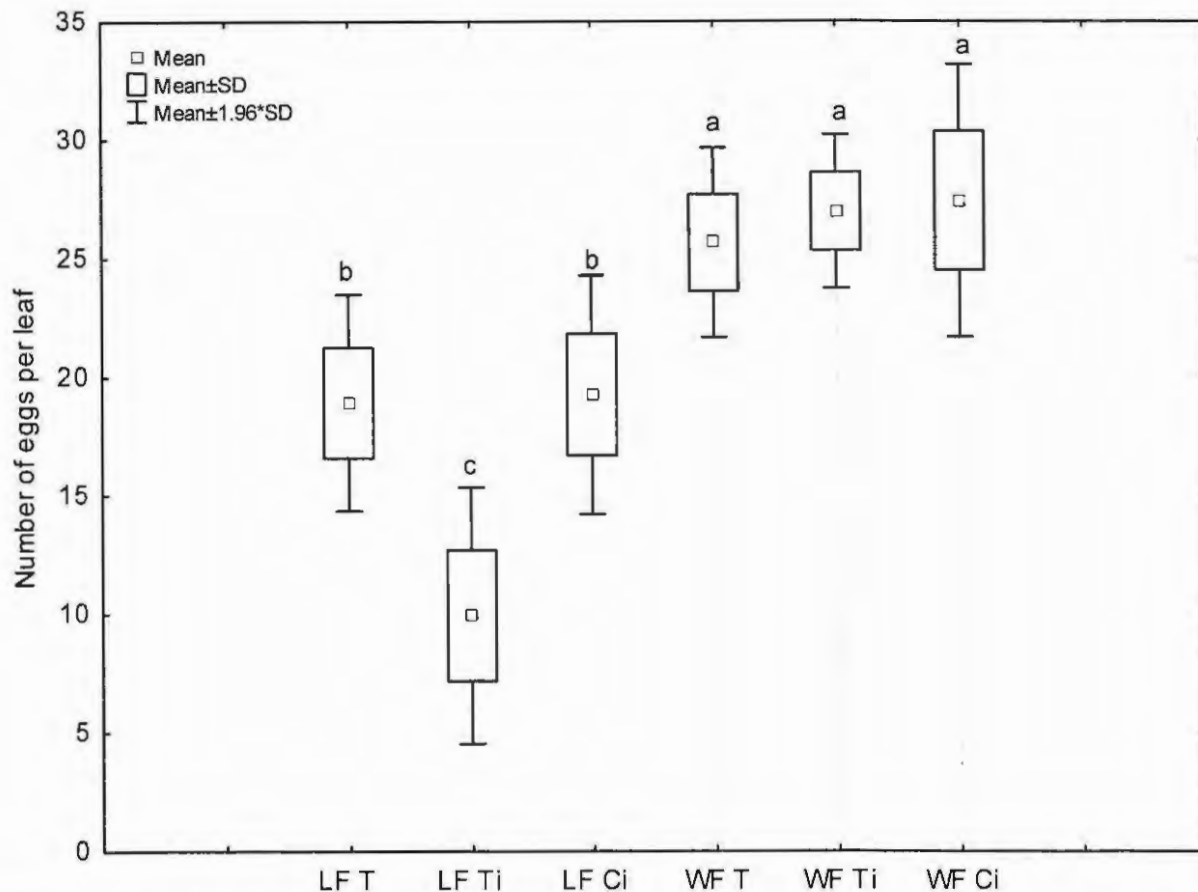
Figure 2.6 Box and whisker plots of *Falconia intermedia* numbers of eggs laid on two *Lantana camara* varieties (Lyndhurst Farm – LF, Whitney Farm – WF) subjected to three treatments (T – un-infested, Ti – induced, Ci – control induced). Plots with the same letter are not significantly different (p > 0.05, paired T - tests).

The proportion of leaves damaged by *F. intermedia* feeding was significantly higher on the Whitney Farm plants than on those found on the Lyndhurst Farm lantana (ANOVA; $F_{(1, 28)}$ = 55.722, p=.00000) (Figure 2.7). The greater number of adults and nymphs at Whitney Farm

translated to a 1.84 factor advantage in the proportion of leaves damaged from the remaining leaves compared to Lyndhurst Farm.
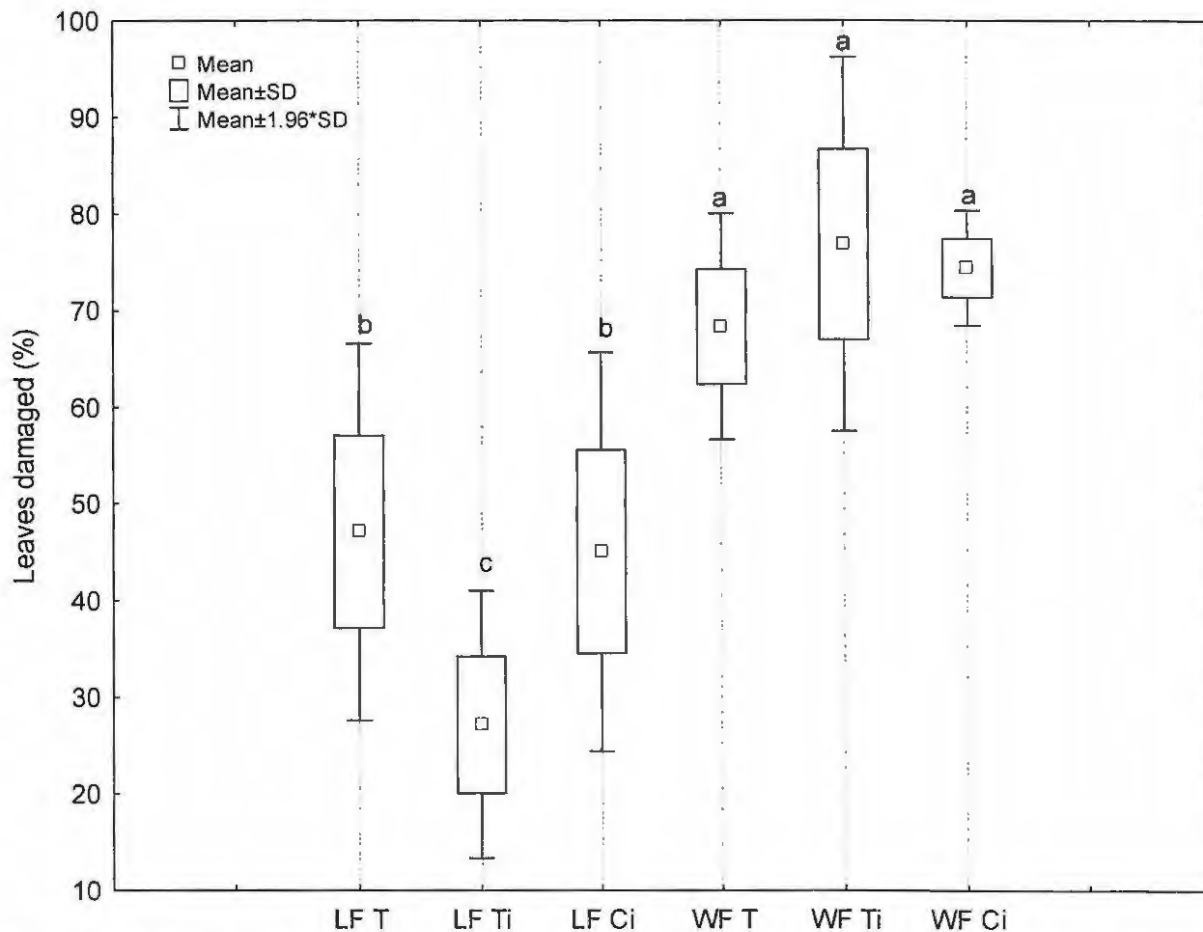
Figure 2.7 Box and whisker plots of the proportion of leaves damaged by *Falconia intermedia* on the plants of two *Lantana camara* varieties (Lyndhurst Farm – LF, Whitney Farm – WF) subjected to three treatments (T – un-induced, Ti – induced, Ci – control induced). Plots with the same letter are not significantly different (p > 0.05, paired T - tests).

There were no significant differences in the percentage of leaves damaged by the mirid between treatments for Whitney Farm (Figure 2.7). For the Lyndhurst Farm treatments though, the induced treatment plants (Ti) had a significantly smaller percentage of leaves damaged than the un-induced test treatment (T; t = -3.392, df = 4, p = 0.027) and the control for induced treatment (Ci, t = 4.669, df = 4, p = 0.009). On the other hand, the two treatments without previous feeding, T and Ci, were not significantly different from one another on the other hand (t = 0.4750, df = 4, p = 0.660) (Figure 2.7).

Falconia intermedia individuals had significantly higher intensity and area of leaves damaged (damage index) on Whitney Farm lantana than on Lyndhurst Farm lantana (ANOVA; $F_{(1, 28)} = 68.230$, $p = 0.0000$) (Figure 2.8). *Falconia intermedia* individuals fed less intensely and on a smaller area of the leaf on the Ti treatment plants of Lyndhurst Farm, leading to a significantly lower statistical effect compared to the un-induced treatment (T; $t = -2.972$, $df = 4$, $p = 0.041$) and the control for induced treatment (Ci; $t = -3.340$, $df = 4$, $p = 0.029$) (Figure 2.8). No significant difference in feeding damage was found between the Lyndhurst Farm T and Ci treatments ($t = 0.775$, $df = 4$, $p = 0.481$) (Figure 2.8).
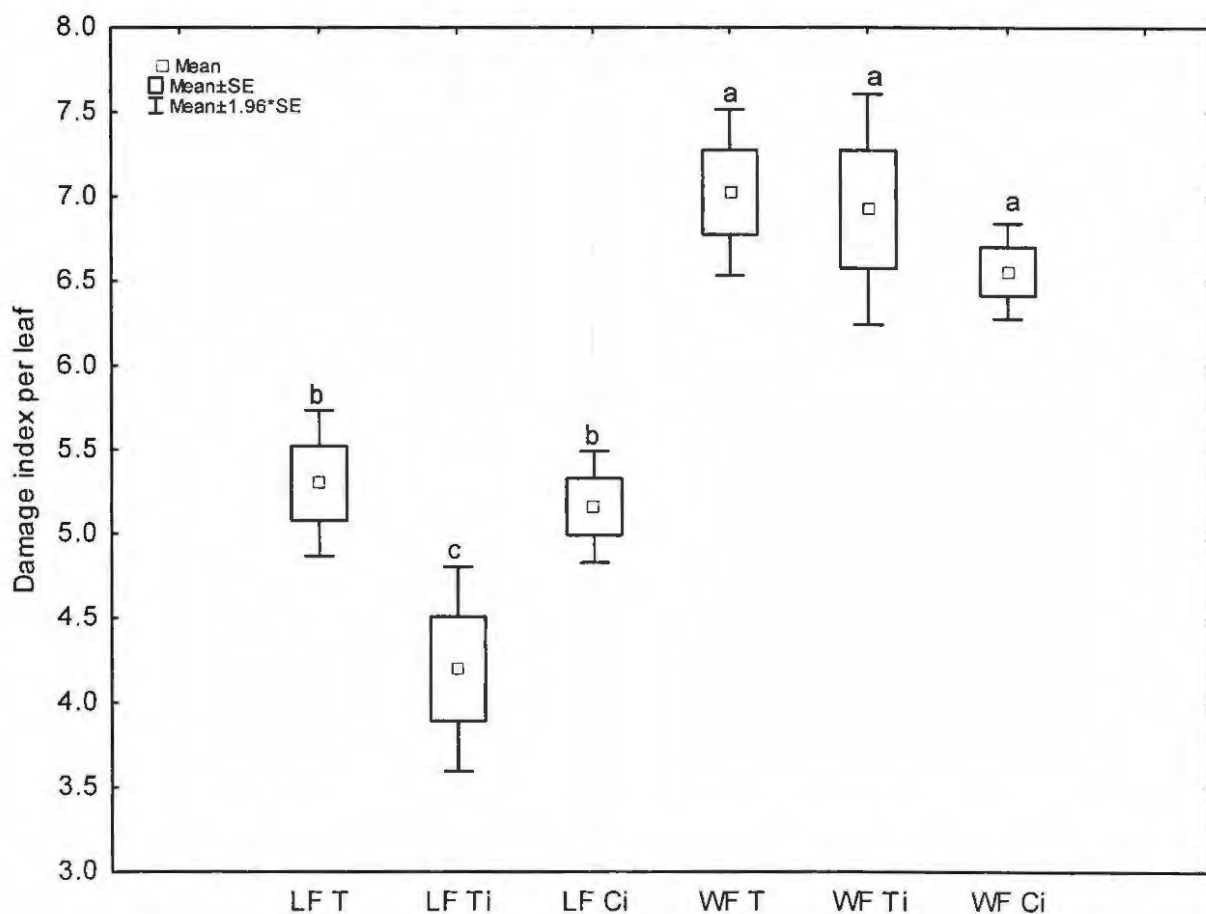
Figure 2.8 Box and whisker plots of *Falconia intermedia* feeding damage on two *Lantana camara* varieties (Lyndhurst Farm – LF, Whitney Farm – WF) subjected to three treatments (T – un-induced, Ti – induced, Ci – control induced). Plots with the same letter are not significantly different ($p > 0.05$, paired T - tests).

Table 2.2 Mean (±SE) relative growth rate (RGR) in the plant parameters of the treatments of two *Lantana camara* varieties (Lyndhurst Farm and Whitney Farm) for the induction trial. Means followed by the same letter in the same column are not significantly different (p > 0.05) (* = p < 0.01, Mann Whitney U-Test). Significance is within treatments of one variety.

Variety	Treatment	N	RGR Plant Height (cm.wk^{-1})	RGR Leading Shoot (cm.wk^{-1})	RGR Side Shoots (nr.wk^{-1})	RGR Leaves (nr.wk^{-1})
Whitney Farm	T	5	0.026 (0.013) *a*	0.003 (0.025) *a*	0.088 (0.032) *a*	0.142 (0.042) *a*
	C	5	0.030 (0.013) *a*	0.043 (0.014) *a*	0.283 (0.089) *a*	0.177 (0.061) *a*
Lyndhurst Farm	T	5	-0.008 (0.016) *a*	0.016 (0.008) *a*	0.092 (0.051) *a*	0.133 (0.048) *a*
	C	5	**0.053 (0.010) *b****	0.028 (0.060) *a*	0.091 (0.043) *a*	0.089 (0.060) *a*

Table 2.3 Mean (±SE) relative growth rate (RGR) in the plant parameters of the treatments of two *Lantana camara* varieties (Lyndhurst Farm and Whitney Farm) for the post-induction experiment. Means followed by the same letter in the same column are not significantly different (p > 0.05, Kruskal-Wallis ANOVA, ranks separated by multiple comparison tests). Significance is within treatments of one variety.

Variety	Treatment	N	RGR Plant Height (cm.wk^{-1})	RGR Leading Shoot (cm.wk^{-1})	RGR Side Shoots index (nr.wk^{-1})	RGR Leaves index (nr.wk^{-1})
Whitney Farm	Ti	5	0.021 (0.014) *a*	0.015 (0.008) *a*	-0.016 (0.004) *a*	0.089 (0.0325) *a*
	Ci	5	0.036 (0.044) *a*	0.018 (0.051) *a*	-0.052 (0.055) *a*	0.154 (0.030) *a*
	Cp	5	0.090 (0.030) *a*	0.083 (0.032) *a*	0.033 (0.033) *a*	0.239 (0.025) *a*
Lyndhurst Farm	Ti	5	0.032 (0.012) *a*	0.574 (0.043) *a*	-0.003 (0.057) *a*	0.160 (0.028) *a*
	Ci	5	0.029 (0.012) *a*	0.037 (0.011) *a*	0.007 (0.027) *a*	0.216 (0.028) *a*
	Cp	5	0.036 (0.009) *a*	0.016 (0.019) *a*	0.023 (0.072) *a*	0.094 (0.092) *a*

2.3.2 *Lantana camara* plant parameters

The relative growth rate of lantana parameters showed little effect in the experiments performed (Table 2.2). The RGR in plant height of the Lyndhurst Farm un-induced plants (T) was significantly less than that of plants of the control (C) treatment. This result was however not repeated in the post-induction treatment equivalents as Ci and Cp were not significantly different (Table 2.3). For the rest of the variables tested, there were no significant differences in changes between the treatments within each of the two varieties. An important trend was observed in the change in variables within treatment plants of Whitney Farm that was absent for Lyndhurst Farm plants (Table 2.3 and 2.4). For the RGR in the Whitney Farm variables, both the treatments with no agents feeding on them (C and Cp) had higher growth rates than treatments with insect feeding and damage (T, Ti, Ci). Of the three treatments with *F. intermedia* feeding effects, the induced treatment (Ti) always had the lowest changes in the variables measured. These trends were however not statistically significant.

Table 2.4 Means (±SE) of reproductive parameters of treatments of two *Lantana camara* varieties (Lyndhurst Farm and Whitney Farm) infested with *Falconia intermedia* adults during the induction experiment. Means followed by the same letter are not significantly different (p > 0.05) (* = p < 0.05, Mann Whitney U Test). Significance is within treatments of one variety.

Variety	Treatment	n	Number of Seeds	Number of Flowers
Whitney Farm	T	5	0.62 (0.39) *a*	2.25 (1.06) *a*
	C	5	3.2 (2.57) *a*	4.5 (4.06) *a*
Lyndhurst Farm	T	5	0 *a*	0 *a*
	C	5	1.6 (1.3) *a*	**3.4 (1.95) *b****

The number of seeds produced was higher for plants of the control (C) treatments of both varieties in the induction experiments. This was however not significant for any of the treatments (Table 2.4). The number of flowers at the control treatment of Lyndhurst Farm was significantly higher than its test treatment. With no flower production at the Lyndhurst Farm test treatment though, this results must be interpreted with caution. It may be that a

58

factor other than the feeding of the mirid, such as effect of enclosure and inadequate sunlight on flower production and other experimental artefacts, is responsible for this.

For the post induction experiment, the number of seed heads produced among the three treatments (Ti, Ci, Co) was not significantly different for either Whitney Farm ($H_{(2, N=30)}$ = 5.190378, p = 0.075) or Lyndhurst Farm ($H_{(2, N=30)}$ =0.000000 p = 1.000). Similarly, there was no significant difference in the number of flowers on the post induction treatments of the Whitney Farm variety ($H_{(2, N=30)}$ = 2.614915 p = 0.271) and Lyndhurst Farm ($H_{(2, N=30)}$ = 0.5689454 p = 0.752) (Table 2.5). Several zero values were recorded in the means of both flowers and seeds in all three of these treatments, and thus the results should be interpreted with caution. They are most certainly not truly reflective of the real effect of the different insect free and insect infested treatments.

Table 2.5 Means (±SE) of reproductive parameters of two *Lantana camara* varieties (Lyndhurst Farm and Whitney Farm) infested with *Falconia intermedia* adults in the post induction experiment. Means followed by the same letter in the same column are not significantly different (p > 0.05, Kruskal Wallis ANOVA). Significance is within treatments of one variety.

Variety	Treatment	n	Seedhead Index	Flower Index
Whitney Farm	Ti	5	0.25 (0.20) *b*	14.75 (6.49) *b*
	Ci	5	0.9 (0.41) *b*	29.9 (9.84) *b*
	Cp	5	0 (0) *b*	10.2 (6.9) *b*
Lyndhurst Farm	Ti	5	0 (0) *b*	14 (6.73) *b*
	Ci	5	0 (0) *b*	19.8 (8.21) *b*
	Cp	5	0 (0) *b*	0 (0) *b*

2.4 Discussion

There is to date no published information on the effect of previous feeding on *L. camara*. Challenging damaged leaves with herbivores is a direct method to prove or disprove induced responses. The current study shows that previous feeding by a sap-sucking agent, *F. intermedia*, induces a response from plants of one of the two *L. camara* varieties tested, Lyndhurst Farm, while no response was evident for plants from the Whitney Farm variety. The variability in response to feeding once again demonstrates the variability of lantana varieties. The dark-pink flowered plants from Lyndhurst Farm demonstrated an ability to reduce feeding and reproductive performance of *F. intermedia* following feeding. On the other hand, previous feeding on the light pink flower variety plants from Whitney Farm produced no significant effect on *F. intermedia* feeding, reproduction and growth under current experimental conditions. The performance of *F. intermedia* in this experiment is consistent with previous laboratory trials where the mirid found Whitney Farm lantana variety more suitable than Lyndhurst Farm lantana plants (Heshula 2005). The reduction in the performance parameters of *F. intermedia* on previously fed plants of Lyndhurst Farm in the current experiment seems to suggest that this variety has the ability to induce resistance to feeding, whereas Whitney Farm plants do not possess this ability.

Flower colour has been used frequently to distinguish different varieties in the biological control of lantana. Although practical and widely used, DNA studies have however shown that flower colour is not an accurate way of differentiating lantana forms or varieties (Scott *et al.* 1997; Scott 1998). Therefore the responses observed cannot be uniformly expected from similar flower-colour lantana varieties and each variety (and flower colour) must be examined individually. The varieties of lantana have a host of different physiological and morphological features that may be contributing towards the observed responses. The field establishment of *F. intermedia*, for example, has been reported to be affected by the hairiness of the lantana leaves (Heystek 2006). On the other hand, a hairy orange flowered variety resulted in no significantly lower preference or performance in laboratory studies conducted by Baars (2002). Thus a number of different factors, singly or in combination, could be responsible for the observed response. The mechanisms by which these changes occur in the

60

L. camara and *F. intermedia* interaction still remain unanswered and are the subject of an investigation in the following chapter.

It is also very important to note the speed with which the responses were induced. The induced response by Lyndhurst Farm lantana occurred in the same season, an example of rapidly induced responses. Even though there is uncertainty in the timing of the onset of responses after feeding, it is clear that by day 31 after initial feeding plants from this variety have a definite response to feeding. Furthermore, the responses were systemic as the performance of the agent was measured even on leaves not previously induced. Therefore any variety with the ability to respond in this dual manner will be able to optimise the speedy response further by quickly distributing it throughout the plant. Systemic responses may demand more energy from the plant than local responses, but may be more effective in instances where releases of a large number of insects are made, such as in biological control releases. Whether the responses by a lantana plant are detected by other lantana plants is unclear and may be the subject of another study.

These results have important consequences for biological control and the weed-agent relations under field conditions. The ability to induce resistance to the detriment of the agent following feeding by any variety renders an advantage to the plant. Such responses lead to a considerable decrease in insect survival, reproduction and preference for the plant (Karban and Baldwin 1997). A case in point may be the colonisation, heavy damage and subsequent disappearance of *F. intermedia* in releases made in the Mpumalanga Province, South Africa, in the absence of chemical exclusion, persistent inclement weather and predation (Heystek, pers. comm.). This chain of events seems to suggest that this variety of *L. camara* induced some response after initial feeding by released agents. Typically of induced resistance, the responses did not directly benefit the plant fitness of the Lyndhurst Farm plants. Despite this, any reduction in injurious feeding by a biological control agent will undoubtedly be beneficial to the induced alien plant. Some of the agents released on varieties with the ability to induce resistance may find it difficult to adapt to these responses after initial feeding. This may be due to the fact that *L. camara* underwent man-mediated genetic changes over a long period of time in the absence of natural enemies. The natural enemies thus did not co-evolve

with *L. camara* and might not have developed measures to counter these responses when they occur.

Varietal characteristics may in turn be more importantly affected by environmental factors. The interplay between these two factors may be very important in determining responses, particularly in terms of the initial damage by the inducer. The onset and even occurrence of responses have been shown to be directly affected by the amount of damage inflicted and the length of feeding time by the inducer (Hódar *et al.* 2004, Kaplan *et al.* 2007). An increase in any of the above factors may alter the responses, but how this may affect the responses of plants, *in situ* and *in vitro*, to feeding needs further study. Unlike in a number of studies where the difference in subsequent performance was mediated by interspecific herbivore interactions, the differences in herbivore performance and impacts on the different varieties reported here were induced by and affected a single herbivore species. Varietal differences in other plant species, such as soybean, have also been shown to affect plant responses to feeding by the same herbivore, *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) (Endo *et al.* 2007). It remains to be examined what effect previous feeding by one biological control agent, such as the widespread lacebug *T. scrupulosa*, might have on the efficacy of other biological control agents, or vice-versa. In this chapter the effect of the recovery period allowed between the induction and the post-induction period on both the performance of the agent and the weed are not yet clear. Insect reproductive and growth parameters, including damage inflicted on lantana, were reduced after induction on one variety while no change was observed on the other. No clear trend emerged among the changes in plant growth and reproduction variables among the treatments at the end of induction and the treatments upon the second release of agents. In the following chapter, mechanisms of the induced defensive responses are investigated.

CHAPTER 3

The interaction of *Lantana camara* leaf quality with the performance of *Falconia intermedia*

3.1   Introduction

Herbivore feeding damage by *F. intermedia* was shown to induce a response in only one of two *L. camara* varieties tested (Chapter 2). The nature of the response by this variety, and the presence and nature of responses on other test varieties, was however not determined. An important niche contested by phytophagous insects and plants are the leaves. Folivorous insects such as *F. intermedia* reproduce, oviposit, and spend their entire life-cycles on the leaves. To plants however, leaves are an important energy generating resource that must be protected. Plants may therefore respond to herbivory by increasing or decreasing the quality of their leaves leading to increased plant resistance or susceptibility. In so doing an increase or decrease in herbivore performance is achieved (Karban and Baldwin 1997). Alternatively, the responses may be directed towards decreasing the impact of feeding damage by increasing plant tolerance, resulting in the plant initiating some level of compensatory growth (Strauss and Agrawal 1999).

Leaf quality factors, the induction of which has been correlated in literature with the performance of herbivores are leaf toughness, trichome length and density, and leaf moisture content (Schoonhoven *et al.* 2005). Damage effected by *Pieris rapae* (L.) (Lepidoptera: Pieridae) and *Trichoplusia ni* (Hubner) (Lepidoptera: Noctuidae) resulted in significant short-term induction of trichome density on leaves of black mustard *Brassica nigra* (L.) Koch. (Traw and Dawson 2002). Along with an increase in a defensive chemical, adding herbivores to thale cress *Arabidopsis thaliana* (L.) Heynh. (Brassicaceae) resulted in an increase in trichome density (Mauricio and Rausher 1997). Prior feeding by adult leaf beetle (*Phratora vulgatissima* L. (Coleoptera: Chrysomelidae) induced increased trichome density in *Salix cinerea* L. (Saliceae) plants and resulted in a corresponding decrease in larval feeding (Dalin and Bjorkman 2003). Similarly, *Urtica dioca* L. plants had significantly higher trichome

63

density after being grazed upon than ungrazed plants in field conditions (Pulin and Gilbert 1989).

In turn, the plant and leaf quality has been shown to affect the performance of herbivores. A biological control agent, *Oxyops vitiosa* Pascoe (Coleoptera: Curculionidae), had greater fecundity, biomass and shorter development time on a variety of *Melaleuca quinquenervia* (Cavanilles) Blake (Myrtaceae) with softer and more nitrogen abundant leaves (Wheeler 2001, 2006). *Hydrellia pakistanae* Deonier (Diptera: Ephydridae) larvae reared on *Hydrilla verticillata* (L.f) Royle plants with low nitrogen and higher leaf toughness had higher mortality, increased development time and decreased female biomass (Wheeler and Center 1996). The growth rates of generalist and specialist Lepidoptera were positively influenced by the higher moisture, nitrogen content and reduced toughness of younger leaves on 40 plant species tested (Coley *et al.* 2006). It therefore appears that in general poor quality plants retard insect development and growth.

Besides the type of plant response to damage, the time it takes for the response to be effective is also important. Responses from the plant may be induced within days after herbivore damage in what is known as rapid induced responses (Karban and Baldwin 1997). On the other hand, delayed induced responses manifested in the following season. Therefore these responses affect the next generation of herbivores. Intermediate delayed induced responses of plants are those that are evident in the latter part of the season. Previous herbivore defoliation under field conditions resulted in intermediate delayed induced and delayed induced resistance in potato and pine; rapidly induced resistance in one of three soybean varieties in greenhouse studies (Hódar *et al.* 2004; Lempa *et al.* 2004; Valkama *et al.* 2005; Endo *et al.* 2007; Kaplan *et al.* 2007). Both the length of feeding and the amount of damage by inducer/s played a significant role in resistance.

In this chapter the mechanisms and effect of the *L. camara* variety responses due to *F. intermedia* were studied. The hypotheses tested were the following:

a) Following feeding damage by *F. intermedia*, there are changes in leaf quality in *L. camara* varieties tested, and these responses show varietal differences.

b) Induced leaf quality changes affect the feeding and reproductive performance of *F. intermedia* on the different varieties.

c) Changes observed affect the growth of *L. camara* positively, thus increasing benefit to the plant, but the induced changes may also come at a cost to the plant and thus growth may be negatively affected

3.2   Materials and Methods

3.2.1   *Falconia intermedia*

A culture of *F. intermedia* was established as outlined in section 2.2.1. Insect cultures were kept in cages on lantana bushes propagated from the site where insects were collected (section 2.2.1) in a glasshouse facility separate from the tunnel facility where experiments were conducted. Plants were watered, fertilised and replaced with new ones when necessary.

3.2.2   Plant material

Five sites from the Eastern Cape which were used in previous laboratory and field studies were chosen for this experiment. Plants were sourced from *L. camara* stands found on these sites (Table 3.1). Two of the chosen varieties (Whitney Farm and Lyndhurst Farm) were tested for the occurrence of induced responses in the previous chapter, while the other three varieties (East London, Port Alfred and Heather Glen Farm) were used in previous *F. intermedia* field releases that were not very successful (Heshula 2005). Plant cuttings were made in the field and prepared as outlined in section 2.2.2.

3.2.3   Feeding trials

Ten plants per original locality, of about the same height, were chosen for this part of the experiment. Five of these plants served as *F. intermedia*-infested treatment plants and were confined in cages in a no-choice design. A mean number of 0.5 adults per lantana leaf pair

65

were released on the five test treatment plants, as was the case in Chapter 2. Five other plants were chosen as control treatments with no manipulation or mirid individuals. Control plants were also confined in cages to control for the effect of the cages. The experiment was conducted from December 2006 to March 2007. All plants from both treatments were watered once daily and fertilised bi-weekly with a liquid fertiliser (Seagro®). The experiments were conducted in a tunnel facility equipped with heaters and fans with temperatures varying between a low 14 °C at night and 38 °C in the daytime. The temperature measurements did not take into account the effect of the microclimate around the plants and on the top and bottom surfaces of the leaf. The temperature around a leaf may be more than 10 °C less than the ambient temperature (Schoonhoven *et al.* 2005). A midday cold water spray over the cages and the floor of the tunnel was applied on hot days to increase humidity and cool down the ambient temperature in the tunnel.

The number of eggs, counted from a random sample of ten damaged leaves per plant and numbers of adults and nymphs were counted at the end of the experiment (March 2007). The ten leaves were scored for *F. intermedia* feeding damage to give an indication of the extent of the agent's feeding activity. Overall damage score was determined as a product of the area of leaf damaged (0-4) and the intensity of the feeding damage (0-3). Leaf area damage ratings ranged from 0% (=0), 1-25% (=1), 26-50% (=2), 51-80% (=3), and >81% (=4), while damage intensity scoring ranged from no damage (0), few and uneven speckles in affected area (=1), uniform white speckling in affected area (=2), and white speckling merging that appear white/yellow in affected area (=3) (as in Chapter 2).

Table 3.1 Distinguishing characteristics and the original locations of *Lantana camara* varieties used during laboratory trials.

L. camara source site	Distinguishing morphological features	Flower and throat colour	Grid reference
Whitney Farm	Leaves large, broad, dark and medium hair; shoots spiny, main stem with large spines	Light pink flower, orange throat	33°40'43"S 26°35'49"E
Lyndhurst Farm	Leaves small, tough and few hair; shoots hairy with small spines; main stem with few small spines	Dark pink flower, orange throat	33°27'11"S 26°53'10"E
East London	Leaves large, broad, dark and medium hair; shoots hairy and spiny; main stem with large spines	Light pink flower, orange throat	33°00'23"S 27°54'47"E
Heather Glen	Leaves large, broad, dark and medium hair; shoots hairy and spiny; main stem with large spines	Light pink flower, orange throat	33°19'28"S 26°47'31"E
Port Alfred	Leaves small, tough and few hair; shoots spiny; main stem with few small spines	Dark pink flower, orange throat	33°36'16"S 26°52'16"E

3.2.4 Plant quality

Ten leaves per plant were sampled in each treatment to evaluate leaf toughness at the end of the experiment (March 2007) and put into tubes. For consistency leaves from the fourth or fifth leaf pairs were chosen from branches randomly sampled around the individual plants. Leaf toughness was determined using an Imada® DPS-1 force gauge mounted on a stand. The mean leaf toughness was measured from three points (leaf tip, middle, and base) with the leaf clamped down on a stand.

Trichome density of each leaf was then immediately evaluated by counting the number of leaf hairs in a 2 mm^2 area from three of the ten leaves sampled from each plant. The leaf hairs were pressed down by rolling a glass bottle on the wet leaves. The three leaves were cut into seven small sections of about 5mm^2, avoiding the leaf mid vein. The sections were coated with a thin conductive film of gold in preparation for the Scanning Electron Microscope (SEM) using a sputtering device (Bulzers Union Ltd). An image from the leaf sections was acquired using the scanning electron microscope (magnification 70x, 20kV, Hi Vacuum). The length (base to tip) and leaf hairs count within the 2 mm^2 area in each section were then generated from the image using Scandium® software.

Ten more leaves from each of the five lantana plants in each of the two treatments, damaged leaves from insect-infested plants and undamaged leaves from un-infested plants were randomly sampled and placed into tubes for leaf moisture evaluation. The weight wet of the leaves was immediately measured using an analytical balance (Adventurer™, Ohaus Ltd). The leaves were dried in an oven at 60 °C for 48 hours and weighed to determine the dry weight. The percentage leaf moisture was then determined as the percentage difference in dry and wet weight using the formula:

$$\% \text{ Leaf Moisture} = \frac{(\text{Wet Weight} - \text{Dry Weight})}{\text{Wet Weight}} \times 100$$

68

The dried leaves used to determine leaf moisture content were then used to determine the content of selected leaf nutrients. The dried leaf samples from the same treatments and variety were pooled and the samples sent away to Production Technology Laboratories, Elsenburg, South Africa for plant analysis. The percentage nitrogen, potassium, phosphorus and magnesium were analysed.

3.2.5 Statistical Analyses

Insect parameters were subjected to parametric one-way ANOVA. Data were acceptably normal with homogenous treatment variances. Percent leaves damaged and percentage change data for plant parameters were subjected to a non-parametric Kruskal-Wallis ANOVA, and medians were separated by multiple comparison tests. Endpoint data were analysed at the 5% significance level on the Statistica $8.1^{®}$ (StatSoft, Inc. 2006). Significantly differently means were separated by using Fisher's Least Square post hoc test. A multiple regression analysis was conducted to determine the relative contribution of the change in plant quality parameters to the success of the insect growth parameters. Plant quality and reproductive parameters were subjected to the non parametric Mann-Whitney U Test.

3.2 Results

3.3.1 *Falconia intermedia* population

All five *L. camara* varieties supported *F. intermedia* populations to varying degrees under the experimental conditions. Consistent with Chapter 2 results, populations confined to the Whitney Farm plants performed well and supported the highest number of adult and nymph individuals per leaf pair (H $_{(4, N = 25)}$ =18.92091, p = 0.0008) (Table 3.2). About forty-three individuals per leaf pair were recorded at this variety, significantly more than at all other varieties except the Heather Glen Farm variety, where about 24 insect per leaf pairs were recorded (Table 3.2). Mirid numbers were not significantly different on the white pink flowered East London and Heather Glen plants compared to the dark pink variety of Port Alfred and Lyndhurst Farm plants.

Table 3.2 Means (± standard errors) of *Falconia intermedia* population parameters for the test treatments of the five Eastern Cape *Lantana camara* varieties. Means in the same column followed by the same letter are not significantly different (p > 0.05, Kruskal-Wallis ANOVA, means separated by multiple comparison mean rank test).

Variety	N	Individuals per leaf pair	Leaves damaged (%)
East London	5	2.48 (0.32) *a*	15.82 (3.32) *b*
Heather Glen Farm	5	24.40 (1.39) *ab*	57.31 (2.73) *b*
Lyndhurst Farm	5	5.20 (3.14) *a*	12.70 (2.12) *b*
Port Alfred	5	9.68 (3.31) *a*	15.35 2.40) *b*
Whitney Farm	5	43.52 (15.72) *b*	**85.39 (5.04)** *a*

70

The *F. intermedia* white pink variety plants of Whitney Farm and Heather Glen Farm had significantly higher oviposition than plants from the other varieties ($F_{(4, 20)}$ = 13.229, p = 0.00002) (Table 3.3). These two varieties had similar numbers of eggs per leaf pair with no statistical significantly difference between them. The reproductive output is a good indicator that the two varieties (Heather Glen and Whitney Farm) are the most suitable for *F. intermedia*. Oviposition suitability is an important step in adult females accepting a plant host, thereby ensuring the best survival chances for its offspring, and aiding in an increase of agent population numbers.

Table 3.3 Means (± standard errors) of *Falconia intermedia* feeding damage intensity and proportion of leaves damaged on five *Lantana camara* varieties. Means in the same column followed by the same letter are not significantly different (p > 0.05, one-way ANOVA, Fisher's Least Square Difference Test).

Variety	N	Damage index per leaf	Eggs per leaf pair
East London	5	2.80 (0.37) *a*	12.96 (1.41) *a*
Heather Glen Farm	5	4.60 (1.17) *ab*	21.92 (1.81) *b*
Lyndhurst Farm	5	3.00 (0.63) *a*	11.80 (2.27) *a*
Port Alfred	5	3.40 (0.87) *a*	7.72 (1.80) *a*
Whitney Farm	5	7.20 (0.73) *b*	21.72 (1.19) *b*

Feeding damage due to the sap sucking activity of *F. intermedia* followed a trend similar to one observed for number of individuals recorded. Whitney Farm plants had the highest proportion of damaged leaves at the end of the experiment with 85.39% ($H_{(4, N= 25)}$ =18.73477, p = 0.0009) (Table 3.2). This value was, with the exception of damage on Heather Glen Farm, significantly higher than values recorded for other varieties, indicating widespread feeding damage on leaves of this variety. The mean damage index of these damaged leaves was also the highest among all the varieties. The Heather Glen

Farm was the only variety with the feeding damage index not significantly lower than Whitney Farm ($F_{(4, 20)}$ = 10.770, p = 0.00008) (Table 3.3). Feeding damage was the least on East London, Lyndhurst Farm and Port Alfred plants. A low level of feeding damage (feeding damage index: 0-3.9) was recorded on East London, Lyndhurst Farm and Port Alfred varieties, with middle level damage (feeding damage index: 4-7.9) at Heather Glen Farm and Whitney Farm.

3.3.2 *Lantana camara* parameters

A general trend of higher relative changes in the measured variables, except for the change in side shoots at Heather Glen and Whitney Farm, on the *F. intermedia* infested treatments was observed on all varieties but East London. Despite this fact, very few of these variables were significantly higher statistically. The change in the length of infested Heather Glen Farm leading shoots was significantly higher than for uninfested plants ($H_{(1, N= 10)}$ = 6.818182, p = 0.0090) (Table 3.4). Lyndhurst Farm plants also had significantly higher change in plant height of infested plants compared to uninfested plants ($H_{(1, N= 10)}$ = 3.962195, p = 0.0465). The percentage change in plant height was not significantly different among any of the other treatments.

Along with the change in leading shoots, the change in the number of leaves on the Heather Glen infested plants was significant at the mirid infested treatment compared to its un-infested treatment ($H_{(1, N= 10)}$ = 5.770909, p = 0.0163). There were no other significant differences in relative change in leaf growth or length of leading shoots in the treatments of other varieties. The relative change in number of side shoots was not significant in the treatments of any varieties. The number of seeds produced at the infested treatment was significantly higher than the un-infested treatment only at the Whitney Farm variety (U = 22.00, Z = 2.108, p = 0.035) (Table 3.5). No other variables had significantly different treatments. Similarly the number of flowers produced was not affected between treatments in any of the varieties. These results indicate that *F. intermedia* damage at the above levels had minimal effect on the reproduction of lantana. As was the trend with the growth data, plants on which the mirids established all had higher number of both flower heads and seed heads than plants without mirids (Table 3.5).

72

Table 3.4 Means (± standard errors) of the percentage relative change of *Lantana camara* plant growth parameters in laboratory trials. Means followed by NS indicate no significant difference between a variety's *Falconia intermedia* infested and un-infested treatments ($p > 0.05$), * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$(Kruskal-Wallis ANOVA, means separated by multiple comparison rank test).

Variety	Treatment	N	Δ Plant Height (%)	Δ Lead Shoots (%)	Δ Side Shoots (%)	Δ Leaf number (%)
East London	Infested	5	0.59 (3.46)	8.57 (3.82)	-4.79 (11.59)	28.72 (7.05)
	Uninfested	5	7.23 (6.47)*NS*	10.22 (1.72)*NS*	11.57 (5.57)*NS*	51.36 (12.90)*NS*
Heather Glen	Infested	5	9.65 (11.46)	25.66 (3.92)	19.44 (10.32)	112.59 (12.74)
Farm	Uninfested	5	1.88 (2.58)*NS*	**4.79 (2.20)****	41.95 (25.30)*NS*	**42.94 (15.65)****
Lyndhurst Farm	Infested	5	49.59 (6.06)	41.11 (9.54)	14.43 (13.35)	94.38 (14.25)
	Uninfested	5	**16.18 (10.61)***	**14.03 (5.27)***	6.14 (11.60)*NS*	46.34 (28.57)*NS*
Port Alfred	Infested	5	21.44 (10.10)	24.35 (9.16)	4.58 (3.22)	59.55 (18.60)
	Uninfested	5	3.30 (3.73)*NS*	14.91 (3.71)*NS*	1.21 (4.33)*NS*	50.16 (25.37)*NS*
Whitney Farm	Infested	5	261.92 (234.99)	33.61 (10.10)	13.19 (10.01)	114.73 (26.68)
	Uninfested	5	6.04 (8.18)*NS*	11.63 (5.56)*NS*	56.61(46.84)*NS*	56.45 (48.32)*NS*

73

The trends observed both in the vegetative and reproductive plant parameters in this chapter were clear, unlike the ambiguous trends observed in chapter 2.

3.3.3 Interaction of plant quality factors with *Falconia intermedia* feeding

There were significant differences in the numbers of trichomes between plants from different locations. Lyndhurst Farm (U = 282.00, Z = 3.493, p = 0.0005), Port Alfred (U = 356.500, Z = 3.004, p = 0.003) and East London (U = 218.500, Z = 4.292, p = 0.00002) insect infested plants all had significantly higher trichome densities than their respective un-infested treatments (Figure 3.1, 3.2 & Table 3.6). *Falconia intermedia* individuals, number of damage leaves, and feeding damage index recorded were the lowest on these varieties (Table 3.2 and 3.3). In contrast, the trichome density was not significantly different for the treatments of Whitney Farm and Heather Glen Farm plants (Table 3.6).

The two treatments with the highest and second highest number of individuals, Whitney Farm and Heather Glen respectively, had the softest leaves among the different varieties in their *F. intermedia* infested treatments (Table 3.6). All leaves under agent attack showed a general increase in their toughness, a trend similar to the one observed for trichome density above. The increases were highly significant between the treatments of all varieties and standard errors in the leaf toughness of un-infested treatment plants were low throughout the varieties, indicating low variation and minimal changes on these treatments throughout the duration of the experiment. Port Alfred, East London and Lyndhurst Farm insect-infested treatments had both trichome density and leaf toughness significantly higher compared to their un-infested treatments.

Leaves from the plants of all five varieties had lower moisture content in their infested treatment leaves compared to the un-infested plants. None of these differences were statistically significant however (Table 3.6). The nitrogen, phosphorus and potassium content of the five varieties were not vastly different from one other (Table 3.8). The results did not show any link in the nutrients' contents and the good performance of the mirid on the Heather Glen Farm and Whitney Farm varieties seen in the feeding trials. The

74

insect-free treatment leaves of all varieties all had higher nutrient contents, indicating essential nutrient uptake by *F. intermedia* on the plants inoculated with insects.

Table 3.5 Means (± standard errors) of the percentage relative change of *Lantana camara* plant reproductive parameters in laboratory trials. Means followed by NS indicate no significant difference between a variety's *Falconia intermedia* infested and un-infested treatments ($p > 0.05$), * = $p < 0.05$ (Mann-Whitney U Test).

Variety	Treatment	n	Number of Seeds	Number of Flowers
East London	Infested	5	5.10 (1.81)	16.5 (7.26)
	Un-infested	5	3.10 (1.35) *NS*	2.40 (1.15) *NS*
Heather Glen	Infested	5	9.30 (3.03)	17.10 (4.99)
	Un-infested	5	5.30 (1.94) *NS*	11.2 (1.92) *NS*
Lyndhurst Farm	Infested	5	19.4 (4.10)	20.1 (6.32)
	Un-infested	5	9.8 (3.82) *NS*	21.7 (5.67) *NS*
Port Alfred	Infested	5	16.7 (7.08)	19.50 (6.98)
	Un-infested	5	9 (2.74) *NS*	11.6 (4.57) *NS*
Whitney Farm	Infested	5	16 (5.78)	19.2 (6.34)
	Un-infested	5	**3.9 (1.48)** *	7.10 (2.62) *NS*

3.3.4   Multiple regression analysis

The results from the regression analysis revealed that leaf toughness and trichome density are the most important contributors in the plant quality traits tested in these experiments. Only trichome density had a statistically significant contribution to the number of *F. intermedia* populations ($R^2 = 0.52339857$, $F_{(3, 21)} = 9.7855$, $p < 0.00031$) (Table 3.7). This suggests than an increase in trichome density is related to a decrease in the number of *F. intermedia*. The percentage leaf moisture was shown to have very little contribution to predicting the performance of *F. intermedia*.
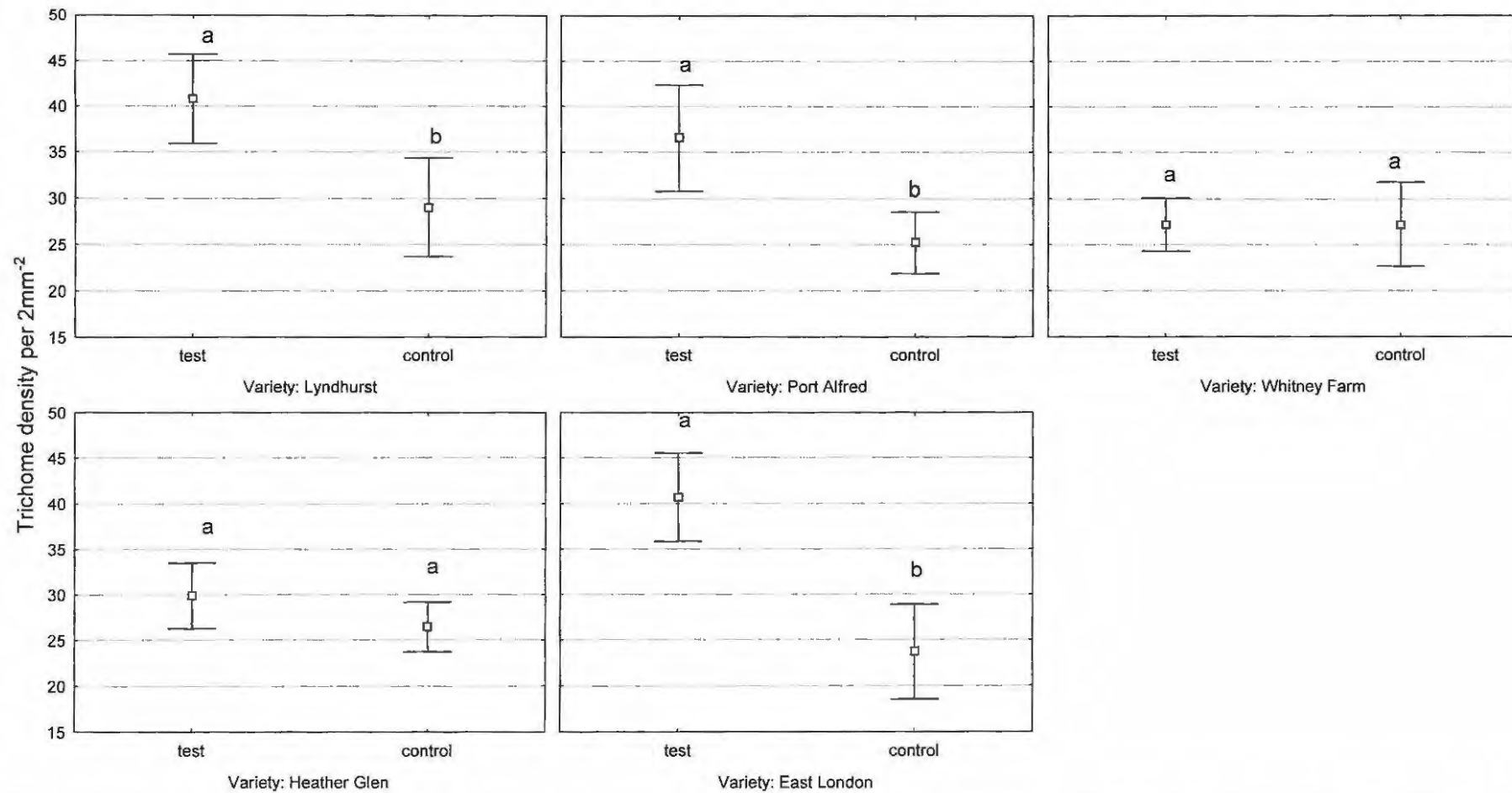
Figure 3.1 Mean number of leaf trichomes in 2mm² for *Falconia intermedia* infested test and un-infested control treatments for *Lantana camara* varieties. Means in one variety followed by different letters are significantly different (p < 0.05, Mann-Whitney U Test). Whiskers denote 95% confidence intervals.

Table 3.6 Means (± SE) of *Lantana camara* plant quality parameters. Means of one variety followed by NS indicate no significant difference (p > 0.05), * = p < 0.05, *** = p < 0.001 (Mann-Whitney U Test).

Variety	Treatment	n	Leaf trichomes (2mm²)	Leaf toughness (g)	Leaf moisture (%)
East London	Infested	5	40.68 (2.38)	22.72 (4.45)	70.10 (2.84)
	Un-infested	5	**23.70 (2.54)*****	**13.05 (0.38)*****	64.70 (3.50)*NS*
Heather Glen	Infested	5	29.88 (1.77)	16.60 (0.40)	59.56 (6.07)
	Un-infested	5	26.46 (1.34)*NS*	**13.21 (0.43)*****	67.36 (3.68)*NS*
Lyndhurst Farm	Infested	5	40.82 (2.42)	19.31 (0.52)	68.24 (1.88)
	Un-infested	5	**29.03 (2.61)*****	**13.15 (0.52)*****	71.82 (1.79)*NS*
Port Alfred	Infested	5	36.57 (2.86)	19.75 (0.61)	65.83 (2.56)
	Un-infested	5	**25.20 (1.66)***	**11.34 (0.40)*****	72.69 (1.46)*NS*
Whitney Farm	Infested	5	27.19 (1.42)	15.96 (0.27)	55.33 (5.55)
	Un-infested	5	27.20 (2.18)*NS*	**12.48 (0.34)*****	69.93 (2.10)*NS*

Table 3.7 Regression analysis for the interaction of the number of *Falconia intermedia* individuals and different plant quality factors.

	Beta	Std Err Beta	B	Std Err of B	t(21)	P-level
Intercept			56.677	13.423	4.222	0.0003
Trichome density	**-0.738**	**0.141**	**-0.667**	**0.12751**	**-5.235**	**0.0000**
Leaf toughness	-0.165	0.151	-0.839	0.771	-1.089	0.2881
Leaf moisture (%)	0.008	0.151	0.006	0.118	0.056	0.9557

(a) East London infested
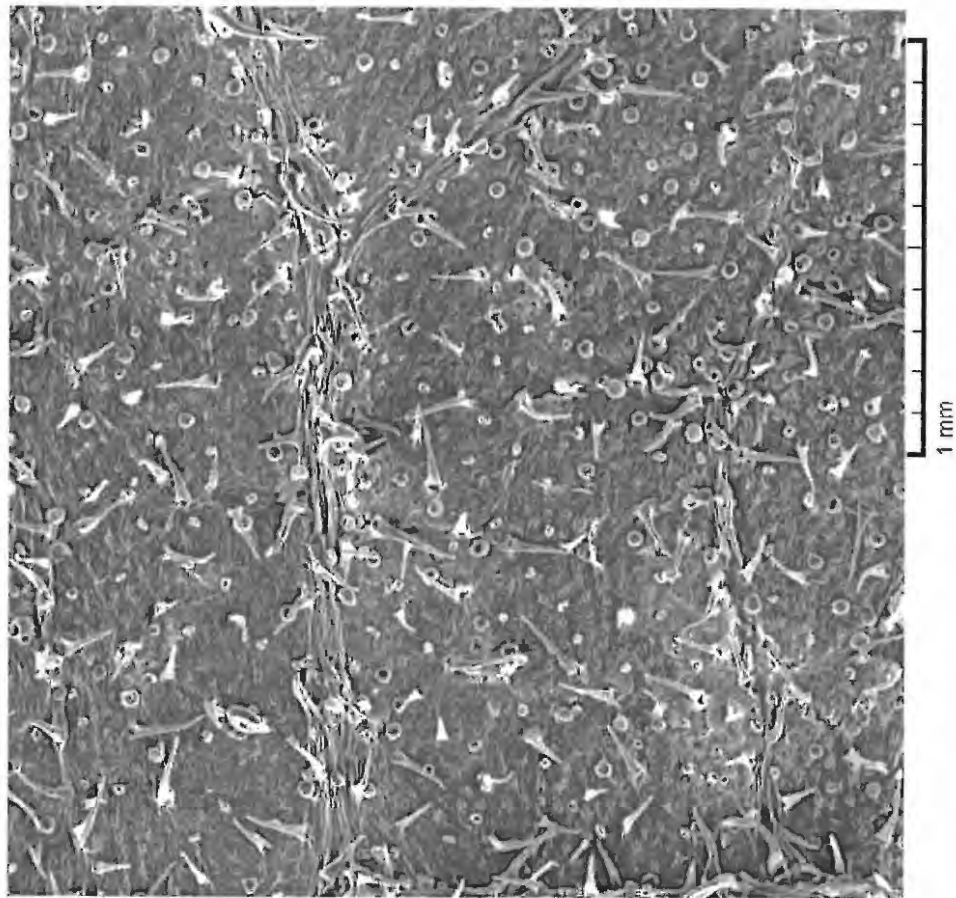
(b) East London uninfested

1 mm

1 mm

78
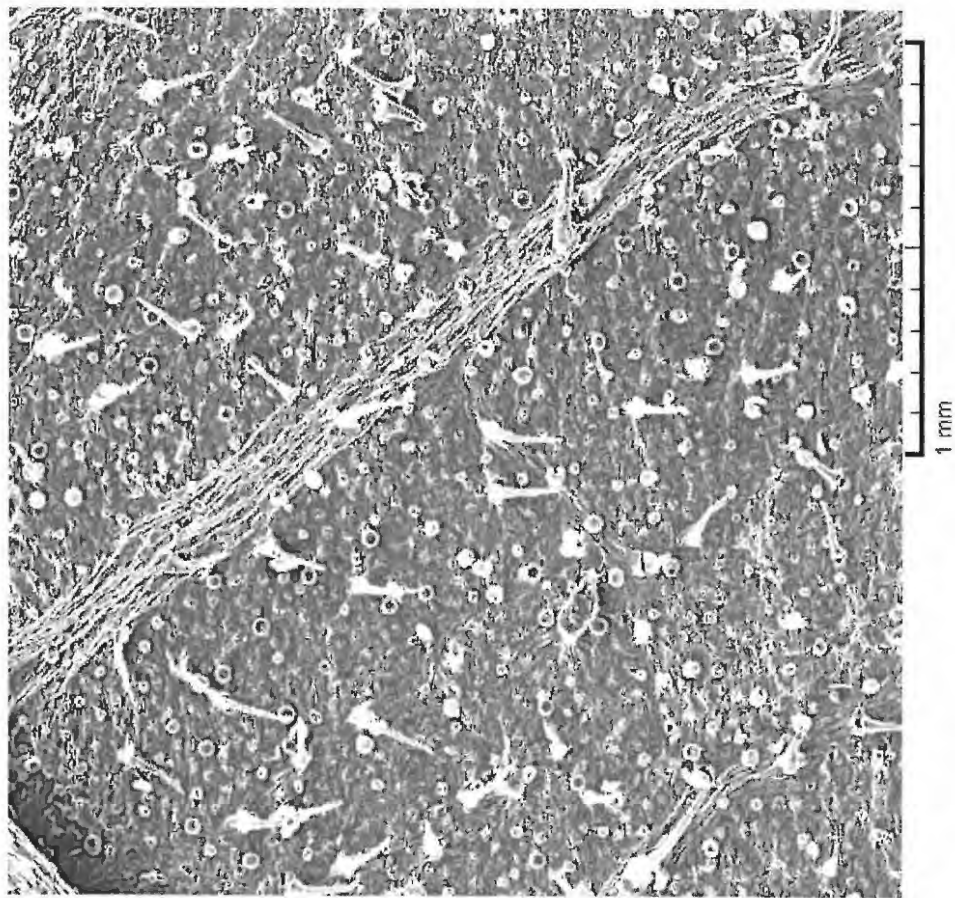
(c) Lyndhurst Farm infested

1 mm
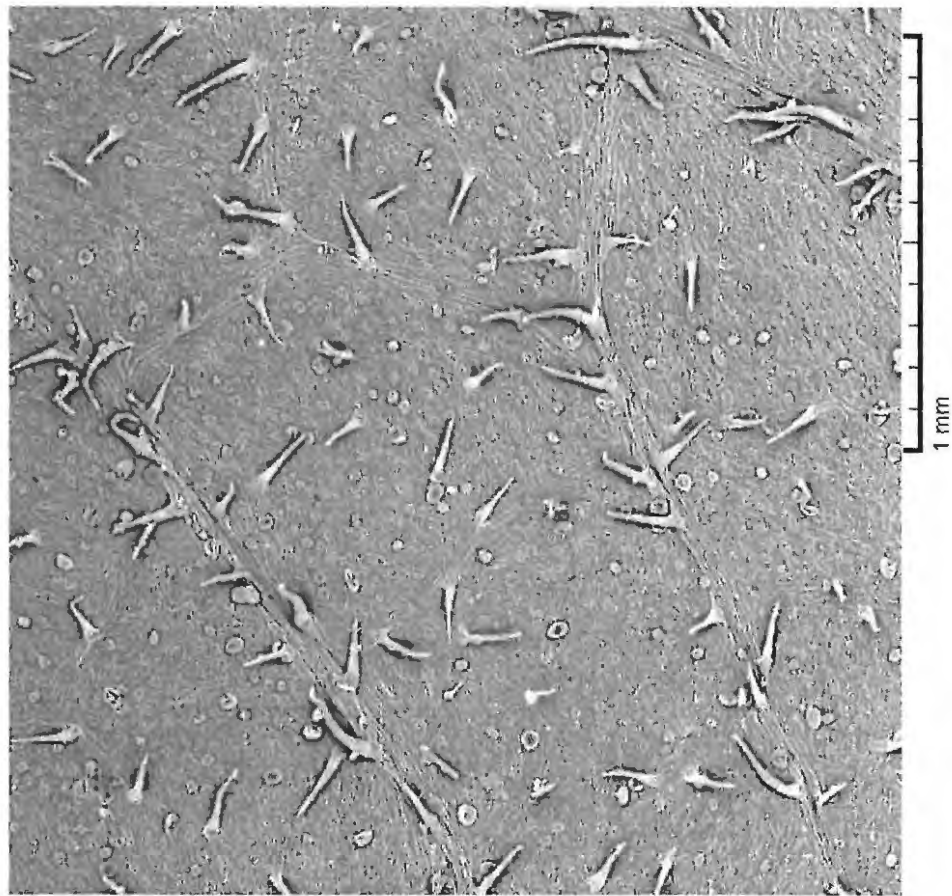
(d) Lyndhurst Farm un-infested

1 mm

79
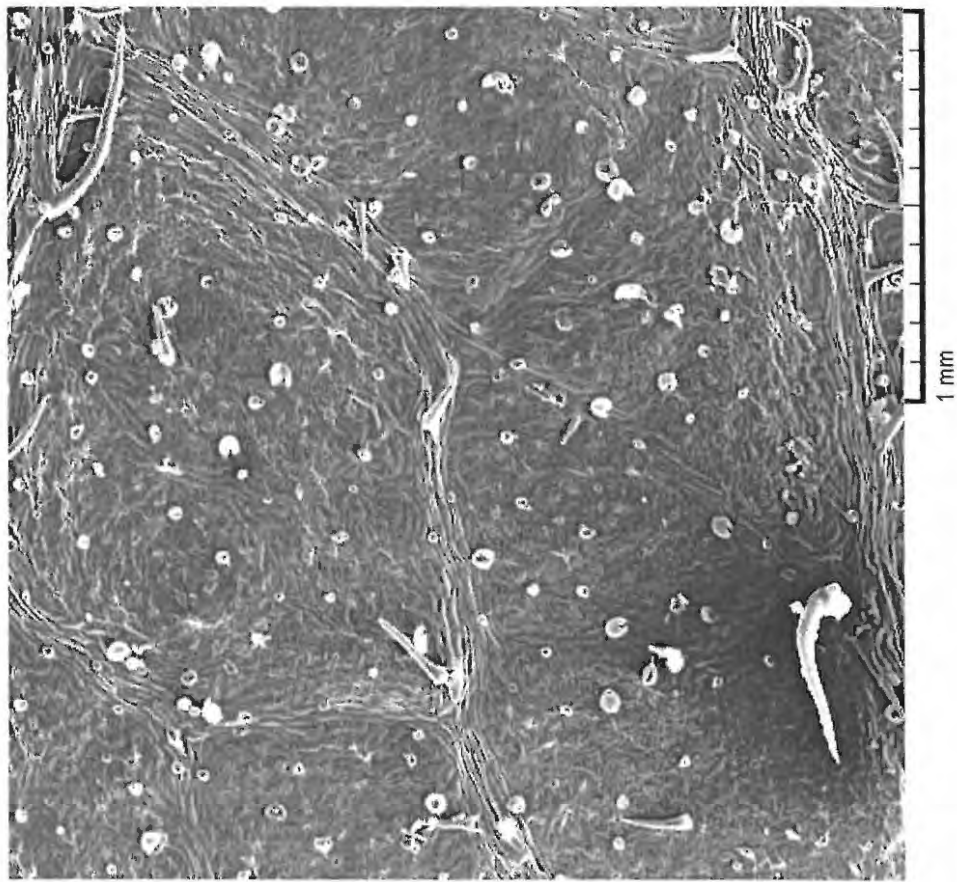
(e) Port Alfred infested
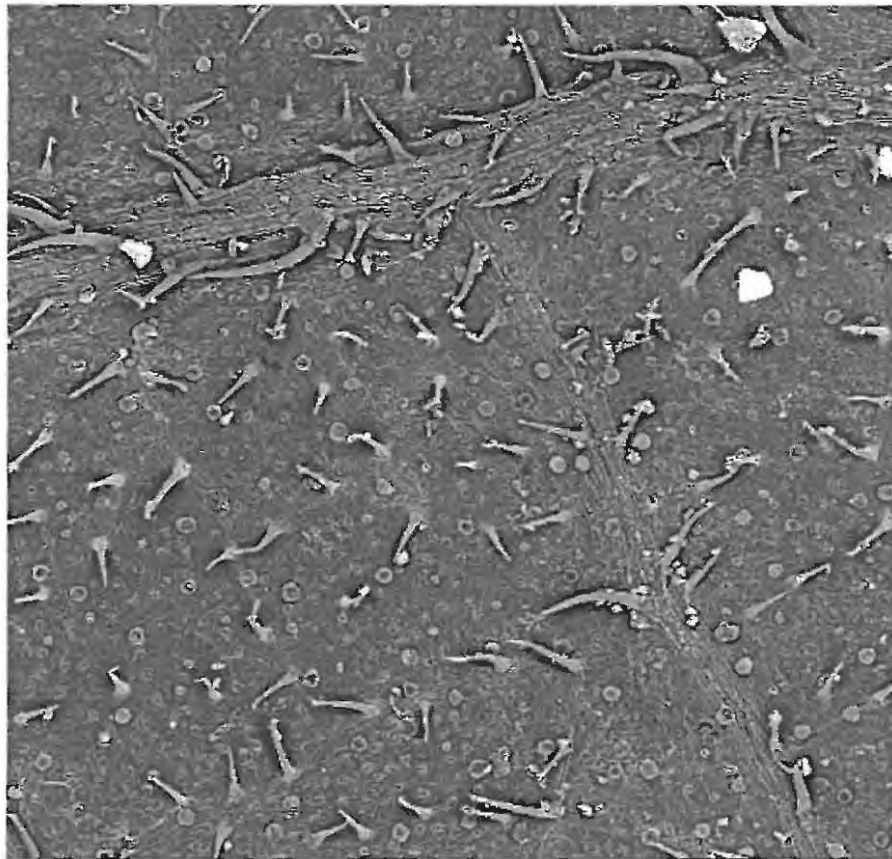
(f) Port Alfred un-infested

500 μm
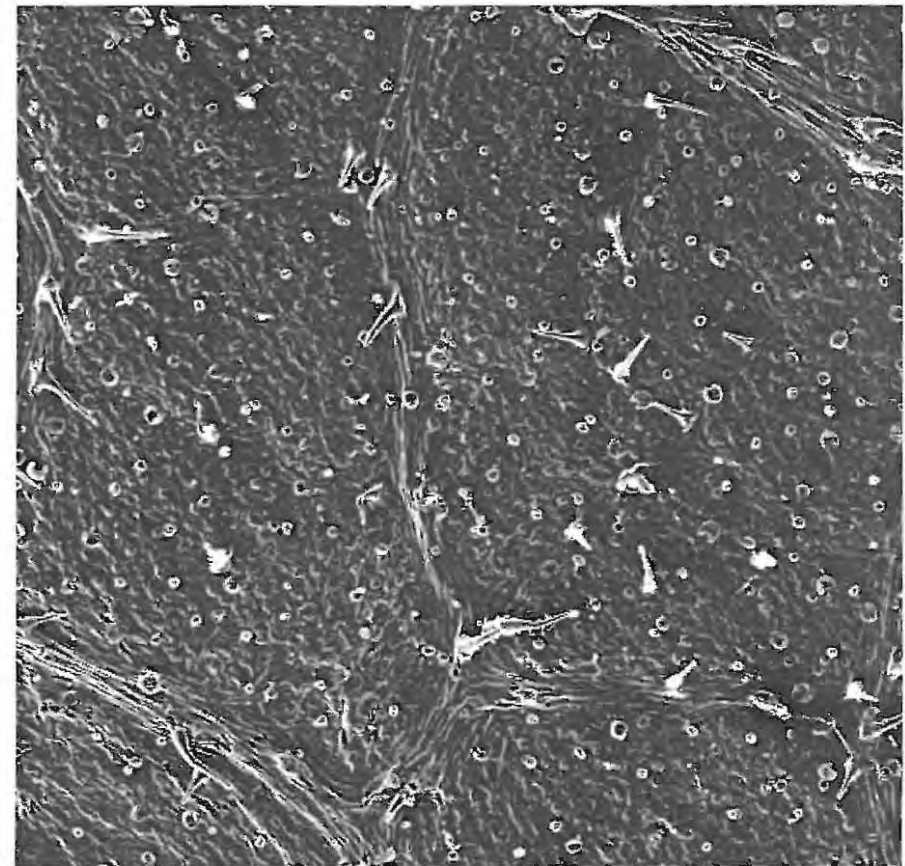
1 mm

Figure 3.2 Scanning Electron Microscopy (SEM) images ((a) to (f)) of a 2mm^2 area of leaf trichomes as seen on three *Lantana camara* varieties un-infested and infested with *Falconia intermedia*.

Table 3.8 Plant nutrient content of damaged and undamaged leaves from the tested varieties of *Lantana camara*.

Variety	%Nitrogen		%Phosphorus		%Potassium		%Magnesium	
	T	C	T	C	T	C	T	C
East London	1.91	2.21	0.32	0.31	2.52	1.44	0.37	0.16
Heather Glen	1.92	2.11	0.39	0.54	2.25	1.30	0.38	0.88
Lyndhurst Farm	2.13	2.45	0.30	0.30	2.60	1.56	0.28	0.16
Port Alfred	1.89	2.35	0.25	0.48	2.15	1.80	0.34	0.98
Whitney Farm	1.94	2.58	0.27	0.35	1.96	1.39	0.35	0.21

3.4   Discussion

In the previous chapter and in previous studies conducted (Heshula 2005, Heystek 2006), both under laboratory and field conditions, *F. intermedia* showed differing preference and performance results on different *L. camara* varieties. Varied performance of herbivores on plant species may be caused by inducible plant responses, the incidence of which is widely reported in plant ecology literature (Karban and Baldwin 1997; Wheeler 2001; 2006; Endo *et al.* 2007). Some responses reduce the feeding and reproductive performance of herbivores (induced resistance), while others serve to benefit the host plant by reducing the effects of feeding and increasing plant fitness (induced defence). At other times feeding may result in better performance by the herbivores, known as induced susceptibility (Karban and Baldwin 1997). No work has yet been conducted to test how *L. camara* induced responses affect the performance of biological control agents. Our results suggest that the plant responses by the five lantana varieties against the imposed rates of herbivory each exhibit one of the following responses: induced resistance (East London, Lyndhurst Farm and Port Alfred), induced defence and tolerance (Lyndhurst Farm, Heather Glen Farm, Port Alfred, Whitney Farm).

81

Three *Lantana camara* varieties (East London, Port Alfred and Lyndhurst Farm) significantly increased both their leaf toughness and trichome density after damage by *F. intermedia*. The physical leaf responses were restricted to only an increase in leaf toughness in the two other white pink varieties of Heather Glen and Whitney Farm infested by the same proportion of the agent. The responses were significant in their effect on the agent's performance. Increases in leaf toughness and trichome density were strong predictors of a decrease in the number of *F. intermedia* individuals, with the latter significantly so, thus suggesting that the latter response is an induced resistance (Karban and Baldwin 1997). The two factors were significant on East London, Port Alfred and Lyndhurst Farm, where the number of individuals were the least, suggesting that leaf toughness and trichome density are both important in significantly reducing the number of *F. intermedia*.

The leaf nutrient analysis conducted in the feeding trials showed very little link between the essential nutrients (N, P, K, and Mg) contents and the performance of *F. intermedia* under the current experimental conditions. The trend observed in content of nutrients also show that none of the nutrient content in any of the varieties could be related to the defences seen in the three varieties above. Leaf toughness and trichome density of *L. camara* varieties therefore appear to be more important factors plant suitability and *F. intermedia* performance than plant nutrients. Toughness of leaves, or sclerophylly, comes about as a result of the deposition of cellulose, lignin, suberin, callose, elongate cells known as sclerenchyma fibers, and other thick-walled cells such as xylem and collenchymas. An increase in leaf toughness therefore acts as a physical barrier to chewing by mandibulate insects, penetration by piercing-sucking insects, and to oviposition. Mouthparts may be also considerably worn by feeding on tougher versus softer foliage and a lot more energy is required for a smaller intake of food (Schoonhoven *et al.* 2005).

Physical defences by means of leaf surface hairs are particularly effective in deterring insect herbivory, with heavy pubescence preventing small piercing-sucking insects from reaching the epidermis with their mouthparts (Southwood 1986). Whether the lantana leaf

trichomes are glandular or not was not investigated in this study. It is a matter that requires investigation in the future. Glandular trichomes may further enhance their protective features to feeding by exuding sticky or noxious substances against insect herbivores. Although never previously reported to occur on *L. camara*, these two leaf quality traits –leaf toughness and trichome development- have been shown to limit herbivore performance and development in studies conducted on other plant species (Wheeler 2001; Agrawal and Spiller 2004). Accordingly, East London, Port Alfred and Lyndhurst Farm lantana supported the least number of individuals and oviposition, proportion of leaves damaged and damage inflicted by *F. intermedia*.

The responses were elicited throughout the whole plant (plant systemic), as revealed by the fact that random sampling to test for these leaf quality traits included damaged and undamaged leaves throughout the plants. A similar systemic response was observed in trials conducted in the previous chapter. This is typical of induced responses, with the responses elicited likely to affect even other susceptible herbivores (Gatehouse 2002). The resistance was rapidly induced and after a four week period proved to reduce the effectiveness of *F. intermedia*.

None of the plants infested with *F. intermedia* derived any statistically significant benefit from their leaf quality responses. However, there were increases in plant height, number of leaves and length of leading shoots were recorded on the *L. camara* herbivore-infested treatments at all varieties except East London. Similarly, all varieties had better reproductive performances on their infested treatment plants versus un-infested. Even though most of these results were mostly not statistically significant, possibly due to inadequate replication, they suggested tolerance for feeding. Tolerance in this context defined as the ratio of the fitness of a plant lineage which has been attacked by herbivores to that of plants from the same lineage grown under identical conditions but without the herbivores (Simms and Rausher 1987; Strauss and Agrawal 1999). Measures of the relative growth of damaged versus undamaged plants may be used to give an indication of plant fitness. Plants from Whitney, Lyndhurst and Heather Glen Farms, and Port Alfred *L. camara* compensated fully for damage, with fitness between damaged and undamaged

83

plants being similar (Strauss and Agrawal 1999). There is therefore still a need to conduct more studies with greater numbers of replicates to further investigate this.

The two white-pink varieties, Heather Glen Farm and Whitney Farm, did not respond to feeding by increased resistance, but supported a significantly higher number of *F. intermedia* individuals and oviposition than other varieties. From results from the previous chapter, individuals released on already damaged Whitney Farm plants did not perform significantly better, thus suggesting that even though this variety is susceptible to the mirid, the response after feeding is not an increase in susceptibility, or induced susceptibility. It must be borne in mind however, that the experimental method in the previous chapter which involved removing the individuals for some time may have an effect on responses. Furthermore, *F. intermedia* on the plants of these varieties damaged a greater proportion of leaves with a significantly higher index of damage. Therefore an assay with longer inducer times and relatively short change-over times may produce different results.

A number of varieties tested employed both tolerance and resistance as a response to feeding. Responses observed on each of these varieties are not mutually exclusive and a plant may choose to employ a combination of these responses in its defence. Plants have been reported to make such ecological trade-offs, such as those between resistance and susceptibility to various natural enemies (Adler and Karban 1994; Rausher 1996), or between resistance and tolerance to herbivory (van der Meijden et al. 1988; Rosenthal and Kotanen 1994; Mauricio et al. 1997). Plants may also extend this to having an ability to use either or both of its constitutive and induced defensive measures (Agrawal *et al.* 1999). In the same way plants may also utilise one of a variety of defensive chemical compounds against herbivores in addition to physical plant quality defensive measures. Such a measure in *L. camara* varieties would probably be activated when the first line of defence, physical plant characteristics, is not effective against herbivore attack.

How increased damage levels may affect the results needs to be studied further. Feeding damage across all varieties was generally low (East London, Port Alfred, Lyndhurst) and medium (Whitney Farm and Heather Glen Farm). It has been shown in previous studies that the impact of the agent on lantana is depended by infestation intensity. Sparse *F. intermedia* infestations increase lantana growth slightly (Heshula 2005), whereas moderate to very heavy *F. intermedia* infestations decrease lantana growth slightly to considerably (Baars 2002; Heystek & Olckers 2003). Linked to the intensity of the damage is the duration of exposure to the inducer insects. The type and intensity of stress and length of interaction has been reported as important in determining the type of response (Karban and Baldwin 1997). Different environmental, soil and plant nutrient, and ecological conditions in the field may serve to alter plant quality characteristics, thus altering outcomes in many field conditions.

The biological control programme against *L. camara* in South Africa, and in some other parts of the world, has had little or no success. Among factors cited for this lack of success is the varied performance of biological control agents on the different varieties of *L. camara*. There is a dearth of published studies examining the feeding - induced leaf quality response of the different varieties of lantana, making this study quite significant. In the previous chapter it was shown that some varieties have the ability to mount a systemic and rapid response following feeding, to the detriment of the feeding herbivore. This study shows from leaf quality characteristics the mechanism some varieties may use to impede the establishment and performance of agents, and how this may affect biological control overall. Some varieties with adequate quality plants may resort to a rapid and systemic compensatory growth as a response to herbivory. On the other hand, some varieties have the ability to rapidly and systemically increase their resistance in response to herbivory. The presence of chemical defences, and the role they may have on the integrated defences of *L. camara* is closely examined in the following chapter.

CHAPTER 4

Effect of *Falconia intermedia* on volatile organic compounds from aerial parts of Eastern Cape *Lantana camara* varieties

4.1 Introduction

Earlier chapters have shown that *L. camara* is able to induce physical defences following feeding by *F. intermedia*. The response options available to any plant to herbivory include not only physical, but also chemical defences. Chemical defences in the form of direct and indirect induced defences are reported among many plants following herbivore damage. Evidence from different experiments has increasingly confirmed the existence of greater emission of volatile organic chemicals and indirect defences in response to herbivory (see reviews by Dicke *et al.* 2003; Arimura *et al.* 2005). Although volatile compounds of numerous essential oils have been isolated and elucidated from different varieties of *Lantana camara* (Sharma *et al.* 2007), the interest in most of this work has arisen from the diverse biological and pharmacological uses of lantana by different communities, and the weed's ubiquitous distribution and its commercial potential. Parts of the plants are used against ailments such as influenza, fevers, asthma, rheumatisms, high blood pressure and bronchitis (Ghisalberti 2000).

In much of the work conducted on the essential oils of *L. camara* varieties a diverse range of volatile terpenes has been isolated. A sample from a variety in India contained germacrene D, γ-elemene and β-caryophyllene as major components from its leaves (Khan *et al.* 2002), while a sample of aerial parts of the plant from South China yielded germacrene D, β-caryophyllene and α-humelene (Sundufu and Shoushan 2004). Leaves and flowers from Iranian plants had sabinene, β-caryophyllene and 1,8-cineole as major components (Sefidkon 2002). On the African continent, Cameroonian and Madagascan samples had *ar*-curcumene, β-caryophyllene, caryophyllene epoxide; and davanone and β-caryophyllene, respectively (Ngassoum *et al.* 1999). However, no study has yet examined

changes in the volatile emissions from *L. camara* due to herbivory feeding in attempting to explore possible indirect induced responses. Feeding induced responses are expressed in the form of quantitatively higher levels of volatiles, and qualitatively different bouquets of compounds being emitted by plants following herbivore damage (Wei *et al* 2006). Most volatile bouquets released in response to feeding by plants were specific, with differences observed due to mechanical versus herbivore damage; fungal or insect damage; and inter-species insect feeding (Stout *et al.* 1998; Cardoza *et al.* 2002; Rodriquez-Soana *et al.* 2003; Mithöfer *et al* 2005).

There are significant insights to be gained by exploring the question of increased volatile emission and induced indirect defences in the biological control of *L. camara*. Of the 13 biological control agents against *L. camara* that have established in South Africa for instance, six are reported to be under some level of parasitism, thus limiting their success (Table 1.2). Volatile chemicals which are increasingly emitted due to herbivore feeding have been reported to assume the role of semiochemicals and aid natural enemies to locate feeding herbivores (induced indirect resistance), as well as facilitate inter- and intra- plant communication (Karban *et al.* 2000; Colazza *et al.* 2004). The presence of feeding-induced volatile responses in lantana opens up the possibility of the existence of the above mentioned interactions.

Although reported to occur in over 100 plant species with mostly terpenoids, phenolic compounds, hydrocarbons, and alkaloids elucidated (Karban and Baldwin 1997), not all herbivory induces increased volatile emission in all plants (Tooker and Moraes 2007). It is thus important to ascertain the existence of these responses in any plant, as well as appreciate the conditions under which they are induced. In this section of the study we attempted to answer the following questions:

a) Does feeding by *F. intermedia* on two *L. camara* varieties result in an increase in production of volatiles from plants of these varieties?

b) What is the identity of the major volatile phytochemicals from the headspace of plants from the two Eastern Cape *L. camara* varieties tested?

87

4.2   Materials and Methods

4.2.1   Induction of *Lantana camara* by *Falconia intermedia* feeding

Two varieties, Whitney Farm and the more resistant East London prepared as previously described in sections 2.2.1 and 3.2.1, were used in this experiment. Five plants from each variety were randomly chosen to be used as *F. intermedia* infested plants while a further five plants were used as un-infested treatment plants. Each plant from the two treatments of each variety was placed in a cage. Adults (0.5 per leaf pair) were released on each of the variety plants chosen as infested treatment plants, with none on the un-infested treatment plants. Feeding was allowed to occur continuously on the infested plants. Plants from both treatments were all concurrently watered once daily and fertilised bi-weekly with a liquid fertiliser (Seagro®). The experiments were conducted in a tunnel facility between a low 14 °C at night and 38 °C in the daytime. The temperature measurements did not take into account the effect of the microclimate around the plants and on the top and bottom surfaces of the leaf. The temperature around a leaf may be more than 10 °C less than the ambient temperature (Schoonhoven *et al.* 2005). A midday cold water spray over the cages and the floor of the tunnel was applied on hot days to increase humidity and cool down the ambient temperature in the tunnel.

Leaves from the different treatment plants were simultaneously harvested at 30, 60 and 90 days after herbivore feeding was commenced. Sampling took place early in the morning (between 8:30am and 10:00am) at all times to prevent variations in emission due to environmental conditions. The profiles generated from the three sampling times were not significantly different, and the data from the three samples were therefore combined. The leaves were placed in marked glass flasks and sealed with new rubber septa immediately to prevent volatiles from escaping. These were taken to the Mass Spectrometer room in the Chemistry Department of Rhodes University for immediate chemical analyses.

4.2.2   Identification techniques

Techniques used in the identification of volatiles traditionally include solvent extraction and steam distillation. These techniques usually require the crushing and grinding of plant

88

material before volatile analysis. Advanced and recent techniques, such as headspace analysis using gas chromatography mass spectrometry (GCMS) used in our experiments, provide for more representative profiling *in vivo* plant tissue than the previously mentioned techniques. Analysis of damaged and undamaged plant samples may now be profiled and analysed to provide a more realistic picture of volatile bouquets at a given time. In solid phase micro extraction static headspace analysis (SPME-SHA), volatiles are adsorbed or absorbed onto an inert fibre, and then desorbed into the GC. The SPME-SHA technique is appropriate for a snapshot survey of volatile organic compounds (VOCs) encountered at a particular time (Tholl *et al.* 2006).

4.2.3   Chromatographic conditions

The major components of the volatile oil from leaves of *L. camara* varieties were detected from head space analysis using solid phase micro extraction (SPME) fibres. Leaves collected from whole plants were put into glass flasks closed with septa. A tiny hole was pierced through the septum and the fibre was exposed to the headspace of the flask. The fibre was exposed with limited success to the headspace for 30 seconds first, and then with more success for 60 seconds. After limited success in getting good chromatographic profiles the collection method was varied by shaking and inverting the flask to allow the heavier volatile gases to settle at the bottom of the flask. When the flask was righted, the septum was immediately pierced and exposed to the headspace. The GC-MS data were acquired using a 30m capillary J&W DB1 GC column. The following oven conditions prevailed during the analyses: 35°C at the start then ramped to 150°C at 15°C per minute. The temperature was maintained at 150°C for seven minutes.

4.2.4   Reference substances

The identities of the compounds were confirmed by authentic standards that were obtained from Sigma Aldrich and by spectroscopic analysis using gas chromatography mass spectroscopy (GC-MS). The mass spectral library facility at the Rhodes University was used to compare chromatogram peaks and retention times. Chemicals were obtained from Sigma Aldrich® Laboratories, South Africa. The β-caryophyllene (98.5% purity) and α-

pinene (99% purity) were Gas Chromatography grade. The α-thujene essential oil (88% pure) was donated by Clive Teubes of Teubes® Essential Oils, South Africa.

4.3   Results

4.3.1 Volatile response to *Falconia intermedia* feeding

Chromatogram profiles from lantana leaves from both varieties were inconclusive when headspace was sampled for 60 seconds on uninverted flasks. Chromatogram peaks from both damaged and undamaged leaves were small, indistinct with a lot of baseline noise due to the weak signal detected (e.g. Figure 4.1 and 4.2 for Whitney Farm). Despite the weak signal chromatograms had similarities in the three main compounds eluting, two at just less than five minutes, and another at around ten minutes. Do to the baseline noise use of this method made it difficult to observe differences in elution profiles between undamaged and damaged leaves for the varieties.

Figure 4.1 Chromatogram profile of uninverted flask headspace emissions by *Falconia intermedia*-damaged *Lantana camara* leaves from Whitney Farm variety. Solid Phase Micro Extraction fibres were exposed for 60 secs. The main chemicals are indicated (1-3).

An improvement of the method outlined above was employed with a better measure of success. Leaf samples from both treatments of Whitney Farm lantana were inverted for three minutes in the glass flasks before exposure to the SPME fibre for 60 seconds. This was done in an attempt to displace the air in the flask and allow the heavier volatile gases to move to the bottom of the inverted flask. When this flask was then restored to the upright position, stronger detection of eluates from the fibres was achieved. The three major components were identified, two eluting at around 4:30 minutes and another at close to 10 minutes for both treatments (Figure 4.3 and 4.4). The chromatogram plots from both treatments registered very similar mass ranges of eluates.

Scan No: 1
Plotted: 1 to 3542
100%

RIC: 28932   Mass Range: 21 - 200
Range: 1 to 3542   100% = 53548

Figure 4.2 Chromatogram profile of uninverted flask headspace emissions by undamaged *Lantana camara* leaves from Whitney Farm variety. Solid Phase Micro Extraction fibres were exposed for 60 secs. The main chemicals are indicated (1-3).

Figure 4.3 Chromatogram profile from inverted flask headspace emissions by undamaged *Lantana camara* leaves from Whitney Farm variety. Solid Phase Micro Extraction (SPME) fibres were exposed for 60 secs. The main chemicals are indicated (1-3).

Two more replicates, using the inverted flask technique, were done for the Whitney Farm treatments both with similar outcomes to the original work shown in Figures 4.3 and 4.4. The profiles from the different replicates all revealed that no differences exist in the quality or type of chemicals eluting from damaged and undamaged Whitney Farm plants. There were some visible differences in the intensity of the main peak labelled 3 indicated by the y-axis, between the Whitney Farm damaged and undamaged chromatogram profiles. The compound designated as number 3 eluted consistently with an intensity of 30-35% when undamaged, but increased to an intensity of between 73-80% for damaged leaf samples (Figure 4.3 & 4.4). The intensities of the peaks for compounds 1 and 2 were similar for both treatments.
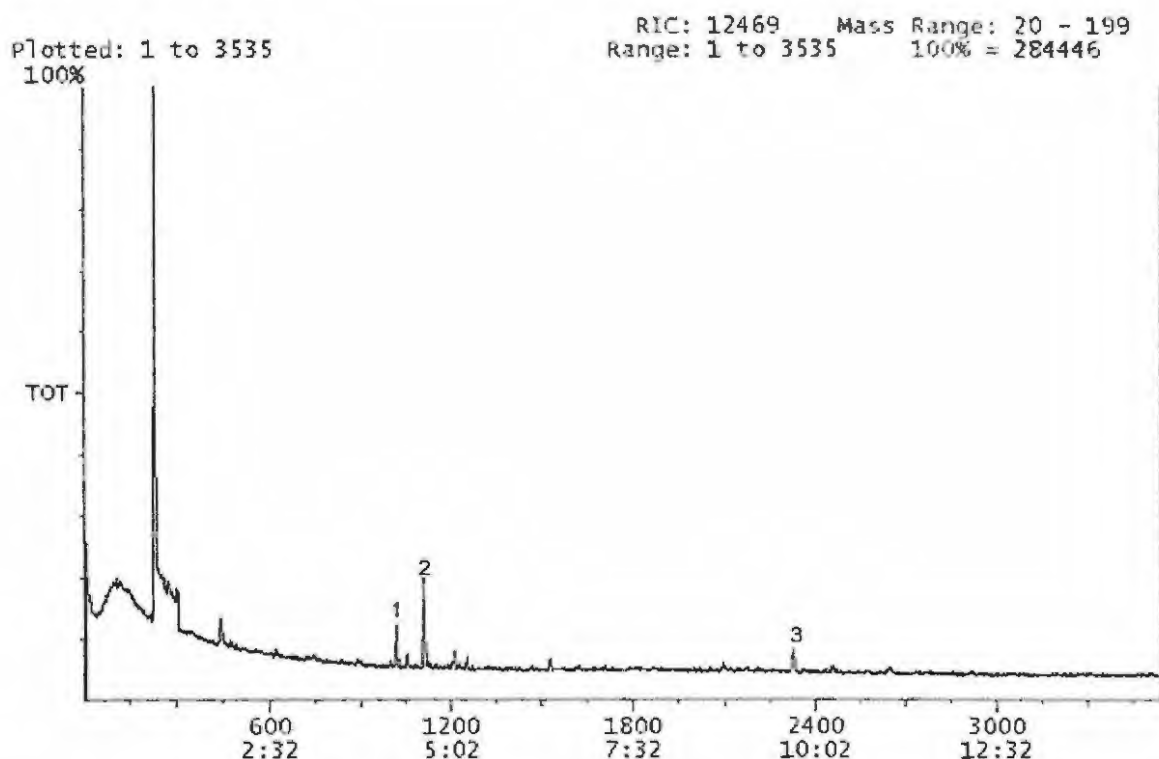
92
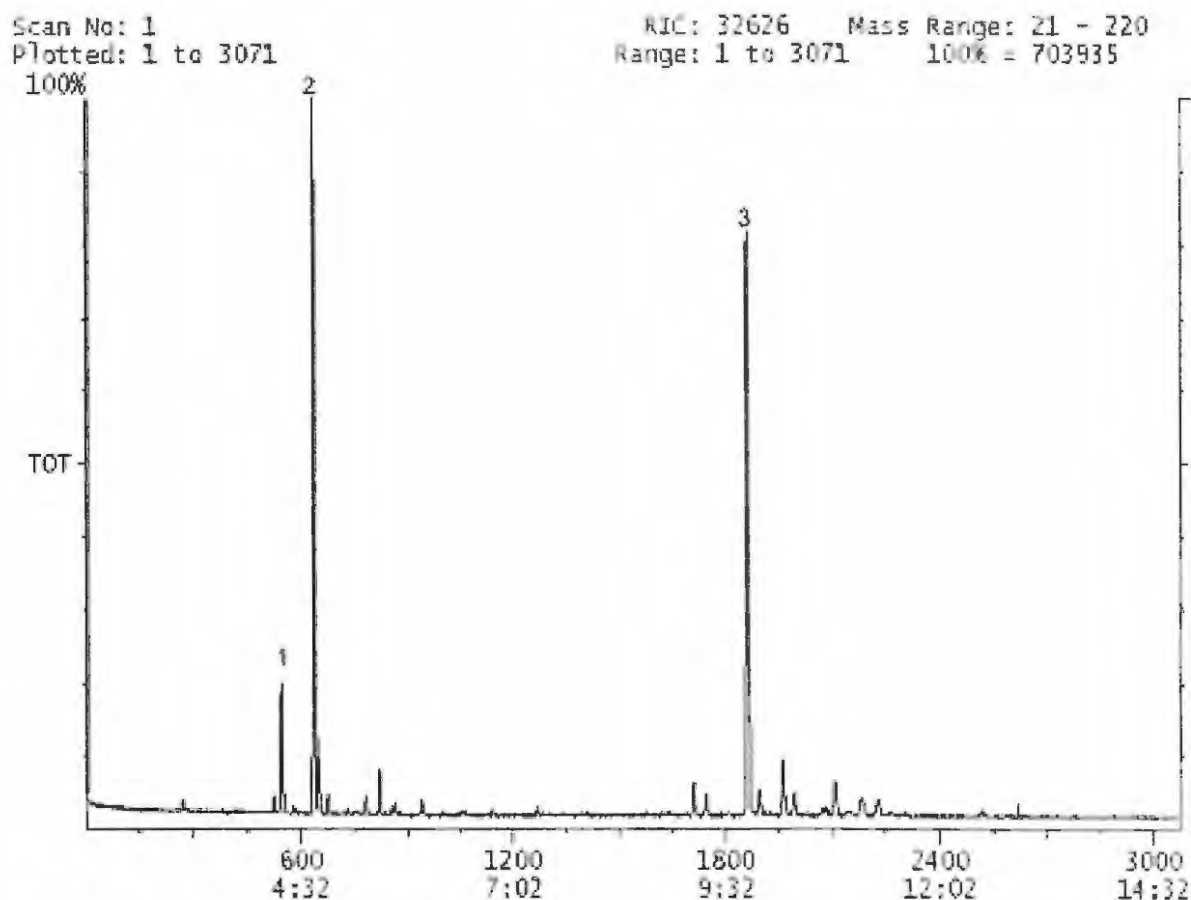
Figure 4.4 Chromatogram profile from inverted flask headspace emissions by damaged *Lantana camara* leaves from Whitney Farm variety. Solid Phase Micro Extraction (SPME) fibres were exposed for 60 secs. The three main chemicals are indicated (1-3).

Following the improved peaks from the inverted flask method used in the Whitney Farm plants above, this technique was used in all the East London volatile chemical analyses. Similar to the Whitney Farm profiles, leaves from the two treatments were also characterised by three main compounds 1, 2 and 3 (Figure 4.5 and 4.6), indicating that these compounds might be common among a number of South African lantana varieties. However this needs to be confirmed by assay or by testing more varieties. Two of these solutes eluted at about 4:24 and 4:34 minutes and another at 9:49 minutes. The mass ranges of eluates were similar between the profiles of the undamaged and damaged treatments. The intensities of peaks from the damaged and undamaged East London leaves were not very different for any of the three main compounds.

93
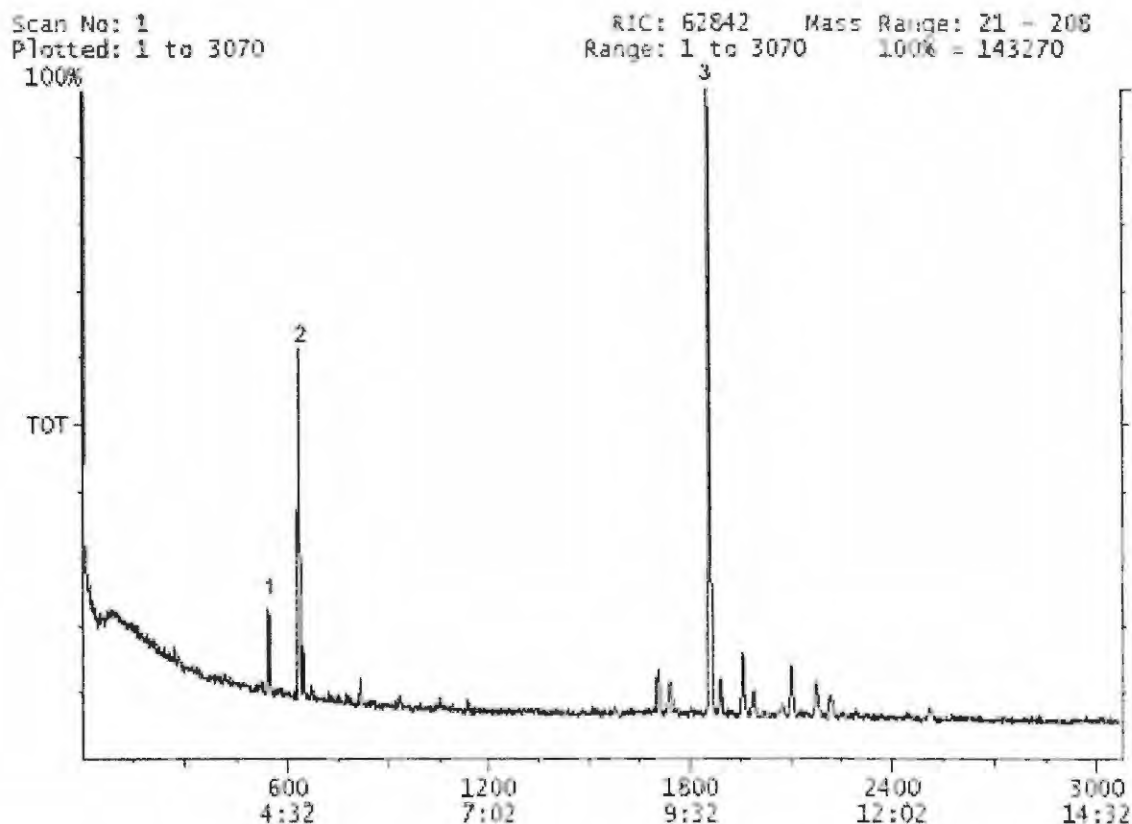
Figure 4.5 Chromatogram profile from inverted flask headspace emissions by undamaged *Lantana camara* leaves from East London variety. Solid Phase Micro Extraction (SPME) fibres were exposed for 60 secs. The main chemicals are indicated (1-3).

4.3.2 Identification and authentication of main compounds

Chromatograms consistently showed peaks indicating three main volatiles in treatment plants from both East London and Whitney Farm sites. A process designed to identify and authenticate these main compounds was initiated. The mass spectra of the peaks with retention times of 4:20 and 4:43 minutes were generated (Figure 4.7 and 4.8). The library search conducted from Rhodes University and from online source showed the possible compounds with the formula of $C_{10}H_{16}$ and a molecular mass of 136 (Tables 4.1 and 4.2). Purified samples (98%) of these compounds were acquired from Sigma and its mass spectrum generated (Figs. 4.10 & 4.11). Comparison of the mass spectrum of the 4:20 peak with both alpha-thujene and alpha pinene peaks mass spectra revealed some differences in these peaks and this confirmed that this was neither of these two standards.
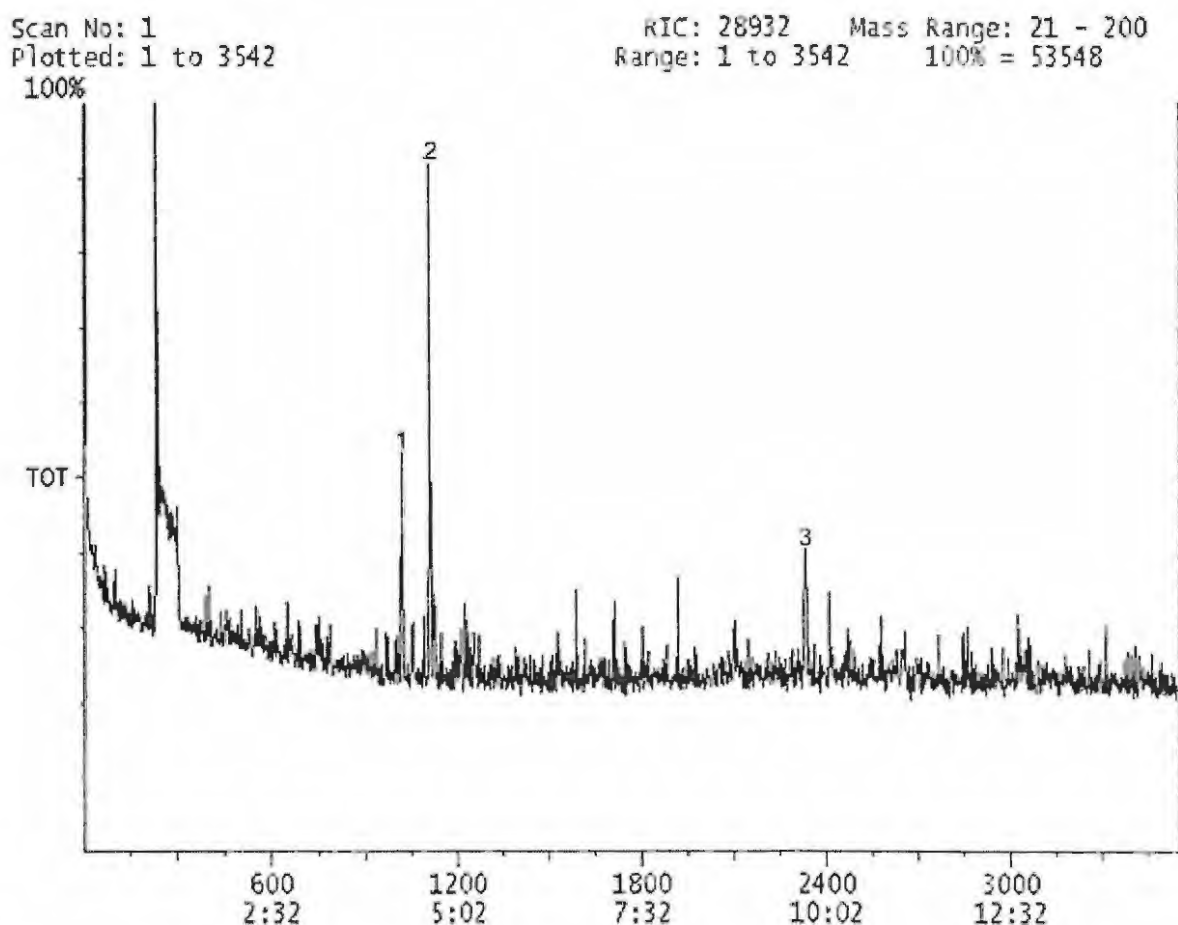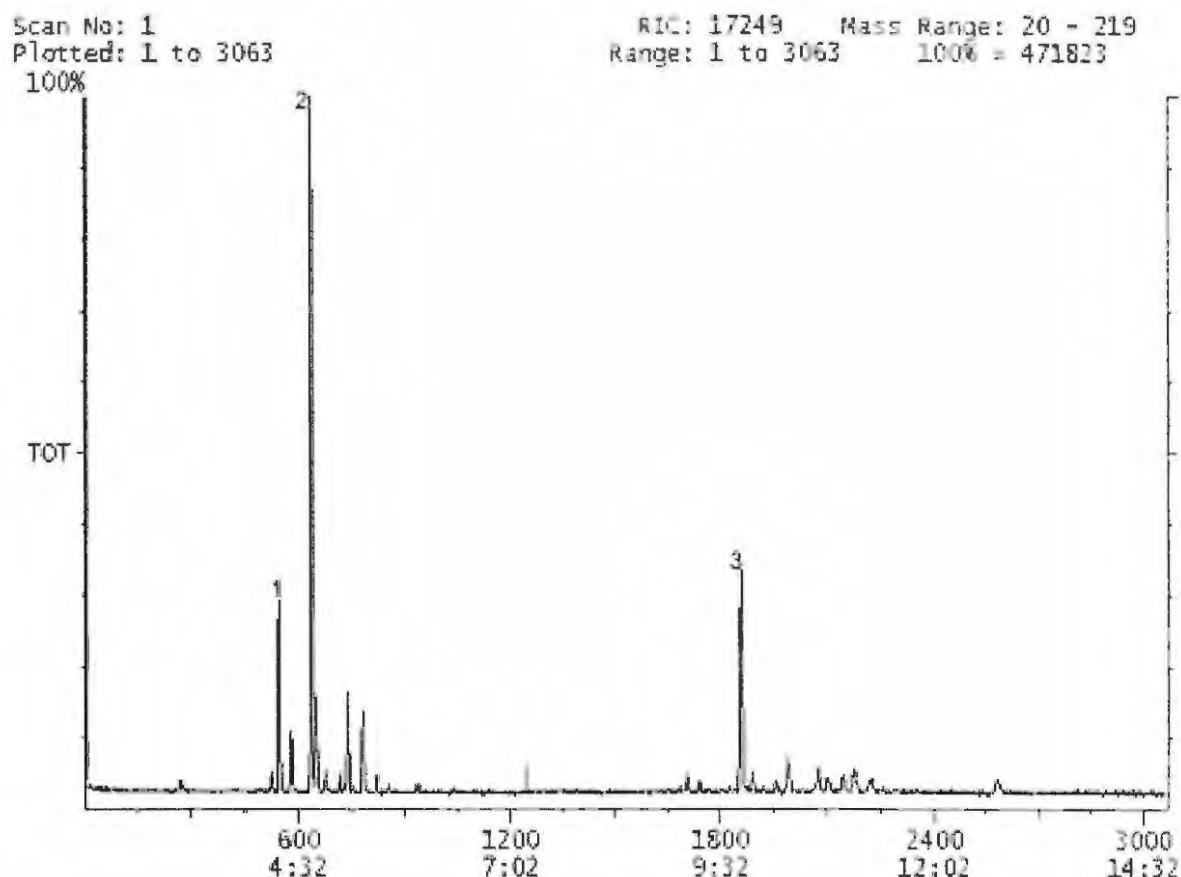
94
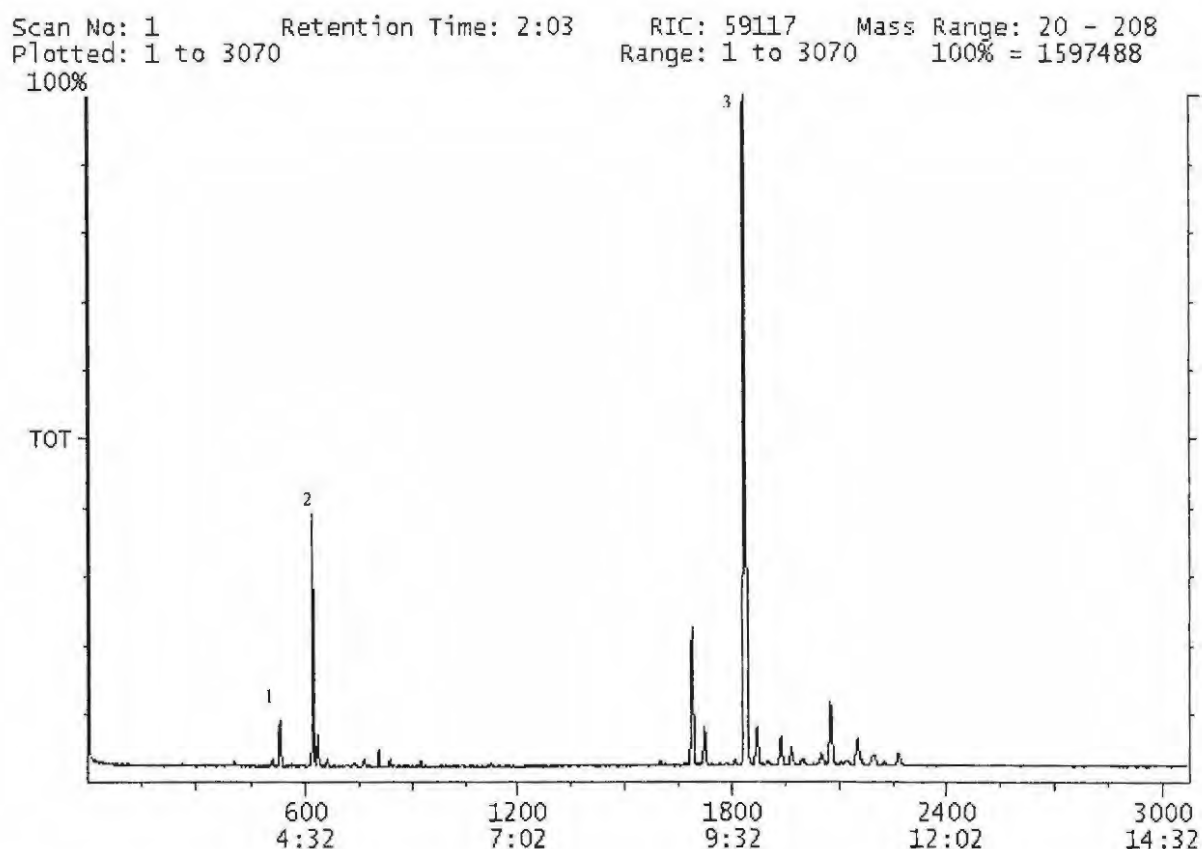
Figure 4.6 Chromatogram profile from inverted flask headspace emissions by damaged *Lantana camara* leaves from East London variety. Solid Phase Micro Extraction (SPME) fibres were exposed for 60 secs. The main chemicals are indicated (1-3).

A similar process was followed to ascertain the identity of compound 3. The mass spectra of the peak with retention times of 9:49 minutes were generated (Figure 4.11). The library search conducted from Rhodes University and from online source showed the possible compounds with the formula of $C_{15}H_{24}$ and a molecular mass of 204 (Tables 4.3). Purified samples (98.5%) acquired from Sigma were used to generate a mass spectrum (Figure 4.12). Comparison of the mass spectrum of the 9:49 peak with the beta-caryophyllene mass spectra confirmed that the 9:49 peak from leaf samples was beta-caryophyllene.
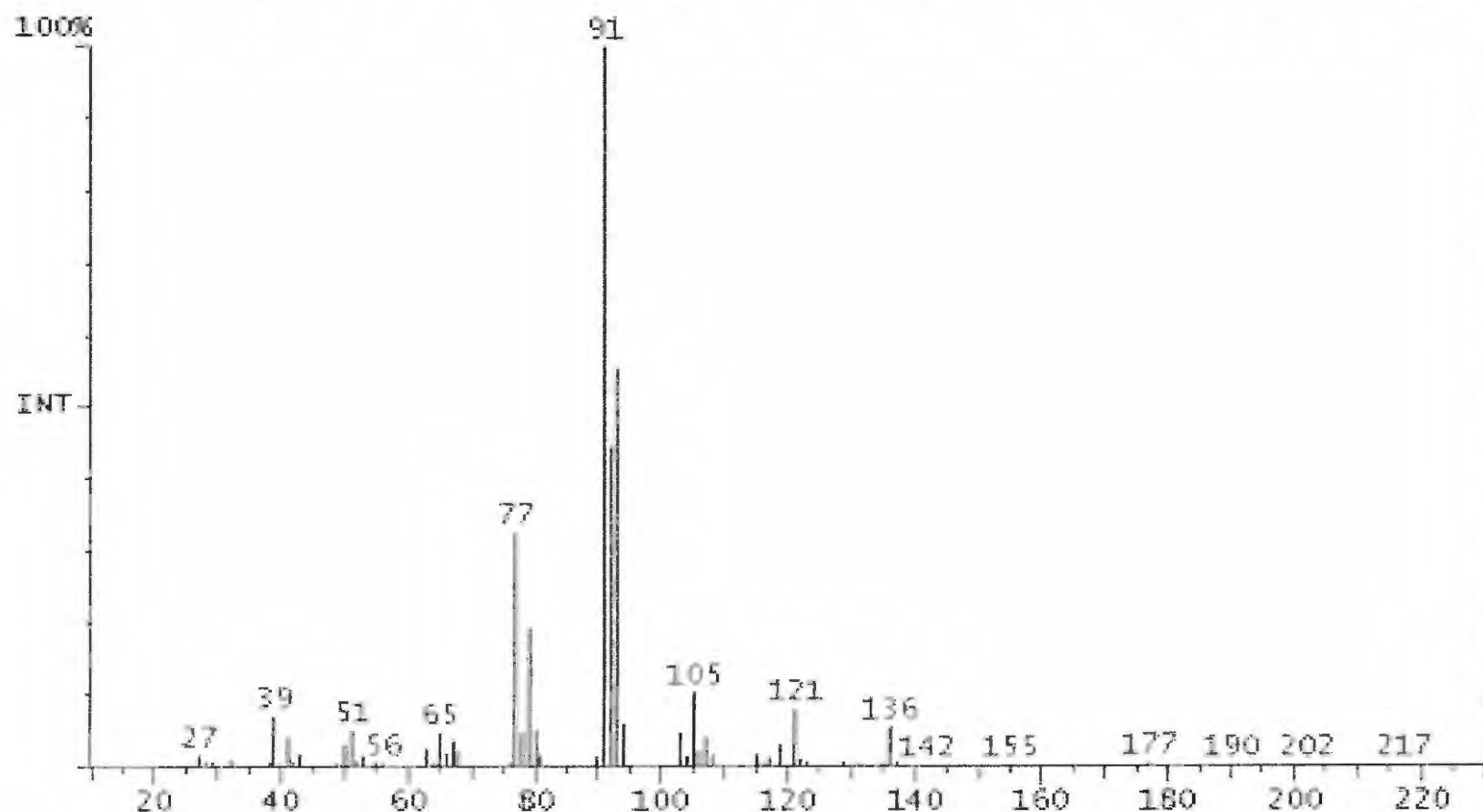
Figure 4.7 Mass spectrum of peak with a retention time of 4:20 minutes

96

Comment: ALPHA THUJENE   EX   TEUBES(88% pure)
Scan No: 503   Retention Time: 4:08   RIC: 433302433 ass Range: 21 - 203
Peaks: 157 Base Pk: 91   Ioniz: 60 us   Int: 99478016   100.00% = 99478016
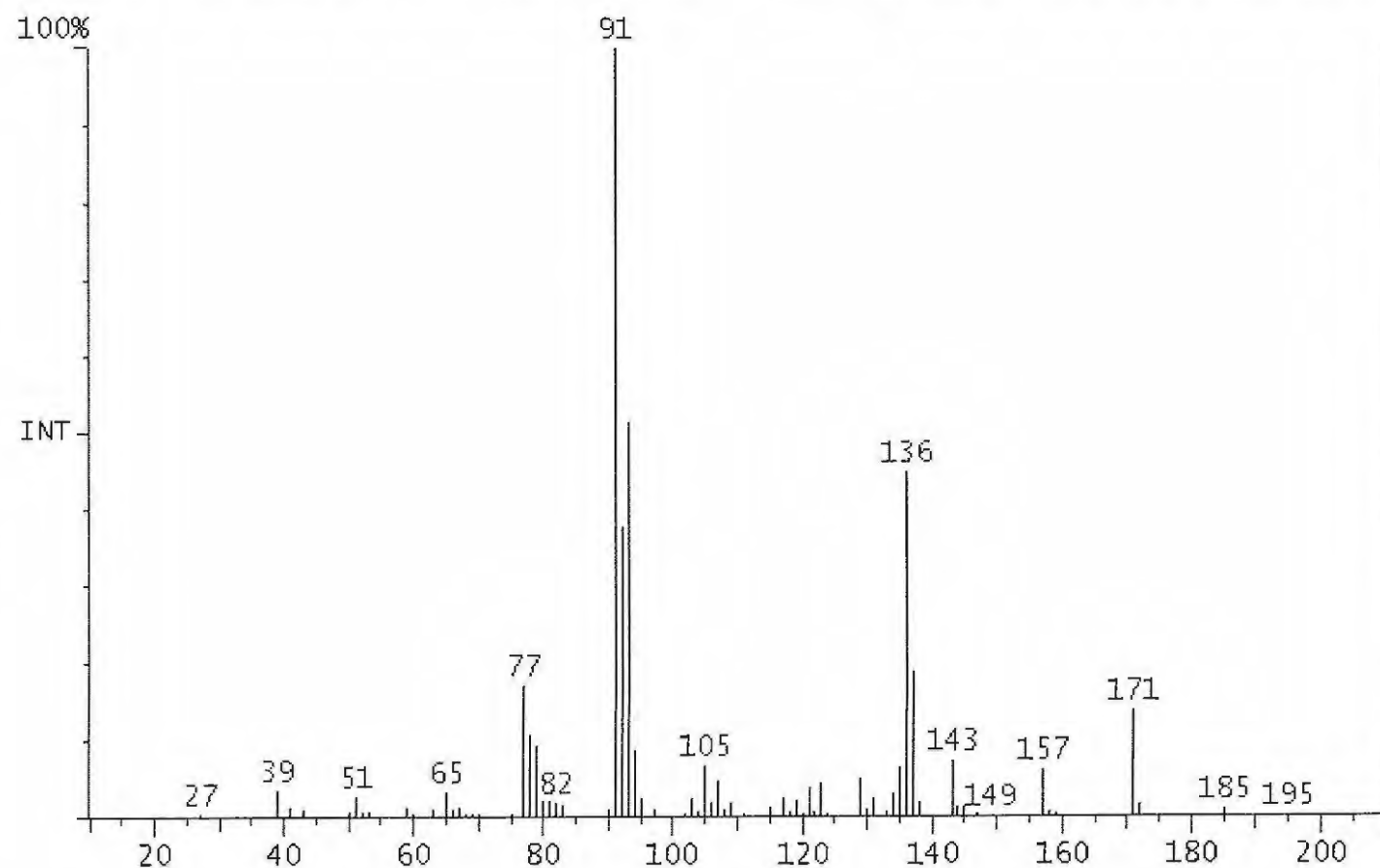
Figure 4.8 Mass spectrum of alpha-thujene standard peak with a retention time of 4:08 minutes.

97
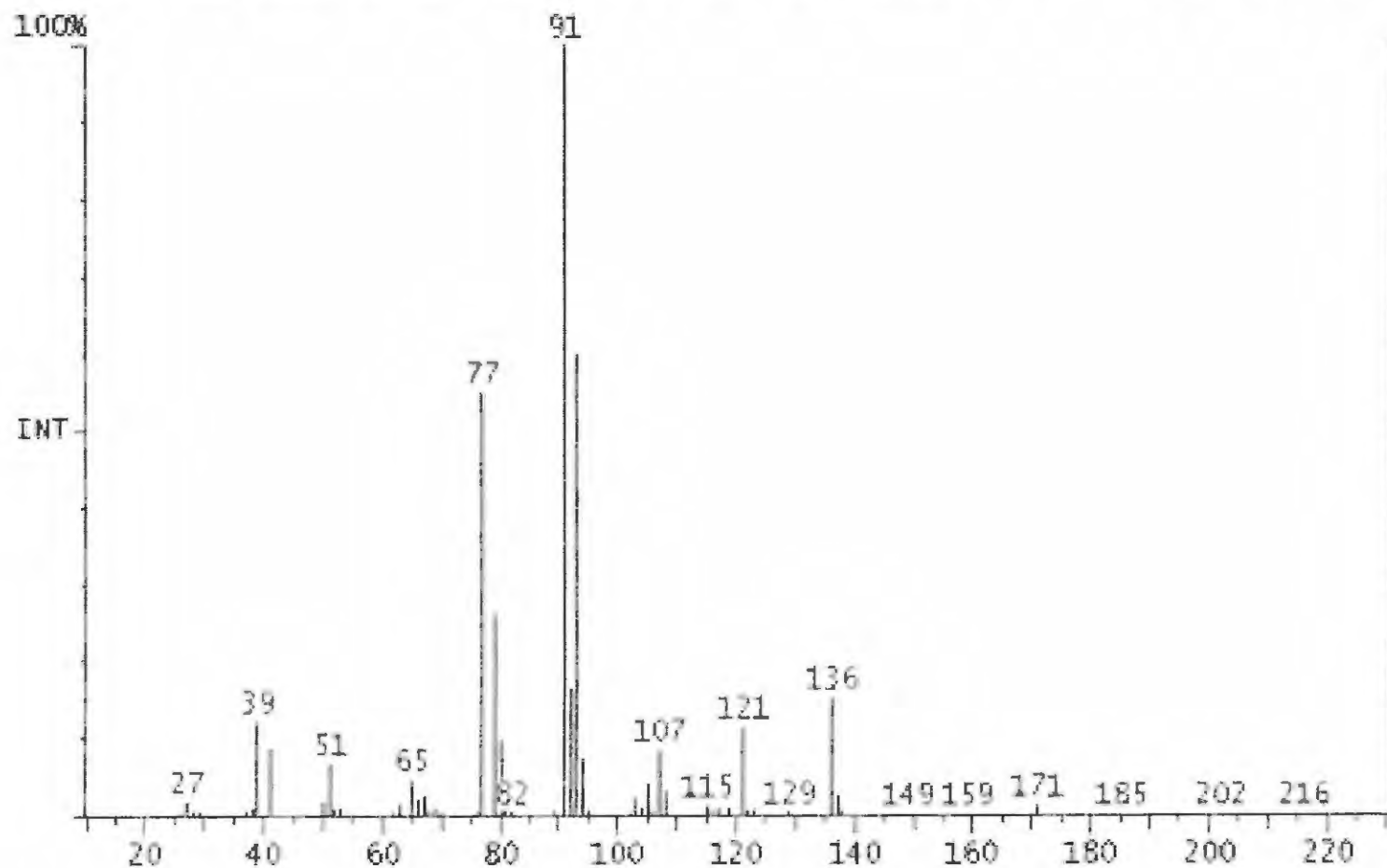
Figure 4.9 Mass spectrum of peak with a retention time of 4:43 minutes

98

Comment: ALPHA PINENE   ALDRICH 98%
Scan No: 524   Retention Time: 4:13   RIC: 189512977 ass Range: 20 - 208
Peaks: 129 Base Pk: 91   Ioniz: 60 us   Int: 45829888   100.00% = 45829888
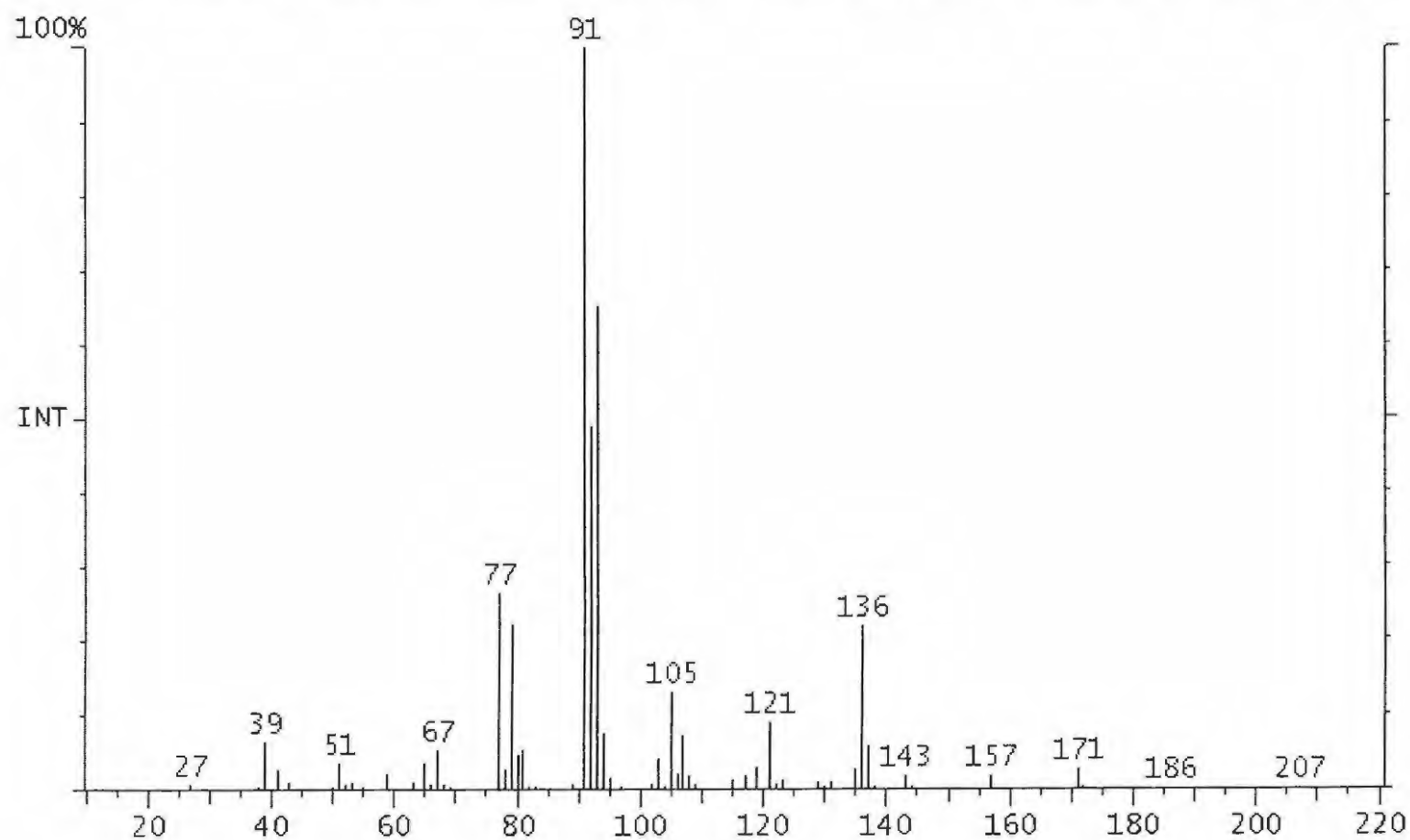
Figure 4.10 Mass spectrum of alpha pinene standard peak with a retention time of 4:13mins
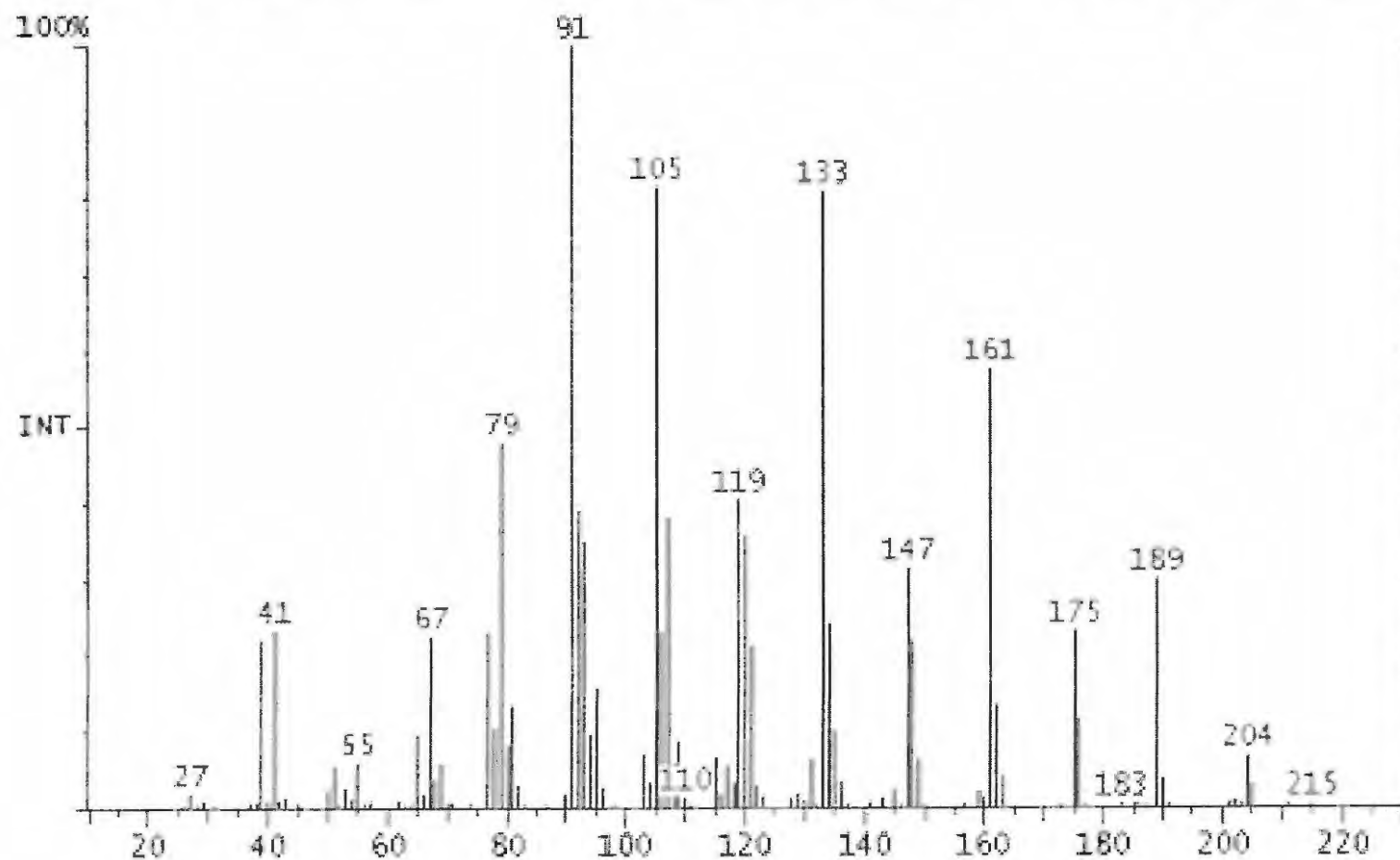
Figure 4.11 Mass spectrum of peak with a retention time of 9:49 minutes

100
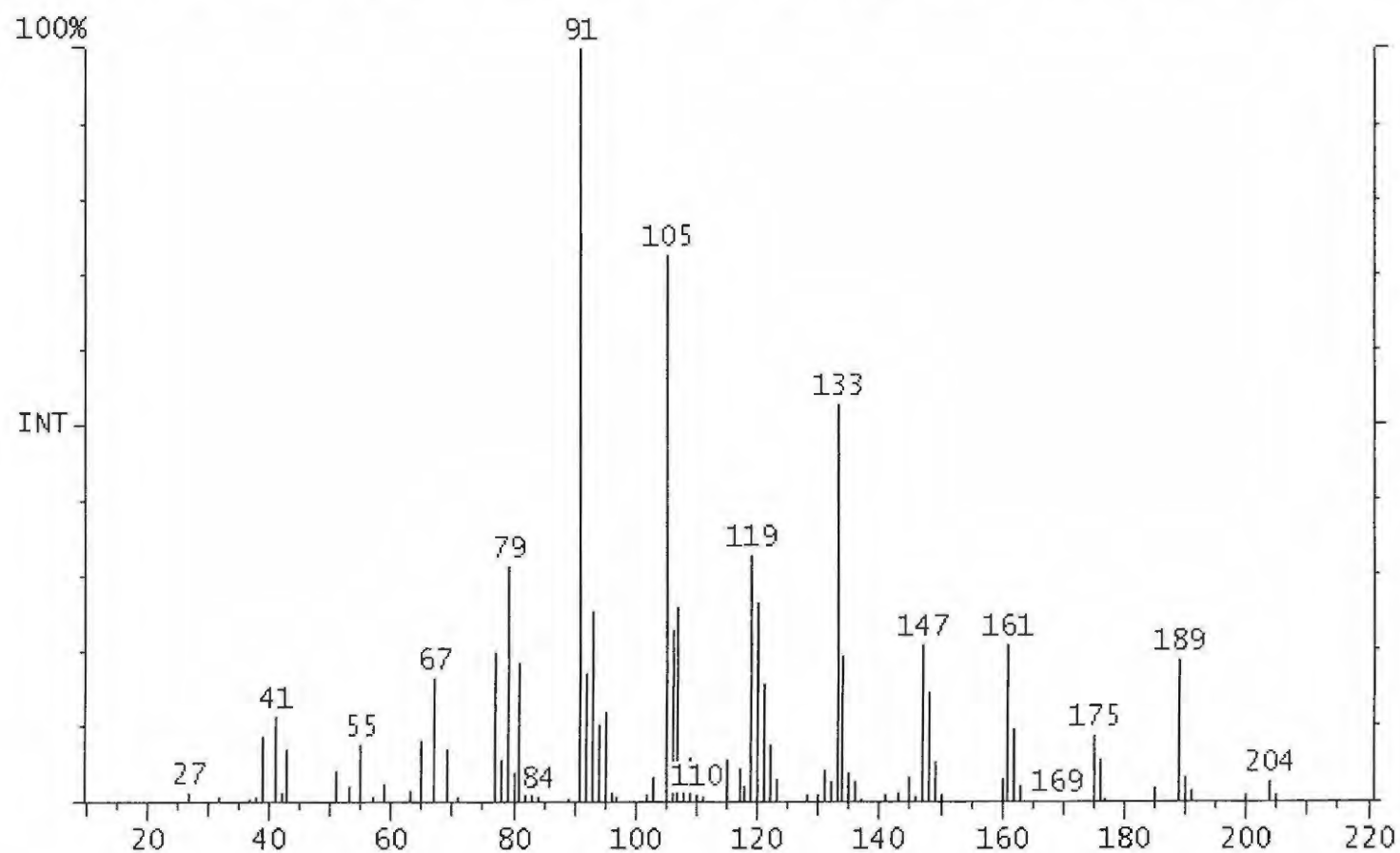
Figure 4.12 Mass spectrum of beta-caryophyllene standard peak with a retention time of 9:49 minutes.

Table 4.1 Library search results for possible compounds for 4:20 mins peak.

Compound Name	Number	Purity	Formula	Fit	Rfit	Molecular mass
Alpha-Thujene	3822	863	$C_{10}H_{16}$	898	873	136
Alpha-Pinene	3801	822	$C_{10}H_{16}$	849	833	136
Gamma-Terpinene	3802	821	$C_{10}H_{16}$	860	827	136
3-Carene	3805	779	$C_{10}H_{16}$	818	817	136
Cis-Ocimene	3803	787	$C_{10}H_{16}$	820	801	136
Alpha-Phellandrene	1296	777	$C_{10}H_{16}$	828	786	136
Tricyclene	3868	772	$C_{10}H_{16}$	807	796	136
Trans-Ocimene	3804	767	$C_{10}H_{16}$	802	788	136
3-Carene	1056	751	$C_{10}H_{16}$	859	760	136
Dipentene	1061	735	$C_{10}H_{16}$	860	735	136

Table 4.2 Library search results for possible compounds for 4:34 mins peak

Compound Name	Number	Purity	Formula	Fit	Rfit	Molecular mass
Alpha-thujene	3822	818	$C_{10}H_{16}$	861	848	136
Terpinolene	3826	797	$C_{10}H_{16}$	827	836	136
Alpha-pinene	3801	790	$C_{10}H_{16}$	828	819	136
3-Carene	3805	779	$C_{10}H_{16}$	818	817	136
Sabinene	3865	775	$C_{10}H_{16}$	814	814	136
A-Phellandrene	1296	767	$C_{10}H_{16}O$	888	785	152
Cis-Ocimene	3803	760	$C_{10}H_{16}$	801	798	136
Tricyclene	3868	753	$C_{10}H_{16}$	790	803	136
3-Carene	1056	751	$C_{10}H_{16}$	853	760	136

Table 4.3 Library results for possible compounds for 9:49 minutes peak

Compound Name	Number	Formula	Purity	Fit	Rfit	Molecular mass
Beta-caryophyllene	3828	$C_{15}H_{24}$	852	912	860	204
Beta-gurjunene	3960	$C_{15}H_{24}$	850	905	850	204
Valencene	3958	$C_{15}H_{24}$	845	887	850	204
Isocaryophyllene	3943	$C_{15}H_{24}$	843	899	852	204
5-Beta-H, 7-Beta, 10-Alpha-Selina-4(19),11-Diene	4022	$C_{15}H_{24}$	840	891	847	204
Beta-patchoulene	4014	$C_{15}H_{24}$	827	881	829	204
Alpha-gurjunene	3956	$C_{15}H_{24}$	824	893	829	204
Beta-chamigrene	4002	$C_{15}H_{24}$	813	877	824	204
Alpha-himachalene	3944	$C_{15}H_{24}$	804	859	808	204
Aromadendrene	3965	$C_{15}H_{24}$	801	853	810	204

4.4   Discussion

Results from our study do not show any increase in the intensity of East London *L. camara* volatile emissions following damage under the current experimental conditions. Other studies have shown that herbivore feeding and damage does not always result in an increase in volatile emissions. The impact of previous feeding by a weevil *O. vitiosa* was reported not to result in significant changes in volatile emissions by *M. quinquenervia* (Wheeler and Ordung 2006). Damage by gall-forming herbivores and the xylem feeding spittlebug also failed to induce volatile emissions from goldenrod plants (Tooker and Moraes 2007). An important point to consider in East London *L. camara* is whether it would have been beneficial to the plant to respond by increasing volatile compounds under the current circumstances.

In the previous chapters *L. camara* varieties, including East London, were shown to have a highly effective leaf quality response to feeding by *F. intermedia*. The induced leaf resistance led to a decrease in the performance of the biological control agent (Chapter 3). It may well be that for this variety more energy is allocated to the physical resistance traits, than the production of indirect defences to repel the herbivore or recruit its natural enemies. Chemical compounds released into the air by plants represent a permanent loss of considerable amounts of energy (Schoonhoven *et al.* 2005). The interaction between the mechanical defences, characterised by induced plant quality traits such as leaf toughness and trichome density, and the chemical defences, characterised by volatile terpenes and constitutive triterpenoids, is important to explore. *Lantana camara* is reported to be laced with antibacterial and feeding-deterrent constitutive triterpenoids, lantadene A and B (Ghisalberti 2000; Sharma *et al.* 2007). Their role in chemically defending plants in this study has not been explored but is likely to be important.

On the other hand profiles of volatiles from the damaged leaf samples of Whitney Farm showed a 2-fold increase in the emission intensity of compound 3 after feeding. Whitney Farm *L. camara* plants were shown to be susceptible to feeding by *F. intermedia* and did not respond to feeding by increasing their physical defences. The results suggest that

104

plants of this variety respond to feeding by increasing volatile emissions instead. This may be beneficial to the plants in one of two ways.

Firstly the increased emission of the compound may serve to attract parasitoids and predators against the feeding herbivores, which inevitably decreases the amount of feeding damage suffered by the plants (Tumlinson *et al.* 1993; De Moraes *et al.* 1998; Dicke and Vet 1999). Indeed the volatile compound increasingly emitted, compound 3 identified as beta-caryophyllene ($C_{15}H_{24}$), has been reported to play an important role in tri-trophic induced interactions. Beta-caryophyllene is often emitted in responses to herbivore damage in several wild relatives of maize (Gouinguene' *et al.* 2001). Parasitic wasps and entomopathogenic nematodes, enemies of *Spodoptera littoralis* larvae feeding on maize, are attracted by the emission of this chemical (Kollner *et al* 2008). Egg parasitoids of *Nezara viridula* are attracted to damaged bean plants with egg masses due to the increased emission of beta-caryophyllene (Colazza *et al.* 2004). Whether a similar response under different conditions, or by different lantana varieties following feeding may result in the attraction of the natural enemies of biological control agents, such as *F. intermedia*, needs further study under carefully designed experimental conditions.

Secondly, plants from this and other varieties may benefit from the increased volatile emissions through intra-specific communication. Volatile compounds emitted by Whitney Farm plants under high levels of herbivory may be received by plants in the vicinity. These messages may in turn help the receiving plants to prime themselves against attack by increasing physical and /or chemical defences (Agrawal 2005; Arimura *et al.* 2001; Dicke *et al.* 2003).

The work done in this chapter is also significant for another reason. This is the first volatile compound elucidated from any variety of *L. camara* in South Africa. Beta-caryophyllene is a natural bicyclic sesquiterpene that is a major constituent of a number of essential oils (e.g. clover, hemp, rosemary). Beta-caryophyllene is the main component in aerial parts of *L. camara* varieties isolated from India (Khan *et al.* 2002), south China

(Sundufu and Shoushan 2004), Iran (Sefidkon 2002), Cameroon and Madagascar (Ngassoum *et al.*1999). It has been used in the treatment of pain and inflammation, and displays local anaesthetic activity (Ghelardini *et al.* 2001; Gertsch *et al.* 2008).

Headspace analysis has been successfully used in the elucidation of a variety of different volatiles. Static headspace analysis is particularly effective in profiling volatiles in static headspaces of containers such as glass. This method may be limited by the inability of the fibre to absorb sufficient amounts of volatiles (Tholl *et al.* 2006), as was the case in chromatograms in figures 4.1 and 4.2. We successfully remedied this methodological limitation. By inverting the flask, we allowed the volatile gases, which are heavier than the air in the headspace, to settle at the bottom of the inverted container, which is the top when container is righted. Volatile absorption was also done by the fibre for a longer time when the container was righted. Shaking the glass container further resulted in an increase in the strength of the volatile signal on the gas chromatography machine, in line with reports that touch, motion and vibrations increase the emission of volatile organic compounds (Cipollini 1997; Arteca and Arteca 1999; Moran and Cipollini 1999). Although excising leaves may have influenced the emission of volatiles, the effect thereof would have been minimised by the leaves being immediately put into containers and the analysis being conducted within a short period of time.

Chapter 5

General Discussion

5.1   Introduction

Induced responses are widely reported in the plant kingdom and play a vital role in plant insect interactions. Of interest to this study were induced responses of an alien invader, *L. camara* due to feeding by a sap sucking insect, *F. intermedia*. The study was motivated by the reported establishment and subsequent collapse of populations of this biological control agent on *L. camara* in the Mpumalanga and Eastern Cape provinces of South Africa. After releases were made *F. intermedia* established on *L. camara* stands at these sites and built up to impressive numbers. Along with this colonisation were reports of widespread and premature defoliation of lantana stands similar to what was observed in laboratory and other field releases (Baars 2002; Heshula 2005; Heystek 2006). Observing the performance of *F. intermedia* many practitioners in the biological control fraternity heralded this agent as the silver bullet that would finally control *L. camara* in South Africa. Without any apparent reason the agent populations soon crashed, or persisted in low numbers.

A number of reasons have been suggested for the failure of *F. intermedia*. Climatic conditions have been suggested as the main reason for the lack of establishment in releases made in South Africa. This was clearly not the reason for the population crash as the mirid initially established in the warmer subtropical areas of lantana's distribution range mentioned above (Baars 2002; Heshula 2005). Differential preference and performance have also been reported for *F. intermedia* on different varieties of *L. camara*. Major differences in *F. intermedia* oviposition were reported in some South African and Australian varieties of lantana tested (Urban and Simelane 1999; Urban *et al*. 2003). Lack of *F. intermedia* establishment and success has also been ascribed to ant and spider predation, as well as low numbers of individuals released (Heystek and Olckers 2003). On the other hand, Baars (2002) argued that the contribution of varietal preference has been overestimated as the reason for the failure of biological control agents. There have been

107

no investigations into the effect of induced defensive responses of *L. camara* on the performance of *F. intermedia* which is the focus of this thesis.

Induced responses may firstly act by benefiting the emitter plant as a deterrent to feeding on one or all parts of the plant. Secondly, these responses may serve to increase feeding activity by the receiver insect herbivore on the plant. Finally, no obvious or discernable change to the plant or insect may occur due to feeding. Such interactions, individually or in combination, have a direct and significant bearing on the outcome of one environmentally important plant-insect interaction: namely, biological control of alien plants.

The current study has investigated and determined that induced responses of *L. camara* varieties due to previous damage by *F. intermedia* exist. Furthermore, I have been able to categorise the nature and effect of the induced responses in the different varieties of *L. camara* tested on *F. intermedia* as;

        a. Physical induced resistance in some varieties

        b. Induced defences in some varieties

        c. Increased volatile chemical emission on one variety, none on another

The above results and their implications are discussed in this chapter with a view to understanding how they may affect the biological control of *L. camara* directly, and possibly of other alien plants indirectly.

5.2   Induced responses of *L. camara* varieties to *F. intermedia* feeding

Results from our studies show clearly that previous *F. intermedia* feeding affects the performance of individuals released subsequently on some *L. camara* varieties (Chapter 2). Individuals on one of the varieties tested, Lyndhurst Farm, had significantly lower reproductive and feeding performance than when they were released on undamaged plants. This result suggests that these plants are able to induce resistance (Karban and Baldwin 1997). Such a response may not directly result in increased plant growth and

reproduction, but reducing the number of herbivores feeding should ultimately be beneficial. On varieties of lantana, some leaf-feeding agents such as *F. intermedia* spend their whole life cycles on host plants. Important physiological processes such as oviposition and feeding all occur on the leaves of these plants. If sufficient feeding pressure is exerted by the initially released or parental generation of agents a negative effect on subsequent feeding down the line might be expected.

Of 22 agents introduced for the control of lantana in South Africa thus far, only 13 have established (Tables 1.2). About 70% of all agents introduced fed on the leaves of *L. camara* by mining, sap-sucking, leaf chewing or petiole galling. It is not clear how common or widespread induced resistance is among the 40 varieties of *L. camara* varieties in South Africa (Graaff 1986). However it is plausible that susceptible insects released on varieties possessing the ability to induce resistance that these responses along with other factors such as excessive predation, parasitism, soil moisture and nutrients, and climatic conditions have the potential to reduce push populations over the edge leading to non-establishment. Other agents previously on lantana varieties (e.g. *T. scrupulosa* and *O. scabripennis*) may also have the ability to induce these and other responses, as a wide range of feeding mechanisms by different insects have induced resistance (Karban and Baldwin 1997). Assays with these agents would have to be conducted to ascertain this.

The number of agents released on *L. camara* also presents another possibility. This is the extent to which feeding induced responses by one agent affect feeding by another. Due to the limited success of biological control against lantana in South Africa, there has been an emphasis on importing additional biological control agents. Induced responses due to agents already established may have a negative influence on feeding by newly-released or dispersing agents. Simelane (2006) reported that feeding at moderate to high densities by an established leaf feeder, *T. scrupulosa*, negatively affected subsequent oviposition and survival of a recently released root-feeding agent, *Longitarsus bethae*. Although that study did not consider induced responses, it confirms the findings in this study that previous feeding on *L. camara* will definitely affect some agents.

Insects also possess the ability to overcome defensive responses by their host plants. Similarly insects may be specialist and always express their adaptation to plant defences, or the adaptations may be induced and only activated when necessary (Gatehouse 2002). *Lantana camara* varieties however have very complex interactions with their biological control agents. Firstly, the weed is a result of deliberate horticultural and field hybridisation over a long time in which it was away from its natural enemies (section 1.2.2). It is therefore unlike the *Lantana* species with which agents have co-evolved in the country of origin. Secondly, there is evidence that an invasive plant increased its toxicity upon re-association with a major herbivore (Zangerl and Berenbaum 2005). Overcoming the above two factors by newly released agents cultured from a genetically narrow pool might be difficult and might make it impossible for future generations to be selected against these responses. Susceptible agent populations are thus always on survival mode in these interactions and any minor ecological effect (predation, parasitism) may drive populations to extinction.

The responses to previous feeding were different on lantana varieties of Whitney Farm from these on the previously described Lyndhurst Farm. While previously damaged Lyndhurst Farm plants resulted in decreased agent performance (induced resistance), no significant differences in plant or agent performance were detected on previously damaged Whitney Farm plants. Thus far lantana varieties are classified by differences in morphology, flowers (size, shape and colour), leaves (size, colour and hairiness), and stems (degree of spinescence) (Smith and Smith 1982), or by their physiology, by differences in rates of growth, general vigour, chromosome number, degree of toxicity to livestock, fertility and cytology (Spies and Stirton 1982 a, b; Spies and du Plessis 1987). It is now also apparent that the differences in induced responses observed from this study are an additional feature that distinguishing varieties of lantana one from another. This is not a feature unique to lantana, as the genotype of other plants have been reported to be important in the type of response induced (McGuire and Johnson 2006, Endo *et al.* 2007). Future evaluations of *L. camara* and its biological control agents may gain very useful knowledge by considering the existence and effects of these reactions.

5.3   Nature of *L. camara* induced responses to feeding

Previous feeding on plants of two lantana varieties was shown to have a significant negative effect on subsequent mirid performance parameters. Following the discovery of such reactions to feeding it was important to characterise them. Extensive feeding by the test agent, *F. intermedia*, on different varieties of *L. camara* produced variable and never before reported induced responses. Three varieties responded by increasing physical barriers – leaf toughness and trichome density- to feeding against the biological control agent (Chapter 3). Performance of insect herbivores has been varied in response to increased leaf toughness, with both increased and decreased performance reported. Feeding, oviposition and survival by *F. intermedia* was significantly reduced on all the plants from these three varieties. Furthermore the population numbers of the agent were significantly negatively correlated with the increased trichome density, indicating a strong relationship in the increase in trichome density and the decrease in population numbers.

Under field conditions in releases made in 2001, *F. intermedia* did not establish in two of the lantana varieties showing induced resistance, Lyndhurst Farm and Port Alfred. Even though the agent established on East London lantana under field conditions, like the current laboratory study the populations persisted in very low numbers compared to Whitney Farm lantana. These responses may be one of the important factors responsible for these field results. The impact of induced resistance responses in combination with other environmental factors needs to be fully investigated under field conditions.

The induced resistance from Lyndhurst Farm and East London occurred within the same season that the damaged was inflicted. By five weeks from the time of initial damage a response was seen in experiments conducted (Chapter 2). This is significant as a response to feeding is only considered a defensive response if it gives benefit to the emitter. This quick response by lantana varieties may put early pressure on any agent released that may be susceptible. Additionally, there is an indication that the responses due to feeding activity were produced throughout the plants affected. The systemic nature of this response allows for whole plants to be protected from the feeding activity of any agent. In such cases, a signalling path is necessary from the point of impact or wounding to the part

111

of the plants not yet fed upon. The mechanisms and signalling pathways for this systemic effect was not examined in this study. In many studies conducted however the signalling pathway involved the use of jasmonic acid and its metabolites as phloem-based signals (Howe and Schaller 2008).

Plants from the Whitney Farm variety did not significantly increase leaf toughness and trichome density due to feeding activity. Plants from this variety not only supported higher numbers of individuals in both laboratory and field experiments previously conducted on the same variety, but showed along with other varieties a general trend of increased reproductive and vegetative growth as a consequence of feeding (Heshula 2005). The susceptibility of Whitney Farm lantana did not increase due to feeding by *F. intermedia* however. Susceptible varieties such as Whitney Farm, that do not have the ability to increase physical barriers, such as leaf toughness and trichome density, will be most suitable in establishment and meaningful suppression and control of alien plants. Under the current experimental conditions, however, a chemical response was produced in response to feeding in the Whitney Farm lantana, as opposed to East London lantana where results suggested no increase in the emission of beta-caryophyllene. This suggests that at least in these interactions there seems to be a relationship between chemical and physical defences. Plants from East London had shown the ability to induce effective physical defences following feeding and did not increasingly emit volatiles, while Whitney Farm plants were not able to induce physical defences and increasingly emit a volatile compound due to feeding.

The emitted volatiles may be important in attracting natural enemies such as predators and parasites of the agents to the location of biological control agents on lantana. Claims from different authors of the effect of predation and parasitism on the success of *L. camara* biological control agents are rarely backed by results from scientific inquiry (Day *et al.* 2003). Indeed besides reports of heavy *F. intermedia* egg predation by ants in a mass rearing facility (Alf Sephton; Alan Urban; PPRI, pers. comm.), and personal observations of *Polyrhachis gagates* Smith (Hymenoptera: Formicidae) ants preying on *F. intermedia* at Whitney Farm, the effect of parasitism and predation on *F. intermedia* has not been

scientifically quantified in South Africa. In the importation of natural enemies to control a weed in its naturalised area it is hoped that it will lose most of its natural enemies. Any heavy predation or parasitism is therefore likely to impact on the success of the agent. It is however clear that further studies are needed to investigate whether the increased emission of beta-caryophyllene significantly increases rates of predation in *L camara* varieties.

Alternatively volatiles from damaged plants that are emitted in the air may be detected by conspecific plants which then may prime themselves either chemically or physically against any oncoming feeding by dispersing agents. Although it appears that increasing physical barriers against plant attack is the first line of defence against feeding, the effect of increased and prolonged damaged, and a difference in the type of damage by the agent on the volatiles emitted needs further investigation. Furthermore the relationship between the physical and chemical responses may also be important in varieties of lantana where both these responses may occur.

This is the first study elucidating the volatile components of any *L. camara* published in South Africa. The identified compound, Beta-caryophyllene, is a major constituent in both East London and the Whitney Farm varieties of lantana tested. This compound has been isolated from several other lantana varieties worldwide and may yet prove to be the most abundant volatile oil constituent in a majority of the lantana found in South Africa. Should feeding increase its production in some other varieties when damaged, this may be an important factor in limiting the success of agents vulnerable to this volatile. Beta caryophyllene was reported to attract natural enemies of *Spodoptera littoralis* and the egg parasitoids of *Nezara viridula* (Colazza *et al.* 2004; Kollner *et al.* 2008), and may yet play this role in indirect defences by lantana varieties.

5.4   Factors affecting plant responses

It is hard to predict establishment and the realised host range of an agent by current pre-release evaluations under laboratory conditions. Predicting the onset, intensity and eventual effect of induced responses on any variety is difficult. A host of different biotic

and abiotic factors under field conditions may be significant in determining a response. The length of time that inducers initially feed on the weed may affect the response observed, as has been shown in other plants studied. Equally important as the duration of feeding on the plant is the intensity of feeding by the inducer, which may be linked to the number of inducers and the type of feeding by the agent. The greater the number of agents released initially, the greater is the damage more likely to be. In our experiments, the low number of insects released at the induction stages resulted in just moderate damage being inflicted by the inducers. Whether increased damage may have led to an intensification of observed induced resistance to the detriment of the herbivore, an increase of induced defence to the benefit of the emitting plants, a sudden or gradual change in emphasis on the type of response (physical to chemical) expressed, or some combination of these, needs further study.

Under field conditions the number of insects may in turn be affected by environmental factors prevailing during field releases. In reviews by Broughton (2000) and Day and Neser (2000) climatic conditions were singled out as the most important factor in the establishment and control by lantana biological control agents. Adverse climatic conditions may act directly on the physiology of released agents by slowing vital physiological processes, as well as indirectly by changing the plant-insect interaction (Baars 2002). Low temperatures have also been reported to reduce the potential rate of population growth in some insects (Harley *et al.* 1979, Cilliers and Neser 1991), leading to an increase in insect mortalities. Drought conditions that place pressure on the plants may result in a decreased likelihood of a response to feeding. More energy may be allocated to other plant functions such as growth, reproduction and survival. Tests conducted under field conditions to determine volatile emission also face major environmental obstacles. Besides feeding by herbivores, a number of other abiotic and environmental factors may elicit volatile emission. Plant responses may be induced by abiotic and environmental factors such as temperature, rainfall, sunlight and even drought. Additionally the responses may be biotically mediated by means of stimuli such as touch and/or vibrations.

The type of damage an agent inflicts on a weed is also quite important in determining the intensity of the damage, and thus its response. Chewing insects, such as caterpillars, that inflict substantial tissue damage, activate different plant response pathways than sap-sucking insects such as aphids and *F. intermedia* (Walling 2000). These factors in the appropriate quantities and combination may collude to surpass an "induction threshold" which may be necessary for a response to be observed as in other varieties.

In conclusion, it is clear from this study that some varieties of *L. camara* have the ability to induce defensive response against *F. intermedia* feeding damage. The ability to induce resistance to feeding by three of the tested varieties; namely East London, Lyndhurst Farm and Port Alfred, and to increasingly emit volatile compounds by the Whitney Farm lantana, is likely to be present in many other varieties of *L. camara*. Even though *F. intermedia* is a sap-sucking biological control agent and the fact that induced responses to various agents are specific (Agrawal 2000), this response is likely to also be triggered by and against chewing insects. It is unclear how many of the lantana varieties in South Africa possess the ability to induce these responses, or how many of the biological control agents are susceptible to these responses. Use of multiple agents on *L. camara* may even further compromise success in instances where one agent induces defences to the detriment of another. Despite the costs associated with inducing these responses, with little pressure from biological control agents on the population of the *L. camara* as a whole, lantana can afford this energy expenditure as the benefits of induction are greater.

Based on these results, it is likely that these responses may have played a role in decreasing the effectiveness of *F. intermedia*, thereby limiting the success of the biological control and management of this alien plant. Although the failure of *F. intermedia* cannot be fully placed at the door of these responses, it is quite clear that this agent is susceptible to some extent to the combination of tougher-leaved and hairier lantana. The report about heavy egg predation by ants also raises the possibility of induced volatiles attracting ants, spiders and other natural enemies to *F. intermedia*. The mechanisms leading to the failure of this biological control agent, now reported to be present in only one site in South Africa, still need to be studied and integrated. Whereas

115

induced responses of plants are an important and a prominent part of plant-insect interactions, studies looking at these issues have mainly been ignored within the biological control fraternity. This results in a gap in our knowledge and understanding of why and how insects succeed or fail. More studies looking at these interactions from a biological control of weeds context are therefore necessary.

CHAPTER 6

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118

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120

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133

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