Impact of biocontrol agents on *Lantana camara* L. (Verbenaceae) in the lowveld region of Mpumalanga, South Africa

By

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Declaration

I declare that this thesis is my own, unaided work. It is being submitted for the Degree of Doctor of Philosophy at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.

1st__day of __June_____2018_____at Johannesburg, South Africa

Abstract

Lantana camara L. (sensu lato) (Verbenaceae) remains one of the worst invasive alien plants in most tropical and subtropical parts of the world, including South Africa. Despite a concerted biological control (biocontrol) effort, with 45 biocontrol agents released against the weed worldwide since the early 1900s to date, *L. camara* control is far from satisfactory in most areas, including the study area. In 2012, during the initial stage of this work, a plant-ecological survey was conducted in riparian areas along the Sabie River, across an altitudinal gradient, and also in the adjacent forest plantation areas, in the province of Mpumalanga (South Africa). As a follow-up to two separate previous studies in the same area (1996/7 and 2005), aimed at determining the effectiveness of the '*Working for Water*'s (WfW) invasive alien plant (IAP) control programme, this work is another milestone in a long-term monitoring study. However, despite 16 years (1996/7-2012) of integrated IAP-control operations in the area, the WfW programme was only able to successfully remove larger overstorey IAPs, which opened-up the canopy and reduced competition, creating a conducive growing environment for an amalgamation of understorey IAPs, including *L. camara*, whose spread and densification were still on the rise. Biocontrol is regarded as a better alternative for long-term, sustainable and environmentally friendly IAP control, compared to the conventional mechanical and chemical methods. Most *L. camara* biocontrol agents introduced into South Africa have not yet had their full impact quantified under field conditions. This work is novel in that, for the first time, it quantifies the combined impact of the 'old plus new' suite of *L. camara* biocontrol agents, on the growth, reproduction and biomass of the weed under field conditions, in an inland area, through an insecticidal exclusion experiment, using carbofuran.

Five prominent biocontrol agents occur on *L. camara* at the study sites, namely the fruit-mining fly, *Ophiomyia lantanae* (Froggatt) (Diptera: Agromyzidae); the shoot-sucking bug, *Teleonemia scrupulosa* Stål (Hemiptera: Tingidae); the defoliating moth, *Hypena laceratalis* Walker (Lepidoptera: Noctuidae); the leaf-mining beetle, *Octotoma scabripennis* Guèrin-Mèneville (Coleoptera: Chrysomelidae); and the fungal leaf-spot pathogen, *cf. Passalora* sp.

(Chupp) U. Braun & Crous var. *lantanae*. During the course of this study, an additional agent, the flower-galling mite, *Aceria lantanae* (Cook) (Acari: Trombidiformes: Eriophyidae), was released and successfully established at lower altitudes (~843 m), showing an affinity for the dark-pink *L. camara* variety over others in the study area, namely light-pink and red-orange.

Agent impact was difficult to measure because the activity of carbofuran in exclusion plants (carbofuran-treated *L. camara* plants) was short-lived; and therefore the impact of biocontrol agents on *L. camara*, which appeared to be negligible, may have been underestimated. Despite failing to maintain the 'exclusion' plants biocontrol agent-free through the application of carbofuran, there were reductions of 28% in the number of side-stems per plant, 31% fewer seeds in the soil seedbank, and 29% lower seed production, in 'biocontrol' plants compared to 'exclusion' plants. Although these differences were not statistically significant, they suggest that the present suite of biocontrol agents slightly reduces the vegetative and reproductive growth of *L. camara*. To achieve significant biocontrol of *L. camara* in inland areas, it seems necessary to introduce additional agents, which are well adapted to inland climatic conditions.

The effects of micro-environmental factors, namely altitude and the degree of shading, were also investigated. Some biocontrol agents, such as *T. scrupulosa*, exhibited feeding phenological plasticity, resulting in it maintaining its presence at different altitudinal levels throughout the seasons. The performance of the suite of biocontrol agents, except *A. lantanae*, was, also, not limited by plant varietal differences. Additional research on biological and integrated control of *L. camara* is required.

Keywords: Biocontrol; Biological invasion; Carbofuran; Insecticidal exclusion; Invasive alien plants; *Lantana camara*; Post-release evaluation.

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"…na sakilili hama kud'eyi Katembo, Mwini Kansas Kazol, Mweni Ngidju, Ngidju ya panshi, yakwiulu ya yeni, ami Mwin'amwana, Chinga namwana Kajiba, Kajiba ka mwinshinda, Ngoni wa milemba ntenkantenka…"

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Glossary

Agent/biocontrol agent

Herbivorous arthropod, either introduced from an IAP's country of origin or naturally occurring in the invaded country, acting as a natural enemy of the IAP

Biocontrol plants

Plants not treated with insecticide, but allowed free insect infestation

cf.

From the Latin 'conferatur', meaning compare with

Clearing

Felling IAPs at ground level combined with simultaneous herbicidal treatment of the cut stumps

Control

Suppressing an IAP population, through mechanical plus chemical means, comprising clearing and subsequent follow-up treatment, and/or the activity of biocontrol agents

Establish

A biocontrol agent is established when its population is self-sustaining on its host plant over several years

Exclusion plants

Plants treated with carbofuran to remove biocontrol agents from them and keep them insect-free

Follow-up treatment

Hand-pulling or foliar herbicide spraying of IAP regrowth after the initial clearing

IAP

Invasive alien plant refers to a non-indigenous plant species posing a threat to indigenous vegetation, biodiversity, agriculture and human livelihoods

Insecticidal exclusion

Use of insecticides to prevent biocontrol agents from surviving on plants

***Lantana camara* variety**

Plant entities distinguishable by the colours of their mature flowers

Performance

Biocontrol agent’s extent of feeding damage

Release

Introducing an agent onto its host plant to act as a natural enemy/for control

Chapter One: General Introduction

1.1 Background

Invasive alien plants (IAPs) are detrimental to biodiversity as they reduce the abundance and diversity of indigenous plant species (Hejda *et al*. 2009; Powell *et al*. 2011), change the structure and functioning of ecosystems (van Wilgen, 2012; van Kleunen et al. 2015), and thus negatively affect ecosystem services, agriculture and human livelihood (Pejchar and Mooney, 2009; Early et al. 2016). Worldwide, efforts have been put in place to mitigate the adverse impacts of IAPs on ecosystems (McNeely *et al*. 2001; Simberloff, 2011); and such efforts address the reduction and/or prevention of new and emerging weeds, while controlling existing ones (van Wilgen *et al*. 2012). In many parts of the world, management strategies to control IAPs are legislated to ensure enforcement (Wittenberg and Cock, 2005; van Wilgen *et al*. 2012). In South Africa, there is just such a nation-wide, government-sponsored IAP control programme known as the "*Working for Water*".

The *Working for Water* (WfW) programme, originally of the South African Department of Water Affairs and Forestry (DWAF); and now of the Department of Environmental Affairs (DEA) under the National Resource Management programme (NRMP), was created in 1995 with the mandate to clear invasive alien plants nationwide (Garner, 2006; Beater, 2006; Beater *et al*. 2008), and in particular riparian woody invaders (Macdonald, 2004). The main objective of the WfW programme is to prevent water loss due to the presence of IAPs. Secondly, through this programme, the government intended to address poverty alleviation by creating jobs (Working for Water, 1999). This goal has successfully been reached with 20,000 job-opportunities created annually (van Wilgen *et al*. 2012). Despite its financial might, with an estimated running cost of 3.2 billion Rands from 1995 to 2008, it has been argued though that WfW has failed to deliver optimally on its primary goal, which is to reduce alien plant invasions and to restore ecosystems countrywide (van Wilgen *et al*. 2012). In the face of an increasing threat to biodiversity due to biological invasions, the use of biological control (biocontrol) in conjunction with conventional control methods, i.e. chemical and mechanical control, in other words integrated control, is advocated

for selected priority alien plants (Zimmermann *et al*. 2004; Moran *et al*. 2005), such as *Lantana camara* L. (Verbenaceae).

1.2 *Lantana camara* L. (sensu lato) (Verbenaceae)

Lantana camara is one of the most ecologically and economically damaging invasive alien plants in tropical, subtropical and temperate parts of the world (Baars and Neser, 1999; Day *et al*. 2003a). *Lantana camara* is reported to occur in over 60 countries worldwide (Parsons and Cuthbertson, 2001). This perennial, floriferous, woody shrub is native to the tropical and sub-tropical south and central regions of the Americas (Kannan *et al*. 2013). It is one of the world's worst introduced understorey weeds (IUCN, 2001) and is highly invasive, occurring in a wide array of habitats under a wide range of climatic and environmental conditions (Thomas and Ellison, 2000). It has distinctive multicoloured inflorescences (flower clusters), which can bloom all year round given warm and wet conditions (Day *et al*. 2003a). *Lantana camara* is a complex of species and hybrids whose centre of origin is not certain, and its taxonomy is extremely difficult to resolve (Day *et al*. 2003a; Sanders, 2006).

1.2.1 *Lantana camara* taxonomy and varieties

The taxonomy of the genus *Lantana* L. (Verbenaceae) is basically as follows. There are four distinct groups or sections within the genus *Lantana*, namely *Lantana* section *Calliorheas, Lantana* section *Sarcolippia, Lantana* section *Rhytocamara,* and *Lantana* section *Camara,* and the first three sections contain species most closely related to *Lippia* (Day *et al*. 2003a), which also occurs in South Africa. The fourth group, *Lantana* section *Camara*, comprises many species, including *L. camara* Linn. and *L. nivea* Vent., which have the propensity to freely hybridize (Sanders, 2006). In the 17th century, species of *L. camara* were first introduced into Europe from Brazil and the West Indies as ornamental plants, and they have since undergone extensive horticultural modification, with more than 650 hybrid varieties now existing throughout the world (Howard, 1969). This work will concentrate on "the weedy taxa" within *Lantana* section *Camara,* which

are collectively referred to as '*Lantana camara* L. (sensu lato)'or 'lantana' (Day *et al*. 2003a; Sanders, 2006; Urban *et al*. 2011).

Lantana camara exists in multiple forms (Parsons and Cuthbertson, 2001), also referred to as cultivars (Howard, 1969), biotypes (Swarbrick, 1986), subspecies or varieties (Anon., 1962), and it comprises different suites of varieties at different geographic locations in its naturalized range, which differ from those in its native range (Smith and Smith, 1982). Factors considered to differentiate one variety from another include toxicity and susceptibility to herbivory (Smith and Smith, 1982), flower colour, hairiness of stems and leaves (Heystek, 2006). Based on DNA analysis done in Australia, Scott *et al*. (2002) found no relationship between flower colour and plant variety. They, however, found more genetic similarity between *L. camara* varieties occurring in the same geographic location, despite their differences in flower colour. This suggests that flower colour alone is insufficient for differentiating *L. camara* varieties, but because of the difficulty in running DNA identifications for each and every *L. camara* plant in the field (Spies, 1984), place of occurrence plus colour of the mature flower in the inflorescence are conveniently used as varietal names (Scott *et al*. 2002; Day *et al*. 2003a).

The work presented in this thesis focuses on *L. camara* plant 'varieties', namely the dark-pink and the light-pink, which occur in abundance in the study area, but also are among the most widespread *L. camara* varieties in Mpumalanga and KwaZulu-Natal Provinces in South Africa (Williams, 2006), having various negative impacts on the environment.

1.2.2 Impacts of *Lantana camara*

Lantana camara has the ability to grow into impenetrable thickets, transform landscapes, displace indigenous species, hinder succession and decrease biodiversity (Day *et al*. 2003a; Gooden *et al*. 2009). Its invasion brings about a noticeable structural and floristic change in the invaded forest landscapes (Day *et al*. 2003a), resulting in a loss of species richness (Fensham *et al*. 1994) and a decline in plant diversity (Foy and Inderjit, 2001).

Lantana camara poses a serious threat to agriculture, especially in Africa, southern Asia and Australasia, where farming activities contribute significantly to the economy (Day *et al*. 2003a). It grows in pastures, forming large, impenetrable thickets, which displace desirable grazing plant species and therefore lower the productivity of stock farming. *Lantana camara* is also poisonous to cattle, buffalo, sheep, goats (Sharma *et al*. 1988), horses and dogs (Morton, 1994), guinea pigs and captive red kangaroos (Johnson and Jensen, 1998). In South Africa, the impact of cattle mortality from *L. camara* was estimated at R1.7 million per year (Kellerman *et al*. 1996).

1.2.3 *Lantana camara* in South Africa

Eighty percent of the *L. camara* infestation in South Africa was found in KZN (Wells and Stirton, 1988), which experiences a subtropical climatic. The weed has extended its range in the provinces of Limpopo (LP), Mpumalanga (MP), Gauteng (GP), North West (NW), KwaZulu-Natal (KZN), Eastern Cape (EC), and the southern part of Western Cape (WC) (Fig. 1.1) (Urban *et al*. 2011). It infests approximately 572 000 ha of the landscape, including riparian areas, and, if condensed, would completely cover 32 000 ha (Kotze *et al*. 2010).

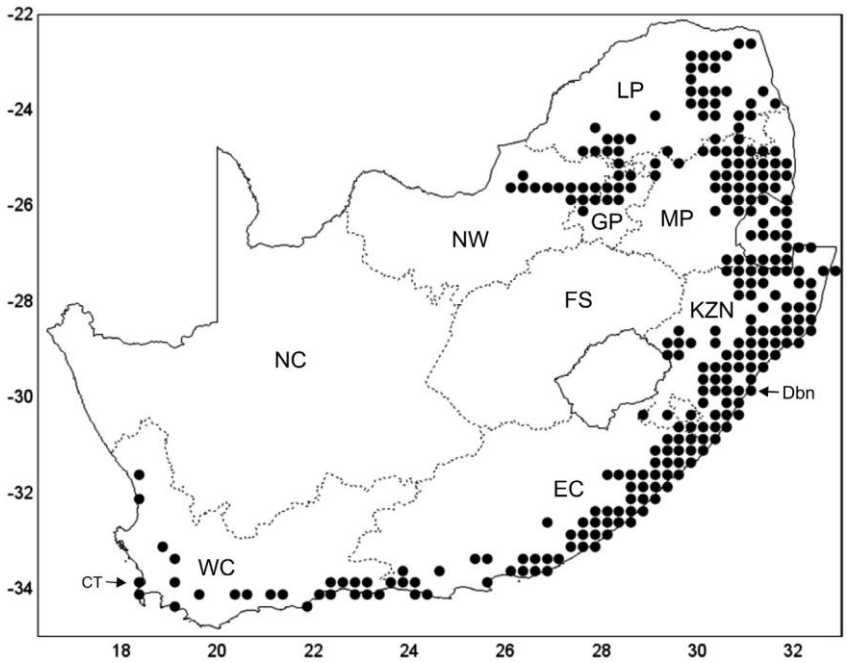

Figure 1.1 Distribution of *Lantana camara* L. (sensu lato) in South Africa, drawn by L. Henderson from the SAPIA database of ARC-PPRI (Urban *et al*. 2011).

Lantana camara can be controlled through chemical, mechanical, and/or biological methods; however, the present study mainly focused on biocontrol. Despite all the established biocontrol agents in many countries worldwide, it is argued that biocontrol of *L. camara* is still ineffective in many parts of the affected regions, i.e. the weed is neither killed nor is its spread halted (Johannsmeier, 2001; Zalucki *et al*. 2007); with the exception of some islands in the Pacific (Muniappan *et al*. 1996). However, it has been argued that in the absence of biocontrol agents, the rate of spread and densification of *L. camara* would have been much greater than it is currently (Cilliers, 1987b; van Wilgen *et al*. 2004; Zalucki *et al*. 2007). Biocontrol is nonetheless seen worldwide as a better alternative pest control method *in lieu* of the use of herbicides (Waterhouse and Sands, 2001).

1.3 Biocontrol

Biocontrol has been re-defined from DeBach's (1974) definition as 'the use of living organisms for the suppression of the population density or impact of a specific pest organism, making it less abundant or less damaging than it would have otherwise been' (Eilenberg, *et al*. 2001). It consists of using live carnivorous or herbivorous agents such as predators, parasitoids or pathogens to control another living organism that is considered to be a pest outside of its natural range. In classical biocontrol the emphasis is placed on the agents' ability to permanently establish and self-sustain for the long-term control of a target pest (Eilenberg, *et al*. 2001). This control approach was first employed in the 1800s, hence the name "classical", in California with the successful control of the cottony cushion scale *Icerya purchasi* Mask. (Homoptera: Margarodidae) using the predatory coccinellid *Rodolia cardinalis* Mulsant (Coleoptera: Coccinellidae) (Greathead, 1994; Eilenberg *et al*. 2001).

Examples of successful biocontrol include the control of the prickly pears, *Opuntia* spp., with the pyralid moth, *Cactoblastis cactorum*, the coconut moth; *Levuana iridescens*, with the tachinid fly, *Bessa remota*, in Fiji (Caltagerone, 1981; Eilenberg *et al*. 2001); Crofton weed, *Ageratina adenophora*, with the stem-galling fly, *Procecidochares utilis* (Tephritidae) in New Zealand (Fowler *et al*.

2000) and to some extent in South Africa (Buccellato *et al*. 2012); and the red water fern, *Azolla filiculoides* (Lamarck (Azollaceae), with the frond-feeding weevil, *Stenopelmus rufinasus* Gyllenhal (Coleoptera: Curculionidae) (McConnachie *et al*. 2003).

One of the drawbacks of biocontrol, however, is the difficulty in predicting the impact of agents on the target species before their release and establishment. Research shows that less than 40% of introduced potential biocontrol agents against weeds and insects yield significant control (Waage and Greathead, 1988; Williamson, 1996). About 130 countries are actively employing biocontrol in the fight against IAPs worldwide, involving a total of 550 biocontrol agents (Winston *et al*. 2014). In South Africa alone 93 biocontrol agents, comprising insects, mites and pathogens, are targeting 59 IAPs for control (Klein 2011, updated 2016: http://www.arc.agric.za/arc-ppri/Documents/Target weed species in South Africa.pdf), placing the country among the five front runners in the practice of biocontrol worldwide (Moran and Hoffmann, 2015). There is, however, a need for extensive experimental studies to measure the impacts of biocontrol on introduced species, especially the most problematic ones, such as *L. camara*.

1.3.1 Biocontrol of *Lantana camara* in South Africa

In 1961, five *L. camara* biocontrol agents, the fruit-mining fly, *Ophiomyia lantanae* (Froggatt) (Diptera: Agromyzidae); the shoot-sucking bug, *Teleonemia scrupulosa* Stål (Hemiptera: Tingidae); the leaf-tier moth, *Salbia haemorrhoidalis* Guenée (Lepidoptera: Crambidae); the leaf-defoliating moth, *Hypena laceratalis* Walker (Lepidoptera: Noctuidae); and the catabena moth, *Neogalea sunia* (Guenée) (Lepidoptera: Noctuidae) were imported into South Africa from Hawaii (Baars and Neser, 1999; Baars *et al*. 2003). It is argued that two of the five agents, *O. lantanae* and *H. laceratalis* were already present in South Africa at that time; *O. lantanae* was introduced unintentionally with the host plant, while *H. laceratalis* was believed to be indigenous to Africa (Cilliers and Neser 1991; Urban *et al*. 2011).

According to Winston *et al.* (2014), *L. camara* biocontrol includes about 45 biocontrol agents worldwide, with 13 of these established in South Africa (Table

1.1). Several biocontrol agents have been reported to inflict considerable damage on *L. camara*, although not necessarily to the extent of bringing the weed under control (Urban *et al*. 2011). For example, *T. scrupulosa*, *Octotoma scabripennis* Guèrin-Mèneville (Coleoptera: Chrysomelidae: Cassidinae – formerly Hispinae) and *Uroplata girardi* Pic (Coleoptera: Chrysomelidae) reduced plant vigour markedly in the coastal region of KZN (Cilliers, 1987b). However, their outbreaks are often localized or sporadic (Baars and Neser 1999; Baars and Heystek, 2003a).

The most prominent biocontrol agents in the present study included the leaf-mining beetle, *O. scabripennis*; the shoot-sucking bug, *T. scrupulosa*, introduced from Hawaii via Australia (Urban *et al*. 2011); the herringbone leaf-mining fly, *Ophiomyia camarae* Spencer (Diptera: Agromyzidae) obtained from Florida, USA (Simelane, 2002, Urban *et al*. 2011); the indigenous moth, *H. laceratalis*; a fungal leaf-spot pathogen, with feeding symptoms similar to those of *cf. Passalora* sp. (Chupp) U. Braun & Crous var. *lantanae*; and the flower-galling mite, *Aceria lantanae* (Cook) (Acari: Trombidiformes: Eriophyidae), native to South and Central America. *Aceria lantanae* was introduced in South Africa in 1989 and released between 2007 and 2012 in the provinces of KwaZulu-Natal (KZN), Limpopo (LP), Mpumalanga (MP) and Gauteng (GP), and its establishment has been recorded in all these provinces (Urban *et al*. 2011; Mukwevho *et al*. 2017).

An earlier impact assessment showed that biocontrol accounted for a 77% reduction in leaf lifespan, and an 85% reduction in seed production of *L. camara* on the coast of KZN (Cilliers, 1987b). Laboratory trials and semi-field experiments have shown how potentially effective some recently released agents can be at reducing *L. camara*'s growth and reproduction; such agents include *Aceria lantanae* (Cook) (Acari: Eriophyidae), *Coelocephalapion camarae* Kissinger (Coleoptera: Brentidae: Apioninae), *Falconia intermedia* (Distant) (Hemiptera: Miridae), *Longitarsus bethae* Savini and Escalona (Coleoptera: Chrysomelidae), and *O. camarae* (Urban *et al*. 2011). As more agents have been added to the suite, field experiments are needed to assess their combined effectiveness in controlling the weed.

Table 1.1 List of *Lantana camara* biocontrol agents worldwide and their establishment status ('Yes' confirms agent is established in at least one country) (Winston et al. 2014).

Biocontrol agents	**Established**
Aceria lantanae Cook (Acari: Eriophyidae)	Yes*
Aconophora compressa Walker (Hemiptera: Membracidae)	Yes
Aerenicopsis championi Bates (Coleoptera: Cerambycidae)	No
Alagoasa parana **Samuelson** (Coleoptera: Chrysomelidae)	No
Apion **sp**. A. (Coleoptera: Brentidae)	No
Apion **sp.** B. (Coleoptera: Brentidae)	No
Autoplusia illustrata Guenée (Lepidoptera: Noctuidae)	No
Calycomyza lantanae Frick (Diptera: Agromyzidae)	Yes*
Charidotis pygmaea Klug (Coleoptera: Chrysomelidae)	No
Coelocephalapion camarae Kissinger (Coleoptera: Brentidae)	Yes*
Cremastobombycia lantanella Busck (Lepidoptera: Gracillariidae)	Yes
Crocidosema lantana Busck (Lepidoptera: Tortricidae)	Yes*
Diastema tigris Guenée (Lepidoptera: Noctuidae)	Yes
Ectaga garcia Becker (Lepidoptera: Oecophoridae)	No
Eutreta xanthochaeta Aldrich (Diptera: Tephritidae)	Yes
Falconia intermedia Distant (Hemiptera: Miridae)	Yes*
Hepialus **sp.** (Lepidoptera: Hepialidae)	No
Hypena laceratalis Walker (Lepidoptera: Erebidae)	Yes*
Lantanophaga pusillidactyla Walker (Lepidoptera: Pterophoridae)	Yes
Leptobyrsa decora Drake (Hemiptera: Tingidae)	Yes
Longitarsus bethae Savini & Escalona (Coleoptera: Chrysomelidae)	Yes*
Neogalea sunia Guenée (Lepidoptera: Noctuidae)	Yes
Octotoma championi Baly (Coleoptera: Chrysomelidae)	Yes
Octotoma gundlachi Suffrain (Coleoptera: Chrysomelidae)	No
Octotoma scabripennis Guérin-Méneville (Coleoptera: Chrysomelidae)	Yes*
Ophiomyia camarae Spencer (Diptera: Agromyzidae)	Yes*
Ophiomyia lantanae Froggatt (Diptera: Agromyzidae)	Yes*
Orthezia insignis Browne (Hemiptera: Ortheziidae)	Yes
Parevander xanthomelas Guérin-Méneville (Coleoptera: Cerambycidae)	No
Passalora lantanae (Chupp) U. Braun & Crous var. *lantanae* (Dothideomycetes: Capnodiales)	No
Phenacoccus parvus Morrison (Hemiptera: Pseudococcidae)	Yes
Plagiohammus spinipennis Thomson (Coleoptera: Cerambycidae)	Yes
Prospodium tuberculatum Arthur (Pucciniomycetes: Pucciniales)	Yes
Pseudopyrausta santatalis Barnes & McDunnough (Lepidoptera: Crambidae)	No
Salbia haemorrhoidalis Guenée (Lepidoptera: Crambidae)	Yes*
Septoria **sp.** (Dothideomycetes: Capnodiales)	Yes
Strymon bazochii Godart (Lepidoptera: Lycaenidae)	Yes
Teleonemia elata Drake (Hemiptera: Tingidae)	No
Teleonemia harleyi Froeschner (Hemiptera: Tingidae)	No
Teleonemia prolixa Stål (Hemiptera: Tingidae)	No
Teleonemia scrupulosa Stål (Hemiptera: Tingidae)	Yes*
Tmolus echion L. (Lepidoptera: Lycaenidae)	Yes
Uroplata fulvopustulata Baly (Coleoptera: Chrysomelidae)	Yes
Uroplata girardi Pic (Coleoptera: Chrysomelidae)	Yes**
Uroplata lantanae *Buzzi* & Winder (Coleoptera: Chrysomelidae)	No

* Agents confirmed established in South Africa

** Agents Established in South Africa (Urban et al. 2011)

1.3.2 Some factors limiting biocontrol

Although the success achieved through *L. camara* biocontrol programmes has been variable thus far, biocontrol is still a very important control method, providing long-term solutions (Day *et al*. 2003b). Its success, however, is often hampered by a number of factors, both abiotic and biotic.

Mismatch between agents and Lantana camara varieties

Biocontrol is largely hindered by an agent's limited geographic range versus the alien plant's ability to colonize a wide range of climatic and edaphic habitats. In the case of *L. camara*, the uncertain taxonomic classification and genetic diversity renders biocontrol even more complex owing to a mismatch between varieties and biocontrol agents (Haseler, 1966; Swarbrick *et al*.1995, Day *et al*. 2003b). Haseler (1966) found that *N. sunia* performed well on the white-pink variety, while *S. haemorrhoidalis* preferred the red variety. Phenotypic preferences were also reported with the tingid, *T. scrupulosa*, which does not perform as well on the common pink as on other varieties (Neser and Cilliers, 1989; Cilliers and Neser, 1991).

Parasitism and predation

Biocontrol of *L. camara* is hampered by the high rate of parasitism encountered by agents in the field. Studies have reported cases of parasitism on agents such as *C. lantanae*, *Cremastobombycia lantanella* Busck (Lepidoptera: Gracillariidae), *O. lantanae*, *Lantanophaga pusillidactyla* Walker (Lepidoptera: Pterophoridae), *S. haemorrhoidalis* and *H. laceratalis* (Cilliers and Neser, 1991; Day and Neser, 2000; Baars and Heystek, 2003a). Other agents such as *T. scrupulosa*, *Uroplata girardi*, *O. scabripennis* and *Falconia intermedia* have been reported to be susceptible to attacks by generalist predators such as spiders, birds, neuropteran larvae, predatory bugs, and ants (Taylor, 1989; Heystek and Olckers, 2003). Parasitism and predation may explain the sporadic outbreaks reported in some biocontrol agents, such as *O. scabripennis*, *T. scrupulosa*, and *U. girardi* (Heystek and Olckers, 2003).

Environment, climate and plant condition

Twenty-nine percent of failures of *L. camara* biocontrol agent establishment are suggested to be attributed to dissimilar climatic conditions between the country of origin and the host country (Broughton, 2000). Climate and altitude are important factors affecting the distribution, not only of *L. camara* but that of its biocontrol agents as well (Urban *et al*. 2011). *Teleonemia scrupulosa* is reported to be more damaging in dry seasons and at low altitudes (Manian and Udaiyan, 1992; Thakur *et al*. 1992). In Australia, *U. girardi* is more effective at high altitudes and in cool conditions than at low altitudes (Thomas and Ellison, 2000). The herringbone leaf-miner, *O. camarae*, was found to be abundant at the coast, but increasingly scarce at higher altitudes, and more-or-less absent at sites above about 900 m above sea level, where the host plant is deciduous (Simelane and Phenye, 2004).

In order to know the true limitations of each biocontrol agent, quantitative field-based trials are required. Rigorous post-release evaluation studies must be conducted in order to measure the success or failure of these agents in controlling *L. camara* in the field.

1.4 Post-release evaluation of biocontrol agents

Many biocontrol programmes are incomplete, such that they mainly focused on the selecting, screening and releasing of agents, with little effort invested in post-release evaluation (McEvoy and Coombs, 2000; Raghu *et al*. 2006). A biocontrol programme can only be declared successful after demonstrating empirically the effectiveness of an agent in causing a reduction in the density of the target weed (Kluge, 2000). 'It is erroneous to equate establishment of agents and demonstrable damage with success because the impact of herbivore damage on the dynamics of plants is often not apparent' (Hoffmann, 1990). Measuring the impact of biocontrol agents in reducing the density of the target weed is not only necessary as an ultimate measure of success or failure, but it is also required by stakeholders to provide direction for future funding of biocontrol programmes (Briese *et al*. 2002; Carson *et al*. 2008). Three approaches can be employed to determine the impact of biocontrol agents on the reproductive and vegetative growth of a weed;

these include before-and-after photography, correlation, or experimental manipulation (Morin *et al*. 2009).

Experimental manipulation is the most complex but reliable approach to evaluate the impact of biocontrol agents on target plants (Goolsby *et al*. 2004; Tipping *et al*. 2008). This approach consists of determining the effect that agents have on weed density through comparisons between plots/quadrats with and without biocontrol agents (Dhileepan, 2003; Morin *et al*. 2009). There are three basic ways of excluding biocontrol agents from plants: controlled releases, exclusion by cages, or exclusion by insecticides. The present study focused on the exclusion by insecticides method of measuring the impact of biocontrol agents on a target plant under field conditions. The impact of biocontrol agents can be effectively evaluated by excluding agents from their host with insecticides, thereby enabling comparisons of host fitness between agent-infested and agent-free plants (Crawley, 1989; Tipping and Center, 2002).

1.5 Choosing insecticides to use in an exclusion experiment

Factors such as efficacy to remove (kill) phytophage arthropods, cost and availability of the product, persistence in the plant system after application, and mammalian toxicity (LD_{50}) have to be considered when choosing insecticides to use in exclusion experiments (Tipping and Center, 2002). As the suite of biocontrol agents established on *L. camara* in South Africa includes chewers, miners and suckers, collectively feeding on leaves, inflorescences, infructescences, shoots and roots, it is wise to use a broad spectrum, systemic insecticide. Aldicarb, a highly toxic and persistent, systemic insecticide, was used successfully in an exclusion experiment measuring the impact of *L. camara* biocontrol agents (Cilliers, 1987b). The use of aldicarb has, however, been prohibited following numerous reports of its harmfulness to the environment (van Zyl, 2012). Aldicarb has also been the cause of several cases of malicious dog poisoning and some of human suicide (Nelson *et al*. 2001; Waseem *et al*. 2010) in South Africa (Arnot *et al*. 2011) and other countries such as the United States of America (Anastasio and Sharp, 2011; Frazier *et al*. 1999) and Spain (Motas-Guzman *et al*. 2003).

As an alternative to aldicarb, another systemic, granular insecticide, carbofuran, has been used in several exclusion experiments at 10 kg a.i./ha (Active ingredient/ha) (Lonsdale *et al*. 1995; Adair and Holtkamp, 1999). The proposition to use carbofuran in the present study is based on its mode of application (soil applied granules), lower toxicity levels compared to aldicarb, high persistence as indicated by withholding period (Table 1.2) (Department of Agriculture, 2007), and availability.

Table 1.2 Toxicity and persistence of some systemic insecticides registered for use in South Africa*

Active ingredient	**Mode of application**	**Toxicity (LD_{50} rat, acute, oral (mg/kg))**	**Persistence (mean withholding period (d))**	**Persistence to Toxicity (P/T) ratio**
Acephate	Foliar	906	21	0.023
Aldicarb	Soil	1	107	107
Benfuracarb	Seed/foliage	138	61	0.442
Carbofuran	Soil	11	94	8.545
Carbosulfan	Seed/soil/foliar	218	84	0.385
Cartap-HCL	Foliar	335	9	0.027
Cyromazine	Foliar/soil	3387	7	0.002
Demeton-S-methyl	Foliar/soil	30	22	0.733
Dimethoate	Foliar/soil	308	20	0.065
Disulfoton	Soil	7	82	11.714
Fenamiphos	Soil	6	96	16
Fosthiazate	Soil	65	80	1.231
Imidacloprid	Soil/seed/foliar	450	86	0.191
Isazophos	Soil	50	56	1.120
Methamidophos	Trunk/foliar	20	18	0.900
Methomyl	Foliar	21	10	0.476
Mevinphos	Foliar	8	4	0.500
Omethoate	Foliar	25	25	1.000
Oxamyl	Soil/foliar	5	72	14.400
Phorate	Soil	3	79	26.333
Spinosad	Foliar	4369	13	0.003
Terbufos	Soil	4	93	23.250
Thiacloprid	Foliar	640	29	0.045
Thiometon	Foliar	96	26	0.271

*Data extracted from: A guide for the control of plant pests. Department of Agriculture, Pretoria (2007)

One of the advantages of a highly persistent insecticide is the reduced frequency of application. However, the potential negative impacts of insecticides on plant pollinators and water (through leaching into nearby rivers) must not be ignored.

There seem to be conflicting reports about the toxicity of systemic insecticides to plant pollinators and/or foraging predators and parasitoids. Totti *et al*. (2006) reported that aldicarb was not toxic to bees even when applied directly, compared to other insecticides such as carbaryl or imidacloprid. Conversely, Bullock and Townsend (1992) found that aldicarb was highly toxic to bees as a contact poison. If the systemic insecticide is soil applied, as is the case of carbofuran, the only contact with pollinators would have to be during nectar or pollen collection. There are no reports on the contamination of *L. camara* pollen and/or nectar by carbofuran, or of its pollinators. In addition, chemical exclusion will not stop seed production because *L. camara* flowers are capable of self-pollination (Sharma *et al*. 2005). This then means that carbofuran can still be used in an exclusion experiment to quantify the impacts of biocontrol agents on *L. camara* growth and reproduction.

1.6 Rationale for the study

Biocontrol can be a costly practice both in monetary terms (Blossey, 1999) as well as the time invested before achieving any result (McFadyen, 1998). Most *L. camara* biocontrol agents that have been released and since established on the plant in South Africa, have not yet had their full impact quantified under field conditions. Consequently a post-release evaluation study was needed to assess the effectiveness of various biocontrol agents on reducing the rate of growth and reproduction of *L. camara* in South Africa. This study was part of a collaborative effort with researchers at the Agricultural Research Council (ARC, South Africa) whose mandate was to measure the joint impact of all established *L. camara* biocontrol agents by insecticidal exclusion both on the coast of KwaZulu-Natal Province (KZN), where relatively higher insect abundance was recorded, and in the inland areas of Mpumalanga Province (MP) (Urban *et al*. 2011). This particular work was conducted in the province of Mpumalanga. In a recent review (Urban *et al*., 2011) it was asserted that the suite of agents established in South Africa was inadequate for control of *L. camara*, and that the most pressing need was to introduce additional agents that would be adapted to inland climatic

conditions. These issues were, therefore, addressed in the present study with the aid of quantitative data.

This work is novel because, for the first time, it quantifies the combined impact of the 'old plus new' suite of *L. camara* biocontrol agents, on the growth, reproduction and biomass of the weed under field conditions, in an inland area, by using insecticidal exclusion manipulations. It also determines the effect of some biotic and abiotic factors on *L. camara* growth, as well as the performance of its biocontrol agents.

1.7 Field sites

This study was conducted at sites located along the Sabie River catchment in Mpumalanga, with over 16 years worth of records (starting from the 1996/7 growing season) of plant species composition, vegetation structure and alien invasion intensity (expressed as plant aerial cover). The high, although seasonal, rainfall in the study area maintains the flow in the perennial Sabie River, with occasional flooding. The province of Mpumalanga has a relatively high *L. camara* biocontrol agent abundance after the provinces of KwaZulu-Natal and Eastern Cape (Heystek, 2006). The choice of Mpumalanga as primary site was, first, because of its relative proximity to the University of the Witwatersrand, which allowed ease of monitoring while cutting down on travel costs; and second, the use of previously monitored sites, stretching across the savanna and grassland biomes, with a well known weed history was also an advantage.

The sites were divided into three different altitudinal levels, i.e. high, medium and low, with temperate to subtropical climate characterized by hot, rainy summers and warm, dry winters (Beater *et al*. 2008; Witkowski and Garner, 2008). Three different *L. camara* colour varieties occurred on the sites, which included 'light-pink', 'dark-pink' and 'red-orange' (per. obs.). The study area is naturally shady since it is found within riparian environments (also referred to as riparian forests); but it is also shaded by the adjacent *Eucalyptus* plantations, particularly at low altitudes. High and medium altitudes, though, experienced a fairly open or semi-open environment, where savanna indigenous Acacias occurred.

1.8 Aims and objectives

The main aim of this study was to measure the combined direct impact of all established *L. camara* biocontrol agents and other *L. camara*-associated phytophagous arthropods on *L. camara* growth and reproduction in the Lowveld area of Mpumalanga, South Africa. This aim was tackled in three phases:

(a) A general field survey was conducted in 2012 to establish the status of alien plant invasions in the study area, and that of *L. camara* in particular. This enabled calculation of the effectiveness of the WfW IAP-clearing programme, using plant ecological data previously collected from the same sites in 1996/7 and 2005.

(b) Chemical exclusion methods were then employed over three years to quantify the combined impact of all established *L. camara* biocontrol agents on the vegetative and reproductive growth of *L. camara* in the study area. The efficacy of the exclusion chemical, carbofuran, was also studied in the laboratory.

(c) Finally, the data collected in the field were related to environmental parameters to determine the impacts of some biotic and abiotic factors on *L. camara* plant growth and its suite of biocontrol agents.

1.8.1 Research questions

(i) How effective has the WfW clearing been at reducing the invasion intensity of IAPs, and *L. camara* in particular, in the Lowveld regions of Mpumalanga?

(ii) What is the combined impact of all established *L. camara* biocontrol agents on the vegetative and reproductive growth of *L. camara* in the Lowveld regions of Mpumalanga, South Africa?

(iii) How do specific biotic and abiotic factors such as the degree of shading and altitude influence the above impact?

1.9 Thesis layout

This thesis is divided into six chapters:

Chapter one: General introduction to the research, providing a general background, a rationale for the study and context for the study

Chapter two: Assessment of the efficacy of WfW's IAP-clearing initiative in the Lowveld region of Mpumalanga, with special reference to *L. camara* invasion

Chapter three: Measurement of the impact of *L. camara* biocontrol agents on the vegetative and reproductive growth of the weed, through the use of chemical exclusion

Chapter four: Laboratory-based study on the efficacy of carbofuran used in the chemical exclusion experiment

Chapter five: Determination of the influence of some biotic and abiotic environmental factors on *L. camara* and its biocontrol

Chapter six: General discussion and conclusions

Chapter Two: Effect of clearing invasive alien plants along the Sabie River, South Africa, from 1996/7 to 2012, on the *Lantana camara* invasion.

This chapter has been prepared for submission to the South African Journal of Botany

2.1 Abstract

The *Working for Water* (WfW)'s invasive alien plant (IAP) clearing programme in the riparian zone along the Sabie River, near the towns of Sabie, Graskop and Hazyview in Mpumalanga, which had been assessed in 1996/7 and 2005, was re-assessed in 2012. The present work was conducted on forty 50x20 m riparian plots, which were also previously used both in the 1996/7 and 2005 studies; and on an additional forty plots located in the adjacent mostly *Eucalyptus grandis* plantations. Invasion intensity (percentage aerial cover of IAPs) was measured using five 20 m line transects across each plot. The overall aerial cover of IAPs was high (>30%) and largely unchanged over 16 years since the initial clearing operation in 1996/7. Records about clearing follow-ups are scant. There were, however, significant changes in plant aerial cover per height class. Prior to the initial WfW clearing, in 1996/7, plots were dominated by >5 m tall plants; in 2005, plots were characterized by 1-2 m tall plants, indicative of recruitment, probably following the 2000 flood; and by 2012 there were no differences in plant cover among height class. The proportion of the total IAP aerial cover occupied by *Lantana camara* L. (Verbenaceae) increased from 26% in 2005 to 39% in 2012. It was very low (~1%) in 1996/7 because it was heavily suppressed by the shading of *Eucalyptus* spp. which dominated the area then. Similarly, *L. camara* density was ~10 times greater in 2005 (2925 plants/ha) compared to 1996/7 (275 plants/ha), but had declined by 2012 (223 plants/ha) to levels similar to 1996/7, as mean *L. camara* plant size increased. In 2012, *L. camara* aerial cover was greater than that of *Solanum mauritianum* Scop. (Solanaceae), at both high (10.5% vs. 4.3%) and low altitude (14% vs. 12%); emphasizing the importance of *L. camara* as a major understorey IAP in the study area. There was no difference in *L. camara* aerial cover between the riparian (10.5%) and plantation zones (9.7%) at higher altitude, but at the lower altitude, *L. camara* aerial cover was greater in the riparian zones (14% vs. 5.7%). The cover of indigenous plants was, as expected, greater in riparian compared to plantation zones both at high (41.6% vs. 7.8%) and low (73% vs. 9.3%) altitudes, possibly because of the closed canopy within the latter zone. Hence, WfW clearing still failed to decrease IAP aerial cover, but likely prevented considerable further increases. To improve effectiveness, more

follow-up clearings and greater use of biological and integrated control methods are paramount.

Keywords: Aerial cover; Biological invasion; Clearing; Invasive alien plants; *Lantana camara*; Plantation zones; Riparian zones; Working for Water.

2.2 Introduction

Invasive alien plants (IAPs) pose a threat to the conservation of biodiversity and the functioning of ecosystems worldwide (Witkowski and Wilson, 2001; van Kleunen et al. 2015). The decline of global biodiversity, with the risk of homogenization, is one of the major negative impacts of biological invasions (Falk-Petersen *et al*. 2006; Mazza *et al*. 2014). Of great concern to conservation biologists is the removal of biogeographic barriers through anthropogenic activities such as global transport and trade, thus exposing susceptible ecosystems to excessive biological invasions (Pyšek *et al*. 2011; van Kleunen et al. 2015). South Africa is not an exception, harbouring a suite of alien plant species since the 1600's with the anchoring of the first European ships, which brought with them hundreds of plant species for various purposes such as horticulture, agriculture and forestry (Olckers *et al*. 1998; Zimmermann *et al.* 2004). The discovery of negative impacts associated with these introduced plants on the scarce water resources in South Africa, has resulted in the establishment of the *Working for Water* (WfW) programme (van Wilgen *et al*. 1998; Esler *et al*. 2008; Turpie *et al*. 2008).

The WfW programme, under the *Natural Resource Management Programme* (NRMP) from the Department of Environmental Affairs (DEA), has been mandated since 1995, with the task of clearing invasive alien plants within 30 m of river reaches in South Africa (van Wilgen *et al*. 1998; Holmes *et al*. 2005). The underlying purpose of this programme is to prevent further water loss via invasive alien plant species, while at the same time addressing poverty alleviation through job creation (Turpie *et al*. 2008).

The method of clearing used by the WfW involves cutting the stem of plants at the base followed by a localized herbicide application to the freshly cut stump

(Witkowski and Garner, 2008). However, in many cases, the same or other invasive plant species rapidly reinvade sites previously cleared (e.g. Beater *et al.* 2008; Witkowski and Garner, 2008; Holmes *et al*. 2008). The control of most IAPs may only be achieved through integrated pest management, combining biocontrol, mechanical and chemical control, which is a management strategy currently adopted by WfW (Zimmermann *et al*. 2004; Moran *et al*. 2005; van Wilgen *et al*. 2012). Nevertheless, whilst some IAPs are under control, others such as *Lantana camara* L. (Verbenaceae), present seemingly intractable obstacles to control by any means.

Lantana camara L. (Verbenaceae), commonly referred to as lantana, is one of the most ecologically and economically damaging invasive alien plants of the tropical, subtropical and warm temperate regions of Africa, southern Asia, Australia and Oceania (Day *et al*. 2003a; Bhagwat *et al*. 2012). *Lantana camara* is reported to occur in over 60 countries worldwide (Parsons and Cuthbertson, 2001), causing the loss of native species (Fensham *et al*. 1994) and reduced plant diversity (Foy and Inderjit, 2001). It covers an estimated area of 560 000 ha in South African, including riparian areas (Urban *et al*. 2011). Beside outcompeting indigenous plant species due to its fast growth rate and allelopathy (Vardien *et al*. 2012; Priyanka and Joshi, 2013), *L. camara* has the propensity to grow into impenetrable thickets (Gooden *et al*. 2009), which can hinder direct access to water sources (Urban, 2010), hence affecting livestock and humans alike.

Since the inception of WfW in 1995, several studies have looked at the impact and effectiveness of IAP clearing in riparian areas (Garner and Witkowski, 1997; Beater *et al*. 2008; Foxcroft *et al*. 2008). Among measures of effectiveness of WfW's clearing efforts, some studies looked at the impact of clearing IAPs on water resources (i.e. extra water generated or increased water flow as a result of clearing) (Dye and Jarmain, 2004; Blignaut *et al*. 2007; Cullis *et al*. 2007), while others on IAP invasion intensity and its effect on indigenous plant cover (Holmes *et al*. 2005; Esler *et al*. 2008). Invasion intensity is a function of the percentage aerial cover of IAPs; and so the greater the percentage aerial cover of a plant, the higher its invasion intensity (Beater *et al.* 2008).

In 1996/7 and 2005, two studies, which form the basis of the present work, were conducted to measure the effectiveness of the WfW IAP-clearing programme along the Sabie River, in Mpumalanga (Garner and Witkowski, 1997; Beater *et al.* 2008; Morris *et al*. 2008; Witkowski and Garner, 2008). They found that the removal of large alien tree species, e.g. *Eucalyptus* spp. and *Pinus* spp., gave rise to a conglomeration of numerous smaller alien shrubs/trees such as *Solanum mauritianum* Scop. (Solanaceae), *Rubus cuneifolius* Pursh (Rosaceae), *Caesalpinia decapetala* Roth Alston (Fabaceae) and *L. camara*, which resulted in no net change in the overall invasion intensity over time.

In the Eastern Cape province, clearings in two river catchments, Krom (1556 km^2) and Kouga (2426 km^2), between 2002 and 2008, reduced IAP cover, except on 36.2% of plots where alien cover increased despite clearing efforts (McConnachie *et al*. 2012). These studies have all cited the lack of proper follow-up clearings as a major reason which explains the overall unchanged invasion intensity of IAPs (Beater *et al*. 2008; McConnachie *et al*. 2012). On the other hand, in the Kruger Nation Park (KNP), Morris *et al*. (2008) showed how effective clearing can be with more regular follow-ups.

The work presented in this chapter was conducted in 2012 as a continuation and synthesis of two previous comprehensive studies carried out along the Sabie River; the first one in 1996/7 and the second one in 2005. These studies aimed to determine the effectiveness of WfW clearings on IAP invasion intensity (Garner, 2006; Beater, 2006; Beater *et al*. 2008). Effectiveness here referred to reduction in IAP percentage canopy aerial cover (Beater, 2006; Beater *et al.* 2008). This study (2012), therefore, aimed to continue measuring the effectiveness of WfW clearing on the invasion intensity of IAP species in general, and *L. camara* in particular, comparing invasions between 1996/7, 2005 and 2012, hence providing a long-term (16 years) evaluation of the effectiveness of the WfW clearing programme along the Sabie River, in Mpumalanga, South Africa. The objectives are: (i) to compare the invasion intensity and height structure of IAP species between 1996/7, 2005 and 2012; (ii) to assess changes in *L. camara* density and aerial cover over time; (iii) to compare *L. camara* cover to that of bugweed during the 2012 survey; (iv) to compare invasive and indigenous plant cover between

riparian and plantation zones in 2012; and (v) to assess the effectiveness of WfW clearing operations from 1996/7 to 2012.

2.3 Materials and methods

2.3.1 Study area

This field work was conducted along the Sabie River, in Mpumalanga, South Africa, between March 2012 and April 2012 on 40 (20x50 m) plots. The Sabie River catchment encompasses an area of about 7096 km^2 (Beater *et al*. 2008). The study plots were situated within 30 m of the river bank and divided into 20 high altitude plots, located near the towns of Sabie (altitude 1133±11 m (mean ± S.E.)) and Graskop (975±9 m), and 20 low altitude plots, near the town of Hazyview (altitude 848±15 m). The original experimental design of 1996/7 was a factorial ANOVA with three factors, i.e. altitude, invasion intensity and clearing (Fig. 2.1.a). In the present study, IAP and indigenous plant aerial cover, and height structure were again assessed in the same riparian plots plus 40 additional plots in the adjacent eucalypt and pine tree plantations beyond the 30 m zone (Fig. 2.1.b). The reason for measuring the invasion intensity of IAPs and indigenous plant cover in the plantation plots was for comparison purposes between the two land uses (riparian and plantation).

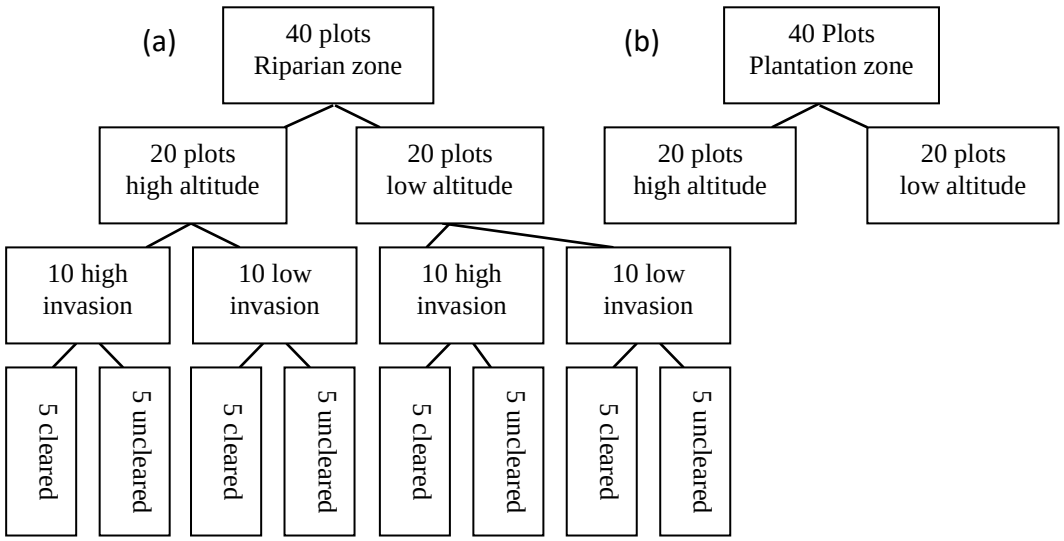

Figure 2.1 Experimental layout showing 40 plots in the riparian zone, organized into a factorial design with three factors (altitude, invasion intensity and clearing) as sampled in 1996/7 and 2005 (a); and 40 plots in the plantation zone sampled in 2012 (b), paired with 40 in the riparian zone, all located along the Sabie River in Mpumalanga.

The Sabie River flows throughout the year and, depending on the intensity of rainfall, flooding events may occur. Numerous floods and droughts have been recorded in this area during the past 70 years (Rogers and O'Keeffe, 2003). In February of 2000, unprecedented heavy rains resulted in severe floods, with devastating consequences on the environment, such as the alteration of river morphologies (Rountree and Rogers, 2004), and the uprooting of trees and shrubs (Parsons *et al*. 2006). The Sabie River is important to agriculture, and forestry in particular, and from an eco-tourism viewpoint (Smithers *et al*. 2001; Beater *et al*. 2008). Its placement is also important as it traverses the KNP and passes through Mozambique en route to the Indian Ocean.

Working for Water has been clearing IAPs since 1995. However, information on clearing efforts in the province of Mpumalanga was only available post-2000. There were large clearing efforts in the region, particularly from 2004 to 2007 (Beater *et al*. 2008), and again from 2012 to 2012 (Holmes *et al*. 2008). However, the frequency of these clearings in the study sites is not known, though it is unlikely to have exceeded one clearing and/or follow-up treatment per year.

2.3.2 Invasion intensity and height class

Experimental design

In 1996/7, a factorial design with three factors, as laid out in Figure (1.a) above, was used to compare the aerial cover of IAPs in relation to clearing, invasion intensity, and altitude. At the time of the 1996/7 study, by design, half of the plots in the study area were cleared (clearing was done by the WfW) and the other half uncleared. Half of the plots were selected as high invasion intensity (>50% IAP aerial cover) and the other half as low invasion intensity (<50% IAP aerial cover) plots. Half of the plots were selected from high altitudes (1133±11 m) and the other half from low altitudes (848±15 m). The abovementioned experimental design was adopted and repeated in the present work. Percentage aerial cover of IAPs was calculated by measuring plant canopy aerial cover of IAPs >1 m in height, using the line intercept method (Beater *et al*., 2008). Five, 20 m line transects, 10 m apart, were sampled per 1000 m^2 (20 x 50 m) plot and plant aerial cover was obtained by measuring the lengths of the canopies of plants for each of

the three height classes, 1-2 m, 2-5 m and >5 m, along each line transect, and combined per plot.

***Lantana camara* cover and density**

Percentage aerial cover of *L. camara* was calculated using the line intercept method as described above. There was no record of *L. camara* aerial cover in 1996/7; therefore comparisons were only made between 2005 and 2012. *Lantana camara* density was compared between 1996/7, 2005 and 2012.

Lantana camara density was expressed as the total count of individual plants, as well as per height class (1-2 m, 2-5 m and >5 m) per plot (20x50 m).

In 2012, the percentage aerial cover of *L. camara* was compared to that of bugweed, both of which were calculated in the same manner described above. Bugweed was singled for comparison because it is also an important IAP occurring in the study area.

Plant cover was also compared between the 40 original plots (20 high and 20 low altitude) found in the riparian zones and an additional 40 plantation plots (20 high and 20 low altitude). In some plots, *L. camara* had grown into impenetrable thickets, hence *L. camara* density was also expressed per canopy width class (1-2 m, 2-5 m, and >5 m).

2.3.3 Statistical analysis

Differences in total and height class percentage aerial cover between treatments were compared using three-way ANOVAs for each time period, as well as three-way ANOVAs with repeated measures (among time periods – 1996/7, 2005 and 2012), followed by LSD Post-hoc tests. Comparisons of IAP vs. indigenous plant aerial cover between riparian and plantation zones, and *L. camara* cover vs. bugweed cover within riparian zones, were done using paired t-tests. Relationships between IAP and indigenous plant species aerial cover, *L. camara* and indigenous plant species aerial cover, and between *L. camara* density and *L. camara* aerial cover were analysed using linear regression. The data were checked for normality, and where necessary data transformation was undertaken. All analyses were conducted at a critical *P* level of 0.05 using Statistica, version 12.

2.4 Results

2.4.1 Impact of WfW clearing

Comparisons of IAP aerial cover among 1996/7, 2005 and 2012

In general, there were no significant differences in the total IAP aerial cover or the aerial cover per height class between years, except in the 'high altitude – low invasion – cleared plots', where total aerial cover increased ~5 fold from 1996/7 to 2005 and by 49% from 2005 to 2012; as well as the 2-5 m and >5 m IAP aerial cover increasing 6 and 5 fold, respectively, from 2005 to 2012 (Table 2.1).

There were no significant overall changes in IAP aerial cover with altitude ($F_{(2, 76)} = 1.14$, $P = 0.32$) (Fig. 2.2.a), invasion intensity ($F_{(2, 76)} = 1.97$, $P = 0.14$) (Fig. 2.2.b) or clearing ($F_{(2, 76)} = 2.95$, $P = 0.05$) (Fig. 2.2.c) among 1996/7, 2005 and 2012. However, there was a trend showing a slight increase in IAP cover in the low invasion plots from 1996/7 to 2012 (Fig. 2.2.b), and in the cleared plots from 1996/7 to 2012 (Fig. 2.2.c). In 1996/7, the main effect that was significant throughout all height classes and the total (all height classes combined) was clearing, however in the 1-2 m height class all main and interaction effects were significant (Table 2.2). By 2005, the clearing effect was no longer significant, and by 2012, all main effects (altitude, invasion intensity and clearing) were no longer significant (Table 2.2).

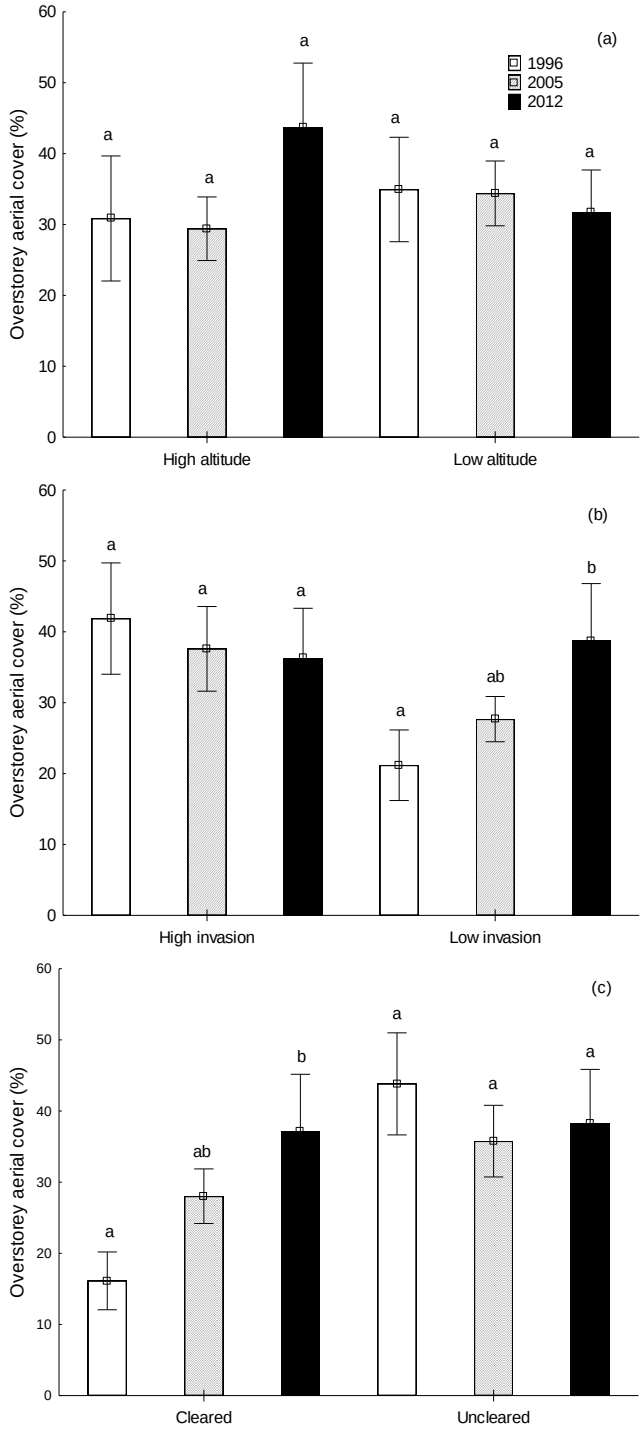

Figure 2.2 Comparisons of percentage aerial cover (mean ± S.E.) of IAPs along the Sabie River among 1996/7, 2005 and 2012, for (a) altitude (high and low altitude), (b) invasion intensity (high and low invasion), and (c) clearing (cleared and uncleared). Means with different letters are significantly different using Fisher LSD Post-hoc tests (n = 20, $P < 0.05$).

Table 2.1 Percentage aerial cover in total and per height class (mean ± S.E) of IAPs in 1996/7, 2005 and 2012. Different superscript letters within rows indicate significant differences between treatments (One-way ANOVA, Fisher LSD, $P < 0.05$). Different subscript letters within columns and in the same class height indicate significant differences between years (three-way ANOVA with repeated measures, Fisher LSD, $P < 0.05$).

		High altitude				**Low altitude**			
		High invasion		**Low invasion**		**High invasion**		**Low invasion**	
		Cleared	**Uncleared**	**Cleared**	**Uncleared**	**Cleared**	**Uncleared**	**Cleared**	**Uncleared**
1-2 m	**'96/7**	$0.90 \pm 1.78^{a}{}_{a}$	$1.30 \pm 1.35^{a}{}_{a}$	$2.93 \pm 1.45^{a}{}_{a}$	$3.0 \pm 2.06^{a}{}_{a}$	$21.58 \pm 2.06^{b}{}_{a}$	$1.79 \pm 2.06^{a}{}_{a}$	$3.07 \pm 1.35^{a}{}_{ab}$	$3.39 \pm 1.35^{a}{}_{a}$
	'05	$12.50 \pm 6.49^{a}{}_{a}$	$20.55 \pm 4.90^{a}{}_{c}$	$25.0 \pm 5.30^{a}{}_{c}$	$10.40 \pm 7.49^{a}{}_{a}$	$11.66 \pm 7.49^{a}{}_{a}$	$7.93 \pm 7.49^{a}{}_{a}$	$11.42 \pm 4.90^{a}{}_{a}$	$12.32 \pm 4.9^{a}{}_{b}$
	'12	$4.01 \pm 3.82^{ab}{}_{a}$	$11.27 \pm 2.89^{a}{}_{b}$	$12.58 \pm 3.12^{a}{}_{b}$	$5.04 \pm 4.4^{ab}{}_{a}$	$10.46 \pm 4.41^{ab}{}_{a}$	$5.66 \pm 4.4^{ab}{}_{a}$	$0.04 \pm 2.89^{b}{}_{b}$	$6.42 \pm 2.89^{ab}{}_{ab}$
2-5 m	**'96/7**	$2.70 \pm 7.73^{a}{}_{a}$	$9.44 \pm 5.84^{a}{}_{a}$	$4.38 \pm 6.31^{a}{}_{ab}$	$15.12 \pm 8.92^{ab}{}_{a}$	$19.54 \pm 8.92^{ab}{}_{a}$	$32.08 \pm 8.92^{b}{}_{a}$	$4.53 \pm 5.84^{b}{}_{a}$	$27.92 \pm 5.84^{b}{}_{a}$
	'05	$0.65 \pm 5.72^{a}{}_{a}$	$11.97 \pm 4.32^{a}{}_{a}$	$3.13 \pm 4.67^{a}{}_{a}$	$4.20 \pm 6.61^{a}{}_{a}$	$7.93 \pm 6.61^{a}{}_{a}$	$45.0 \pm 6.61^{b}{}_{a}$	$12.88 \pm 4.32^{a}{}_{a}$	$10.90 \pm 4.32^{a}{}_{b}$
	'12	$10.70 \pm 9.03^{a}{}_{a}$	$18.18 \pm 6.82^{ab}{}_{a}$	$19.97 \pm 7.37^{ab}{}_{b}$	$32.24 \pm 10.43^{ab}{}_{b}$	$30.43 \pm 10.43^{ab}{}_{a}$	$41.92 \pm 10.43^{b}{}_{a}$	$11.98 \pm 6.82^{a}{}_{a}$	$16.81 \pm 6.82^{ab}{}_{ab}$
>5 m	**'96/7**	$15.87 \pm 11.19^{abc}{}_{a}$	$39.73 \pm 8.46^{c}{}_{a}$	$0.66 \pm 9.14^{a}{}_{a}$	$27.75 \pm 12.92^{abc}{}_{a}$	$1.33 \pm 12.92^{a}{}_{a}$	$38.87 \pm 12.92^{bc}{}_{a}$	$3.0 \pm 8.46^{a}{}_{a}$	$8.53 \pm 8.46^{ab}{}_{a}$
	'05	$1.25 \pm 3.20^{a}{}_{a}$	$6.97 \pm 2.42^{ab}{}_{b}$	$6.90 \pm 2.61^{ab}{}_{a}$	$0 \pm 3.70^{a}{}_{b}$	$13.76 \pm 3.70^{b}{}_{a}$	$15.40 \pm 3.70^{b}{}_{ab}$	$3.21 \pm 2.42^{a}{}_{a}$	$3.92 \pm 2.42^{a}{}_{a}$
	'12	$2.06 \pm 7.94^{a}{}_{a}$	$3.05 \pm 6.0^{a}{}_{b}$	$36.65 \pm 6.49^{b}{}_{b}$	$17.49 \pm 9.17^{ab}{}_{a}$	$6.01 \pm 9.17^{a}{}_{a}$	$12.96 \pm 9.17^{a}{}_{b}$	$5.18 \pm 6.0^{a}{}_{a}$	$4.13 \pm 6.0^{a}{}_{a}$
Total	**'96/7**	$19.53 \pm 16.39^{ab}{}_{a}$	$50.48 \pm 12.38^{bc}{}_{a}$	$7.38 \pm 13.98^{a}{}_{a}$	$45.87 \pm 18.92^{abc}{}_{a}$	$42.45 \pm 18.92^{abc}{}_{a}$	$72.75 \pm 18.92^{c}{}_{a}$	$10.6 \pm 12.38^{a}{}_{a}$	$39.85 \pm 12.38^{abc}{}_{a}$
	'05	$14.4 \pm 8.37^{a}{}_{a}$	$39.50 \pm 6.32^{b}{}_{a}$	$35.03 \pm 6.83^{ab}{}_{a}$	$14.60 \pm 9.66^{a}{}_{a}$	$33.36 \pm 9.66^{ab}{}_{a}$	$68.33 \pm 9.66^{c}{}_{a}$	$27.52 \pm 6.32^{ab}{}_{a}$	$27.15 \pm 6.32^{ab}{}_{a}$
	'12	$16.77 \pm 15.77^{a}{}_{a}$	$32.51 \pm 11.92^{a}{}_{a}$	$69.22 \pm 12.88^{b}{}_{b}$	$54.77 \pm 18.21^{ab}{}_{a}$	$46.89 \pm 18.21^{ab}{}_{a}$	$60.54 \pm 18.21^{ab}{}_{a}$	$17.21 \pm 11.92^{a}{}_{a}$	$27.36 \pm 11.92^{a}{}_{a}$

Table 2.2 Results of three-way ANOVAs showing main and interaction effects of treatments on the canopy percentage aerial cover of IAPs at different time periods (1996/7, 2005 and 2012) and per height class (1-2 m, 2-5 m and >5 m). Degrees of freedom are 1, 32 throughout. 'Signif.' stands for significance. * $P < 0.05$, ** $P < 0.01$, NS = not significant.

	F	***P***	**Signif.**	**F**	***P***	**Signif.**	**F**	***P***	**Signif.**	**F**	***P***	**Signif.**
Effect (1996/7)		**1-2m**			**2-5m**			**>5m**			**Total**	
Altitude	19.94	0.00	**	6.22	0.02	*	19.92	0.00	**	42.28	0.00	**
Invasion	7.37	0.01	*	0.32	0.58	N.S	1.13	0.30	N.S	0.88	0.35	N.S
Clearing	15.32	0.00	**	6.45	0.02	*	3.37	0.08	N.S	3.30	0.08	N.S
Altitude*Invasion	18.05	0.00	**	1.59	0.22	N.S	9.55	0.00	**	8.31	0.01	*
Altitude*Clearing	16.83	0.00	**	0.77	0.39	N.S	0.00	0.96	N.S	1.19	0.28	N.S
Invasion*Clearing	16.58	0.00	**	0.50	0.48	N.S	0.07	0.80	N.S	0.04	0.84	N.S
Altitude*Invasion*Clearing	17.72	0.00	**	0.10	0.75	N.S	0.90	0.35	N.S	0.02	0.90	N.S
Effect (2005)												
Altitude	2.03	0.16	N.S	13.31	0.00	**	5.91	0.02	*	5.40	0.03	*
Invasion	0.14	0.71	N.S	4.90	0.03	*	7.18	0.01	*	5.08	0.03	*
Clearing	0.28	0.60	N.S	9.31	0.00	**	0.02	0.89		2.98	0.09	N.S
Altitude*Invasion	0.01	0.92	N.S	2.35	0.13	N.S	5.65	0.02	*	3.53	0.07	N.S
Altitude*Clearing	0.04	0.83	N.S	2.13	0.15	N.S	0.16	0.69	N.S	1.73	0.20	N.S
Invasion*Clearing	1.04	0.31	N.S	10.05	0.00	**	2.41	0.13	N.S	12.64	0.00	**
Altitude*Invasion*Clearing	2.39	0.13	N.S	3.43	0.07	N.S	1.80	0.19	N.S	0.20	0.66	N.S
Effect (2012)												
Altitude	0.99	0.33	N.S	0.67	0.42	N.S	2.06	0.16	N.S	0.25	0.62	N.S
Invasion	0.50	0.49	N.S	0.68	0.42	N.S	3.33	0.08	N.S	0.08	0.78	N.S
Clearing	0.02	0.90	N.S	2.16	0.15	N.S	0.32	0.57	N.S	0.34	0.56	N.S
Altitude*Invasion	1.33	0.26	N.S	7.43	0.01	*	7.38	0.01	*	10.30	0.00	**
Altitude*Clearing	0.03	0.86	N.S	0.02	0.89	N.S	1.24	0.27	N.S	0.28	0.60	N.S
Invasion*Clearing	0.12	0.73	N.S	0.01	0.94	N.S	1.70	0.20	N.S	0.62	0.44	N.S
Altitude*Invasion*Clearing	6.26	0.02	*	0.22	0.64	N.S	0.32	0.58	N.S	0.39	0.54	N.S

Changes in IAP aerial cover per height class

At high altitudes, the cover of 1-2 m IAPs increased from 1996/7 to 2005, and subsequently decreased by 2012, but was still greater than in 1996/7 ($F_{(2, 38)} = 14.70$, $P < 0.01$) (Fig. 2.3.a). The aerial cover of 2-5 m plants was not different between 1996/7 and 2005 but was greater in 2012 than in both 1996/7 and 2005 ($F_{(2, 38)} = 5.38$, $P < 0.01$). The aerial cover of >5 m tall plants significantly decreased from 1996/7 to 2005 but the cover in 2012 was not significantly different from 1996/7 and 2005 ($F_{(2, 38)} = 2.53$, $P = 0.09$). Comparing height classes between years, 1996/7 was dominated by >5 m plants ($F_{(2, 57)} = 5.58$, $P < 0.01$), whereas in 2005, 1-2 m plants were more abundant ($F_{(2, 57)} = 8.78$, $P < 0.01$), and by 2012 there were no significant differences in plant cover per height class ($F_{(2, 57)} = 1.42$, $P = 0.24$) (Fig. 2.3.a).

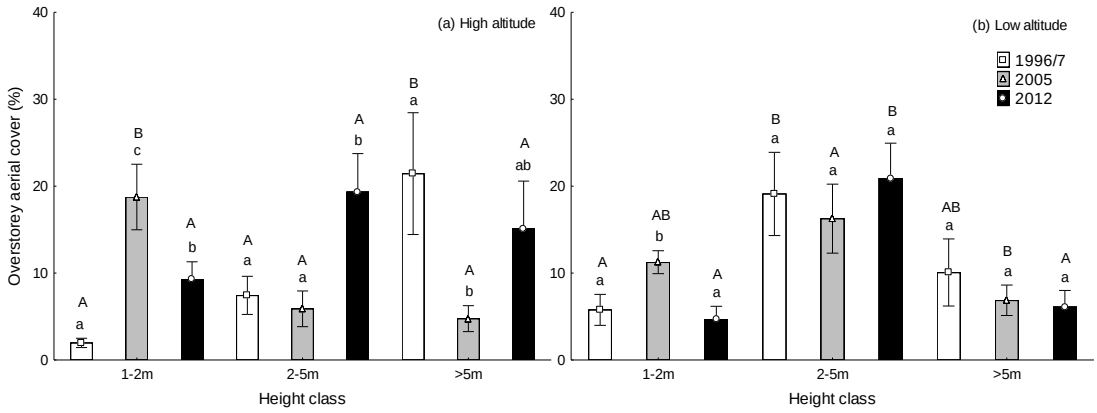

Figure 2.3 Change in aerial cover within IAP height classes (mean ± S.E.) among 1996/7, 2005 and 2012 along the Sabie River at (a) high and (b) low altitudes. Means with different lowercase letters within each height class and uppercase letters within each year are significantly different using Fisher LSD Post-hoc tests (n = 20, $P < 0.05$).

At low altitudes, aerial cover of 1-2 m IAPs was higher in 2005 compared to 1996/7 and 2012 ($F_{(2, 38)} = 5.61$, $P < 0.01$), however, there were no significant changes in the cover of 2-5 m ($F_{(2, 38)} = 0.51$, $P = 0.60$) and >5 m plants over time ($F_{(2, 38)} = 0.71$, $P = 0.49$) (Fig. 2.3.b). Overall, 2-5 m was the dominant height class in 1996/7 ($F_{(2, 57)} = 3.39$, $P = 0.04$), 2005 ($F_{(2, 57)} = 3.22$, $P = 0.04$), and 2012 ($F_{(2, 57)} = 11.07$, $P < 0.01$) (Fig. 2.3.b).

Changes in Lantana camara aerial cover and density

At high altitude, of the total IAP aerial cover, there were no changes in the proportion of the cover occupied by *L. camara* between 2005 (29%) and 2012 (28%) ($t_{19} = 0.08$, $P = 0.93$); however, at low altitudes, it increased significantly from 26% in 2005 to 39% in 2012 ($t_{19} = 2.19$, $P = 0.04$) (Fig. 2.4).

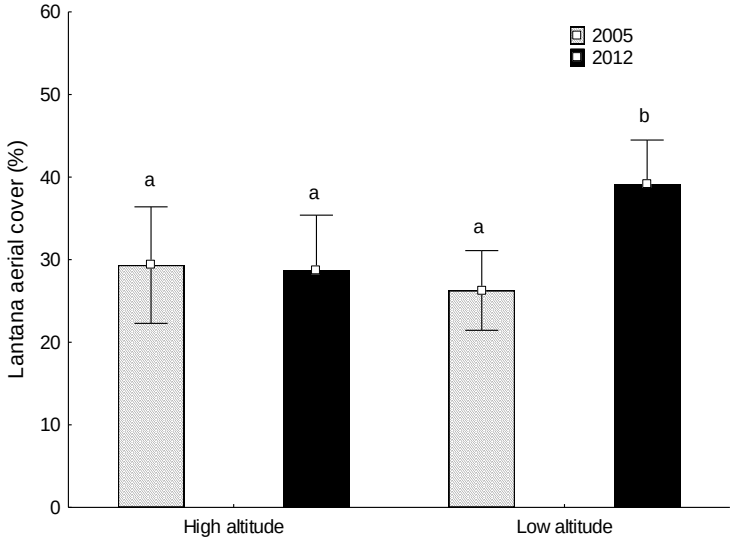

Figure 2.4 *Lantana camara* proportion of cover relative to the total cover of IAPs at high and low altitude over time. Columns with different letters within the same altitude indicate significant differences, using paired t-tests (d.f. = 19, n = 20, P < 0.05).

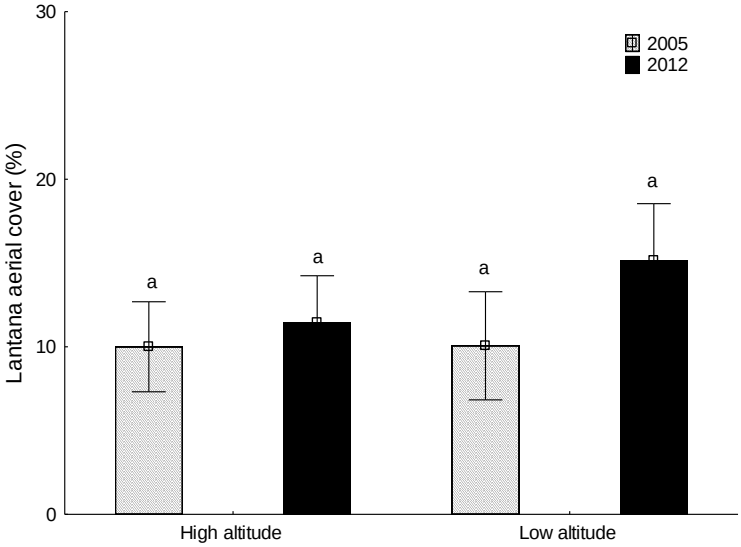

Figure 2.5 Change in *L. camara* aerial cover (mean ± S.E.) between 2005 and 2012, along the Sabie River at high and low altitudes. Columns with different superscript letters are significantly different, using paired t-tests (d.f. = 19, n = 20, P < 0.05).

Overall, there were no changes in *L. camara* aerial cover between 2005 and 2012 both at the high (t_{19} = 0.45, *P* = 0.64) and low altitudes (t_{19} = 1.54, *P* = 0.13) (Fig. 2.5).

However, some changes in terms of height class were found, more so at low than high altitudes. At high altitudes, there were no differences in the cover of 1-2 m *L. camara* plants between 2005 and 2012 (t_{19} = 1.64, *P* = 0.11) (Fig. 2.6.a), whereas at low altitudes it decreased significantly (t_{19} = 3.83, *P* < 0.01) (Fig. 2.6.b).

The cover of 2-5 m *L. camara* plants increased significantly from 2005 to 2012, both at high altitudes (t_{19} = 3.06, *P* < 0.01) (Fig. 7.a) and low altitudes (t_{19} = 3.70, *P* = 0.01) (Fig. 2.6.b).

There were no significant differences in the cover of >5 m tall *L. camara* plants between 2005 and 2012 both at high (t_{19} = 1.25, *P* = 0.22) (Fig. 2.6.a) and low altitudes (t_{19} = 1.70, *P* = 0.10) (Fig. 2.6.b).

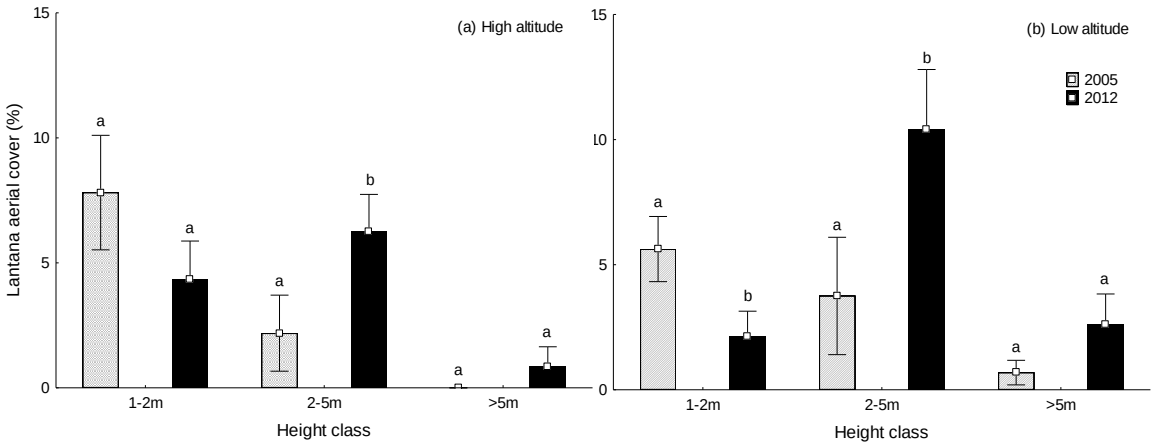

Figure 2.6 Change in *L. camara* aerial cover per height class (mean ± S.E.) between 2005 and 2012, along the Sabie River at (a) high and (b) low altitudes. Columns with different letters within the same height class are significantly different, using paired t-tests (d.f. = 19, n = 20, P < 0.05).

Overall, *L. camara* density was significantly greater (~7 times greater) in 2005 compared to 1996/7 and in 2012 it declined significantly to levels almost similar to 1996/7. At high altitudes, *L. camara* density was significantly greater in 2005 compared to both 1996/7 and 2012 ($F_{(2, 57)}$ = 9.26; *P* < 0.01), however at low altitudes

there were no differences between 1996/7, 2005 and 2012 ($F_{(2, 57)} = 1.59$; $P = 0.21$) (Fig. 2.7).

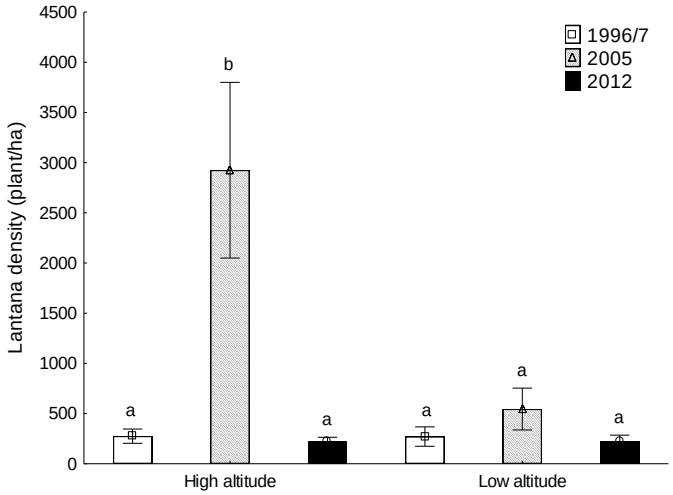

Figure 2.7 Comparison of *L. camara* density (all height classes combined) (mean ± S.E.) along the Sabie River between 1996/7, 2005 and 2012 at high and low altitudes. Means with different letters are significantly different, using Fisher LSD Post-hoc tests (n = 20, P < 0.05).

There was a positive relationship between *L. camara* aerial cover and *L. camara* density ($R^2 = 0.58$; $P < 0.01$) at high altitudes, in 2005; whereas in 2012, these were unrelated (Fig. 2.8.a). At low altitudes, there was no relationship between *L. camara* aerial cover and *L. camara* density, either in 2005 or 2012 (Fig. 2.8.b).

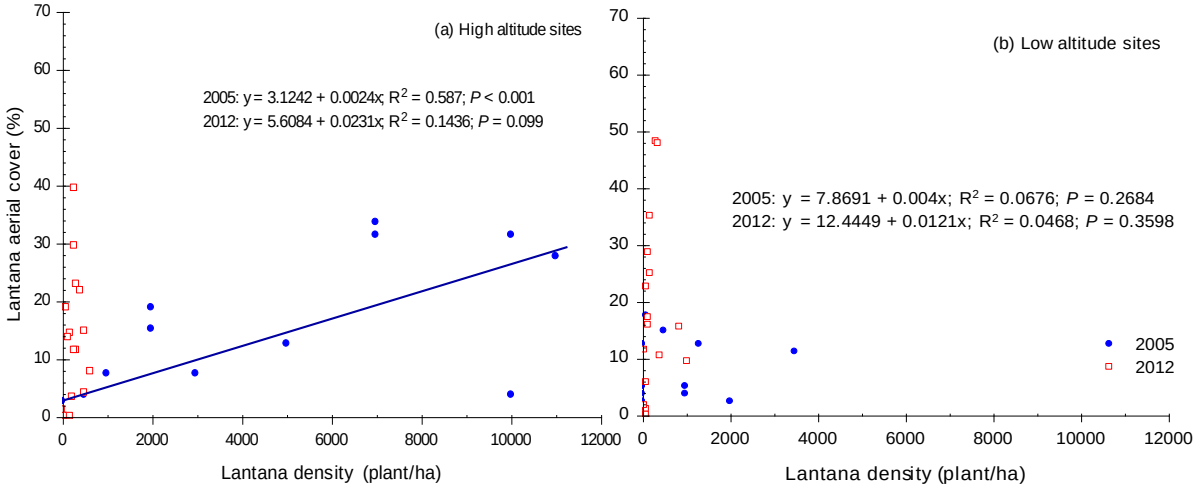

Figure 2.8 Regressions between *L. camara* aerial cover and density in 2005 and 2012 at (a) high and (b) low altitudes, along the Sabie River in Mpumalanga.

Comparing Lantana camara and bugweed aerial cover in 2012

Lantana camara aerial cover was significantly greater compared to bugweed, both at high ($t_{19} = 2.64$, $P = 0.01$) and low altitude ($t_{19} = 2.16$, $P = 0.04$), and overall ($t_{19} = 3.19$; $P < 0.01$) (Fig. 2.9).

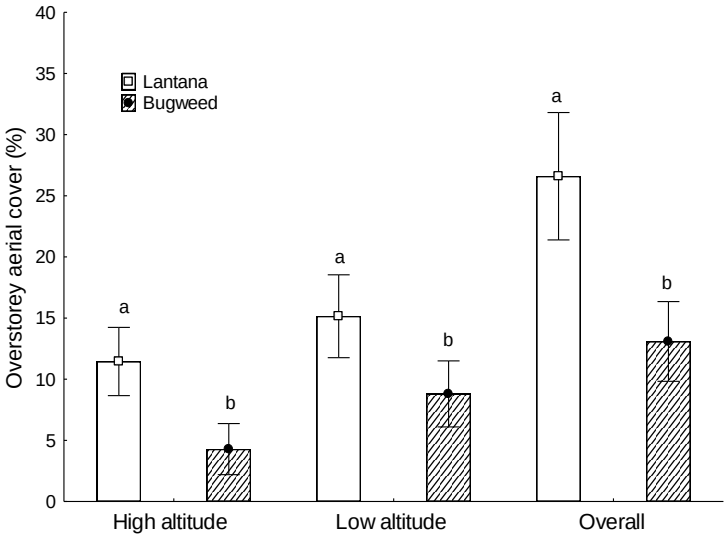

Figure 2.9 Comparisons in total percentage aerial cover between *L. camara* and bugweed, at high altitude, low altitude and overall, along the Sabie River in 2012. Columns with different letters within the same group are significantly different, using paired t-tests (d.f = 19, n = 20, P < 0.05).

2.4.2 Plant aerial cover in 2012: riparian vs. plantation zones

At high altitudes, there was no difference in *L. camara* aerial cover between riparian and plantation zones ($t_{19} = 0.50$, $P = 0.61$), however the cover of all IAPs was greater in the plantation zone ($t_{19} = 5.84$, $P < 0.01$), while that of indigenous plants was greater in the riparian zone ($t_{19} = 3.99$, $P < 0.01$) (Fig. 2.10.a).

At low altitudes, *L. camara* and indigenous plant covers were greater in the riparian compared to plantation zone ($t_{19} = 2.90$, $P < 0.01$; $t_{19} = 7.02$, $P < 0.01$, respectively), whilst the cover of all IAPs was greater in plantations compared to riparian zones ($t_{19} = 11.10$, $P < 0.01$) (Fig. 2.10.b). The overstorey cover of IAPs within plantation zones was very high because plantation plants were included in the measurement.

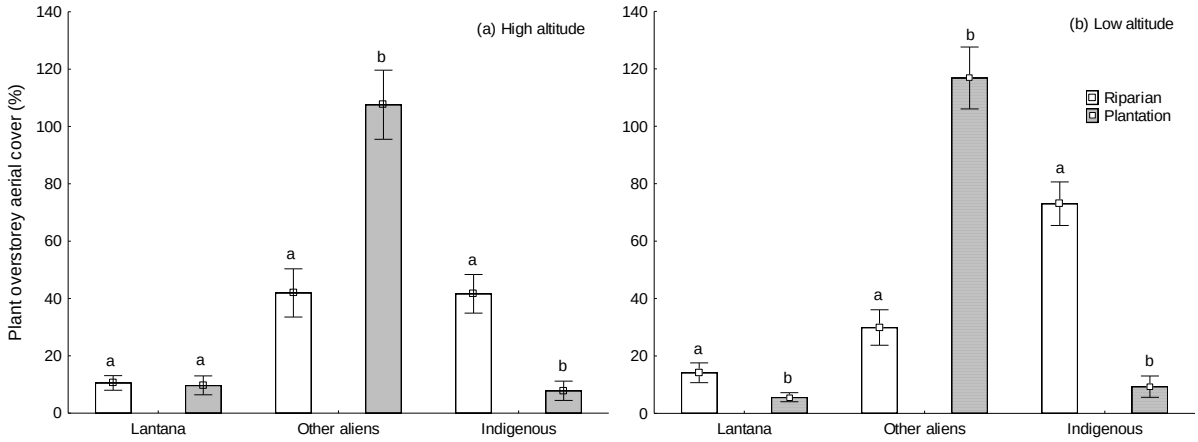

Figure 2.10 Comparisons in total percentage aerial cover of *L. camara*, other alien, and indigenous plants between riparian and plantation plots, at high (a) and low (b) altitude sites along the Sabie River in 2012. Columns with different letters within the same plant type are significantly different, using paired t-tests (n = 20, P < 0.05).

At high altitudes, there were no differences in *L. camara* cover for height classes 1-2 m, 2-5 m and >5 m between riparian and plantation zones ($t_{19} = 0.19$, $P = 0.84$), ($t_{19} = 0.08$, $P = 0.93$), and ($t_{19} = 1.09$, $P = 0.28$), respectively (Fig. 2.11.a). At low altitudes, there was no difference in the cover of 2-5 m *L. camara* plants between riparian and plantation zones, ($t_{19} = 1.65$, $P = 0.11$), whereas the cover of 1-2 m and >5 m plants was greater in the riparian compared to the plantation zone ($t_{19} = 2.11$, $P = 0.04$) and ($t_{19} = 2.21$, $P = 0.04$), respectively (Fig. 2.11.b).

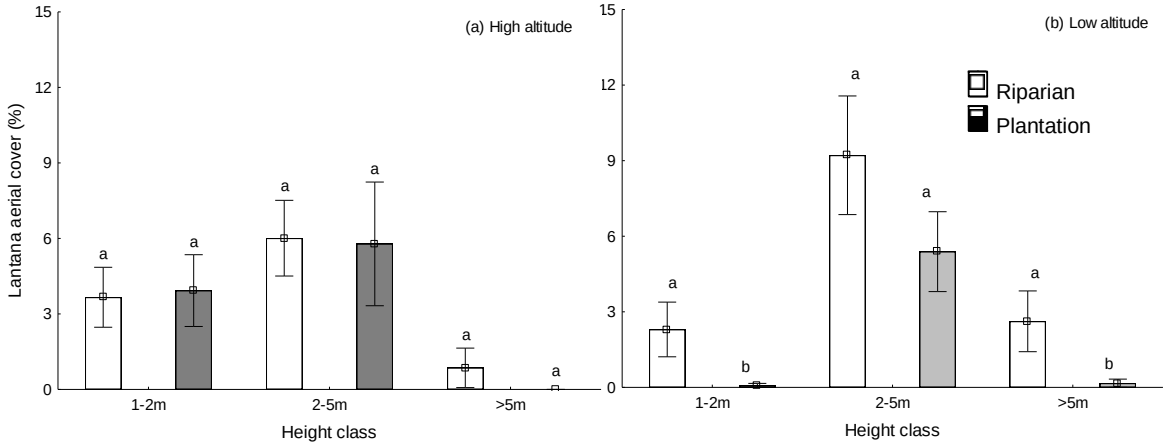

Figure 2.11 Differences in *L. camara* aerial cover per height class between riparian and plantation zones, and at high (a) and low (b) altitude sites, along the Sabie River in 2012. Columns with different letters within the same height class are significantly different, using paired t-tests (d.f. = 19, n = 20, P < 0.05).

2.4.3 *Lantana camara* density per height and width class in 2012

There were strong positive relationships between *L. camara* density per height class and *L. camara* density per width class, except in the >5 m class, at high and low altitude in the riparian zone (see R^2 values on Figures).

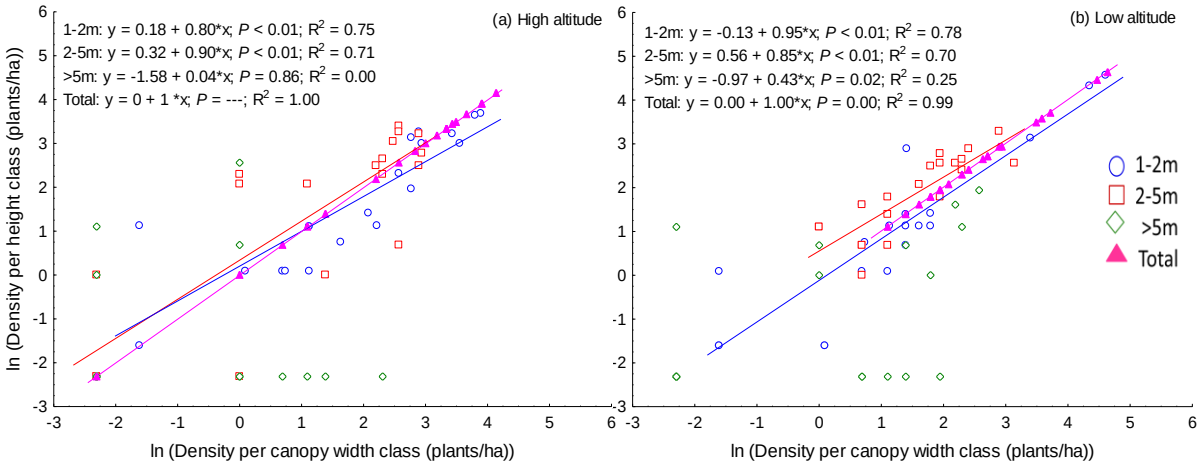

Figure 2.12 Relationship between *L. camara* density per height class and *L. camara* density per width class (canopy diameter) in the riparian zone at high (a) and low altitudes (b), along the Sabie River in 2012 (n = 20).

Similarly, in the plantation zone, strong positive relationships existed between *L. camara* density per height class and *L. camara* density per width class, except in the >5 m class, both at high and low altitude (Fig. 2.13.a.b).

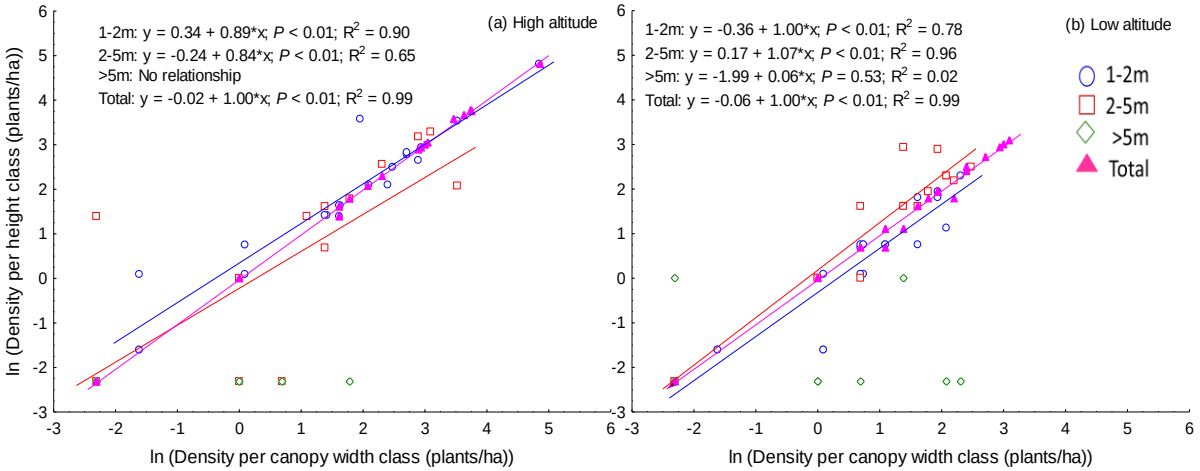

Figure 2.13 Relationship between *L. camara* density per height class and *L. camara* density per width class (canopy diameter) in the plantation zone at high (a) and low altitudes, along the Sabie River in 2012 (b) (n = 20).

2.4.4 Aerial cover in 2012: indigenous vs. invasive alien plants

There was a negative but weak relationship between the aerial cover of all IAPs and that of indigenous plants at high altitude, but not at low altitude (see Fig. 2.14.a), and no relationship between *L. camara* and indigenous plants both at high or low altitude (Fig. 2.14.b).

At low altitude, there was no linear relationship between indigenous plant cover and *L. camara* density; whereas at high altitude, it was positive but weak (Fig. 2.14.c).

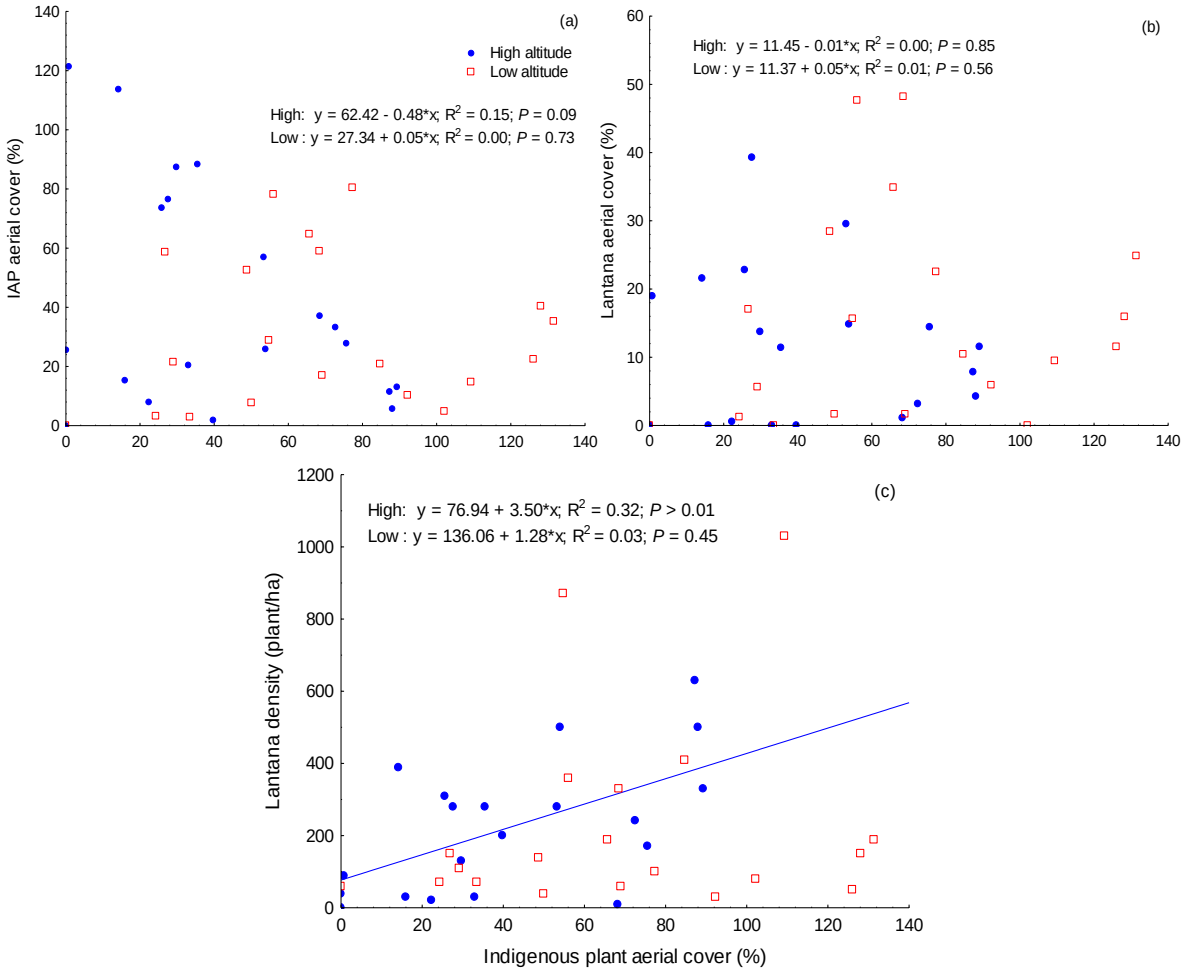

Figure 2.14 Relationship between indigenous plant cover and IAP cover (a), *L. camara* cover (b) and *L. camara* density (c), at high altitude and low altitudes, along Sabie River, in 2012.

2.5 Discussion

The overall aerial cover of all IAPs, both at high and low altitude plots did not change significantly from 1996/7 to 2012. The effectiveness of the WfW clearing programme may be questioned at this point because after 16 years of clearing operations, with possibly at least an annual clearing and several follow-up clearings, a significant decrease in the overall invasion intensity should be expected. Nevertheless, since the 1996/7 study, the overall IAP cover has not increased either, and this is surely an indication that WfW clearings have been effective in at least keeping IAPs under some level of control and therefore alleviating their impacts (e.g. on the water resources).

Le Maitre *et al*. (2002) estimated that it would only take 20 to 30 years for IAPs in the Sabie River catchment to increase and reach 100% canopy cover if the invasion was not controlled. They also estimated the cost of clearing IAPs at USD 4 to 13 million per year, with a projected estimate of USD 11 to 278 million if IAP invasions were not managed (Le Maitre *et al*. 2002). Data collected from flow gauges on the Sabie-Sand River catchment have revealed no significant variations in the flows of the river from 1996/7 to 2012 (van Wilgen and Biggs 2011; Vieira, 2015). *Working for Water*, through its IAP-clearing operations, has certainly contributed to the prevention of significant reductions in the Sabie River flow projected by Le Maitre *et al*. (2002).

Although the overall aerial cover of IAPs remained unchanged from 1996/7 to 2012, results showed significant variations in their height structures, both at high and low altitudes. The dominant IAP height class in the study area was 2-5 m, especially at low altitude. Foxcroft and Freitag-Ronaldson (2007) noted that the infrequent clearing of IAPs in the upper Sabie River catchment constituted an impediment to the management efforts made in the lower catchment in the KNP. Three phases are identified in the control of IAPs: one, initial control, involving major clearing resulting in significant reduction of the weed population; two, follow-up control, where previously cleared sites are visited to remove seedlings and coppices; and three, maintenance control, which is an ongoing control aimed at keeping IAPs at low

numbers and preventing resurgences (Department of Water Affairs, 2018). Results show a difference in height structure, with 2-5 m tall plant dominating the area. This implies a lack of regular follow-up control. Removing smaller plants, i.e. seedlings and coppices, during the follow-up control phase is crucial as it helps deplete seedbank as well as prevent seed formation (Morris *et al*. 2008). For the 1996/7 study, half of the 40 plots had been cleared, but all areas had been cleared prior to the 2005 study. Therefore WfW clearings and follow-ups were carried out sporadically between 2005 and 2012, throughout the study area.

Results of this study showed a transformation in IAP height classes from being dominated by >5 m plants in the early stages of the WfW clearing programme, to showing significant recruitment in 2005, indicated by the prevalence of 1-2 m tall plants, and being characterised by 2-5 m plants in 2012. The initial 1996/7 WfW alien plant clearing operations were successful at removing the majority of large alien plants (Beater *et al*. 2008; Witkowski and Garner, 2008). In 2005, *L. camara* aerial cover, in particular, was largely dominated by 1-2 m closely followed by 2-5 m tall plants. This strong presence of a relatively juvenile *L. camara* population at the study sites could have emerged from the gaps and patches caused by the 2000 flood (Beater *et al*. 2008, Foxcroft *et al*. 2008). In the KNP, Petit and Naiman (2005) found higher seedling abundance and richness in the riparian vegetation under flood induced debris piles.

The devastating consequences of the flood in the riparian vegetation, i.e. digging out of most alien and indigenous vegetation, and depositing sediment and propagules, would have reset the ecosystem in the affected zone, resulting in new and vigorous recruitment of indigenous but also alien plant species (Holmes *et al*. 2005; Foxcroft and Freitag-Ronaldson, 2007). It has been shown that canopy gaps resulting from disturbances provided favourable establishment grounds for *L. camara* invasions (Day *et al*. 2003a; Raizada *et al*. 2008). In the KNP, however, Vardien *et al*. (2012) noted that *L. camara* density did not increase considerably following the 2000 flood, particularly owing to continuous management efforts (clearings) in the area. van Wilgen *et al*. (2012) reported a considerable decline in the area invaded by

L. camara in South Africa, between 1995 and 2008, also owing to WfW management efforts. On the contrary, the present study showed an increase in *L. camara* aerial cover from 2005 to 2012, possible resulting from the open condition (open overstorey canopy as opposed to shade) created upon removing larger plants, such as *Eucalyptus* spp. and *Pinus* spp. since 1996/7.

According to the WfW clearing records post the 2000 flood, IAPs in and around the present study area were cleared annually followed by several follow-ups. However, after the handing over of clearing and maintenance responsibilities of specific lands to their respective users, the frequency of successive follow-up clearings has drastically declined (Marais and Wannenburgh, 2008); suggesting either a lack of proper coordination between the WfW and land-users or unwillingness of land-users to clear IAPs. The large number of *L. camara* plants recorded in 2005 may have simply been a reflection of the increased, post-flood plant density prior to subsequent clearings. Consequently, subsequent clearings and probably the process of self-thinning might have brought the density down to the levels observed in 2012.

The 2012 surveys showed no differences in the percentage aerial between invasive and indigenous plants, particularly at high altitudes; whereas, at low altitudes, indigenous plant cover (particularly >5 m tall plants) was greater than invasive plant cover. It is expected that plant communities with abundant and diverse indigenous species should have less invasive alien plants (Levine, 2000; Kennedy *et al*. 2002), but that is not always the case (Lavorel *et al*. 1999). Huston (2004) reported that both alien and native plant species derived the same benefits from favourable environmental conditions, such that a positive, rather than a negative, correlation should be expected between native and alien plant species (Stohlgren *et al*. 2001). The 2012 results suggested that indigenous trees were progressively becoming canopy dominants, particularly in low altitude plots where there was evidence of clearings. Thus WfW clearings have been effective, at least at stopping alien plants from completely outcompeting indigenous plant populations.

In conclusion, the overall aerial cover of IAPs in the study area was above 30% and largely unchanged over 16 years after the initial WfW clearing operation. In

2012, the height class structure that had changed greatly in response to the 2000 flood, had simply reverted back to the original 1996/7 situation, except that the study area was now invaded with large numbers of understorey (e.g. *L. camara* and *S. mauritianum*) rather than overstorey IAPs such as *Eucalyptus grandis*.

By 2012, *L. camara* cover was probably the highest of all understorey IAPs in the study area, at least results showed it was greater compared to bugweed. The proportion of IAP cover occupied by *L. camara* increased significantly from representing <5% in 1996/7, to 26% in 2005 and 39% of the total IAP cover in 2012. While WfW clearings successfully removed larger overstorey IAPs, they also opened the canopy creating a growing environment conducive for an amalgamation of understorey IAPs, which collectively still put an enormous amount of pressure on the South African water resources (Le Maitre *et al*. 2002; Le Maitre *et al*. 2016). Le Maitre et al. (2002) estimated that IAPs reduced water flow of the Sabie River by ~2192 m^3 per year, and of this reduction eucalypts accounted for 24%; pines, 18%; *S. mauritianum*, 14%; and *L. camara*, 13%. There was reduction in the national estimated total water flow from 3 300 million m^3 per year (Versveld *et al*. 1998) to 1 444 million m3 per year (Le Maitre *et al*. 2016); and of the total flow reduction, *Acacia* spp. accounted for 34%; *Pinus* spp., 19%; *Eucalyptus* spp., 16%; and *S. mauritianum*, 4% (Le Maitre *et al*. 2016).

Coupled with their regenerative capacity, IAPs grow faster than most native plants (Morris, 2008); and clearing can be considered another form of disturbance with the potential to facilitate their spread even further (Witkowski and Wilson, 2001). Therefore the only way to keep the invasion under control is to ensure responsive follow-up clearings and adopt a preventive approach by targeting emerging weeds. Clearings of IAPs must not only be focused in the riparian but also in the adjacent plantation zone, such that neither of these zones harbours IAP that could potentially serve as sources of re-infestation.

Such long-term monitoring studies are of great value to both WfW and land-users as they contribute to the improvement of monitoring and evaluation strategies for the control of IAPs. For such a reputable programme as WfW, the monitoring and

evaluation of its clearing efforts should be conducted on a short as well as long-term basis to keep the levels of alien invasions under control. Knowing that *L. camara* has also been targeted for biocontrol, WfW management must stress to their clearing teams as well as land-owners the importance of leaving a proportion of an infested land uncleared in order to serve as a refuge and source of spread for the suite of biocontrol agents already established. Keeping an established suite of biocontrol agent on a small weed population will help save the cost of rearing and re-releasing agents when the weed resurges.

Chapter Three: Field-based evaluation of biocontrol of *Lantana camara* through chemical exclusion.

This chapter has been prepared for submission to Biological Control

3.1 Abstract

Despite intensive biological control efforts, *Lantana camara* L. (sensu lato) (Verbenaceae) has remained an invasive alien plant of significant concern in many tropical and subtropical countries worldwide, including South Africa. This work aimed to quantify the combined impact of all established biocontrol agents on the vegetative and reproductive growth of *L. camara* under field conditions, in an inland area, across an altitudinal gradient, over two consecutive growth seasons; and monitor and evaluate the release, establishment and spread of the *L. camara* flower gall mite, *Aceria lantanae* (Cook) (Acari: Eriophyidae) in the study area. An insecticidal exclusion method using carbofuran was employed, and comparisons of plant growth parameters and agent performance were made between 'insect-exclusion' plants (carbofuran-treated plants) and 'biocontrol' plants (untreated plants). Despite failing to maintain the exclusion plants completely biocontrol agent-free, side-stem production was 37%, 24% and 28% less in biocontrol plants compared to insect-exclusion plants; and leaf damage was 19%, 20% and 40% greater in biocontrol compared to insect-exclusion plants, at high, medium and low altitude, respectively. There were, however, no differences in lantana dry biomass increase from 2012 to 2017 between biocontrol and exclusion plants. *Aceria lantanae* was released successfully in 2013 and persisted until the end of the study in 2017. Its population, however, established in low numbers; and only in 20% of the study sites, mainly at low altitude sites (~848 m). Although there were no significant differences in the germinable seedbank between soil underneath biocontrol and exclusion plants, both at high altitude (100 ± 78.33; 91.11 ± 66.80) and low altitude (95.56 ± 35.94; 140 ± 40.92), respectively; the number of germinable seeds/m^2 was considerably high, which indicates *L. camara*'s potential to re-invade sites over long periods. This study highlighted some of the difficulties of employing insecticidal techniques to measure the impact of biocontrol agents on a target host under field conditions, but also demonstrated that the established suite of biocontrol agents is having an impact on lantana in inland areas.

Keywords: *Aceria lantanae*; Biocontrol; *Hypena laceratalis*; insecticidal exclusion; Seedbank; *Teleonemia scrupulosa*.

3.2 Introduction

Biological invasion results from some introduced species having a competitive advantage over native ones, enabling them to expand their range beyond their natural frontiers (van der Velde *et al*. 2006; Valery *et al*. 2008). Invasive alien plants (IAPs) have led to biodiversity loss through habitat destruction (Mooney and Hobbs, 2000), having negative effects on both livestock and humans (Mack *et al*. 2000; Hulme, 2006). Habitat loss and IAPs are regarded as the two most important drivers of biodiversity loss (Lowe *et al*. 2000). An example of an IAP with damaging consequences on biodiversity is *Lantana camara* L. (sensu lato) (Verbenaceae). This work examined the contribution of biocontrol of *L. camara* in an integrated management strategy, which includes other control methods such as manual clearing and herbicide applications.

Lantana camara has become one of the worst IAPs in recorded history (Urban *et al*. 2011; Bhagwat *et al*. 2012), having ecologically and economically damaging impacts in tropical, subtropical and temperate regions of the world (Day *et al*. 2003a). It has invaded millions of hectares of land in more than 60 countries worldwide including countries such as Australia, India and South Africa (Day *et al.* 2003a; Bhagwat *et al*. 2012). *Lantana camara*'s invasiveness has been attributed to its wide ecological tolerance, occurring at altitudes from sea level up to 2,000 m, and under rainfall conditions ranging from 750 to 5,000 mm per annum (Day *et al*. 2003a; Urban *et al*. 2011). In addition to the ecological tolerance, *L. camara*, just like many other invasive plants (Chin, 2001), owes its invasiveness to its high seed production (Day *et al*. 2003a; Urban *et al*. 2011).

In Australia, each *L. camara* infructescence (cluster of fruits) was reported to comprise up to eight fruits (Barrows, 1976), while in India it ranges between 25 and 28 (Sharma *et al*. 2005). Despite enclosing between one and two seeds (1-2 mm long) per fruit (Sharma *et al*. 2005), which are capable of germinating throughout the year

under adequate light, temperature and moisture conditions (Duggin and Gentle, 1998; Parsons and Cuthbertson, 2001), *L. camara* germination percentage has been reported to be very low (4-45%) (Sharma *et al*. 2005). The low germination rate caused by *L. camara* seed dormancy and low seed viability is, however, compensated for by an equally low rate of seedling mortality (Duggin and Gentle, 1998; Sahu and Panda, 1998).

Seedbank ecology is an important life history stage which guarantees continued invasion of IAPs (Vivian-Smith and Panetta, 2009), yet little is known about the seedbank ecology of *L. camara* (Day *et al*. 2003a; Vivian-Smith *et al*. 2006; Vivian-Smith and Panetta, 2009). Studies in Australia have reported various soil seedbank densities, from five seeds/m^2 (Gentle and Duggin, 1998) to between 79 and 402 seeds/m^2 (Vivian-Smith *et al*. 2006), and Fensham *et al*. (1994) recorded 10.5 to 25.3 seedlings/m^2 of soil in north Queensland. In Ecuador, 43 seeds/m^2 were recorded (Myster, 2004), and 1,049 to 2,690 seeds/m^2 in Ghana (Oppong *et al*. 2003). Aided by frugivorous birds on one hand, and various pollinators such as bees on the other (Johansen and Mayer, 1990), *L. camara* has the propensity of invading pristine ecosystems, transforming native vegetation into mono-stands of impenetrable thickets, thus impeding crop production, animal production, forestry and water resource availability (Day *et al*. 2003a). In South Africa, it is estimated that *L. camara* covers about 560,000 ha of land including riparian zones (Kotze *et al*. 2010).

Riparian zones, also referred to as 'critical transition zones' (Ewel *et al*. 2001), play important ecological functions to both humans and animals, including the provision of food, the control of nutrient flux, creation of a buffer zone between terrestrial and aquatic environments, stabilization of stream water temperatures as well as stream banks (Hood and Naiman, 2000; Richardson *et al*. 2007). Invasive alien plants, such as *L. camara*, have been reported to reduce stream flows, restrict access to rivers for both animals and humans, and most importantly threaten the already limited water resources in South Africa (Richardson *et al*. 2007). To cope with the threat to the ecosystem and biodiversity posed by the myriad of IAPs in South Africa, a nationwide initiative, termed the *Working for Water* (WfW)

programme, was created in 1995, with the primary aim of controlling IAPs in an integrated pest management strategy along riparian zones, but also to respond to the social challenge of unemployment through job creation (van Wilgen *et al*. 1998; Turpie *et al*. 2008). Integrated pest management is used, involving the combination of two or more control methods, such as manual, mechanical, chemical and biocontrol.

Biocontrol of IAPs has proven to offer good value for money (Zimmermann *et al*. 2004), and is regarded as a better alternative for long-term, sustainable and environmentally friendly IAP control compared to the conventional mechanical and chemical methods (van Wilgen and De Lange, 2011; Zachariades *et al*. 2017). Classical biological control consists of releasing biocontrol agents with the aim to suppress targeted pest species on a long-term basis and in a sustainable manner, keeping their populations at acceptably low levels (Sujii *et al*. 1996). For over a century, various countries have been attempting to control *L. camara* using the biological method (Swarbrick *et al*. 1995, Day *et al*. 2003a, Urban *et al*. 2011). Globally, 45 biocontrol agents have been released for the control of *L. camara*, and of the 45, South Africa counts 13 established biocontrol agents (Urban *et al*. 2011; Winston *et al*. 2014), yielding variable results, but mostly failing to adequately suppress the target weed (Crawley, 1989; Urban *et al*. 2011). The term 'biocontrol agent' hereinafter will refer to all arthropods introduced for classical biological control, which are established in an introduced range, plus the target weed's other associated phytophagous insects. In South Africa, from its inception in 1961 to date, *L. camara* counts 17 biocontrol agents (Baars *et al*. 2003; Urban *et al*. 2011; Klein, 2011). At the study sites in Mpumalanga, the most common agents were:

(i) The *L. camara* flower gall mite, *Aceria lantanae* (Cook) (Acari: Eriophyidae), native to South and Central America in countries around the Gulf of Mexico, was introduced into South Africa in the year 1989 (Urban *et al*. 2011). It was cultured at ARC-PPRI, Pretoria, from flower galls collected from orange and pink *L. camara* varieties (Urban *et al*. 2011). The mite feeds, reproduces and multiplies within the developing flower gall (Urban *et al*. 2011). Reproduction in eriophyid mites is by spermatophore transfer and arrhenotoky (Oldfield and Michalska, 1996), and they are

capable of doubling their populations within approximately 10 days (Sabelis and Bruin, 1996). They are dispersed by wind and by phoresy on flower-visiting insects (Sabelis and Bruin, 1996). By feeding on the undifferentiated flower buds, *A. lantanae* induces the formation of large galls comprising a cluster of very small green leaves (Cook, 1909); this prevents flowering and consequently fruiting. *Aceria lantanae* was released between 2007 and 2012 in the provinces of KwaZulu-Natal (KZN), Limpopo (LP), Mpumalanga (MP) and Gauteng (GP), and its establishment has been observed in all these provinces (Urban *et al*. 2011; Mukwevho *et al*. 2017). The flower-galling mite has been reported to reduce inflorescence and infructescence production by >95% along the humid coastal regions of KZN, where climatic conditions are most favourable to the agent (Urban *et al*. 2011; Mukwevho *et al*. 2017). One of the advantages of using mites as biocontrol agents is the fast rate at which they are capable of increasing their populations, plus their rapid, visible effect on seed production (Day *et al*., 2003a; Smith *et al*. 2010). However, their impact on *L. camara* in the field needs to be further quantified inland.

(ii) The leaf-chewing moth, *Hypena laceratalis* Walker (Lepidoptera: Noctuidae), is indigenous to South Africa and is found in Kenya and Zimbabwe (Day *et al*. 2003a); but also reported to be native to Asia and Australia (Cilliers and Neser, 1991). It has only been recorded from *L. camara* species, and is the only *L. camara* biocontrol agent which does not originate from the Americas (Day *et al*. 2003a). The adult moth feeds on flowers and lays eggs on the underside of leaves, where the larvae feed while leaving the upper epidermis undamaged. *Hypena laceratalis* development from egg to adult takes about 30 days (Day *et al*. 2003a). In many countries, it is known to have sporadic outbreaks, often in summer (Baars and Neser, 1999; Day *et al*. 2003a). In South Africa, the moth is spread throughout the country and reported to be among the most abundant *L. camara* biocontrol agents (Urban *et al*. 2011), however its ability to defoliate the plant is believed to be hindered by native parasitoids (Cilliers and Neser, 1991).

(iii) The *L. camara* leaf-mining beetle, *Octotoma scabripennis* Guèrin-Mèneville (Coleoptera: Chrysomelidae), is of Mexican origin, where it was found on *L. camara*

and *L. urticifolia* (Palmer and Pullen, 1995). It is deemed to be one of the most damaging *L. camara* biocontrol agents (Cilliers and Neser, 1991; Day *et al*. 2003a). Adult beetles use the upper surface of leaves to feed and oviposit, whereas larvae mine the leaves causing blotches (Day *et al*. 2003a). Damage has been reported to prevail in late spring and summer, resulting in leaf abscission and a reduction in flower and seed production (Cilliers, 1987a; Baars and Neser, 1999; Day *et al*. 2003a). *Octotoma scabripennis* is not restricted by varietal preference (Day *et al*. 2003a). In South Africa, the beetle thrives in inland as well as coastal areas, i.e. KwaZulu-Natal-Natal, Eastern Cape (Heystek, 2006), and Mpumalanga (Urban *et al*. 2011).

(iv) The *L. camara* herringbone leaf-mining fly, *Ophiomyia camarae* Spencer (Diptera: Agromyzidae), which is morphologically similar to the fruit-mining fly, *Ophiomyia lantanae* (Froggatt) (Diptera: Agromyzidae) and the leaf-mining fly, *Calycomyza lantanae* (Frick) (Diptera: Agromyzidae) (Day *et al*. 2003a), was imported into South Africa from Florida (USA) in 1997. It is believed to have originated from Central and South America (Palmer and Pullen, 1995; Baars and Neser, 1999), and was released into South Africa in 2001 (Simelane, 2002). *Ophiomyia camarae* adults feed on the nectar in flowers. Eggs are laid in the leaf tissue and the larvae tunnel along the veins through to the midrib forming herringbone-shaped mines on the leaves (Simelane, 2002). Larval tunneling interferes with translocation, eventually leading to leaf abscission (Simelane, 2002). The adult fly ranges in length from 1.5 to 2.0 mm, with a black body, shining abdomen, and dark red compound eyes (Simelane, 2002). The fly was found to be abundant mostly in KZN, followed by MP (Heystek, 2006). *Ophiomyia camarae* utilizes most *L. camara* varieties (Heystek, 2006), but has shown a preference for the light pink *L. camara* variety which is probably the most common variety in South Africa (Simelane and Phenye, 2005). Semi-field impact studies (i.e. in cages over plants in the ground) showed that the herringbone leaf-miner reduced *L. camara* leaf density, flower density and above-ground biomass of smallish plants by 73%, 99% and 49%, respectively, within a single growth season (Simelane and Phenye, 2005).

(v) A fungal leaf-spot pathogen with damage symptoms similar to those of *Passalora lantanae* (Chupp) U. Braun and Crous var. *lantanae* (Mycospherellales: Mycospherellaceae) (Day *et al*. 2003a) was present at the study sites. Its damage symptoms include chlorosis with grey lesions visible on the adaxial surface of leaves, necrosis of flower buds and stalks, which can result in defoliation and consequently a reduction in the vegetative and reproductive growth of the plant. The pathogen was observed to be attacking *L. camara* of various colour varieties.

(vi) The *L. camara* leaf-sucking bug, *Teleonemia scrupulosa* Stål (Hemiptera: Tingidae) is not only among the first insects to be used as a biocontrol agent of *L. camara*, but it is also the most widespread and successful agent, established in over 25 countries (Winston et al. 2014). Early records trace the origin of *T. scrupulosa* from Mexico, Central and South America (Harley and Kassulke, 1971; Waterhouse and Norris 1987). In 1902, *T. scrupulosa* was introduced into Hawaii from Mexico (Distant, 1909), and from Hawaii via Fiji, it was introduced into Australia in 1935 (Harley and Kassulke, 1971). Subsequent introductions were made throughout most *L. camara* infested areas worldwide using material mostly originally from Mexico (Winston et al. 2014). Adult females of *T. scrupulosa* preferably insert a batch of up to 30 eggs on the abaxial surface of young leaves, however they do also deposit in stems or flowers. The eggs can overwinter; and they take seven to eight days to hatch in summer, and 11 to 18 day for nymphs to reach the adult stage. Both nymphs and adults live and feed in colonies mainly on the undersurface of leaves but also on flowers and tips of emerging stems (Fyfe, 1937). Adults live approximately seven to eight weeks (Harley and Kassulke, 1971). Feeding by both the nymphs and adults has been found to have some systemic phytotoxic effects resulting in chlorosis, causing necrotic lesions, foliar deformation (Waterhouse and Norris, 1987) and abscission, and ultimately leading to stem dieback (Harley and Kassulke, 1971).

The impact of biocontrol agents on plant fitness, under field conditions, can be measured by performing some pre- and post-release comparisons of the target plant's abundance and growth (Carson *et al*. 2008), but such comparisons become difficult if agents are already established on the target weed. This challenge can, however, be

surmounted through the use of experimental manipulations such as, controlled release (Carson *et al*. 2008; Urban *et al*. 2011), exclusion by cages (McFadyen, 1998; Dhileepan, 2003; Carson *et al*. 2008; Morin *et al*. 2009), and chemical exclusion (Crawley, 1989; Tipping and Center, 2002). Experimental manipulation is the most complex but reliable approach to evaluate the impact of biocontrol agents on target plants (Goolsby *et al*. 2004; Tipping *et al*. 2008). This approach consists of determining the effect that agents have on weed density through comparisons between plots/quadrats with and without biocontrol agents (Dhileepan, 2003; Morin *et al*. 2009). The latter experimental manipulation, namely chemical exclusion, has been successfully used to evaluate the effectiveness of biocontrol in *Sida acuta* (Lonsdale *et al*. 1995), *Mimosa pigra* (Lonsdale and Farrell, 1998), *Parthenium hysterophorus* (Dhileepan and McFadyen, 2001), *Echium plantagineum* (Sheppard *et al*. 2001), *Chrysanthemoides monilifera* (Adair and Holtkamp, 1999), and *L. camara* (Cilliers, 1987b; Mukwevho *et al*. 2017). The impact of biocontrol agents can be effectively evaluated by excluding agents from their host with insecticides, thereby enabling comparisons of host fitness between agent-infested and agent-free plants (Crawley, 1989; Tipping and Center, 2002).

Insect pests on plants are controlled using contact or systemic insecticides, often, applied to the foliage either as a spray or dust (Juraske *et al*. 2009); however soil application is also employed in many instances. One such soil applied insecticide is carbofuran (2,3-dihydro-2,2-dimethylbenzofuran-7-yl methylcarbamate), which is a broad-spectrum, systemic insecticide, nematicide, and acaricide, commonly used in homes and agriculture (Dobsikova, 2003). Its application to grain sorghum at 1kg, 2 kg and 3 kg a.i./ha (active ingredient) reduced maize stalk borer, *Busseola fusca*, shoot fly, *Anatrichus erynasius*, and maize aphid, *Rhopalosiphum maidis*, infestations (van Rensburg *et al*. 1978). It was also used at a rate of 10 kg a.i./ha (10% a.i.), to quantify the impact of the chrysomelid beetle, *Calligrapha pantherina* Stål, on seed production of the tropical weed, *Sida acuta* Burm. F. (Malvaceae). Results showed a significantly higher number of seeds in exclusion plants compared to *C. pantherina*-infested plants, which signified that carbofuran effectively excluded *C. pantherina*

from the plants (Lonsdale *et al*. 1995). Aldicarb (15% a.i.), a highly toxic and persistent, systemic insecticide, was used at a rate of 400kg/ha, and it successfully removed *L. camara* biocontrol agents (Cilliers, 1987b). The use of aldicarb has, however, been prohibited following numerous reports of its harmfulness to the environment (van Zyl, 2012).

An earlier study (Cilliers, 1987b) quantified the impact of an 'old' suite of biocontrol agents, namely the *O. scabripennis*, *Uroplata girardi* Pic (Coleoptera: Chrysomelidae) and *T. scrupulosa*, on the growth and reproduction of the pink flowering *L. camara* variety on the east coast of KwaZulu-Natal, in South Africa; and found the agents to be damaging to the weed. Since that time, several 'new' agents have been introduced, and studies have assessed their efficacy under laboratory and semi-field conditions (Simelane and Phenye, 2005; Simelane, 2010; Urban *et al*. 2011); however, laboratory results may not always accurately predict performance in the field (Urban and Phenye, 2005). This is the first study to quantify the combined impact of the 'old plus new' suite of biocontrol agents on the vegetative and reproductive growth of *L. camara* in the field, in an inland area, through the use of the chemical exclusion method. The three specific objectives were:

(i) To determine the impact of all established biocontrol agents on the vegetative and reproductive output of *L. camara*.
(ii) To measure the impact of these agents on the regeneration capacity of *L. camara*.
(iii) To release and monitor *A. lantanae* at the study sites and determine its impact on the reproductive output of *L. camara*.

3.3 Materials and Methods

3.3.1 Study sites

This work was conducted along the Sabie River catchment in Mpumalanga, South Africa, as part of a long-term study on sites with over a decade-worth of history of IAP monitoring (Garner, 2006; Beater *et al*. 2008). Rising from the Drakensberg, at an altitude of 2200 m, the Sabie River flows eastward for 210 km crossing the Kruger National Park to Mozambique at an altitude of 150 m (Foxcroft *et al*. 2008). Study

sites were divided into three altitude levels, high (Sabie), medium (Graskop) and low altitude (Hazyview). Graskop receives 25% more rain than the other areas (Table 3.1). The climate at these sites is temperate to subtropical, with hot, rainy summers and mild, dry winters (Garner, 2006; Beater *et al*. 2008), which is conducive to an array of IAPs, including *L. camara* and its suite of biocontrol agents (Urban *et al*. 2011). The study sites are situated within the riparian zone, immediately adjacent to eucalypt and pine plantations.

Table 3.1 Altitude, slope, annual precipitation and daily minimum/maximum temperatures (mean ± S.E.) of the riparian study sites within the Sabie, Graskop and Hazyview areas (n = 10) (Modified from Beater *et al*. 2008). Values with different superscript letters within rows are significantly different (Tukey HSD, $P < 0.05$).

Environmental Parameter	High altitude Sabie	Medium altitude Graskop	Low altitude Hazyview
Altitude (m asl)	1133±11[a]	975±9[b]	848±15[c]
Slope inclination (degrees)	6.2±2.1[b]	6.2±2.1[b]	12.8±1.7[a]
Annual precipitation (mm/annum)	1012.5±9.9	1286.5±20.2	1056.9±3.9
January daily maximum temperature (°C)	26.0±0.0	26.5±0.2	27.0±0.0
July daily maximum temperature (°C)	19.0±0.0	20.5±0.2	21.0±0.0
January daily minimum temperature (°C)	15.0±0.0	16.0±0.0	16.4±0.1
July daily minimum temperature (°C)	4.0±0.0	5.0±0.0	5.0±0.0

The plantation zone, however, was omitted from the present study due to the frequent disturbances, from the active physical and chemical control of IAPs (using slashing and various herbicides at various frequencies, especially before tree canopy closure) to other physical disturbances such as pruning, thinning, felling and replanting of eucalypts and pines, all of which introduce an unacceptable amount of uncontrollable variation within the plantation zone.

3.3.2 Experimental plots and treatments

This study was conducted on sites previously used by Garner (2006) and then Beater *et al*. (2008) (Chapter two). During a general survey (Chapter two), lantana density and aerial cover were determined throughout the study area; and plots with similar lantana plant density and aerial cover were selected for the present experiment. The

general survey was also used to identify members of the suite of *L. camara* biocontrol agents occurring in the study area.

Field trials were conducted from summer 2012/13 to summer 2015, covering two full growth seasons, and additional experiments were completed in 2016 and 2017. In September of 2012, ten plots (20x50 m) were arranged, with two near Sabie, three near Graskop, and five near the town of Hazyview. Within each plot, a pair of 4x4 m quadrats, ~10 m apart, was set up with one containing exclusion plants, which were carbofuran-treated and intended to be free of phytophagous arthropods; and the other one, containing chemical-free biocontrol plants allowing the infestation of biocontrol agents. Initially, all plants within these quadrats were cleared by cutting the stems at ground level and removing the plant. The impact of biocontrol agents was monitored on pre-existing plants, cut down to the ground (with only < 5cm stumps left) and allowed to coppice.

Two *L. camara* stumps, centrally located within the quadrats were tagged and monitored seasonally while other plant species were cleared to the ground to allow easy access to the growing *L. camara* plants within the quadrats. Monitoring of plant growth and insect performance was conducted on 20 biocontrol and 20 exclusion plants (Fig. 3.1).

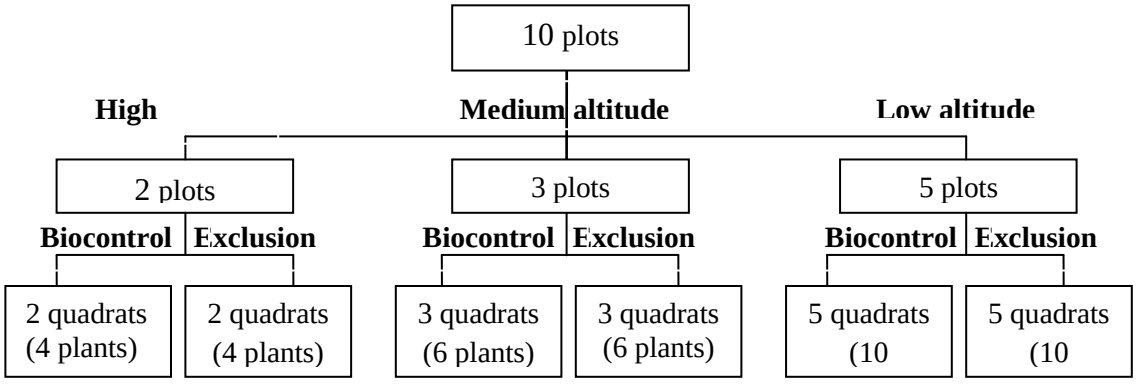

Figure 3.1 Experimental layout showing plots at high, medium and low altitude sites in Mpumalanga, with biocontrol and exclusion plants.

Initial biomass (in 2012) of the targeted *L. camara* plants within each quadrat was determined by weighing the cut plant materials separated into leaves (including seed and flowers) and stems to the nearest gram. Biomass obtained in 2012 was compared

with that obtained in 2017, weighed in the same manner as the initial biomass (see detailed method below in *L. camara* biomass sub-section).

Exclusion plants were treated with carbofuran, a broad-spectrum, systemic carbamate insecticide (100 g a.i. per kg granules i.e. 10% a.i.). Carbofuran granules were initially spread and worked into the soil around exclusion *L. camara* stumps (which grew into whole plants over the seasons) at a dosage of 70 g/m^2 (i.e. 7 g a.i./m^2) in December of 2012 (three months after the plants were cleared) before being watered to facilitate the initial uptake through the plants' root system. Stumps from biocontrol quadrats received an equal amount of plain water as that applied to exclusion plants to avoid bias. In spring 2013, one growth season later, the carbofuran dosage was doubled (140 g/m^2) (i.e. 14 g a.i./m^2) to maintain the efficacy of the chemical as plant biomass kept increasing. Carbofuran was applied every two months at a dosage of 70 g/m^2 (i.e. 7 g a.i./m^2) from summer 2012/13 to spring 2013, which was later increased to (140 g/m^2) (i.e. 14 g a.i./ m^2) from spring 2013 to summer 2015. Due to unforeseen technical reasons, insecticide applications were halted after the summer 2015's application and only resumed later in summer 2016, a year later.

The combined impact of all established *L. camara* biocontrol agents was compared between biocontrol and exclusion plants; and plant growth parameters were measured at three different altitudinal levels, high, medium and low.

3.3.3 Vegetative and reproductive growth

Plant height and canopy area cover

Plant height was measured as the vertical distance from the ground to the highest living part of the plant (Anderson and Ingram, 1993).

Canopy cover was estimated by measuring two diameters perpendicular to each other and using their mean to find the cover in the following formula: Canopy cover = $\pi (D/2)^2$, where D is mean diameter (Mueller-Dombois and Ellenberg, 1974).

Stem length and number of side-stems

Three centrally located stems per plant were selected and tagged. Their length was measured using a tape-measure, and the number of side-stems per tagged stem was counted.

Number of leaves, inflorescences and infructescences

The number of leaves (>15 mm long), inflorescences and infructescences was counted on the distal 500 mm of tagged stems. When the distal part of these stems became too high and unreachable, their side-stems were used.

Lantana camara biomass

In the summer of 2017, *L. camara* wet biomass was measured through destructive sampling, separated into leaves (including inflorescences and infructescences) and stems, and compared between biocontrol and exclusion plants. Dry biomass was estimated using a wet to dry weight ratio developed from a laboratory experiment (see Chapter Four). Total above ground biomass (leaf + stem) gained between 2012 and 2017 was measured as 2012 biomass minus 2017 biomass.

3.3.4 *Lantana camara* regeneration capacity

Seedling and soil seedbank density comparisons were done at high (high and medium altitude combined) and low altitude sites, using five quadrats per altitude (n = 5). High and medium altitude quadrats were combined in order to have a large enough sample size for statistical analysis. Seed rain density, on the other hand, was observed at three altitude levels, high (4 plants), medium (6 plants) and low (10 plants).

Seedling and seed rain densities

Seedlings growing within all the quadrats (4x4 m) were counted and then pulled out at every seasonal sampling period.

Four seed traps (inverted, cut–off, plastic 2L Coca-Cola bottles), each measuring 100 mm in diameter and 180 mm high, with a combined surface area of ~

0.035 m^2, were placed underneath a plant's canopy at the four cardinal compass directions (on a 500 mm radius around the main stem) and collected seeds per plant were counted. The traps were secured 50 mm above the ground with 8 mm steel pegs (fence droppers). The bottle neck part was fitted with <1 mm stainless wire mesh to allow rain water to escape while keeping seeds trapped. The traps were inspected and mesh replaced when needed after every two months. Seeds were harvested in summer (November/December) of 2014, 2015 and 2016. Summer was chosen because seed production was greater at this time of the year. For convenience, seed herein, unless stated otherwise, refers to a single fruit from a fruit cluster or infructescence.

Soil seedbank trial

In summer 2014 (December), soil samples were collected from underneath biocontrol and exclusion plants, using a small steel square quadrat (300x300 mm) at a depth of 0-30 mm, after scraping off the top layer of leaf litter. Twenty samples in total were collected randomly, 10 from within the biocontrol plant quadrats and 10 from the exclusion plant quadrats. Soil samples were returned to the greenhouse at the University of the Witwatersrand, where they were spread out in germination trays (450x200 mm, 50 mm depth). Irrigation within the greenhouse was done twice a day for 15 minutes; and the germination trays were exposed to full sun (light condition). Minimum/maximum temperatures were 21^{o}C and 26 ^{o}C, respectively, with an average relative humidity of 58%.

The seedbank was estimated through germination tests which ran for over two months. Germination was confirmed when a cotyledon was visible above the soil. Emerged seedlings were removed after being recorded. To increase the chance for all the seed to germinate, the soil was turned at day 57. Observations were made daily. The experiment was terminated when no additional seed germinated. Observation of the germination trays continued for 10 months after termination of the experiments, and there was no further germination occurred.

Seed production

In summer of 2015, infructescences (fruit clusters) were counted per plant on the 40 plants, 20 biocontrol and 20 exclusion. The number of fruits was counted from five infructescences randomly selected per plant and the mean number calculated from the five infructescences decorticated per plant. Five hundred fruits, half from each treatment, were dissected to determine the number of seeds per fruit. Total seed production per plant was calculated as follows: seeds/plant = (infructescences/plant) (fruits/infructescence) (seeds/fruit)

3.3.5 Biocontrol agent abundance and performance

Most biocontrol agents cause obvious foliar damage symptoms (Even though inflorescences and infructescences are also attacked), and so by separating the symptoms of individual biocontrol agents, the percentage of leaves damaged by each biocontrol agent was used to determine individual agent's performance. The number of leaves (>15 mm long) counted on the distal 500 mm of tagged stems was used as a standard sample of leaves to estimate the percentage leaf damage from the agents: $\frac{\text{Number of damaged leaves}}{\text{Total number of leaves}} * 100.$

3.3.6 *Aceria lantanae* release and monitoring

Aceria lantanae was released on all biocontrol plants, while kept away from their respective, paired exclusion ones. The mites were initially released in summer 2013, followed by three subsequent releases in autumn 2013, spring 2013 and spring 2014. Mite inoculates in the form of *L. camara* flower galls (~540 galls) were collected from Tzaneen, Limpopo (S 23° 59.355 E 30° 14.973, Elevation: 673m). Releases were done by tying a flower gall as close as possible to the tip of an actively growing stem, to enable the emerging mites to locate the undifferentiated inflorescence buds in which they breed (Urban *et al*. 2011; Urban and Mpedi, 2012).

The impact of *A. lantanae* on the reproductive output of *L. camara* plants was measured from two plots at the low altitude sites, chosen based on observations showing consistent presence of galls throughout the study time. *Aceria lantanae*

flower galls were, however, counted per plant in the entire study area and presented in a table as absolute numbers from summer 2013 to autumn 2016. Each plot had two biocontrol and two exclusion plants. The extent to which *A. lantanae* inflicted damage on *L. camara* was measured by counting the number of galls in comparison to that of inflorescences, expressed as a percentage (Mukwevho *et al*. 2017).

3.3.7 Carbofuran residue analysis

Leaf samples from the biocontrol and exclusion plants were sent to an independent laboratory, Hearshaw and Kinnes Analytical Laboratory (HKAL) (Pty) Ltd., in Cape Town for insecticide residue analysis using the LCMS (liquid chromatography plus mass spectrometry) analytical method. Two leaf samples were collected per plant from a total of three plants per treatment, i.e. six biocontrol and six exclusion leaf samples. Each leaf sample weighed a minimum of 200 g. Three different sets of samples were sent for analysis; the first one, two weeks after carbofuran was applied; the second one, four weeks later; and third one, six weeks on. These samples were collected between March and May 2017 from newly grown plants, i.e. growing from stumps of plants previously felled. The carbofuran-treated plants received (70 g/m^2) (i.e. 7 g a.i./m^2) which is the initial dosage applied at the beginning of the exclusion experiment.

3.3.8 Statistical analysis

Comparisons of the vegetative and reproductive growth of *L. camara* between exclusion and biocontrol plants, and impacts of biocontrol agents on growth from 2012/13 to 2015 were done using one-way ANOVAs with repeated measures followed by an LSD post-hoc tests. A student's t-tests was also performed to compare plant biomass at the beginning and the end of the experiment. Regression analyses were performed to assess relationships between agent herbivory and plant growth. The data were checked for normality, and where necessary data transformation was undertaken. All analyses were conducted at a $P < 0.05$ using Statistica, version 12.

3.4 Results

3.4.1 Vegetative and reproductive growth

Plant height and canopy aerial cover

Across all seasons, there were no significant differences in height between biocontrol and exclusion plants at high ($F_{(1, 6)} = 0.92$; $P = 0.37$), medium ($F_{(1, 8)} = 0.67$; $P = 0.44$) and low ($F_{(1, 18)} = 0.17$; $P = 0.68$) (Fig. 3.2.A) altitudes; whereas canopy cover was greater in exclusion quadrats compared to biocontrol quadrats at high altitudes ($F_{(1, 6)} = 6.39$; $P = 0.04$), while not different at medium ($F_{(1, 8)} = 3.92$; $P = 0.08$) or low altitudes ($F_{(1, 18)} = 0.01$; $P = 0.91$) (Fig. 3.2.B). Fisher LSD post-hoc tests showed that canopy cover was greater in exclusion plants compared to biocontrol plants in spring 2014 at high altitudes, and in autumn 2013, summer 2014 and autumn 2014 at medium altitudes (Fig. 3.2.B). There were significant changes between seasons in plant height and canopy aerial cover at high, medium and low altitudes (Table 3.2).

Stem length and number of side-stems

There were no significant differences in stem length between biocontrol and exclusion plants at low ($F_{(1, 58)} = 0.004$; $P = 0.95$) and high altitudes ($F_{(1, 22)} = 2.63$; $P = 0.12$) (Fig. 3.3.A); however, LSD post-hoc tests revealed that, at high altitudes, stem length was greater in exclusion plants compared to biocontrol plants in autumn 2014 and winter 2014. At medium altitudes, on the other hand, stem length was significantly greater in exclusion compared to biocontrol plants, as shown in autumn 2013, winter 2013 and spring 2013 ($F_{(1, 31)} = 8.22$; $P < 0.01$) (Fig. 3.3.A).

There were significant interactions between treatment and time (season) at high altitudes, meaning that the stem length differed between treatments only in some seasons and not others; and significant changes between seasons at all altitude levels (Table 3.2).

The number of side-stems was greater in exclusion compared to biocontrol plants at high altitudes ($F_{(1, 22)} = 5.34$; $P = 0.03$), particularly in summer 2014, autumn 2014, and summer 2015; at medium altitudes ($F_{(1, 31)} = 14.01$; $P < 0.01$) in autumn 2013, winter 2013 and spring 2013, and autumn 2014 and spring 2014; and at low

altitudes ($F_{(1, 58)}$ = 3.89; P = 0.05) in autumn 2014, spring 2014 and summer 2015 (Fig. 3.3.B).

There were significant interactions between treatment and season at high and low altitude sites; and significant seasonal changes at all altitude levels (Table 3.2).

Number of leaves, inflorescences and infructescences

There were no differences in the number of leaves per plant between biocontrol and exclusion plants at high ($F_{(1, 22)}$ = 0.05; P = 0.83) and low altitude sites ($F_{(1, 57)}$ = 0.42; P = 0.52), however at medium altitude sites in winter 2013, the number of leaves was greater in exclusion plants compared to biocontrol plants ($F_{(1, 31)}$ = 8.07; P = 0.01) (Fig. 3.4.A). LSD post-hoc tests also revealed that there were more leaves in exclusion plants than biocontrol plants at low altitudes in spring 2013 and spring 2014. There were significant seasonal effects at all altitude levels; and a significant interaction effect between treatment and season at low altitudes (Table 3.2).

There were no significant differences in the number of inflorescences ($F_{(1, 22)}$ = 0.61; P = 0.44), ($F_{(1, 31)}$ = 0.99; P = 0.33), ($F_{(1, 58)}$ = 2.02; P = 0.16) (Fig. 3.4.B) and infructescences ($F_{(1, 22)}$ = 1.43; P = 0.25), ($F_{(1, 34)}$ = 0.74; P = 0.40), ($F_{(1, 58)}$ = 3.62; P = 0.06) (Fig. 3.4.C) between biocontrol and exclusion plants at high, medium and low altitude sites, respectively. Post-hoc tests, however, showed more inflorescences in exclusion plants than biocontrol plants at high altitudes in summer 2014, at medium altitudes in spring 2013, and at low altitudes in spring 2014; and more infructescences in exclusion than biocontrol plants at high altitudes in summer 2014, at medium altitudes in autumn 2013, and at low altitudes in autumn 2014, spring 2014 and summer 2015.

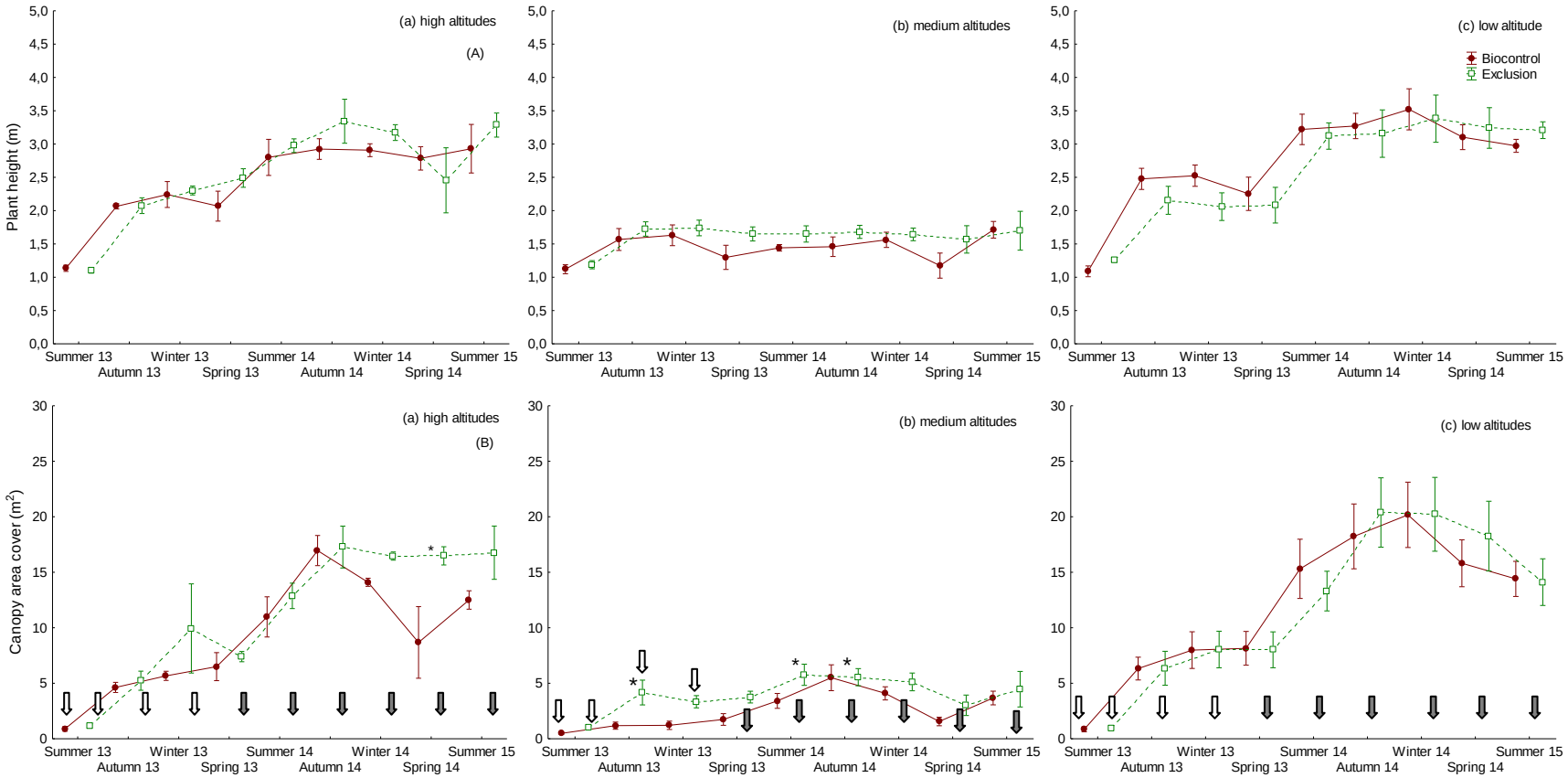

Figure 3.2 Comparisons of plant height (A) and canopy cover (B) between biocontrol and exclusion plants at high (a), medium (b) and low altitudes (c) from summer 2013 to summer 2015 (mean ± SE). Biocontrol/exclusion pairs with an asterisk are significantly different using Fisher LSD post-hoc tests ($P < 0.05$). Insecticidal application dates are indicated by plain arrows at 70 g/m^2 and shaded arrows at 140 g/m^2.

Table 3.2 Summary of the effect of insecticidal exclusion (treatment) and season on various plant growth parameters of *Lantana camara* L. (Verbenaceae) growing along the Sabie River, in Mpumalanga province, South Africa, at high, medium and low altitude sites. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, NS = not significant.

Altitude	Parameters	Factor variables	d.f.	F-value	*P*-value	Significance
High altitude	Plant height (m)	Treatment	1	0.92	0.37	NS
		Season	8	23.17	<0.001	***
		Treatment*Season	8	0.84	0.58	NS
	Canopy area cover (m^2)	Treatment	1	0.05	0.83	NS
		Season	8	21.90	<0.001	***
		Treatment*Season	8	2.56	0.02	*
	Stem length (m)	Treatment	1	2.63	0.12	NS
		Season	8	83.67	<0.001	***
		Treatment*Season	8	2.41	0.02	*
	Number of stems	Treatment	1	5.34	0.03	*
		Season	8	54.95	<0.001	***
		Treatment*Season	8	2.02	0.04	*
	Number of leaves	Treatment	1	0.05	0.83	NS
		Season	8	18.81	<0.001	***
		Treatment*Season	8	0.98	0.45	NS
	Number of inflorescences	Treatment	1	0.61	0.44	NS
		Season	8	9.53	<0.001	***
		Treatment*Season	8	2.17	0.03	*
	Number of infructescences	Treatment	1	1.43	0.25	NS
		Season	8	6.88	<0.001	***
		Treatment*Season	8	1.62	0.12	NS
	Leaves damaged (%)	Treatment	1	14.58	<0.001	***
		Season	8	16.57	<0.001	***
		Treatment*Season	8	0.89	0.53	NS
	Inflorescences damaged (%)	Treatment	1	1.33	0.26	NS
		Season	8	9.43	<0.001	***
		Treatment*Season	8	0.95	0.48	NS
	Infructescences damaged (%)	Treatment	1	1.07	0.31	NS
		Season	8	11.16	<0.001	***
		Treatment*Season	8	2.26	0.02	*
Medium altitude	Plant height (m)	Treatment	1	0.67	0.44	NS
		Season	8	5.30	<0.001	***
		Treatment*Season	8	0.68	0.71	NS
	Canopy area cover (m^2)	Treatment	1	3.92	0.08	NS
		Season	8	14.28	<0.001	***
		Treatment*Season	8	1.28	0.27	NS
	Stem length (m)	Treatment	1	8.22	0.007	**
		Season	8	27.70	<0.001	***
		Treatment*Season	8	1.18	0.31	NS
	Number of stems	Treatment	1	14.01	<0.001	***
		Season	8	25.07	<0.001	***
		Treatment*Season	8	1.17	0.32	NS

	Number of leaves	Treatment	1	8.07	0.007	**
		Season	8	31.5	<0.001	***
		Treatment*Season	8	0.89	0.52	NS
	Number of inflorescences	Treatment	1	0.99	0.33	NS
		Season	8	27.34	<0.001	***
		Treatment*Season	8	1.46	0.17	NS
	Number of infructescences	Treatment	1	0.74	0.39	NS
		Season	8	24.76	<0.001	***
		Treatment*Season	8	2.96	0.003	**
	Leaves damaged (%)	Treatment	1	25.14	<0.001	***
		Season	8	46.09	<0.001	***
		Treatment*Season	8	0.66	0.72	NS
	Inflorescences damaged (%)	Treatment	1	1.41	0.24	NS
		Season	8	6.92	<0.001	***
		Treatment*Season	8	0.90	0.51	NS
	Infructescences damaged (%)	Treatment	1	2.00	0.17	NS
		Season	8	50.10	<0.001	***
		Treatment*Season	8	3.07	0.002	**
Low altitude	Plant height (m)	Treatment	1	0.17	0.68	NS
		Season	8	32.99	<0.001	***
		Treatment*Season	8	0.81	0.60	NS
	Canopy area cover (m^2)	Treatment	1	0.01	0.91	NS
		Season	8	45.38	<0.001	***
		Treatment*Season	8	0.47	0.88	NS
	Stem length (m)	Treatment	1	0.00	0.94	NS
		Season	8	170.6	<0.001	***
		Treatment*Season	8	0.67	0.71	NS
	Number of stems	Treatment	1	3.89	0.05	NS
		Season	8	66.45	<0.001	***
		Treatment*Season	8	2.67	0.007	**
	Number of leaves	Treatment	1	2.52	0.11	NS
		Season	8	18.16	<0.001	***
		Treatment*Season	8	2.80	<0.001	***
	Number of inflorescences	Treatment	1	2.02	0.16	NS
		Season	8	17.48	<0.001	***
		Treatment*Season	8	0.93	0.49	NS
	Number of infructescences	Treatment	1	3.62	0.06	NS
		Season	8	12.32	<0.001	***
		Treatment*Season	8	3.39	<0.001	***
	Leaves damaged (%)	Treatment	1	17.47	<0.001	***
		Season	8	46.81	<0.001	***
		Treatment*Season	8	1.44	0.17	NS
	Inflorescences damaged (%)	Treatment	1	7.77	0.007	**
		Season	8	14.41	<0.001	***
		Treatment*Season	8	0.94	0.48	NS
	Infructescences damaged (%)	Treatment	1	3.30	0.07	NS
		Season	8	28.80	<0.001	***
		Treatment*Season	8	2.51	0.01	*

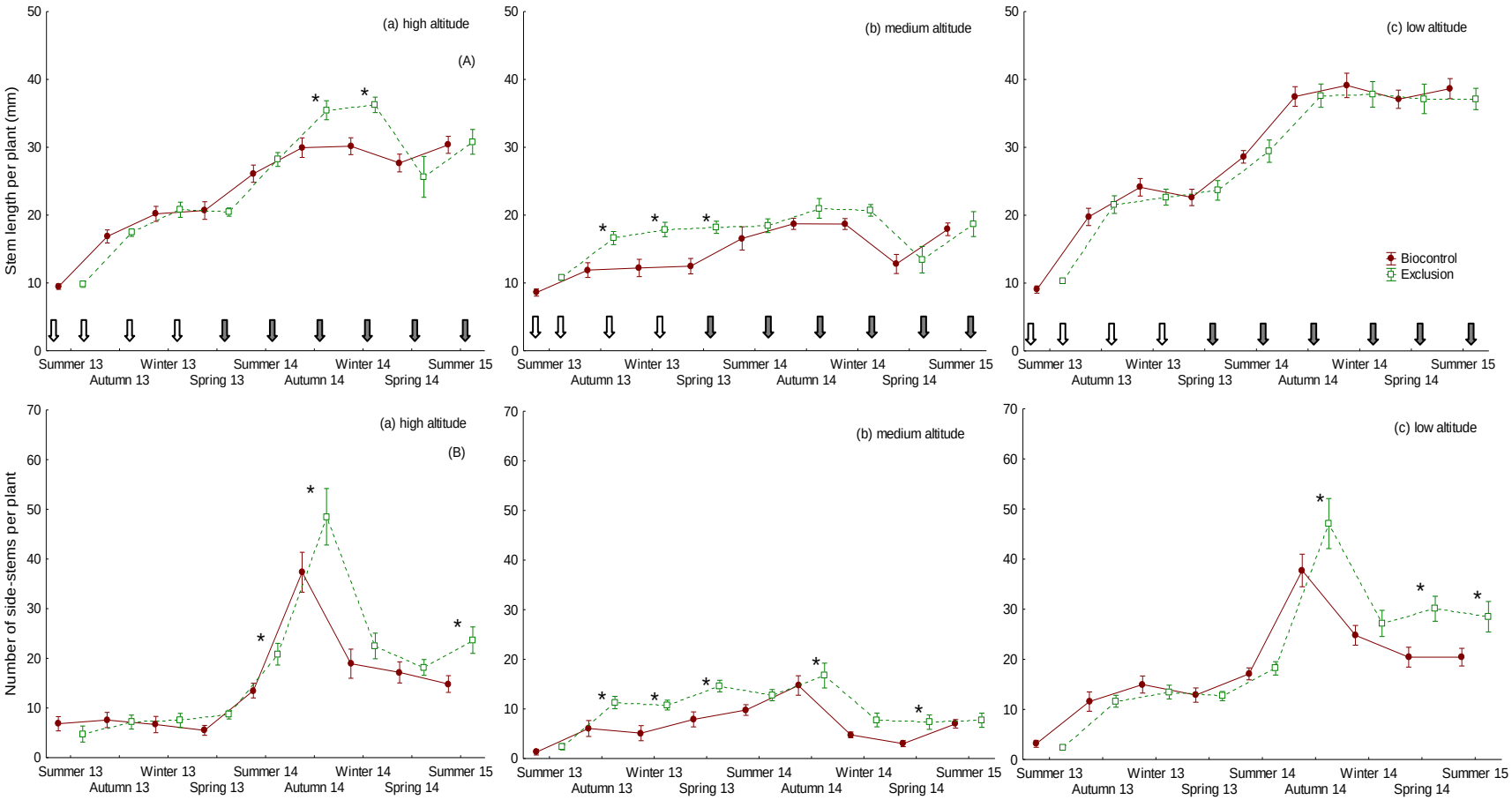

Figure 3.3 Comparisons of stem length (A) and number of side-stems (B) between biocontrol and exclusion plants at high (a), medium (b) and low altitudes (c) from summer 2013 to summer 2015 (mean ± SE). Biocontrol/exclusion pairs with an asterisk are significantly different using Fisher LSD post-hoc tests ($P < 0.05$). Insecticidal application dates are indicated by plain arrows at 70 g/m^2 and shaded arrows at 140 g/m^2.

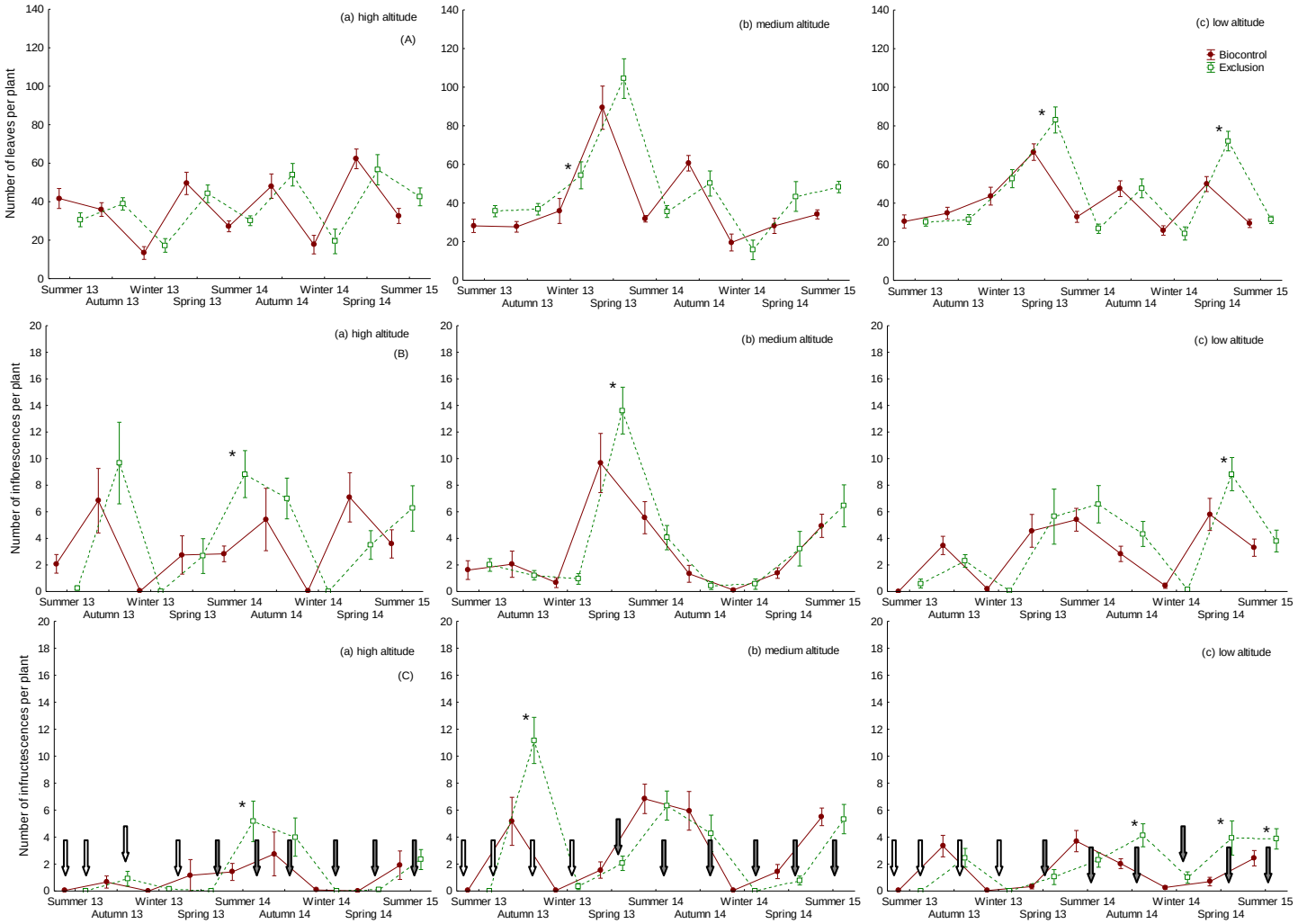

Figure 3.4 Comparisons of the number of leaves (A), inflorescences (B) and infructescences (C) between biocontrol and exclusion plants at high (a), medium (b) and low altitudes (c) from summer 2013 to summer 2015 (mean ± SE). Biocontrol/exclusion pairs with an asterisk are significantly different using Fisher LSD post-hoc tests ($P < 0.05$). Insecticidal application dates are indicated by plain arrows at 70 g/m^2 and shaded arrows at 140 g/m^2.

There were seasonal changes both in the number of inflorescences and infructescences at all altitude levels; and interactions between treatment and season in the number of inflorescences at high altitude sites and in the number of infructescences at medium and low altitude sites, implying that both the number of infructescences and inflorescences differed between biocontrol and exclusion plants only in some seasons and not others (Table 3.2).

Number of leaves, inflorescences and infructescences damaged by agents

The number of leaves damaged by all established biocontrol agents combined was significantly greater in biocontrol compared to exclusion plants ($F_{(1, 22)} = 14.58$; $P < 0.01$), ($F_{(1, 31)} = 25.14$; $P < 0.01$), ($F_{(1, 58)} = 17.47$; $P < 0.001$) at high, medium and low altitude sites, respectively (Fig. 3.5.A). Fisher LSD post-hoc tests revealed significant differences in autumn 2014 and winter 2014 at high; summer 2014, autumn 2014, winter 2014 and spring 2014 at medium; and in winter 2013, spring 2013, winter 2014, spring 2014 and summer 2015 at low altitude sites.

There were no significant differences in the number of damaged inflorescences between biocontrol and exclusion plants at high ($F_{(1, 22)} = 1.33$; $P = 0.26$) and medium altitudes ($F_{(1, 34)} = 1.41$; $P = 0.24$) (Fig. 3.5.B), except in summer 2014, at high altitudes, where it was greater in biocontrol than exclusion plants according to the LSD post-hoc tests. At low altitudes, in autumn 2014, the number of inflorescence damaged was greater in biocontrol compared to exclusion plants ($F_{(1, 58)} = 7.77$; $P = 0.01$) (Fig. 3.5.B).

There were also no differences in the number of infructescences damaged at all altitude levels ($F_{(1,22)} = 1.07$; $P = 0.31$), ($F_{(1,34)} = 2.00$; $P = 0.17$), ($F_{(1,58)} = 3.30$; $P = 0.07$), respectively (Fig. 3.5.C). LSD post-hoc tests, however, revealed that, at high altitudes, the number of infructescences damaged was unexpectedly greater in exclusion compared to biocontrol plants in summer 2014 but in autumn 2014 it was the opposite; at medium altitudes, it was also greater in exclusion compared to biocontrol plants in autumn 2013, but again in summer 2014 and summer 2015 it was

the opposite; and at low altitudes, it was, as expected, greater in biocontrol compared to exclusion plants in autumn 2013, summer 2014 and autumn 2014 (Fig. 3.5).

There were significant seasonal changes in the number of damaged leaves, inflorescences and infructescences, as well as a significant interaction effect between treatment and season in the number of damaged infructescences at all altitude levels (Table 3.2).

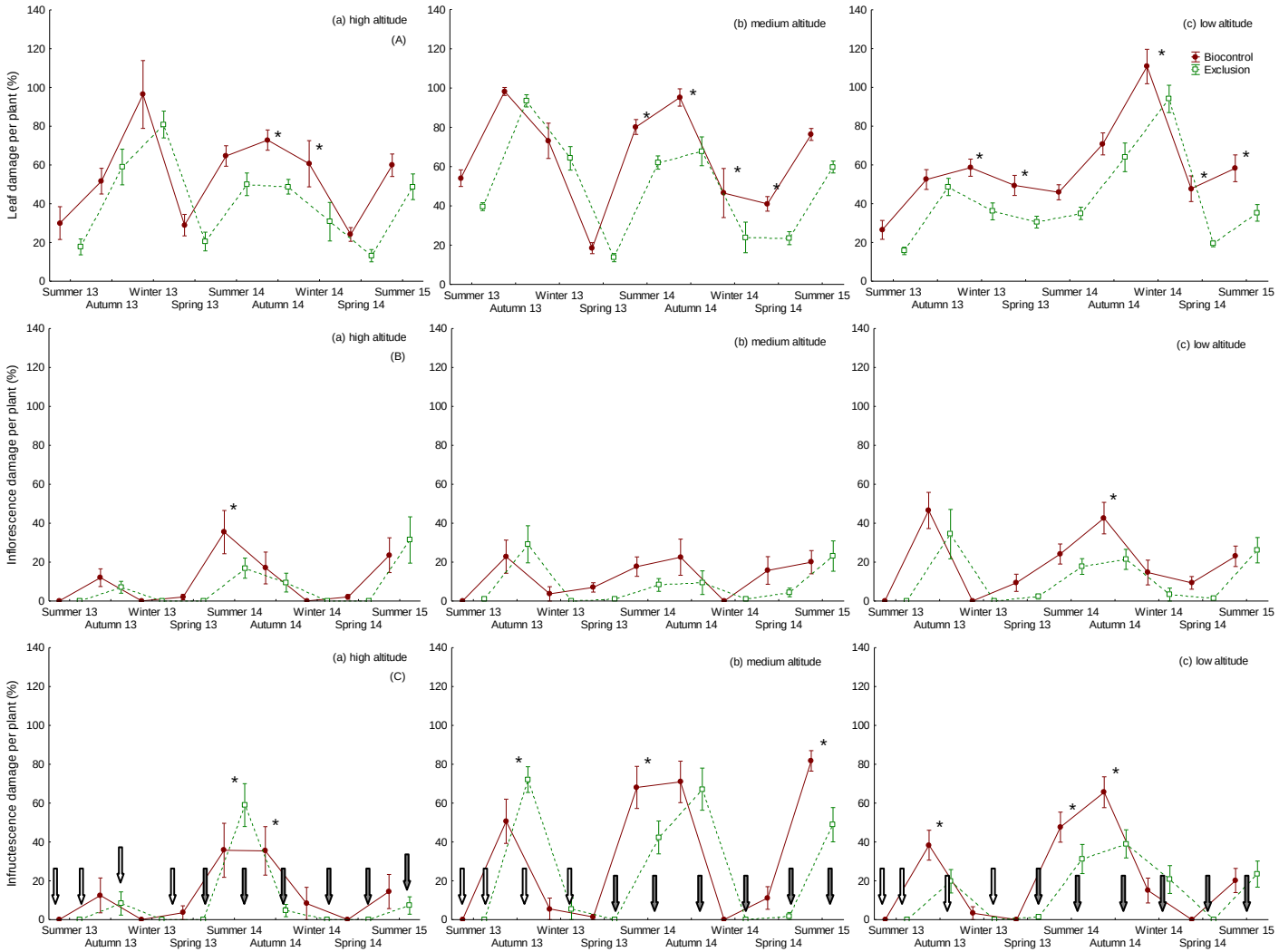

Figure 3.5 Comparisons of the number of leaves (A), inflorescences (B) and infructescences (C) damaged between biocontrol and exclusion plants at high (a), medium (b) and low altitudes (c) from summer 2013 to summer 2015 (mean ± SE). Biocontrol/exclusion pairs with an asterisk are significantly different using Fisher LSD post-hoc tests ($P < 0.05$). Insecticidal application dates are indicated by plain arrows at 70 g/m^2 and shaded arrows at 140 g/m^2.

Lantana camara biomass

There were no differences in leaf (leaves, inflorescences and infructescences combined) (t_3 = 1.54; *P* = 0.22), (t_5 = 1.75; *P* = 0.14), (t_9 = 0.03; *P* = 0.97) (Fig. 3.6.a) and stem biomass (t_3 = 1.54; *P* = 0.22), (t_5 = 1.75; *P* = 0.14), (t_9 = 0.03; *P* = 0.97) (Fig. 3.6.b) in 2017; and total above-ground biomass gained from spring 2012 to summer 2017 (t_3 = 0.13; *P* = 0.91), (t_5 = 0.74; *P* = 0.49), (t_9 = 2.10; *P* = 0.16) (Fig. 3.6.c) between biocontrol and exclusion plants at high, medium and low altitude sites, respectively.

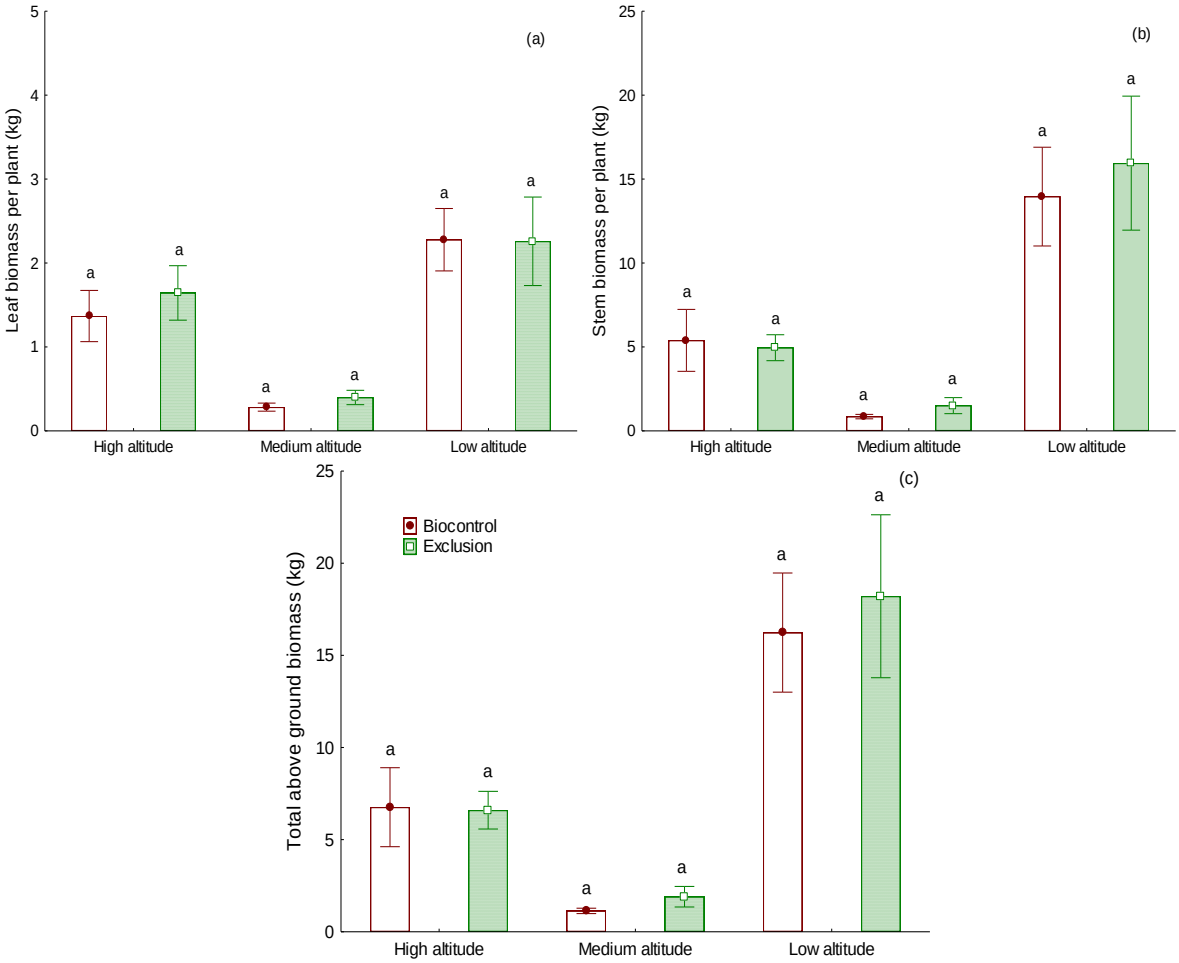

Figure 3.6 Comparisons of leaf (a) and stem biomass (b) in 2017, and total above ground biomass gained from 2012 to 2017 (c) (mean ± SE) between biocontrol and exclusion plants, at high (n = 4), medium (n = 6) and low (n = 10) altitudes. Means with different letters within the same altitudinal level are significantly different using paired t-tests (*P* < 0.05).

3.4.2 Regeneration capacity

Seedling and seed rain density

There were no significant differences in the number of seedlings between biocontrol and exclusion quadrats at high ($F_{(1, 8)} = 0.01$; $P = 0.89$) (Fig. 3.7.a) and low altitude sites ($F_{(1, 8)} = 0.01$; $P = 0.89$) (Fig. 3.7.b). There were, however, significant seasonal changes at high ($F_{(8, 64)} = 2.11$; $P = 0.04$) and low ($F_{(8, 64)} = 7.76$; $P < 0.01$) altitude sites.

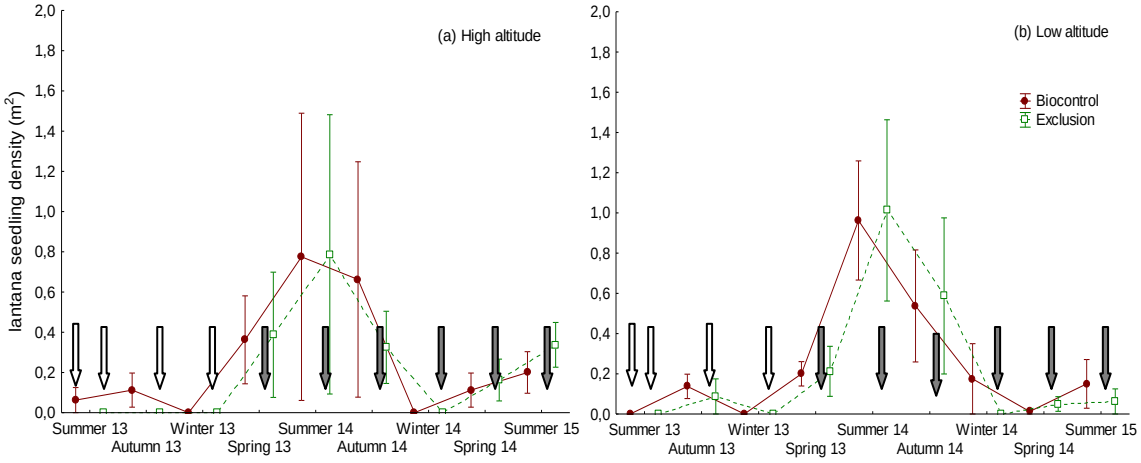

Figure 3.7 *Lantana camara* seedling density/m^2 between biocontrol and exclusion quadrats (4x4 m) from summer 2013 to summer 2015 at high (high and medium altitudes combined) and low altitude sites (mean ± SE). No significant differences were noted between biocontrol/exclusion pairs using Fisher LSD post-hoc tests (n = 5, $P < 0.05$). Insecticidal application dates are indicated by plain arrows at 70 g/m^2 and shaded arrows at 140 g/m^2.

Seed rain (seeds/m^2) was not different between biocontrol and exclusion plants at high ($t_3 = 1.44$; $P = 0.24$), ($t_3 = 0.35$; $P = 0.75$), ($t_3 = 0.51$; $P = 0.64$) (Fig. 3.8.a) and low altitude sites ($t_9 = 0.42$; $P = 0.68$), ($t_9 = 1.17$; $P = 0.26$), ($t_9 = 0.32$; $P = 0.75$) (Fig. 3.8.c) in summer 2014, 2015 and 2016, respectively; except at medium altitude sites, where it was higher in exclusion compared to biocontrol plants in summer 2014 ($t_5 = 2.71$; $P = 0.04$), while not significantly different in summer 2015 and 2016 ($t_5 = 0$; $P = 0.64$), ($t_5 = 0.44$; $P = 0.67$) respectively (Fig. 3.8.b).

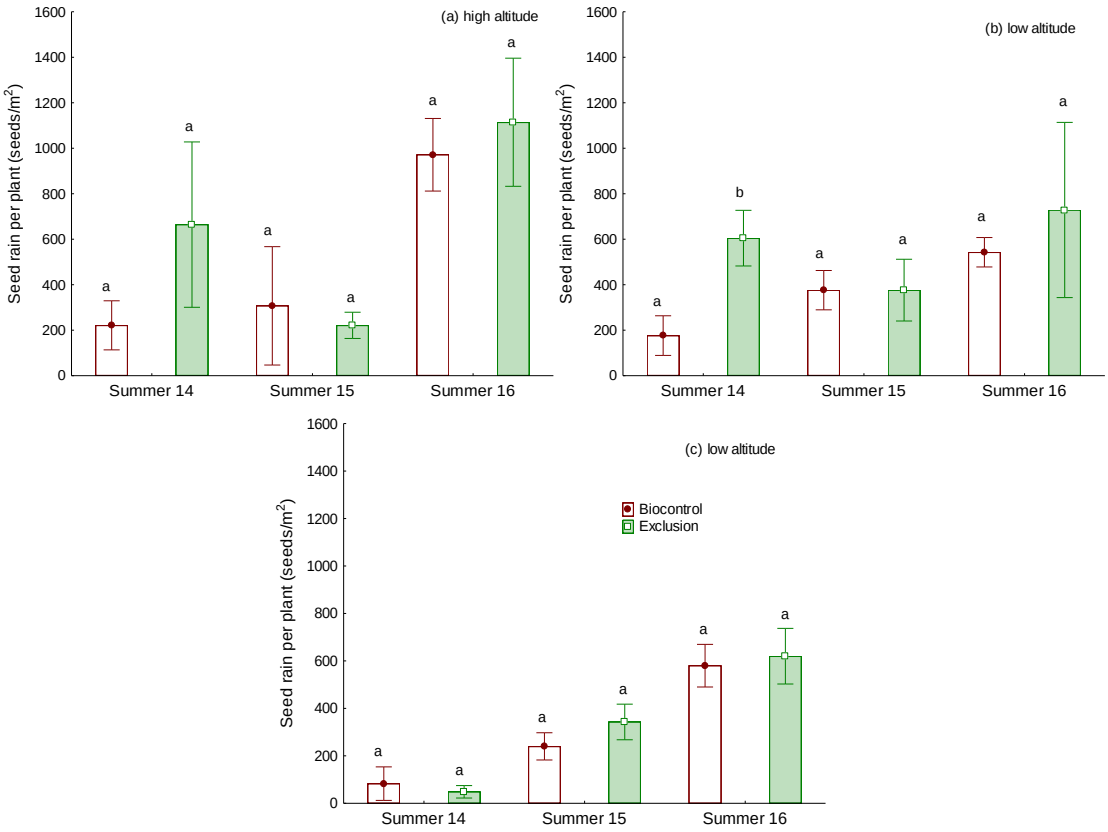

Figure 3.8 Comparisons of seed rain density per plant between biocontrol and exclusion plants at high (a) (n = 4), medium (b) (n = 6) and low altitude sites (c) (n = 10) in summer 2014, 2015 and 2016 (B) (mean ± SE). Means with different letters within the same year are significantly different using paired t-tests ($P < 0.05$).

Seedbank germination trial

There were no significant differences in the cumulative number of germinable seeds between soil samples collected from biocontrol and exclusion quadrats at both high ($F_{(21, 168)} = 0.03$; $P = 1.0$) (Fig. 3.9.a) and low altitudes ($F_{(21, 168)} = 0.64$; $P = 0.88$) (Fig. 3.9.b); but in biocontrol quadrats, at low altitudes, there was a 31.75% reduction in germinated seeds. The soil seedbank was, nevertheless, considerably higher in biocontrol and exclusion quadrats, both at high (100 ± 78.33; 91.11 ± 66.80) and low altitudes (95.56 ± 35.94; 140 ± 40.92), respectively. There were no differences in 'days to germination' between seeds collected from biocontrol (42.78 ± 0.28) or exclusion quadrats (42.44 ± 0.63) ($t_{16} = 0.48$; $P = 0.63$).

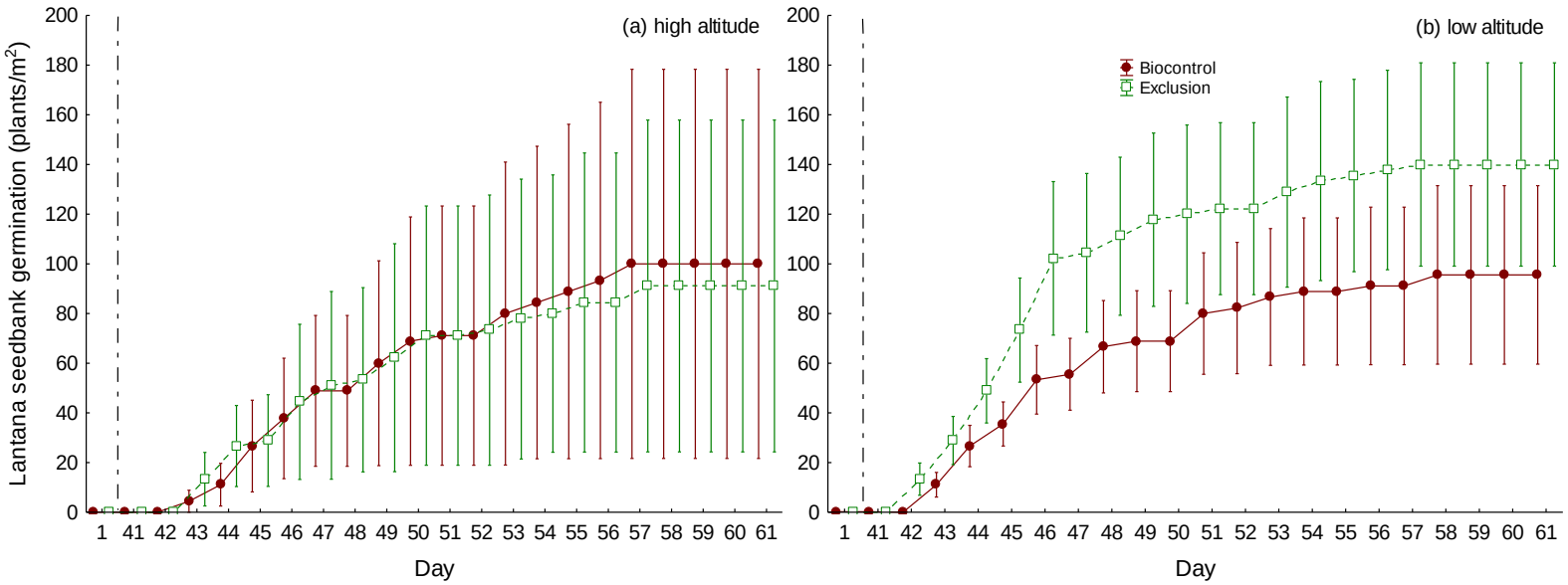

Figure 3.9 Comparisons of cumulative numbers of seedbank seeds germinated (plants/m^2) between biocontrol and exclusion quadrats at high (high and medium altitudes combined) and low altitude sites (mean ± SE). No significant differences were noted between biocontrol/exclusion pairs using Fisher LSD post-hoc tests ($P < 0.05$) (n = 5). No seeds germinated during the first 40 days.

Seed production

There were no significant differences in the number of seeds produced per plant between biocontrol and exclusion plants at high (t_3 = 1.16; *P* = 0.28), medium (t_5 = 0.32; *P* = 0.75) and low altitudes sites (t_9 = 0.44; *P* = 0.67) (Fig. 3.10). There was, however, a 29% reduction in seed production in biocontrol plants at low altitude (Fig. 3.10).

Of the 500 fruits dissected, only one in 100 fruits had more than one, namely two seeds.

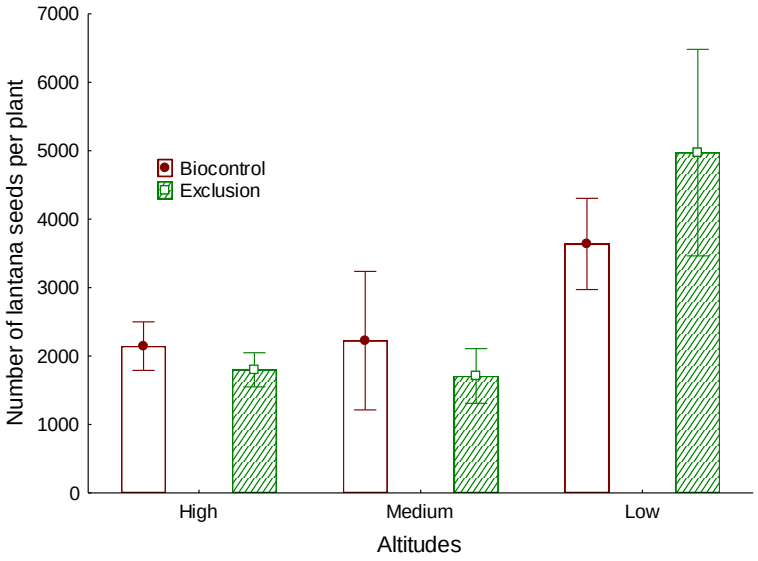

Figure 3.10 Comparisons of seed production per plant between biocontrol and exclusion plants at high (n = 4), medium (n = 6) and low altitude sites (n = 10) in summer 2014 (mean ± SE). No significant differences were noted between biocontrol/exclusion pairs using paired t-tests (*P* < 0.05).

3.4.3 Biocontrol agent abundance and performance

Agent abundance

Teleonemia scrupulosa and the leaf-spot pathogen, *cf. Passalora* sp. were the most abundant agents in the entire study area throughout the study period, followed by *H. laceratalis*, and the least abundant ones were *O. scabripennis* and *O. camarae*. However, the overall most abundant agent at high and low altitude was *T. scrupulosa*, and the leaf pathogen, *cf. Passalora sp.* at medium altitude (Table 3.3).

Table 3.3 Comparisons of abundance between biocontrol agents (percentage of leaves damaged by each agent) on biocontrol plants within different seasons, and at different altitudes (mean ± SE). Values with different superscript letters within same rows (one way ANOVA) denote significant differences at $P < 0.05$.

	Octotoma scabripennis	***Ophiomyia camarae***	***Teleonemia scrupulosa***	***Hypena laceratalis***	***cf. Passalora* sp.**
			High altitude		
Summer 13	0.05±0.02^{a}	0.05±0.02^{a}	0.05±0.02^{a}	0.05±0.02^{a}	0.05±0.02^{a}
Autumn 13	17.01±2.1^{b}	0.05±0.02^{a}	33.34±4.95^{c}	0.05±0.02^{a}	0.05±0.02^{a}
Winter 13	16.55±4.96^{c}	13.67±5.53^{bc}	30.32±8.29^{d}	1.65±0.92^{ab}	8.46±3.39^{abc}
Spring 13	3.82±1.2^{a}	0.05±0.02^{a}	19.52±4.92^{b}	2.28±0.96^{a}	3.33±1.36^{a}
Summer 14	8.64±3.28^{a}	0.05±0.02^{a}	53.51±7.23^{b}	0.52±0.52^{a}	1.97±1.07^{a}
Autumn 14	20.44±5.4^{b}	1.09±0.67^{a}	46.59±6.01^{c}	4.22±1^{a}	0.49±0.35^{a}
Winter 14	2.64±1.46^{a}	2.47±2.08^{a}	11.55±6.34^{ab}	18.03±8.2^{bc}	25.94±8.64^{c}
Spring 14	2.02±1.03^{ab}	0.05±0.02^{a}	14.05±1.89^{bc}	3.87±1.16^{bc}	4.2±1.21^{d}
Summer 15	0.3±0.3^{a}	0.05±0.02^{a}	53.85±4.69^{b}	4.41±1.54^{a}	1.3±0.69^{a}
Overall	71.48±5.19^{b}	17.54±6.79^{a}	262.78±16.22^{c}	35.08±9.16^{a}	45.79±10.70^{ab}
			Medium altitude		
Summer 13	0.03±0.01^{a}	0.03±0.01^{a}	0.03±0.01^{a}	0.03±0.01^{a}	0.03±0.01^{a}
Autumn 13	21.24±3.68^{b}	1.19±0.95^{a}	42.67±7.3^{c}	0.03±0.01^{a}	31.64±10.88^{bc}
Winter 13	3.24±1.25^{ab}	0.14±0.14^{a}	3.7±1.39^{ab}	9.85±3.03^{b}	56.2±8.2^{c}
Spring 13	0.91±0.32^{a}	0.71±0.51^{a}	9.29±2.01^{d}	2.18±0.44^{ab}	5.37±2.2^{bc}
Summer 14	4.59±1.18^{a}	0.37±0.37^{a}	20.12±4.43^{b}	0.36±0.36^{a}	54.75±3.01^{c}
Autumn 14	1.42±0.64^{ab}	0.96±0.43^{a}	3.87±1.17^{ab}	13.88±1.98^{c}	74.64±3.99^{d}
Winter 14	2.8±1.08^{ab}	0.25±0.17^{ab}	12.44±6.9^{abc}	16.94±10.92^{c}	14.12±4.96^{bc}
Spring 14	7.38±1.82^{cd}	0.03±0.01^{a}	19.62±3.02^{e}	7.68±2.22^{bcd}	6.35±1.67^{d}
Summer 15	5.48±2.54^{bc}	0.03±0.01^{a}	3.57±1.23^{abc}	6.61±1.64^{b}	60.72±3.41^{d}
Overall	47.09±5.48^{a}	3.70±1.45^{b}	115.30±11.37^{c}	57.54±12.60^{a}	303.82±16.24^{d}
			Low altitude		
Summer 13	0.04±0.01^{a}	0.04±0.01^{a}	0.04±0.01^{a}	0.04±0.01^{a}	0.04±0.01^{a}
Autumn 13	1.11±0.45^{b}	11.42±1.67^{a}	3.9±0.89^{a}	0.04±0.01^{a}	0.04±0.01^{a}
Winter 13	3.61±0.8^{a}	2.23±0.64^{a}	18.19±3.18^{d}	15.38±2.73^{cd}	9.97±2.6^{bc}
Spring 13	0.65±0.31^{ab}	0.95±0.43^{ab}	42.41±4.7^{c}	5.17±0.98^{a}	0.22±0.13^{b}
Summer 14	20.83±2.35^{d}	0.04±0.01^{a}	18.8±3.33^{c}	3.08±0.88^{ab}	3.18±0.97^{ab}
Autumn 14	6.96±1.84^{ab}	1.66±0.43^{a}	21.91±4.06^{cde}	24.52± 2.81^{ce}	15.26±2.98^{bcd}
Winter 14	2.11±1.29^{a}	0.36 0.26^{a}	47.03±5.33^{d}	30.93±4.01^{bc}	27.12±4.4^{b}
Spring 14	1.44±0.72^{a}	0.17±0.13^{a}	26.28±3.33^{d}	11.31±2.14^{bc}	8.52±2.89^{b}
Summer 15	11.06±3.88^{bc}	3.42±0.72^{a}	13.55±2.7^{b}	14.27±2.45^{b}	14.63±4.31^{b}
Overall	47.81±6.80^{c}	20.29±2.73^{b}	192.11±15.32^{d}	104.74±8.13^{a}	78.98±9.43^{a}

Agent performance

It is important to note that the leaf pathogen *cf. Passalora* sp. was not affected by carbofuran, which means that any differences in its performance between biocontrol and exclusion plants could have been as a direct effect of the quality of leaves,

together with environmental parameters, such as rainfall, relative humidity and dew point; which are unlikely given the proximity of plots to each other.

Octotoma scabripennis performance (percentage leaves damaged by the agent per plant) was significantly greater in biocontrol plants compared to exclusion plants at high ($F_{(1, 22)} = 14.70$; $P < 0.01$) (Fig. 3.11.A.a), medium ($F_{(1, 31)} = 7.67$; $P < 0.01$) (Fig. 3.11.A.b) and low altitudes ($F_{(1, 58)} = 8.26$; $P < 0.01$) (Fig. 3.11.A.c). The LSD post-hoc tests revealed significant differences in summer 2014 and autumn 2014 at high altitudes (Fig. 3.11.A.a), in autumn 2013, spring 2014 and summer 2015 at medium altitudes (Fig. 3.11.A.b), and in summer 2014, autumn 2014 and summer 2015 at low altitudes (Fig. 3.11.A.c).

Both *O. camarae* and *cf. Passalora* sp. performances were significantly greater in biocontrol compared to exclusion plants at high ($F_{(1, 22)} = 5.21$; $P = 0.03$) (Fig. 3.11.B.a), ($F_{(1, 22)} = 6.22$; $P = 0.02$) (Fig. 3.11.E.a), but not at medium ($F_{(1, 34)} = 0.77$; $P = 0.38$) (Fig. 3.11.B.b), ($F_{(1, 34)} = 3.50$; $P = 0.06$) (Fig. 3.11.E.b) or low altitudes ($F_{(1,58)} = 0.04$; $P = 0.84$) (Fig. 3.11.B.c), ($F_{(1,58)} = 1.34$; $P = 0.25$) (Fig. 3.11.E.c), respectively. The LSD post-hoc test revealed that *O. camarae* performance was greater in biocontrol compared to exclusion plants in winter 2013 at high altitudes (Fig. 3.11.B.a), but in autumn 2013 at low altitude sites, it was the opposite (Fig. 3.11.B.c).

There were significant changes between seasons at all altitude levels; and significant interactions between treatment and season at high and low altitude sites, meaning that there were differences in their performance between biocontrol and exclusion plants only in some seasons and not others (Table 3.4).

Similarly, *cf. Passalora* sp. performance was greater in biocontrol compared to exclusion plants at high altitude in winter 2013 and winter 2014 (Fig. 3.11.E.a), and in autumn 2014 at medium altitude sites (Fig. 3.11.E.b); however in autumn 2014 and winter 2014 at low altitude sites, it was higher in carbofuran treated-plants (Fig. 3.11.E.c).

There were significant changes between seasons at all altitude levels, in both *O. camarae* and *cf. Passalora* sp. performance; and significant interactions between

treatment and season at high and low altitude sites, meaning performance was different between biocontrol and exclusion plants only in some seasons and not others (Table 3.4).

Teleonemia scrupulosa and *H. laceratalis* performances were significantly greater in biocontrol than exclusion plants at medium ($F_{(1, 31)} = 7.56$; $P < 0.01$), ($F_{(1, 31)} = 6.52$; $P = 0.01$) (Fig. 3.11.C.b) (Fig. 3.11.D.b) and low altitudes ($F_{(1, 58)} = 16.07$; $P < 0.01$), ($F_{(1, 58)} = 16.88$; $P < 0.01$) (Fig. 3.11.C.c) (Fig. 3.11.D.c), but not so at high altitudes ($F_{(1, 22)} = 1.65$; $P = 0.21$), ($F_{(1, 22)} = 0.17$; $P = 0.68$) (Fig. 3.11.C.a) (Fig. 3.11.D.a), respectively.

Table 3.4 Summary of the effect of insecticidal exclusion (treatment) and season on the performance of some *Lantana camara* biocontrol agents (percentage of leaves damaged) on plants growing along the Sabie River, in Mpumalanga province, South Africa, at high, medium and low altitude sites. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, NS = not significant.

Altitude	Parameters	Factor variables	d.f.	F-value	*P*-value	Significance
High altitude	*Octotoma scabripennis*	Treatment	1	14.70	<0.001	***
		Season	8	13.60	<0.001	***
		Treatment*Season	8	3.07	0.002	**
	Ophiomyia camarae	Treatment	1	5.21	0.03	*
		Season	8	6.18	<0.001	***
		Treatment*Season	8	4.23	<0.001	***
	Teleonemia scrupulosa	Treatment	1	1.65	0.21	NS
		Season	8	24.20	<0.001	***
		Treatment*Season	8	1.25	0.26	NS
	Hypena laceratalis	Treatment	1	0.17	0.68	NS
		Season	8	5.55	<0.001	***
		Treatment*Season	8	0.78	0.61	NS
	cf. Passalora sp.	Treatment	1	6.22	0.02	*
		Season	8	9.71	<0.001	***
		Treatment*Season	8	3.35	0.001	**
Medium altitude	*Octotoma scabripennis*	Treatment	1	7.67	0.009	**
		Season	8	24.01	<0.001	***
		Treatment*Season	8	1.03	0.40	NS
	Ophiomyia camarae	Treatment	1	0.77	0.38	NS
		Season	8	2.23	0.02	*
		Treatment*Season	8	0.33	0.95	NS
	Teleonemia scrupulosa	Treatment	1	7.56	0.009	**
		Season	8	21.29	<0.001	***
		Treatment*Season	8	0.57	0.79	NS
	Hypena laceratalis	Treatment	1	6.52	0.01	*
		Season	8	3.56	<0.001	***
		Treatment*Season	8	1.43	0.18	NS
	cf. Passalora sp.	Treatment	1	3.50	0.06	NS
		Season	8	46.39	<0.001	***
		Treatment*Season	8	0.80	0.60	NS
Low altitude	*Octotoma scabripennis*	Treatment	1	8.26	0.005	**
		Season	8	31.18	<0.001	***
		Treatment*Season	8	2.43	0.013	*
	Ophiomyia camarae	Treatment	1	0.04	0.84	NS
		Season	8	36.60	<0.001	***
		Treatment*Season	8	2.47	0.012	*
	Teleonemia scrupulosa	Treatment	1	16.07	<0.001	***
		Season	8	42.88	<0.001	***
		Treatment*Season	8	2.05	0.03	NS
	Hypena laceratalis	Treatment	1	16.88	<0.001	***
		Season	8	34.90	<0.001	***
		Treatment*Season	8	2.68	0.006	**
	cf. Passalora sp.	Treatment	1	1.34	0.25	NS
		Season	8	28.60	<0.001	***
		Treatment*Season	8	2.77	0.005	**

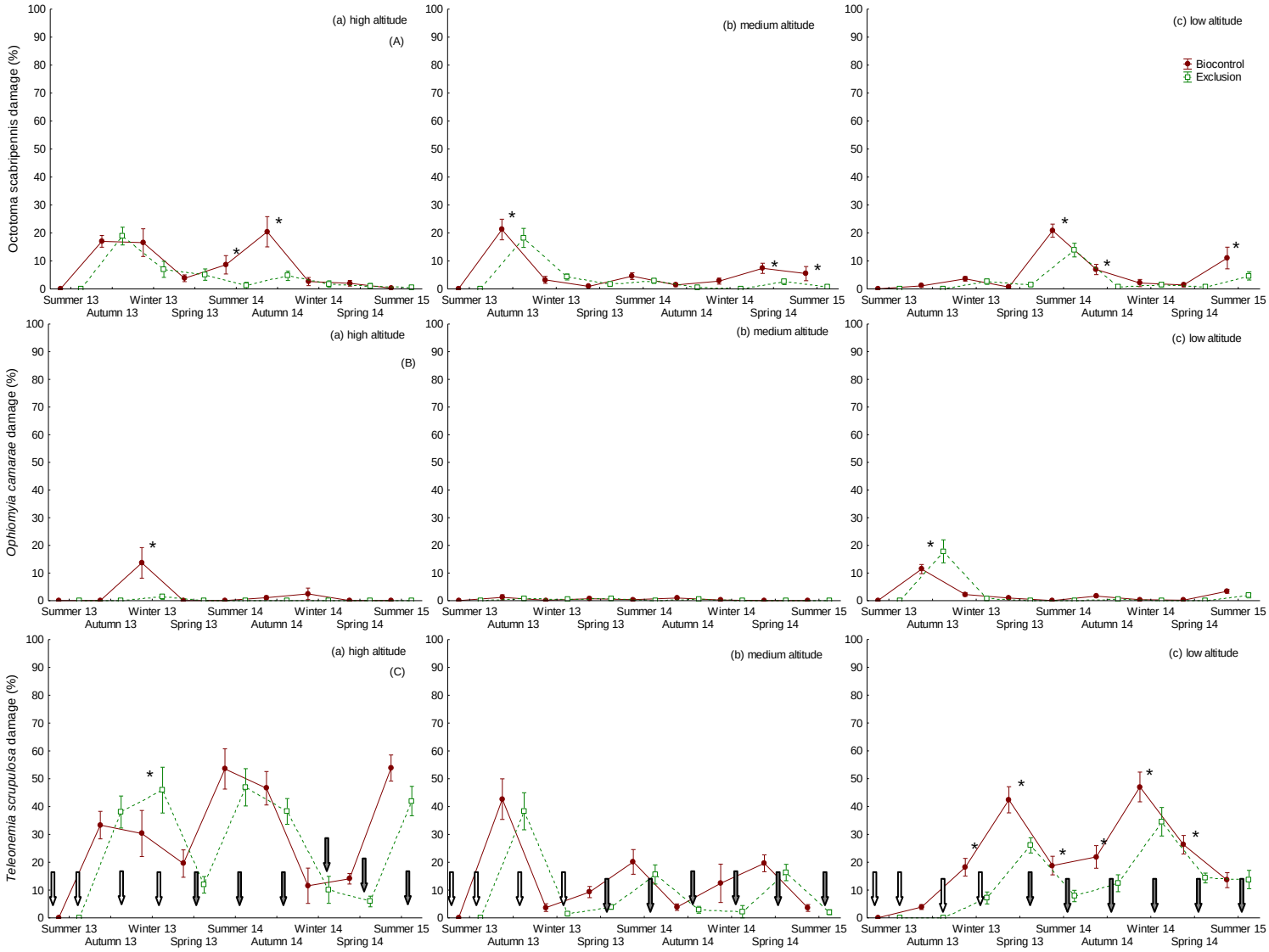

Figure 3.11 Comparisons of *O. scabripennis* (A), *O. camarae* (B), *T. scrupulosa* (C). Full legend over-leaf

Fig. 11 Cont…

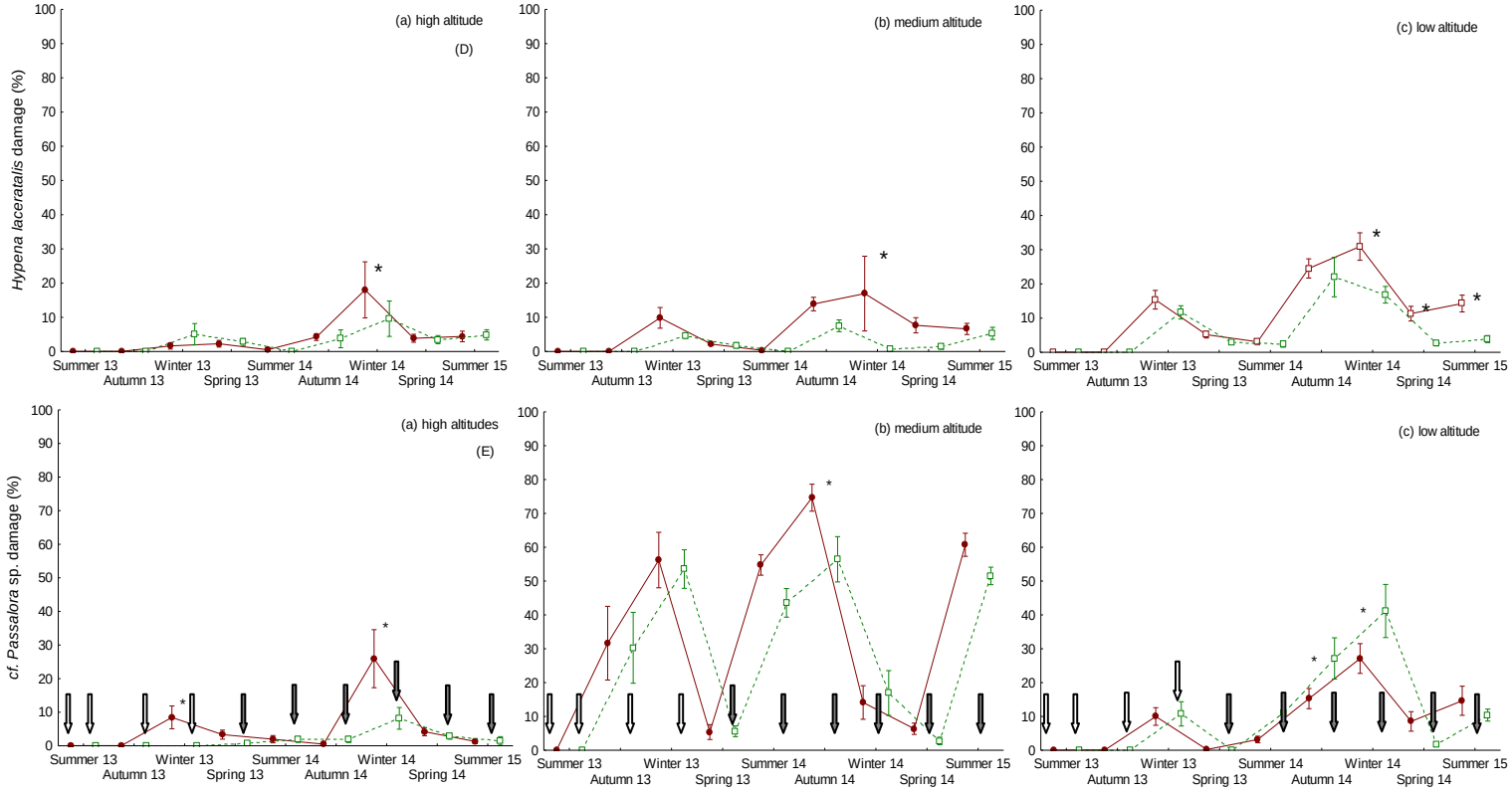

Figure 3.11 Comparisons of *Octotoma scabripennis* (A), *Ophiomyia camarae* (B), *Teleonemia scrupulosa* (C), *Hypena laceratalis* (D) and *cf. Passalora* sp. (E) performance between biocontrol and exclusion plants at high (a), medium (b) and low altitudes (c) from summer 2013 to summer 2015 (mean ± SE). Biocontrol/exclusion pairs with an asterisk are significantly different using Fisher LSD post-hoc tests ($P < 0.05$). Insecticidal application dates are indicated by plain arrows at 70 g/m^2 and shaded arrows at 140 g/m^2.

Although most agents performed well on biocontrol compared to exclusion plants, none of their performances translated into significant changes in the plant's vegetative or reproductive growth, as measured through correlation (Fig. 3.12).

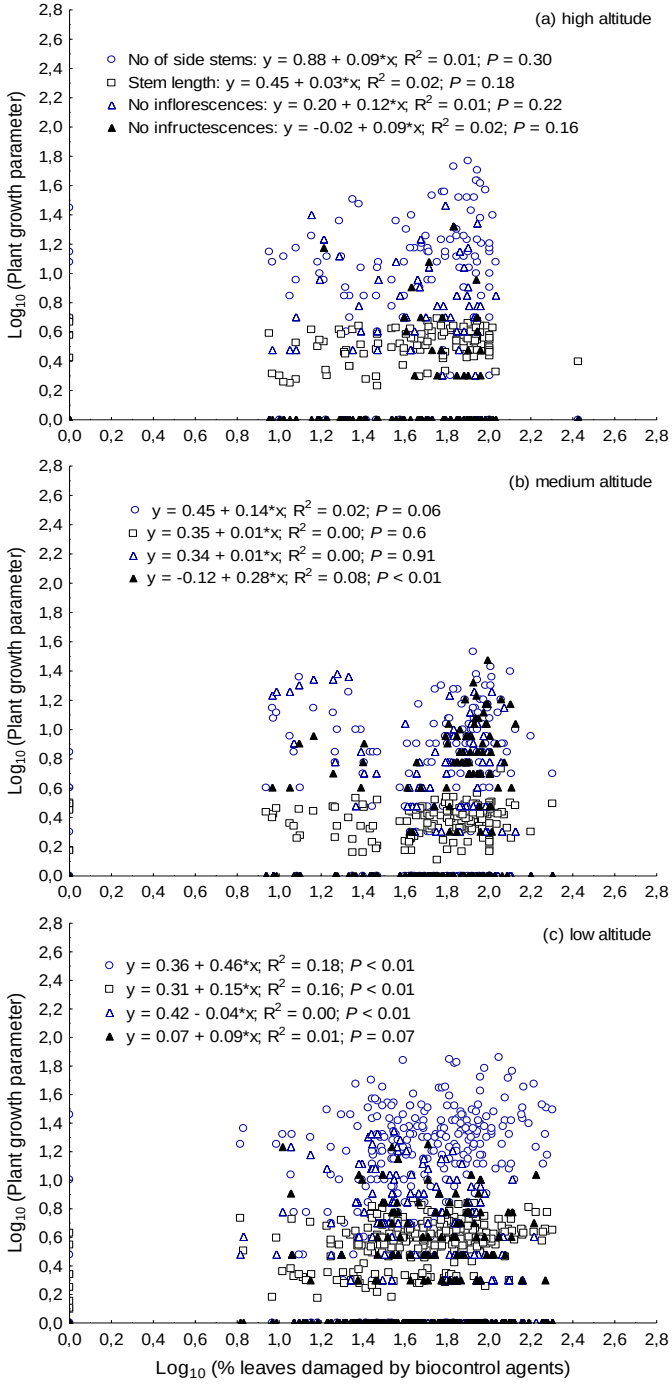

Figure 3.12 Number of side-stems (blue open circle), stem lengths (black open square), number of inflorescences (blue open triangle) and number of infructescences (blue close triangle) per plant from 2013 to 2015 as a function of the performance of all biocontrol agents combined (percentage of leaves damaged) at high (a), medium (b) and low altitude (c) sites.

3.4.4 *Aceria lantanae* release and monitoring

Aceria lantanae establishment on biocontrol plants

Aceria lantanae established a small population; and it only occurred at 20% of the study sites, all found at low altitude. The overall number of galls was greater at low compared to medium and low altitudes ($F_{(2, 30)} = 6.75$, $P < 0.01$) (Fig. 3.13).

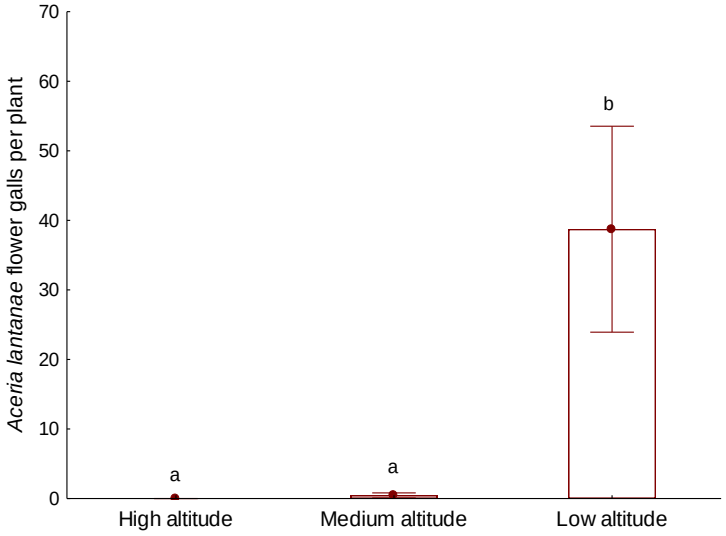

Figure 3.13 Comparisons of the overall number of flower galls among high, medium and low altitude sites from 2013 to 2016 (mean ± SE). Means with different letters are significantly different using Fisher LSD post-hoc tests ($P < 0.05$).

Impacts of A. lantanae on L. camara reproduction

Aceria lantanae flower galls occurred on biocontrol plants from autumn 2013 until autumn 2016 at the end of the study's monitoring period. Aceria lantanae flower galls, unexpectedly, occurred on the exclusion plants in summer 2016 and autumn 2016 but in low numbers compared to biocontrol plants ($F_{(1, 4)} = 12.89$; $P = 0.02$) (Fig. 3.14), probably because carbofuran was not applied between summer 2015 and summer 2016. Carbofuran applications were completed every season. However, after it was applied in summer 2015, carbofuran was only reapplied in summer 2016, a year later, leaving the 'exclusion plants' unprotected from biocontrol agents.

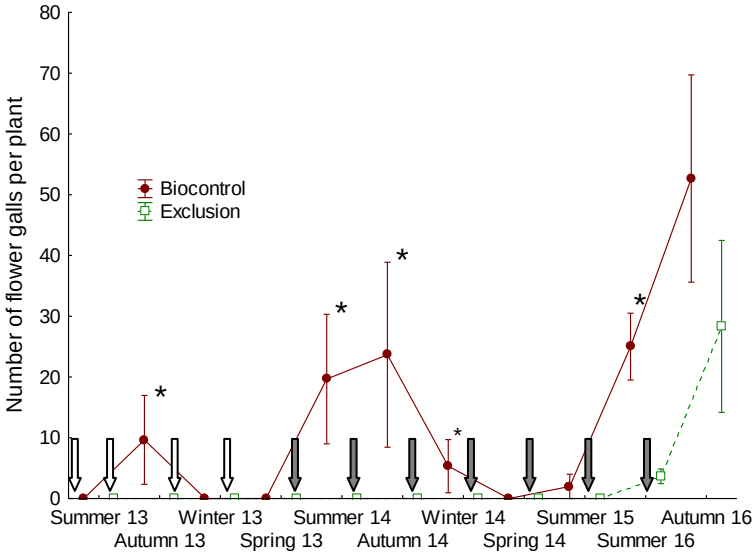

Figure 3.14 Comparisons of the number of flower galls between biocontrol and exclusion plants from summer 2013 to autumn 2016 (mean ± SE). Biocontrol/exclusion pairs with an asterisk are significantly different using Fisher LSD post-hoc tests ($P < 0.05$).

While *A. lantanae* showed signs of persistence on biocontrol plants, there was no relationship between the number of flower galls and the number of inflorescences produced; however a positive but weak relationship existed between the number of flower galls and the number of infructescences produced per plant (Fig. 3.15).

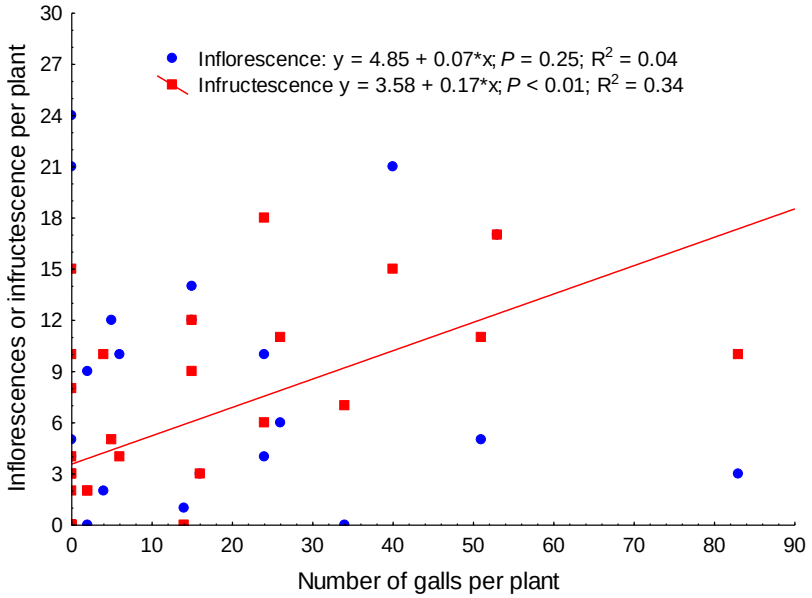

Figure 3.15 Number of inflorescences and infructescences produced per plant as a function of the number of flower galls per plant, along the Sabie River, Mpumalanga.

3.4.5 Carbofuran residue analysis

In autumn 2017 (between March and May 2017), trace levels of residues of carbofuran were detected within leaf samples from treated exclusion plants while nothing was detected in leaves from biocontrol plants (Table 3.5).

Table 3.5 Carbofuran residue levels (ppm) in *L. camara* leaves from carbofuran-treated exclusion plants and untreated biocontrol plants (mean ± SE) in autumn 2017. Young plants (coppicing from plants previously cut back to the ground level) received carbofuran at 7g a.i./m^2 through a soil application. Means with different superscript letters (one-way ANOVA) denote significant differences at $P < 0.05$, $n = 3$.

Time after carbofuran application	Biocontrol	Exclusion
Two weeks	Undetected	0.15 ± 0.08^{a}
Four weeks	Undetected	$<0.01 \pm <0.01^{b}$
Six weeks	Undetected	0 ± 0^{b}

3.5 Discussion

According to Smith and DeBach (1942), a biocontrol programme is considered successful when a pest population decreases in the presence of its natural enemies. In this study, significant and sustained trends emerged showing greater stem production in the exclusion compared to biocontrol plants, presumably attributable to the pressure exerted by the combination of all established biocontrol agents. Ultimately, biological control success or lack of it can only be evaluated based upon whether or not additional control methods (other than biocontrol) are required, and if yes, to what extent, to reduce a weed population to lower or non-invasive levels (Hoffmann, 1995; Klein, 2011). Based on Klein (2011) and Zachariades *et al*.'s (2017) definition of a successful biocontrol programme, it can be concluded that the impacts of *L. camara* biocontrol in the study area were negligible, i.e. requiring the integration of other control methods, such as chemical and manual control (physical clearing), to reduce *L. camara* growth and reproduction to acceptable levels, despite a fairly strong presence of well-established agents. It is, however, argued that lantana biocontrol is still rewarding, due to the fact that it helps reduce the frequency and consequently the cost involved in using alternative conventional control methods (Urban *et al*. 2011).

Despite seasonal fluctuations, lantana plants were never without pressure exerted by biocontrol agents at any time throughout the study period. Most agent populations peaked in summer and autumn, corroborating Cilliers' (1987b) findings. Leaf damage, however, varied over the seasons, and the resumption of the agents' activity in spring clearly indicates that these agents were able to overwinter, and quickly recover to peak by summer. Such trends in insects and the impacts on their host plants can only be recorded with the aid of quantitative long-term post-release evaluation studies.

Post-release evaluation studies for the biological control of lantana are scarce, however; where such were conducted, they mostly focused on a few agents (Cilliers, 1987b), or a single one at a time, and over a relatively short period (Harley *et al*. 1978; Mukwevho *et al*. 2017). While lantana biocontrol agents have been found to reduce plant growth and reproduction by employing insecticidal exclusion methods (Cilliers, 1987b; Mukwevho *et al*. 2017), the difference in plant growth between exclusion and biocontrol plants was not maintained throughout the present study period, suggesting that the insecticidal exclusion experiment failed to completely remove, or at least significantly reduce the number of agents from the exclusion plants. The impact of lantana biocontrol agents on plant growth was therefore difficult to fully assess, given problems with keeping insecticidal exclusion plants insect-free over long time periods at these distant field sites.

Carbofuran treatment at 7 g and subsequently 14 g a.i./m^2 every 2 months was short-lived (~ three weeks), protecting the 'insect-exclusion' plants for only about 31% of the time/season/year. This, therefore, implies that in the interval between one insecticide application and the next, biocontrol agents would have re-colonized their host (see Table 3.5 and Chapter four). Interestingly, while the insecticidal effect in the *L. camara* experiment was short-lived, carbofuran applications to *Solanum mauritianum* Scop. (Solanaceae), in the same study area and applied at the same dosage as in the lantana experimental trials, significantly reduced both biocontrol agents, *Gargaphia decoris* and *Anthonomus santacruzi*'s feeding damage on exclusion plants (Sasa, 2018). Little is known about the persistence of systemic insecticides, such as carbofuran, in plants.

The disparity in the results between this study and previous ones, apart from the obvious, i.e. difference in insecticides used at different dosages, is the geographic locations of study areas. The impact of lantana biocontrol agents has been demonstrated to be greater in humid, frost-free, coastal regions compared to inland regions (Urban and Phenye, 2005; Baars *et al*. 2007; Zalucki *et al*. 2007; Urban *et al*. 2011). The lantana flower-galling mite, *Aceria lantanae* (Cook) (Acari: Trombidiformes: Eriophyidae), for example, has shown to be more damaging to the reproductive output of the weed in coastal regions than inland (Urban *et al*. 2011; Mukwevho *et al*. 2017).

Because *A. lantanae* established in low numbers, it did not significantly reduce the reproductive output of plants; instead, a weak positive correlation emerged between the number of galls and infructescences, suggesting that *L. camara* plants with a more vigorous reproductive output (for a combination of genetic and micro-environmental reasons), produce more undifferentiated inflorescence buds, and by implication, would result in having more *A. lantanae* flower galls. *Aceria lantanae* establishes and breeds best on plants that are growing most vigorously (A. Urban, pers. comm.). In general, it could be argued that the combined impacts of *L. camara* biocontrol agents have resulted in the reduction of the growth rate of the plant but not its spread and densification (Zimmermann *et al*. 2004; Vardien *et al*. 2012). Although the focus was on the combined impacts of the entire suite of *L. camara* biocontrol agents present in the study area, some agents exerted more damage on the plant than others.

Teleonemia scrupulosa was the most abundant and damaging agent of the whole suite, followed by *H. laceratalis* and the leaf-spot pathogen *cf. Passalora* sp. Remarkable success of *H. laceratalis* has been recorded in Hawaii (Day *et al*. 2003a), and in recent years, studies in South Africa have shown the indigenous moth, *H. laceratalis*, to be among the most abundant agents in major provinces invaded by *L. camara*, including Mpumalanga (Heystek, 2006; Urban *et al*. 2011). Plant variety differences and parasitism (Day *et al*. 2003a), or failure to establish (Denton *et al*. 1991) have been cited as possible causes for the limited success of agents in *L. camara* biocontrol (Cilliers and Neser, 1991). In the present study, the majority of plants were of one colour variety, the pink-flowered variety, therefore

varietal limitation was not measured (see Chapter Five). There were, however, some altitudinal limitations, particularly with the establishment of *A. lantanae*; only 20% of the plots, all found at low altitude sites, were moderately susceptible to *A. lantanae,* whereas those in the other 80% were either highly resistant or immune.

Yellow-grey leaf-spot lesions of the pathogen *cf. Passalora* sp. were found throughout the entire study area, at all three altitude levels, throughout all seasons. It was more abundant at medium altitude sites compared to high and low altitude sites (Fig. 3.11.E and Chapter five), which is probably because the rainfall is 22-27% higher at the medium altitude (Table 3.1), and also relative humidity slightly higher compared to high and low altitudes.

The proliferation and spread of invasive alien plants such as *L. camara* can be attributed to many factors, including their propensity to produce a large amount of seeds (Witkowski and Wilson, 2001). Seed production, seed rain and seedbank germination results showed no significant differences between biocontrol and exclusion treatments throughout the study area. However, at low altitudes, there was a 29% reduction in seed production (Fig. 3.10), and a 31% reduction in the seedbank density of germinable seeds (Fig. 3.9.b) in biocontrol compared with exclusion plots, although these differences were not statistically significant. However, seedling, seed rain and seedbank densities measured at these sites were considerably lower compared to other studies. Fensham *et al*. (1994) recorded 10.5 to 25.3 seedlings/m^2 in north Queensland, Australia, compared to 0.8 to 1.01 seedlings/m^2 at the present study's high and low altitude sites respectively. *Lantana camara* seed dormancy also contributes to difficulties in controlling this weed. A soil seedbank of between 100 and 140 non-dormant, germinable seeds/m^2 under biocontrol and exclusion plants, respectively (Fig. 3.9.a & b), is worth noting, as it shows *L. camara*'s high potential to re-populate sites over long periods.

In as much as the number of germinable seeds was high (100-140 seeds/m^2) in the laboratory germination trial, these numbers did not translate into seedlings in the field (as above mentioned: 0.8-1.01 seedlings/m^2). Seed dormancy is the primary inhibitor of *L. camara* germination (Sharma *et al*. 2005). Nonetheless, the

low germination rate and low seed viability are compensated for by an equally low rate of seedling mortality (Duggin and Gentle, 1998; Sahu and Panda, 1998). In addition to environmental factors such as light, temperature and moisture which can limit germination (Duggin and Gentle, 1998; Parsons and Cuthbertson, 2001), it seems, *L. camara* is not immune to the growth inhibitory effects of its own allelochemicals. Some perennial plant species, including *L. camara* (Sharma *et al*. 2005) and *Chromolaena odorata* (Asteraceae) (Sahid and Sugau, 1993), have the capacity of releasing allelochemicals to the soil, which prevent other plants (e.g. the fern *Cyclosorus dentatus* Forsk. (Pteridophyta), wheat, corn, and soybean) occurring in their vicinity from thriving (Achhireddy *et al*. 1985; Sharma *et al*. 2005). *Lantana camara*, it seems, inhibits its own germination and seedling recruitment through light deprivation underneath the plant canopy, but also presumably through its allelochemicals.

Results showed that the impact of the suite of *L. camara* biocontrol agents on the *L. camara* plants was negligible, as per Klein's (2011) definition. This observation, however, does not necessarily suggest that biocontrol methods are ineffective, especially if one considers the extent to which IAPs, *L. camara* in particular, would have spread in the absence of whatever pressure, big or small, is exerted by biocontrol agents (van Wilgen *et al*. 2004).

In conclusion, *L. camara* biocontrol agent impact was difficult to measure because the activity of carbofuran in exclusion plants was short-lived. Despite failing to maintain the 'exclusion' plants biocontrol agent-free through the application of carbofuran, there were reductions of up to 37% in stem production and 31% in soil seedbank in biocontrol compared to exclusion plants, and leaf damage was up to 40% greater in biocontrol compared to exclusion plants. Therefore the present suite of biocontrol agents tended to reduce the growth of lantana. The efficacy of carbofuran treatments in excluding lantana biocontrol agents was investigated further under laboratory conditions (Chapter four). The growth data presented in this study are not directly comparable to other studies; therefore they are of great potential value as a reference for future research. To achieve significant biocontrol of lantana in inland areas, it seems to be necessary to introduce additional agents, which are adapted to inland climatic conditions.

Chapter Four: Use of carbofuran for insecticidal exclusion: *Lantana camara* and its biocontrol agent, *Teleonemia scrupulosa*, under laboratory conditions

4.1 Abstract

Insecticides, such as carbofuran, are sometimes used in chemical exclusion experiments to measure the impacts of biocontrol agents on their host plants. This laboratory-based study sought to determine the efficacy of using carbofuran in an exclusion experiment aimed at assessing the impacts of biocontrol agents on *Lantana camara* L. (sensu lato) (Verbenaceae). Two separate experiments were conducted, the first one on insect-free plants, to determine the effects of carbofuran solely on plant growth; and the second one, on *Teleonemia scrupulosa* Stål (Hemiptera: Tingidae) infested plants, with the objective of determining the impact of carbofuran on this biocontrol agent, as well as its impacts on plant growth. Carbofuran granules (10% a.i.) were applied at 7 g/m^2 a.i. to the potting medium, which is the same dosage used on small plants in a field experiment (Chapter Three). It was found that carbofuran did not have a significant effect on plant growth. Total removal of *T. scrupulosa* from exclusion plants (carbofuran-treated plants) was not achieved; however the low level of leaf feeding lesions on those plants indicated that carbofuran had considerably reduced the insect's population density. Results from a bioassay showed 100% and 40% *T. scrupulosa* mortality on leaves collected from carbofuran-treated and control plants, respectively, within three weeks of exposure. Analysis of chemical residue levels in the leaf material revealed that carbofuran was detectable at trace levels (<0.1 mg/kg) for the duration of the experiment (three weeks), and its potency only persisted for about three weeks. It was therefore concluded that carbofuran was effective at reducing the population of *T. scrupulosa* on its host plant, but only briefly. The chemical should be applied at least once every three weeks or at a higher dosage in order to maintain a low insect population for the duration of an experiment or to achieve total exclusion. For better removal, one should consider combining carbofuran and foliar insecticides.

Keywords: Active ingredient; Carbofuran dosage; Insecticide residue; Insect survival; Persistence; Plant growth.

4.2 Introduction

Under field conditions, it is difficult to measure the impacts of biocontrol agents because of the absence of naturally occurring, insect-free plants to act as a control for comparison. Chemical exclusion methods have therefore been widely adopted for measuring such impacts (Cilliers, 1987b; Crawley, 1989; Carson *et al*. 2008; Mukwevho *et al*. 2017), provided that the insecticides used do not act as a plant fertilizer or as a herbicide. One of the alien invasive plants requiring the impacts of its biocontrol agents to be quantified through chemical exclusion methods is *Lantana camara* L. (Verbenaceae).

Lantana camara continues to have the reputation of being one of the most ecologically and economically damaging invasive alien plants in many tropical, subtropical and warm temperate regions of the world (Day *et al.* 2003a, Moran *et al*. 2013), including South Africa (Urban *et al*. 2011). This perennial woody shrub is native to the tropical and sub-tropical regions of the Americas (Day *et al*. 2003a), and was the first weed to have been targeted for biocontrol, with several insect species released against it worldwide from 1902 onwards (Urban *et al*. 2011; Winston *et al*. 2014).

The present work focused on the *L. camara* leaf-sucking bug, *Teleonemia scrupulosa* Stål (Hemiptera: Tingidae), which is originally from Mexico, Central America and South America, and was released into South Africa in 1971 (Winston *et al*. 2014). *Teleonemia scrupulosa* was chosen for two main reasons: first, it is available throughout the country, wherever *L. camara* occurs (Urban *et al*. 2011), and secondly, it is considered the most damaging of all *L. camara* biocontrol agents due to its short generation time (three to four weeks) and long adult lifespan (up to three months) resulting in the build-up of large populations (Baars and Heystek, 2003a; Urban *et al*. 2011). In South Africa, *T. scrupulosa* has about 9 to 11 generations per year (Cilliers, 1987a). *Teleonemia scrupulosa* adults and nymphs feed gregariously, mainly on the underside of leaves, but also occasionally on inflorescences and the apex of growing stems (Day *et al*. 2003a). The chemical exclusion of insects with more than one feeding guild, such as *T. scrupulosa*, requires the use of systemic insecticides.

In an early study, aldicarb, a systemic insecticide, was used successfully in an exclusion experiment at a rate of 40 g/m^2 (15% a.i.), to measure the impact of *L. camara* biocontrol agents (Cilliers, 1987b). This highly toxic insecticide has since been prohibited following numerous reported cases of honeybee mortality (Nigg *et al*. 1991), malicious dog poisoning and human suicide (Nelson *et al*. 2001; Waseem *et al*. 2010; van Zyl, 2012) in many parts of the world (Anastasio and Sharp, 2011; Frazier *et al*. 1999; Motas-Guzman *et al*. 2003), including South Africa (Arnot *et al*. 2011). As a result, carbofuran was used as an alternative insecticide for this study.

Carbofuran (2,3-dihydro-2,2-dimethylbenzofuran-7-yl methylcarbamate) is a broad-spectrum, systemic insecticide, nematicide, and acaricide of agricultural importance worldwide (Dobsikova, 2003). It is commonly used in homes, gardens and agriculture to protect plants from insect pests such as *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae), *Chortophila brassicae* Bouche (Diptera: Muscidae), *Phyllotreta nemorum* L. (Coleoptera: Chrysomelidae), *Atomaria linearis* Stephens (Coleoptera: Cryptophagidae), and *Phorodon humuli* Schrank (Homoptera: Aphididae) (Dobsikova, 2003). Insects get exposed to the insecticide indirectly by feeding on treated plants.

The type of insecticides, rate of application and host plant targeted (leguminous, herbaceous, shrub or woody plant) are all an important factor to consider when designing chemical exclusion experiments. According to the carbofuran (Curaterr 10GR) pamphlet from 'Bayer CropScience' (RSA/0610/Curaterr 10GR/pamphlet, code: 03294068D), the recommended field application rate of carbofuran (10% a.i.), for grain sorghum, cabbage, tobacco and potatoes ranges from 2 to 4 g/m^2 (this assuming the furrow is 100 m in length and 0.5 m wide); sugarcane, 2.5 to 3 g/m^2; and wheat, 1 g/m^2. Carbofuran normally require a single application throughout the entire cropping season (Mora *et al*. 1996). The recommended carbofuran application to grain sorghum at 0.1, 0.2 and 0.3 g/m^2 resulted in a reduction of maize stalk borer, *Busseola fusca* Fuller (Lepidoptera: Noctuidae), shoot fly, *Anatrichus erynasius* Loew (Diptera: Chloropidae), and maize aphid, *Rhopalosiphum maidis* Fitch (Hemiptera: Aphididae), infestations (van Rensburg *et al*. 1978). The present study used

carbofuran (10% a.i.) at a rate of 7 g/m^2, with the aim to exclude *L. camara* biocontrol agents from their host.

Carbofuran is an anti-cholinesterase, which acts as an inhibitor of cholinesterase enzymes, resulting in impairment of the transmission of nerve impulses (Dobsikova, 2003). Plants do not have a nervous system; therefore they are not affected by the anti-cholinesterase activity of insecticides. Hence, if insecticides do have any effects on plant growth, these effects have some other basis. For example, when an insecticide has a growth-stimulating effect on an infested plant, that effect is probably mainly 'indirect', i.e. achieved by killing the insects that were suppressing the growth of the plant.

The use of insecticides in agriculture has had both negative and positive effects on plant growth. Early work with leguminous crops demonstrated that, depending on the type and dosage employed, some insecticides such as phorate and carbofuran can improve nitrogen fixation in the soil via affecting nitrogen-fixing bacteria, thus promoting root formation and consequently plant growth (Mundade *et al*. 1980; Benjamini, 1986). Das and Mukherjee (2000) reported a significant increase in the density of microorganisms in soil treated with carbofuran (3% a.i.) applied at a field rate of 1.0 kg/ha a.i.

Conversely, carbofuran has elsewhere been reported to have negative effects on plant growth in alfalfa, sweet clover (Shin *et al*. 1972), and sugarcane (Sachan and Manchanda, 1979). Lonsdale and Farrell (1998) reported a decline in fruit production on carbofuran-treated *Mimosa pigra* plants, possibly due to the effect of carbofuran on pollinators more than a direct effect on the plant, they argued. Insecticides used to protect plants from insect pests are not intended to have any effects on plant growth, but to kill target pests. Carbaryl, dimethoate, fluvalinate, benomyl and carbofuran were effective at removing *Comostolopsis germana* larvae from *Chrysanthemoides monilifera* without affecting the plant's shoot growth or seed production (Adair and Holtkamp, 1999). Generally, in chemical exclusion experiments plant growth is expected to be slightly higher on insecticide-treated plants compared to untreated ones, simply because the former plants are freed from the growth-suppressing effect of all insects.

The present study, under laboratory conditions, aimed to determine the effectiveness of using carbofuran in an insecticidal exclusion experiment to measure the impact of *T. scrupulosa* on *L. camara*. This study was conducted in response to a similar field-based experiment (Chapter Three), which measured how the carbofuran application affected both the suite of biocontrol agents, and their impact on the growth and reproduction of *L. camara*. The objectives of the present study were to determine: (i) the effect of carbofuran on *L. camara* growth, (ii) the impact of *T. scrupulosa* on plant growth, (iii) the impact of carbofuran on *T. scrupulosa* survival through bioassays, and (iv) the frequency of carbofuran application required for optimal exclusion of such a biocontrol agent.

4.3 Materials and methods

This study was conducted at the University of the Witwatersrand, Johannesburg, South Africa (S 26^{o} 11.240', E 28^{o} 01.588'). Plants were grown in plastic pots (height: 320 mm, diameter: 460 mm). Granular carbofuran (100g a.i. per kg granules i.e. 10% a.i.), manufactured by Bayer Crop Science under the commercial name Curaterr 10 GR, was applied to the soil around plants intended to be treated, and raked into the soil before being watered to encourage initial uptake. Control pots were only raked and watered. Carbofuran was applied at a rate of 7 g/m^2 active ingredient (a.i) (equivalent to 70 kg/ha a.i). This dosage was the same as that used in the field initially in 2013 (Chapter Three). To address the objectives of this study, experiments were conducted on two sets of plants, insect-free and insect-colonized plants. Since the maximum duration of these experiments was two months, carbofuran application was done once in accordance with field applications (Chapter Three). Nitrosol fertilizer (N 8%; P 2%; K 5.8%) was applied to all pots to ensure healthy growth of plants prior to carbofuran treatment trials; and they were irrigated daily, twice a day at 08:00 and 15:00, for 15 minutes. Plants were grown and kept under full sun conditions.

4.3.1 Effect of carbofuran on *Lantana camara* plant growth

Insect-free potted *L. camara* plants of the dark pink-flowered variety were grown in a glasshouse at the University of the Witwatersrand. To avoid intra-specific

variations as well as to ensure uniformity in size, plants were grown from cuttings obtained in the field from a single mother plant. Seven plants were treated with carbofuran (referred to as exclusion plants), and another seven were untreated (referred to as control plants). Carbofuran was applied to the treated plants on the first day of the experiment, and the first measurements were taken one week later. The experiment ran for nine weeks over January and February 2014. The following plant growth parameters were measured daily and compared between control and exclusion plants: plant height, plant canopy diameter, total number of leaves and inflorescences, stem diameter, leaf chlorophyll content, and plant biomass. Stem diameter was compared between control and exclusion plants twice during the trial, at the start (week one) and at the end (week nine).

Plant biomass was measured at the end of the experiment through destructive sampling. Leaves (leaves + inflorescence + infructescence), stems and root were placed separately in brown paper bags; and oven dry at 60 ^{o}C for 48 hours. Chlorophyll content was measured on weeks five, six and seven using a 'Single Photon Avalanche Diode' (505) Chlorophyll Meter (Minolta, Osaka 542, Japan) on six randomly selected leaves per plant. These measurements were taken between 12:30 and 13:30 under full sun conditions.

4.3.2 Impact of *Teleonemia scrupulosa* on *Lantana camara* growth

This experiment mimicked the situation in the field where plants are readily colonized by insects (Chapter Three). The impact of *T. scrupulosa* on plant growth was measured using the chemical exclusion method. Ten potted *L. camara* plants of the dark pink-flowered variety obtained from Plant Protection Research Institute (PPRI), in Pretoria, were kept within a semi-open glasshouse at the University of the Witwatersrand. Plants were watered daily once a day for 15 minutes. In spring of 2013 they were pruned and by summer of 2014 (February, 2014) they had re-grown and had a near uniform size. There was an outbreak of *T. scrupulosa* within the glasshouse, which meant that no additional releases were required since all plants were already infested at the time of the experiment.

Plants were divided into biocontrol (5 plants) and exclusion (5 plants). Exclusion plants were treated with carbofuran (at the rate described above) at

week zero and first measurements taken from week one; while biocontrol plants remained untreated. The experiment ran for seven weeks. The following *L. camara* plant growth parameters were measured and compared between biocontrol and exclusion plants: plant height, plant canopy diameter, stem length, number of leaves damaged by *T. scrupulosa*, total number of leaves and inflorescences.

4.3.3 Bioassay: *Teleonemia scrupulosa* survival

Six potted *L. camara* plants, obtained from a culture kept at the University of the Witwatersrand, were divided into carbofuran-treated (three plants) and untreated (control) (three plants). Plants used in this experiment were heavily infested with *T. scrupulosa* as a result of an outbreak within the plant culture. Carbofuran was applied at week zero, and one week later (Day 1); four leaves and 10 adult insects were picked from each of the six plants and placed into six respective petri dishes (three carbofuran and three controls) fitted with a filter paper each. There was a total of 60 insects, with half (30) collected from treated plants and the other half (30) from control plants. On a daily basis, leaves within each petri dish were replaced with fresh ones picked from respective plants, carbofuran-treated and untreated; and the filter paper kept moist by adding ~ 8 ml of water. The survival of the 10 insects per petri dish was monitored daily. This experiment ran for four weeks and insect survival was compared between carbofuran-treated and untreated leaves. Since *T. scrupulosa* feeds on cell contents of leaves (Day *et al*. 2003a), the cut foliage was suitable for this trial.

4.3.4 Carbofuran residue analysis

Leaf samples from the survival experiment above were sent to an independent laboratory, Hearshaw and Kinnes Analytical Laboratory (HKAL) (Pty) Ltd., in Cape Town for insecticide residue analysis using the LCMS (liquid chromatography plus mass spectrometry) analytical method. Three sets of samples (~200 g each) were sent for analysis on the first week after carbofuran was applied, and two subsequent consignments on the second and third week.

4.3.5 Statistical analysis

One-way ANOVAs with repeated measures followed by LSD Post-hoc tests were used to compare plant growth parameters between carbofuran-treated and untreated plants. Comparisons of plant diameter, leaf chlorophyll content and plant biomass between treatments were done using a student's t-tests. All analyses were conducted at a critical *P* level of 0.05 using Statistica, version 8.0.

4.4 Results

4.4.1 Effect of carbofuran on *Lantana camara* plant growth

There were no significant differences in plant height ($F_{(1, 12)} = 0.42$; $P = 0.52$), canopy diameter ($F_{(1, 12)} = 1.35$; $P = 0.26$), number of leaves ($F_{(1, 12)} = 0.01$; $P = 0.89$) and number of inflorescences ($F_{(1, 12)} = 0.01$; $P = 0.90$) between control and carbofuran-treated plants (Fig. 4.1.a.b.c.d, respectively). There were, however, significant differences between weeks in each of the above plant parameters as plants were growing (Table 4.1).

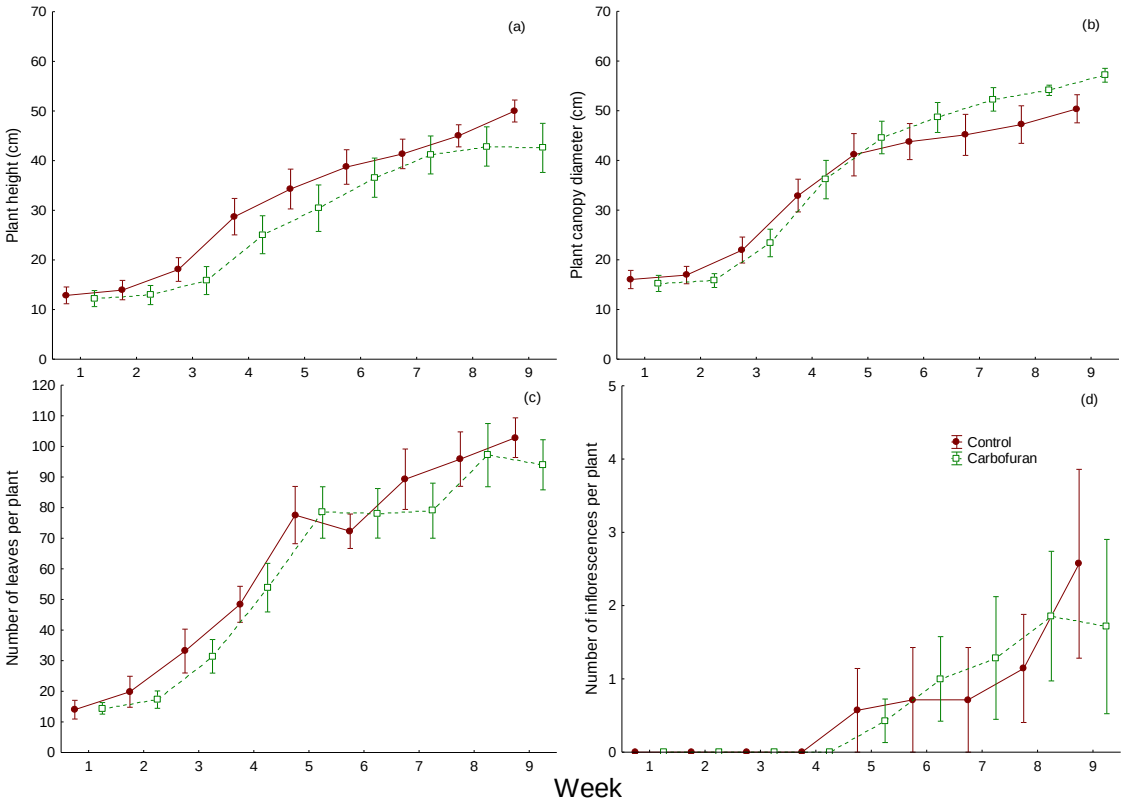

Figure 4.1 Comparisons of plant height (a), canopy diameter (b), number of leaves (c) and number of inflorescences (d) between control and carbofuran-treated plants (mean ± SE). No significant differences were noted between control and carbofuran plants ($P < 0.05$) (n = 7).

Table 4.1 Summary of the effects of treatments (carbofuran treated and untreated plants), time (week) and interactions (treatment*week) on the vegetative and reproductive growth of *L. camara* between January and March 2014 in (i) insect-free plants and (ii) *Teleonemia scrupulosa* infested plants.

Experiments	Parameters	Factor variables	d.f.	F-value	*P*-value	Significance
Experiment (i) Insect-free plants	Plant height	Treatment	1	0.42	0.52	NS
		Week	8	107.3	<0.001	***
		Treatment*Week	8	0.75	0.65	NS
	Canopy diameter	Treatment	1	1.35	0.26	NS
		Week	8	108.5	<0.001	***
		Treatment*Week	8	1.21	0.29	NS
	Number of leaves	Treatment	1	0.01	0.89	NS
		Week	8	92.58	<0.001	***
		Treatment*Week	8	0.67	0.71	NS
	Number of inflorescences	Treatment	1	0.01	0.90	NS
		Week	8	4.28	<0.001	***
		Treatment*Week	8	0.36	0.93	NS
Experiment (ii) Insect-infested plants	Plant height	Treatment	1	0.09	0.76	NS
		Week	6	21.11	<0.001	***
		Treatment*Week	6	1.83	0.11	NS
	Canopy diameter	Treatment	1	0.06	0.80	NS
		Week	6	14.99	<0.001	***
		Treatment*Week	6	0.77	0.59	NS
	Stem length	Treatment	1	0.93	0.36	NS
		Week	6	70.04	<0.001	***
		Treatment*Week	6	2.16	0.06	NS
	T. scrupulosa damaged leaves	Treatment	1	18.26	0.002	**
		Week	6	63.74	<0.001	***
		Treatment*Week	6	6.85	<0.001	***
	Number of leaves	Treatment	1	0.01	0.92	NS
		Week	6	12.45	<0.001	***
		Treatment*Week	6	0.93	0.47	NS
	Number of inflorescences	Treatment	1	0.36	0.56	NS
		Week	6	2.17	0.06	NS
		Treatment*Week	6	0.12	0.99	NS

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, NS = not significant.

Although, there were no significant differences in stem diameter in week one at the beginning of the experiment ($t_{12} = 0.37$; $P = 0.71$), and week nine at the end of the experiment ($t_{12} = 1.13$; $P = 0.28$) (Fig. 4.2.a); leaf chlorophyll content in week five ($t_{12} = 0.32$; $P = 0.75$), week six ($t_{12} = 0.80$; $P = 0.43$) and week seven ($t_{12} = 1.36$; $P = 0.19$) (Fig. 4.2.b); and leaf ($t_{12} = 0.02$; $P = 0.97$), stem ($t_{12} = 0.34$; $P = 0.73$) and root biomass ($t_{12} = 0.60$; $P = 0.55$) between control and carbofuran-treated plants (Fig. 4.2.c); there were, however, trends indicating that carbofuran inhibited *L. camara*'s root biomass growth (Fig 4.2.c), chlorophyll synthesis (Fig. 4.2.b), and stem diameter growth (fig. 4.2.a).

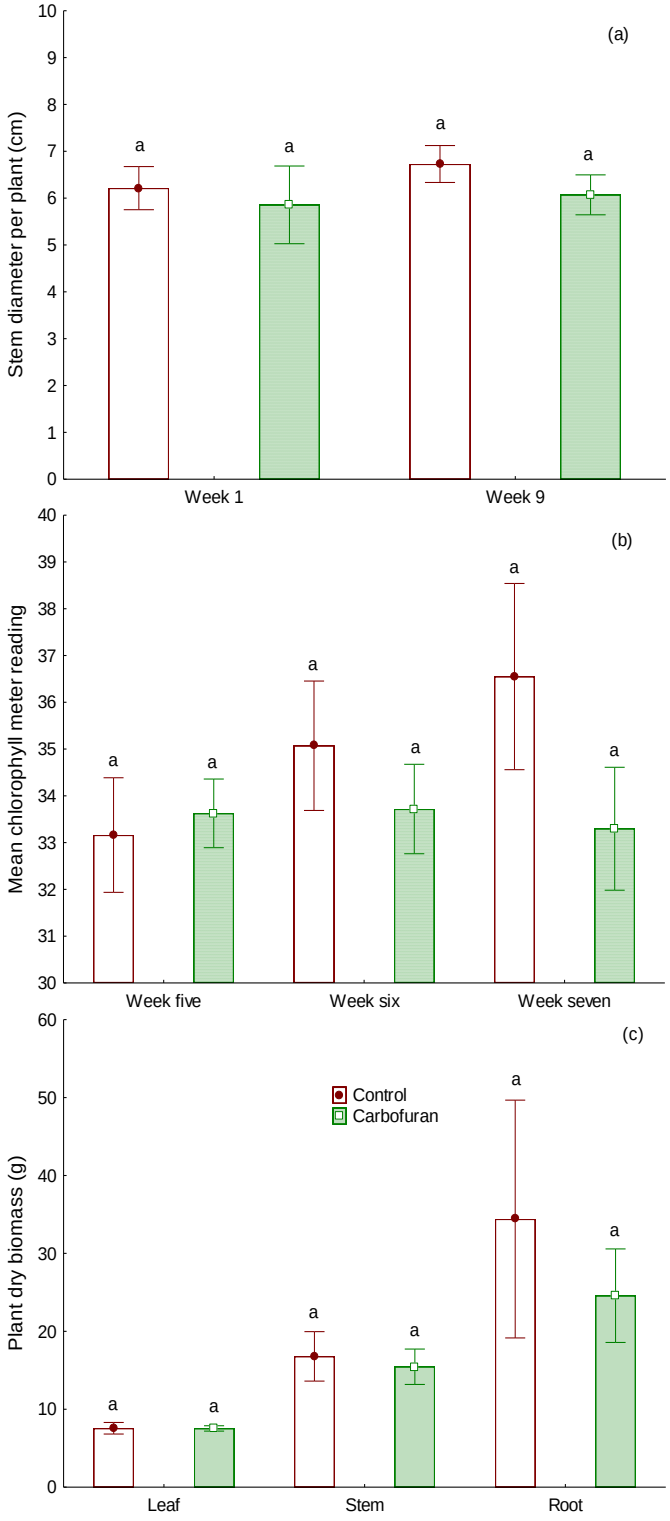

Figure 4.2 Comparisons of stem diameter (a), chlorophyll content (b), and plant biomass between control and carbofuran-treated plants (mean ± SE). Means with different letters within same week and plant parts are significantly different using a student's t-test ($P < 0.05$; n = 7).

4.4.2 Impact of *Teleonemia scrupulosa* on *Lantana camara* plant growth

There were no significant differences in plant height ($F_{(1, 8)} = 0.09$; $P = 0.76$), plant canopy diameter ($F_{(1, 8)} = 0.06$; $P = 0.80$), stem length ($F_{(1, 8)} = 0.93$; $P = 0.36$), number of leaves ($F_{(1, 8)} = 0.01$; $P = 0.92$) and number of inflorescences ($F_{(1, 8)} = 0.36$; $P = 0.56$) (Fig. 4.3.a.b.c.e.f) between biocontrol and exclusion plants from week one to week seven. However, the number of leaves damaged by *T. scrupulosa* was significantly greater in biocontrol plants compared to exclusion plants ($F_{(1, 8)} = 18.26$; $P < 0.01$) (Fig. 4.3.d).

There were significant changes between weeks, and a significant week/treatment interaction in the number of leaves damaged by *T. scrupulosa*, showing that the agent's damage was significantly greater in biocontrol than exclusion plants in most weeks (Table 4.1).

There was also an indication that the effect of carbofuran, applied at this dose rate, on *T. scrupulosa* lasted only for about three weeks, from week 0 (when carbofuran was applied) to week 3 (Fig. 4.3.d).

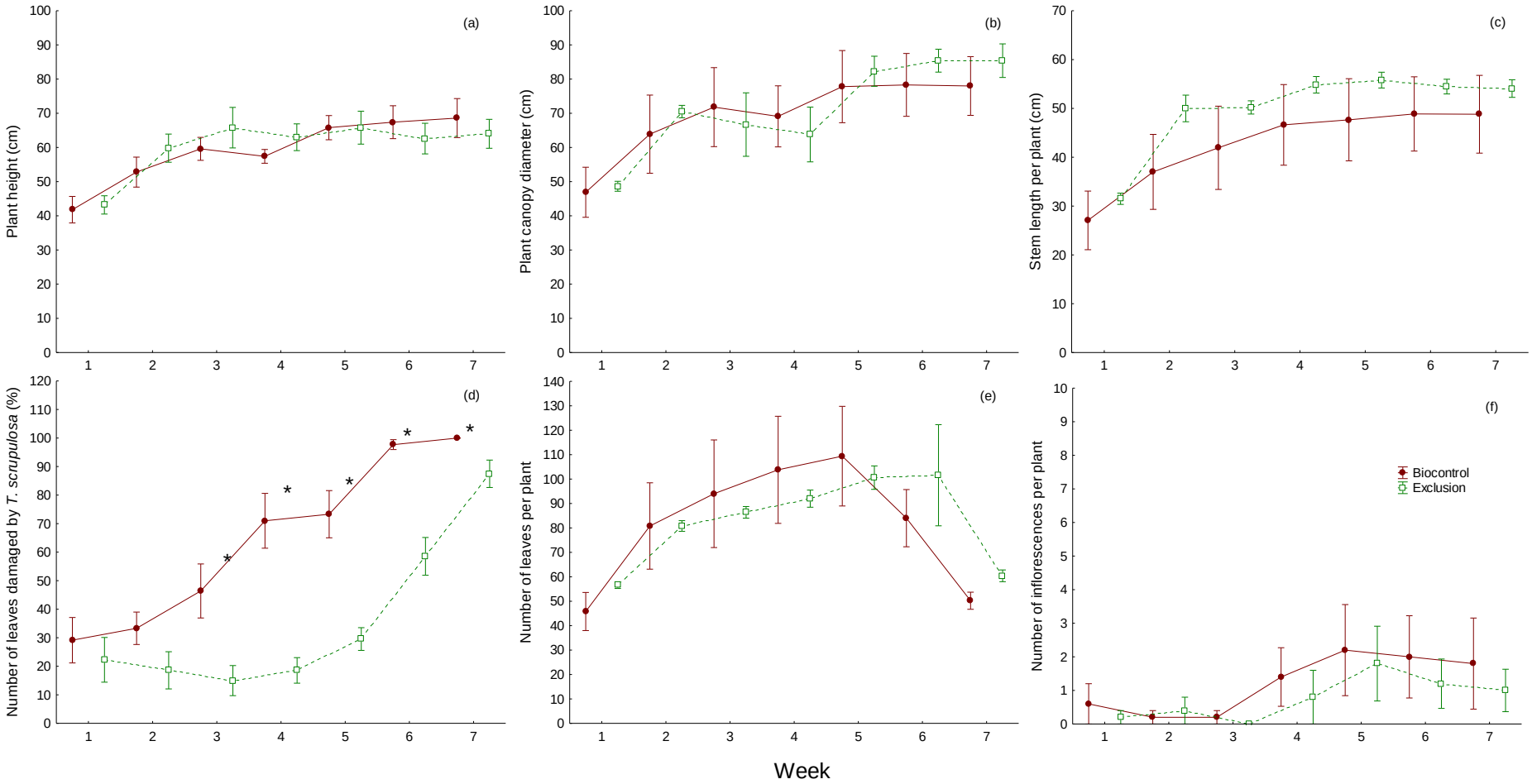

Figure 4.3 Comparisons of plant height (a), canopy diameter (b), stem length (c), leaves damaged by *Teleonemia scrupulosa* (d), number of leaves (e), and number of inflorescences (f) between biocontrol and exclusion plants (mean ± SE). Biocontrol/Exclusion pairs with an asterisk are significantly different using Fisher LSD post-hoc tests ($P < 0.05$) (n = 5).

4.4.3 *Teleonemia scrupulosa* survival (bioassay)

Teleonemia scrupulosa survival was significantly lower on carbofuran-treated leaves compared to the control ($F_{(1, 4)} = 10.03$, $P = 0.03$) (Fig. 4.4).

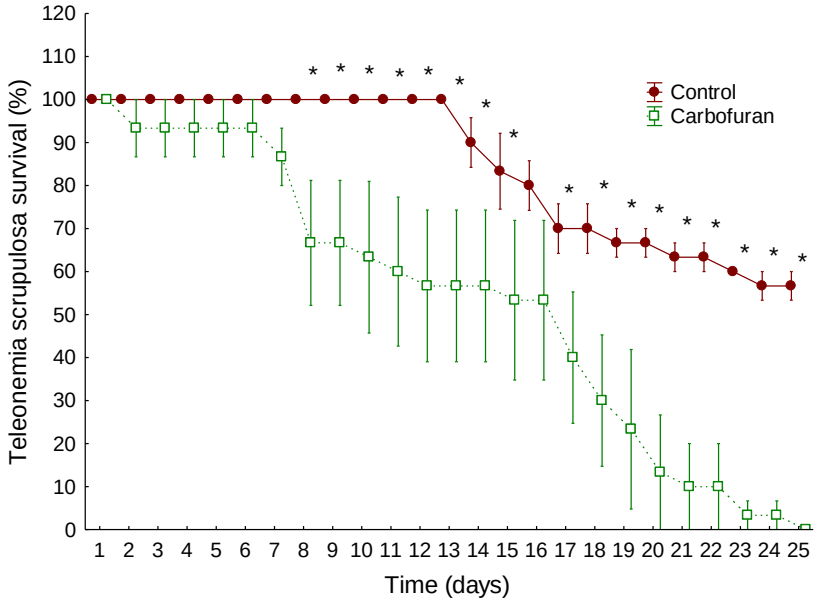

Figure 4.4 *Teleonemia scrupulosa* survival on control and carbofuran-treated plants over 25 days (mean ± SE). Control/carbofuran daily pairs with an asterisk are significantly different using Fisher LSD post-hoc tests ($P < 0.05$) (n = 10).

4.4.4 Carbofuran residue analysis

Carbofuran residues were detected in leaves from carbofuran-treated plants but were undetectable in the control as expected (Table 4.2). The insecticide was applied at week zero and measurements were taken from week one. The residue level significantly declined two weeks after carbofuran application.

Table 4.2 Carbofuran residue levels (ppm) in *L. camara* leaves from carbofuran-treated plants (exclusion plants) and untreated plants (biocontrol plants) (mean ± SE). Potted plants received carbofuran at 7 g a.i./m^2 through a soil application. Means with different superscript letters (one-way ANOVA) denote significant differences at $P < 0.05$. n = 3

Time after carbofuran application	Biocontrol	Exclusion
Week one	Undetected	0.02 ± 0.01^{a}
Week two	Undetected	0.07 ± 0.02^{b}
Week three	Undetected	$<0.01 \pm 0^{a}$

4.5 Discussion

Although there were no statistical differences in *L. camara* growth between carbofuran-treated plants and control plants, there were tendencies for reduced growth in the plant height (14.9%) and the number of leaves (8.6%) in the former plants (carbofuran-treated), indicating that carbofuran had some inhibiting effects on *L. camara* growth, more so towards the last week of the experiment (Fig. 4.1.a.c). Therefore, while carbofuran can be used in an exclusion experiment intended to measure impacts of biocontrol agents on *L. camara* growth, its inhibiting effect on plant growth should not be ignored.

Tiyagi *et al*. (2004) found a significant improvement in chickpea growth parameters, namely length, weight, number of pods, chlorophyll content and root nodulation, of plants treated with insecticides compared to untreated ones. They found that carbofuran was the best plant growth promoter of the insecticides they tested followed by aldicarb, phorate, fenamiphos and fensulfothion. Several other studies, however, have demonstrated that organophosphate and carbamate insecticides do not directly affect plant growth (Shin *et al*. 1972; Adair and Holtkamp, 1999), and therefore have been considered for use in chemical exclusion experiments seeking to measure impacts of biocontrol agents on the growth of their host plants.

Although the carbofuran application did not exclude or totally remove *T. scrupulosa* from the exclusion plants, the number of leaves damaged by this agent was greater in the biocontrol plants compared to the exclusion plants, as would be expected. The low number of damaged leaves within exclusion plants indicated that carbofuran had considerably reduced *T. scrupulosa* population density throughout the experimental period. These findings corroborate field results (Chapter Three) which showed that the number of leaves, inflorescences and infructescences damaged by the suite of biocontrol agents present in the study area was generally greater in biocontrol compared to exclusion plants. In another field study, carbofuran was used in an exclusion experiment at a rate of 1 g/m^2 (10% a.i.) to quantify the impact of the chrysomelid beetle, *Calligrapha pantherina* Stål, on seed production of the tropical weed, *Sida acuta* Burm. f. (Malvaceae). It was shown that the number of seeds was significantly greater in

exclusion plants compared to *C. pantherina* infested plants, indicating that carbofuran was effective at excluding *C. pantherina* from the plants (Lonsdale *et al*. 1995).

Results of the bioassay revealed that *T. scrupulosa* survival was low on leaves from carbofuran-treated plants compared with those on the controls, as expected. These finding support the assertion that carbofuran could be used in an exclusion experiment aimed at measuring impacts of biocontrol agents on their host plants. In the case of the present study, however, the suppressing effect of carbofuran on herbivory by *T. scrupulosa* as well as all agents combined (Chapter Three) was short-lived, despite the fact that the dose applied both in the field and laboratory studies, i.e. 7 g/m^2, was within lethal ranges by way of comparison with other studies (DeBarr *et al*. 1982; Adair and Holtkamp, 1999). Survival of the shoot tip-feeding, *Comostolopsis germana* Prout (Lepidoptera: Geometridae) on an evergreen shrub or small tree, *Chrysanthemoides monilifera* L. Norlindh (Asteraceae) declined significantly three months after carbofuran application at a rate of 2.5 g/m^2 (Adair and Holtkamp, 1999).

The present study is not directly comparable to previous chemical exclusion studies because the dose and host plant used are not similar. Mortality of *Gargaphia decoris* and *Anthonomus santacruzi* on *Solanum mauritianum* was significantly greater on carbofuran-treated plants at a dose of 14 g/m^2 (same as *L. camara* field dosage, Chapter three) than on untreated plants (Sasa, 2018). In this study, the carbofuran residue in the plant (leaves) peaked at week four and only started declining by week six after application, compared to *L. camara* where carbofuran residues were undetectable by week four. This suggests that the uptake of carbofuran is highly dependent on the type of plant, and in this case, *L. camara* was more resistant than bugweed, presumably due to its secondary metabolites. This aspect should be further investigated in future research.

Carbofuran levels in *L. camara* leaves peaked three weeks after it was applied onto the soil, but then declined significantly afterwards. A similar trend was observed on plant obtained from a field experiment (Chapter Three). The fast metabolic breakdown of carbofuran explains why its residues in plants are often detected at trace levels (Eisler, 2000). The current study showed a very short time

effect of carbofuran on the number of leaves damaged by *T. scrupulosa* (Fig. 4.3d); nevertheless leaf damage was still significantly greater (45.9%) on the biocontrol compared to the exclusion plants throughout the study period, suggesting that even at trace levels carbofuran had a suppressing effect on *T. scrupulosa* feeding. This finding suggests that whilst the biocontrol agents slightly suppress the growth of *L. camara* in the 'biocontrol' treatment, so does carbofuran in the 'exclusion' treatment, thus minimizing the agents' apparent impact.

In conclusion, it was revealed that even at trace levels, carbofuran was able to reduce *T. scrupulosa* survival. Although, the insect exclusion population took a while to recover, there was an indication that they were slowly catching up with the biocontrol population. The removal of *L. camara* biocontrol agents was very brief and not complete, as they continued to exert some pressure on the 'exclusion' plants. It is therefore recommended that (1) follow-up applications of carbofuran should be done at an interval of three weeks to maintain low insect populations, (2) the carbofuran application should be supplemented with a foliar spray to achieve optimum insect removal from 'exclusion plants', and (3) in the case of emerging weeds, pre-release surveys of plant density should be conducted; but this is unlikely because in most cases alien plants are only given attention once they have reached disturbing/damaging proportions.

Chapter Five: Effects of biotic and abiotic environmental factors on *Lantana camara* growth and the performance of its biocontrol agents

5.1 Abstract

Biotic and abiotic environmental factors such as elevation, light regime and plant variety can influence plant growth and consequently have an impact on the performance of its associated phytophagous arthropods. This study aimed to determine the effects of such factors on *L. camara* growth and the resultant impacts on the performance of its suite of biocontrol agents. The study sites were divided into three altitude levels, high (1133 m), medium (975 m) and low (848 m); three light regimes, shade, semi-shade and full sun; and two *L. camara* varieties, light-pink flowered and dark-pink flowered varieties. The spread and abundance of *Aceria lantanae* (Cook) (Acari: Eriophyidae), one of the new additions to the suite of *L. camara* biocontrol agents, was determined along the altitudinal gradient. Plant growth was generally two to five times greater at low altitude compared to high and medium altitudes, under shaded conditions and in the light-pink varieties. Agents' performance, however, varied not only between altitudes and degrees of shade but also within seasons. A fungal leaf-spot pathogen, *cf. Passalora* sp. (Chupp) U. Braun & Crous var. *lantanae*, showed a strong preference for environmental conditions at the medium altitude sites and it didn't perform well on shaded plants. Light regime preferences between plants and agents revealed a paradoxical trend; while plant growth was most vigorous under shady conditions, the opposite was true for the performance of biocontrol agents. Biocontrol agents did not show varietal preferences, with the exception of *cf. Passalora* sp., which generally performed well on the dark-pink *L. camara* variety. The upper altitudinal limit of *A. lantanae* at the study sites increased from 780 m in 2015 to 950 m in 2016, suggesting successful spread. In conclusion, *L. camara* plant growth and its biocontrol agents were affected by altitude, light regime and plant variety, as well as season. There was a 'complementary relationship' between biocontrol agents, coupled with an apparent shift in their phenological peaks with altitude; thus maintaining a strong presence of the suite of agents at all altitudinal levels, throughout the seasons. Some consideration must be given to the introduction of more shade-adapted agents or strains of agents, to better suppress the growth of plants growing under shady conditions.

Keywords: Altitude; Phenological plasticity; Micro-environment; Biocontrol; Degree of shading; Plant variety.

5.2 Introduction

Lantana camara L. (Verbenaceae), native to the tropical and subtropical south and central regions of the Americas (Kannan *et al.* 2013), is one of the world's worst understorey weeds (Bhagwat *et al*. 2012), and one of the most ecologically and economically damaging invasive alien plants occurring in Africa, southern Asia, Australia and Oceania (Baars and Neser, 1999; Day *et al*. 2003a). This highly invasive weed occurs in a wide range of climatic and environmental conditions; having the ability to colonize a wide array of habitats (Vardien *et al*. 2012). Its ability to invade new environments was not recognized until after it had become widely spread (Myers and Bazely, 2003). *Lantana camara*'s wide geographic distribution is one of the attributes ranking it among the 100 worst invasive alien plants in the world (IUCN, 2001; Sharma *et al*. 2005). Adding to its wide geographic range, *L. camara* is a complex of species and hybrids with a taxonomy difficult to resolve (Day *et al*. 2003a; Sanders, 2006).

The biocontrol of some plants, particularly *L. camara* and others that reproduce sexually, can be difficult due to their genetic variations (Burdon and Marshall, 1981); which often improve the plant's adaptability to varying environmental conditions, but also enhance its resistance to natural enemies (Urban *et al*. 2011; Mukwevho *et al*. 2017). *Lantana camara* flower colour is one of the features conventionally used in the field to identify different plant varieties (Day *et al*. 2003a; Urban *et al*. 2011) despite evidence showing genetic relatedness being more linked to geographic proximity than flower colour (Scott *et al*. 1997). In other words, *L. camara* plants occurring within the same geographic location tend to be genetically related despite their differences in flower colours. Since the 16th century, *L. camara* has undergone extensive horticultural modification having more than 650 hybrid varieties existing throughout the world (Howard, 1969). As a result, it is a complex of species and varieties whose origin is not clear (Thomas and Ellison, 2000, Day *et al*. 2003a). This certainly has significant implications for biocontrol of *L. camara* as varieties

in their native range from where biocontrol agents are collected may be genetically different to those occurring in introduced ranges, resulting in agents having limited impacts, or none at all, on their new host (Neser and Cilliers 1989; Willson, 1993).

The term "variety" in this work is henceforth referring exclusively, unless stated otherwise, to flower colour varieties. Several *L. camara* biocontrol agents have displayed varietal preferences, namely *Falconia intermedia* (Distant) (Hemiptera: Miridae) (Urban and Simelane 1999; Urban *et al*. 2004), *Teleonemia scrupulosa* Stål. (Heteroptera: Tingidae) (Mkasi, 2016), and *Aceria lantanae* Cook (Acari: Eriophyidae) (Urban *et al*. 2004). *Aceria lantanae* has shown preferences for pink-flowered varieties, both dark-pink and light-pink (Urban *et al*. 2011; Mukwevho *et al*. 2017; Katembo, pers. obs.). Although the *A. lantanae* flower galls have been spotted on *L. camara* plants of different flower colours in the same area, the mite performs significantly better on one *L. camara* variety than the others (A. Urban, pers. comm.; Katembo, pers. obs.). Other factors, such as variable habitat conditions, particularly light regime or the degree of shading may also affect the insect-plant interaction (Ruban, 2009; Mkasi, 2016; Cowie *et al*. 2016).

The two prevailing natural environments in the field in relation to light regime are shade and full sun, or closed canopy and open canopy (Witkowski and Wilson, 2001; Roberts and Paul, 2006; Ruban, 2009). Temperatures are higher in the latter compared to the former habitats, due to their continuous exposure to sunlight; and different plants and their associated phytophagous arthropods are adapted differently to either of the habitats (Roberts and Paul, 2006; Patrick and Olckers, 2014; Cowie *et al*. 2016). Under low light conditions, plants produce less carbohydrates, which renders them less palatable to phytophagous animals (Elsayed, 2011). Vivian-Smith and Panetta (2009) reported that, not only does *L. camara* vegetative and reproductive growth increase in summer; it is also higher under unshaded or open canopy conditions. *Lantana camara* growth is also greatly affected by temperature. During the cold winter, *L. camara* stops growing and experiences loss of leaves and side-branches; while in summer rapid growth is attained (Winder, 1980). Although parasitism and predation have been noted on

some *L. camara* biocontrol agents such as *Calycomyza lantanae* (Frick) (Diptera: Agromyzidae) (Baars and Neser, 1999; Baars and Heystek, 2003a), *Uroplata girardi* Pic (Coleoptera: Chrysomelidae), *T. scrupulosa* (Cilliers, 1987a), and *F. intermedia* (Heystek and Olckers, 2003), they do not account for much of the limited success in some *L. camara* biocontrol programmes compared to factors such as climate and altitude (Hill and Hully, 1995).

The effects of climate and elevation on biocontrol agents can either be direct, by restricting their ranges, or indirect through limiting the growth of their host plants (Broughton, 2000). Several *L. camara* biocontrol agents, including *C. lantanae*, *Leptobyrsa decora* (Drake) (Hemiptera: Tingidae), *Octotoma scabripennis* (Guérin-Méneville) (Coleoptera: Chrysomelidae) and *U. girardi* are affected by climate and elevation (Cilliers and Neser, 1991; Swarbrick *et al*. 1995). Over the last decades, the majority of researchers have reached a consensus on the relatedness of climate change with shifts in seasonal phenology (Walther *et al*. 2002; Root *et al*. 2003; Visser and Both, 2005). Seasonal changes have an impact on leaf nutrients and allelochemicals (secondary plant metabolites), which in turn significantly influence phytophagous insects' feeding (Elsayed, 2011). Phytophagous insects tend to increase their feeding in early spring in association with higher water and nutrient availability in their host plants (Feeny, 1992). Success of a biocontrol agent does not entirely depend on the feeding biology of the arthropod selected, but it also relies strongly on how well-adapted is the agent to its new environment, i.e. climate, altitude, parasites and/or predators (Blossey, 1995; Gassmann and Schroeder, 1995). Nevertheless, a climate match does not automatically guarantee success because some agents have performed better than expected outside of their predicted climatic range (Gassmann and Schroeder, 1995), for example the gall fly *Rhopalomyia californica* Felt (Diptera: Cecidomyiidae) introduced into Australia from the USA for the control *Baccharis halimifolia* L. (Compositae) (McFadyen, 1998).

The effects of biotic and abiotic factors, such as elevation, competition, hybridization of host plants, shade, water, and soil nutrient content, on plant growth and herbivory activities (Inbar *et al*. 2001) have been reported in many studies (Roy and Stantont, 1999; Wilsey *et al*. 1998). The present study aimed to

measure the effects of altitude, light regime and plant biotype or variety on *L. camara* growth as well as the performance of some of its biocontrol agents. Agent performance here was taken as the percentage of leaves damaged by biocontrol agents.This study also looked, particularly, at the relationship between *A. lantanae* abundance and altitude. *Aceria lantanae* was singled out because it was a relatively recently released agent compared with the suite of agents already present at the study sites, and hence a particular interest existed in determining its spread.

5.3 Materials and methods

5.3.1 Site description

This work was conducted along the Sabie River catchment in Mpumalanga, South Africa. The study sites were divided into three altitude levels, high (mean ± S.E.: 1133±11 m), medium (975±9 m) and low (848±15 m) (Garner, 2006; Beater, 2006; Beater *et al*. 2008); three light regimes, namely shade, semi-shade and full sun; and two *L. camara* varieties identifiable by the colour of their inflorescences, light-pink and dark-pink. The overstorey aerial cover in and around the study area, particularly at low altitude, consisted of *Eucalyptus* spp. adjacent to most plots, as well as indigenous *Acacia* spp. found within some plots in riparian zones, which influenced the light regime around the study sites. While low altitude sites were characterized by a closed canopy, high and medium altitude sites were exposed to a more open canopy.

Temperature and relative humidity were recorded daily from 2013 to 2015, using iButtons (FairBridge Technologies, Sandton, South Africa) (Table 5.1).

The climate at the sites is temperate to subtropical, with hot rainy summers and mild dry winters (Garner, 2006; Beater *et al*. 2008). Daily rainfall data obtained from the South African Weather Services located in Sabie, which is about 24 km away from the study sites at 1109 m (GPS coordinates: -25.1000, 30.7830), showed differences in the total annual precipitation (Fig. 5.1).

Table 5.1 Daily maximum and minimum understorey temperatures and relative humidity recorded underneath plant canopies at three different altitudes and three light regime levels in Mpumalanga from 2013 to 2015. Means (means ± S.E.) with different superscript letters within the same row (one-way ANOVA) denote significant differences ($P < 0.05$; n = 20; LSD)

	Altitudes		
	High	Medium	Low
Daily mean maximum	25.66 ± 0.26[a]	25.96 ± 0.25[a]	24.58 ± 0.17[b]
Daily mean minimum	4.63 ± 0.19[a]	6.89 ± 0.18[b]	6.11 ± 0.15[c]
	Light regime		
	Full shade	Semi-open	Full sun
Daily mean maximum	24.96 ± 0.19[a]	25.59 ± 0.24[a]	25.14 ± 0.33[a]
Daily mean minimum	5.06 ± 0.17[a]	6.48 ± 0.15[b]	5.73 ± 0.14[c]
Relative humidity	High altitude 56%[a]	Medium altitude 74%[b]	Low altitude 65%[c]

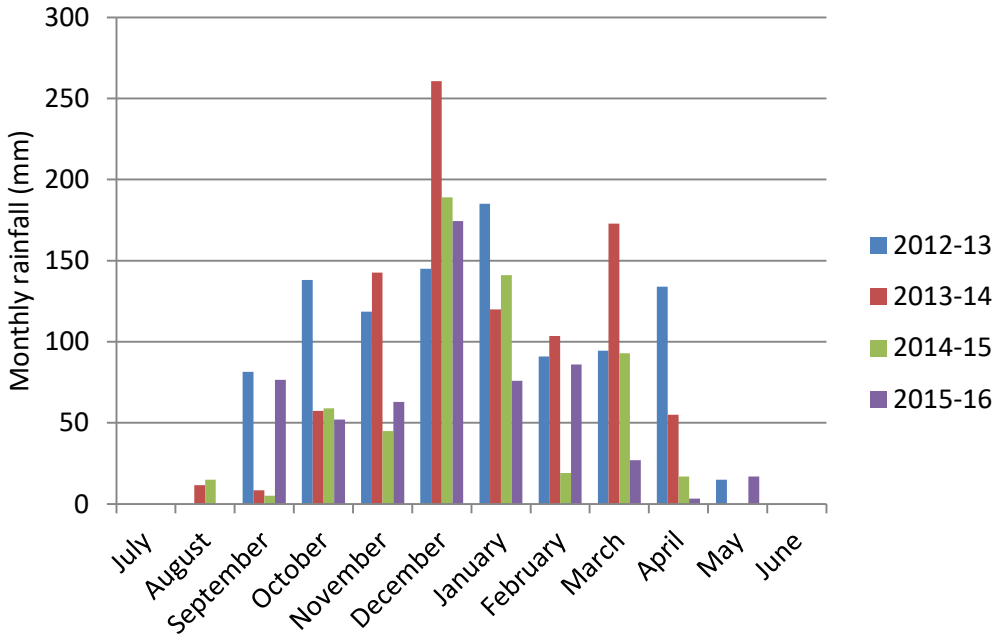

Figure 5.1 Total monthly rainfall in Sabie, Mpumalanga, from 2012-13 to 2015-16. Data obtained from The South African Weather Services.

The most dominant invasive alien plants (IAPs) in the study area are *L. camara* and *Solanum mauritianum* Scop. (Solanaceae), both of which can form mono-specific stands in the understory of *Eucalyptus* plantations or on the adjacent riversides and roadsides (Witkowski and Garner, 2008). More details of the description of the study sites are given in chapters two and three.

Effects of altitude, light regime and plant variety on *L. camara* and its associated biocontrol agents were measured from newly grown plants or 'new growth', coppicing from plants previously cut at ground level. This implies that agent colonization of these plants was unbiased by plant size, and secondly the differences in plant growth and agent performance would have been a function of the environment under which different plants occurred, i.e. altitude and light regime. For this experiment, only carbofuran-untreated plants were used (refer to Chapter Three).

5.3.2 Effects of altitude, light regime and *Lantana camara* variety

Altitude

The impact of altitude on plant growth and agent herbivory was measured by comparing parameters between plants growing at high altitude (n = 4), medium altitude (n = 6) and low altitude sites (n = 10) from summer 2013 to summer 2015. Plant growth and agent herbivory parameters included plant height, canopy aerial cover, stem length, and percentage of leaves damaged by biocontrol agents present at the study sites.

Light regime

The impact of light regime on plant growth and biocontrol agent performance was compared at three levels, i.e. 'full sun' (<30% shade) (n = 8 plants), 'semi-shade' (30-75% shade) (n = 8) and 'full shade' (>75% shade) (n = 4) from summer 2013 to summer 2015.

In autumn of 2016, leaf surface area was compared between plants growing under full shade (six plants) and full sun conditions (six plants) (N.B: semi-shade and full sun plants were combined here to represent 'sun plants'). Three centrally located stems were selected and tagged per plant (Chapter three). On each tagged stem, three leaves (>15 mm long) were picked from around the distal 500 mm of tagged stems, and the outline of each leaf was immediately drawn on paper. The surface area of each leaf as a cut-out outline, was measured in the laboratory at the University of the Witwatersrand using a leaf area meter (cm^2) (Model: LI-3100 Area Meter / LI.COR, inc. Lincoln, Nebraska USA).

Lantana camara variety

Two varieties prevailed within the study area, namely 'light-pink' (n = 8) and 'dark-pink' (n = 11), and therefore plant growth and biocontrol agent performance were compared between the two from summer 2013 to summer 2015. The majority of *L. camara* plants in the study area were of the dark-pink variety (65%), followed by the light-pink variety (35%).

5.3.3 Effect of altitude on *Aceria lantanae* abundance

During the survey, it was observed that *A. lantanae* flower galls were present along the roadsides in the vicinity (~50 km radius) of the release plots. Their abundance in relation to altitude was measured in summer 2015 and summer 2016 on the dark-pink *L. camara* variety. *Aceria lantanae* abundance, which is here a function the intensity of flower galls per plant, was estimated using a scoring system (Table 5.2).

Table 5.2 Scores indicating estimated abundance of *Aceria lantanae* flower galls per plant, with 0 representing absolute absence and 5 a very strong presence of the agent.

Intensity of *Aceria lantanae* flower gall infestation on plant	**Score**
No Flower galls found on plant	0
Flower galls present but hard to spot (one or two on the entire plant)	1
Flower galls found in very insignificant numbers, covering < 25% of the plant	2
Noticeable presence of flower galls, covering ~50% of the plant	3
Flower galls noticeable on large portions of plant parts, plant sections showing signs of stress. Galls covering ~75% of the plant	4
Plant heavily infested, noticeably stressed, galls covering > 75% of the plant	5

Eleven roadside sites stretched along the R536 from Sabie to Hazyview, and the R40 from Hazyview through White river to Nelspruit.

Altitude along each roadside site was measured using a Digital Altimeter (TL-3524 altimeter, TL electronic Inc., Czech Republic) and it (roadside altitude) ranged from 510 – 920 m.

5.3.4 Statistical analysis

One-way ANOVAs with repeated measures were employed to compare plant and biocontrol agent parameters between altitudinal ranges (high, medium and low

altitude), light regime (shade, semi-shade and full sun), and plant variety (light-pink and dark-pink) from summer 2013 to summer 2015. Comparisons of leaf surface area and the number of damaged leaves between shade and full sun plants were made using student's t-tests. Regression analyses were conducted to measure relationships between altitude and *A. lantanae* abundance. The data were checked for normality, and where necessary data transformation was undertaken. All analyses were performed using the computer statistical programme, Statistica (Version 8.0).

5.4 Results

5.4.1 Effects of altitude on plant growth and agents' performance

Plant canopy aerial cover ($F_{(2, 17)} = 11.10$; $P < 0.01$), plant height ($F_{(2, 16)} = 28.09$; $P < 0.01$) and stem length per plant ($F_{(2, 57)} = 81.52$; $P < 0.01$) were generally significantly greater at low altitude than at high, and much lower at medium altitude (Fig. 5.2). There were significant seasonal changes, and interactions between altitude and season in plant canopy aerial cover; meaning plant canopy cover differed between altitudes in some seasons and not others ($F_{(8, 136)} = 30.79$; $P < 0.01$) ($F_{(16, 136)} = 4.93$; $P < 0.01$), plant height ($F_{(8, 128)} = 18.29$; $P < 0.01$) ($F_{(16, 128)} = 3.97$; $P < 0.01$), and stem length per plant ($F_{(8, 456)} = 101.76$; $P < 0.01$), ($F_{(16, 456)} = 13.18$; $P < 0.01$), respectively (Fig. 5.2.a.b.c).

There were no consistent significant differences in the percentage of leaves damaged by biocontrol agents combined, between altitudes ($F_{(2, 57)} = 2.22$; $P = 0.11$) (Fig. 5.3.a), but there were significant seasonal changes ($F_{(8, 456)} = 22.24$; $P < 0.01$), as well as interactions between season and altitude ($F_{(16, 456)} = 10.60$; $P < 0.01$); meaning leaf damage differed between altitudes in some seasons and not others. The Fisher LSD post-hoc tests, however, showed a greater percentage damage at medium than at high or low altitude sites in summer 2013, autumn 2013, summer 2014, autumn 2014, and summer 2015; and a greater percentage damage at low altitude than medium or high in spring 2013 and winter 2014 (Fig. 5.3.a).

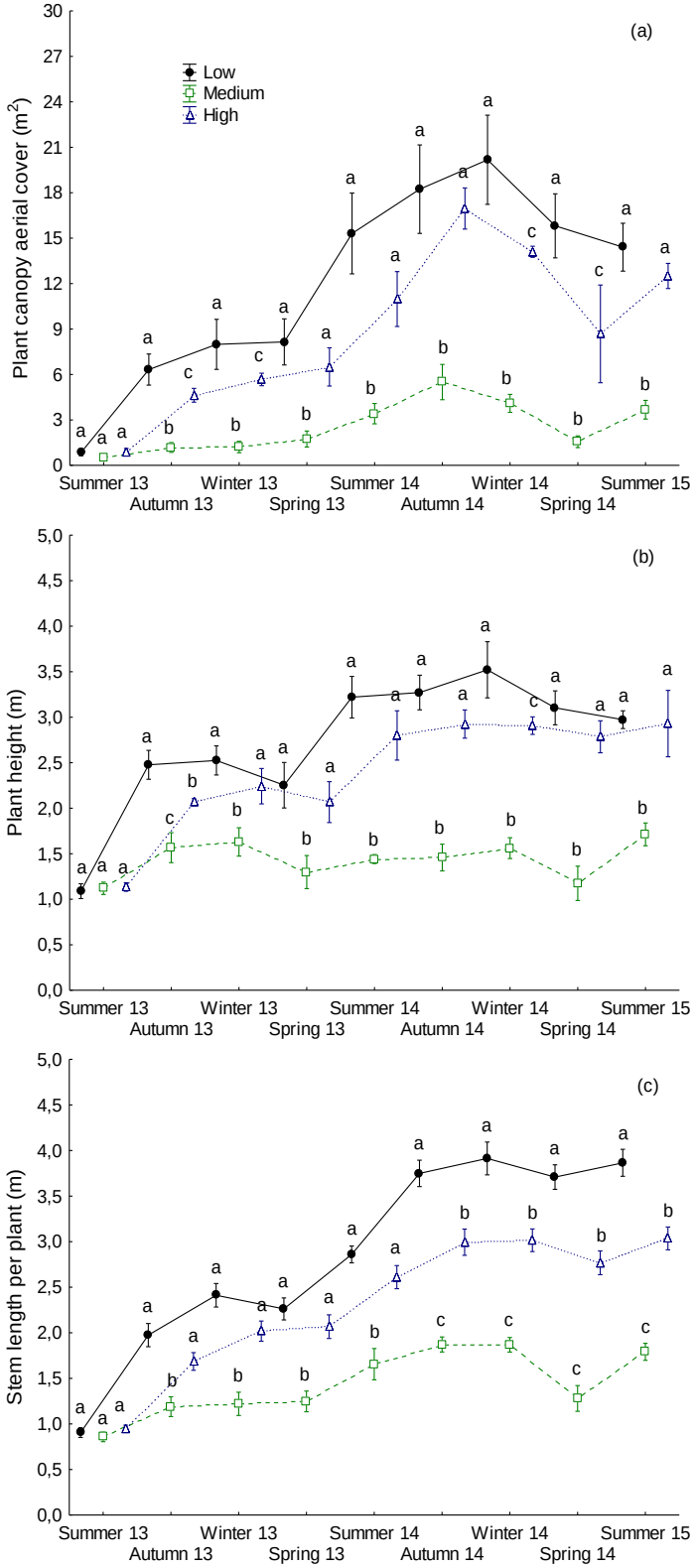

Figure 5.2 Plant canopy aerial cover (a), plant height (b) and stem length (c) compared between high (n = 4), medium (n = 6) and low altitude sites (n = 10) from summer 2013 to summer 2015 in Mpumalanga (mean ± S.E). Means ('low, medium, high') with different letters within the same season are significantly different using the Fisher LSD post-hoc tests ($P < 0.05$).

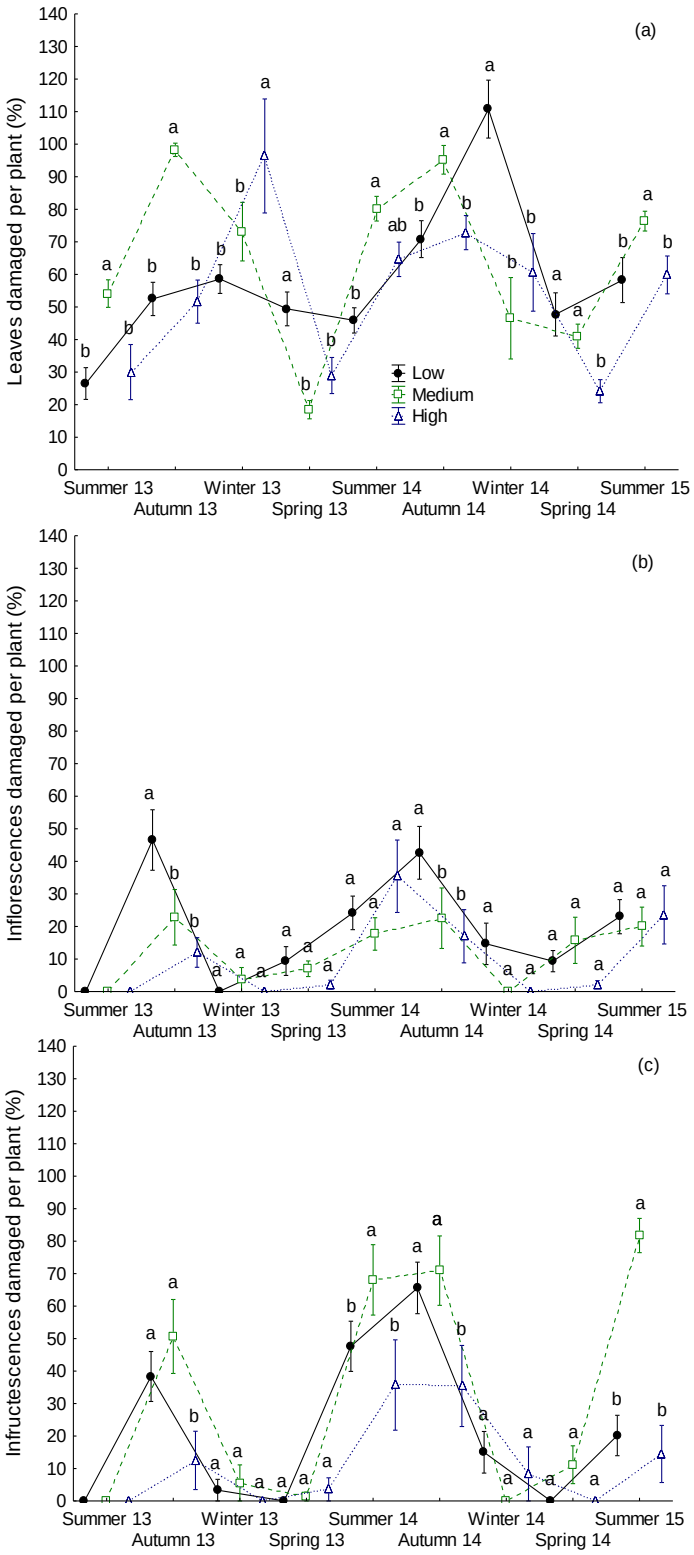

Figure 5.3 Percentage of leaves (a), inflorescences (b) and infructescences (c) damaged by the suite of biocontrol agents present at the study sites in Mpumalanga, compared between high (n = 4), medium (n = 6) and low altitude sites (n = 10) from summer 2013 to summer 2015 (mean ± S.E). Means ('low, medium, high') with different letters within the same season are significantly different using the Fisher LSD post-hoc tests ($P < 0.05$).

There were significant differences in the percentage of inflorescences ($F_{(2, 57)} = 4.17$; $P = 0.02$) and infructescences damaged between altitudes ($F_{(2, 57)} = 10.09$; $P < 0.01$) (Fig. 5.3.b.c). Fisher LSD post-hoc tests showed that the percentage of inflorescences damaged was greater at low than at high or medium altitudes in autumn 2013 and autumn 2014 (Fig. 5.3.b); and the percentage of infructescences damaged was greater at medium than at high or low altitude in summer 2014 and summer 2015 (Fig. 5.3.c). There were also significant seasonal changes and interactions between season and altitude in the percentage of inflorescences ($F_{(8, 456)} = 10.22$; $P < 0.01$), ($F_{(16, 456)} = 1.82$; $P = 0.02$) and infructescences damaged ($F_{(8, 456)} = 33.64$; $P < 0.01$), ($F_{(16, 456)} = 4.31$; $P < 0.01$), respectively; meaning inflorescence and infructescence damage differed between altitudes in some seasons and not others.

There were no significant differences in the percentage of leaves damaged by *O. scabripennis* between high, medium and low altitudes ($F_{(2, 57)} = 2.96$; $P = 0.06$) (Fig. 5.4.a). However, significant seasonal changes ($F_{(8, 456)} = 13.81$; $P < 0.01$) and interactions between season and altitude existed ($F_{(16, 456)} = 10.98$; $P < 0.01$). The LSD post-hoc tests, however, showed that *O. scabripennis* percentage damage was significantly lower at low than at medium or high altitude, except in summer 2014 and summer 2015 when it was significantly greater at low than at medium or high altitude (Fig. 5.4.a).

Ophiomyia camarae Spencer (Diptera: Agromyzidae), *T. scrupulosa* and *cf. Passalora* sp. (Chupp) U. Braun & Crous var. *lantanae*, were significantly affected by altitude ($F_{(2, 57)} = 6.93$; $P < 0.01$), ($F_{(2, 57)} = 16.51$; $P < 0.01$), ($F_{(2, 57)} = 115.36$; $P < 0.01$), with significant seasonal changes ($F_{(8, 456)} = 12.00$; $P < 0.01$), ($F_{(8, 456)} = 12.97$; $P < 0.01$), ($F_{(8, 456)} = 23.11$; $P < 0.01$), and interactions between season and altitude ($F_{(16, 456)} = 12.04$; $P < 0.01$), ($F_{(16, 456)} = 16.96$; $P < 0.01$), ($F_{(16, 456)} = 19.16$; $P < 0.01$), respectively (Fig. 5.4.b.c.e). *Ophiomyia camarae* damage was greater at low altitude in autumn 2013 and summer 2015; and at high altitude only in winter 2013 (Fig. 5.4.b).

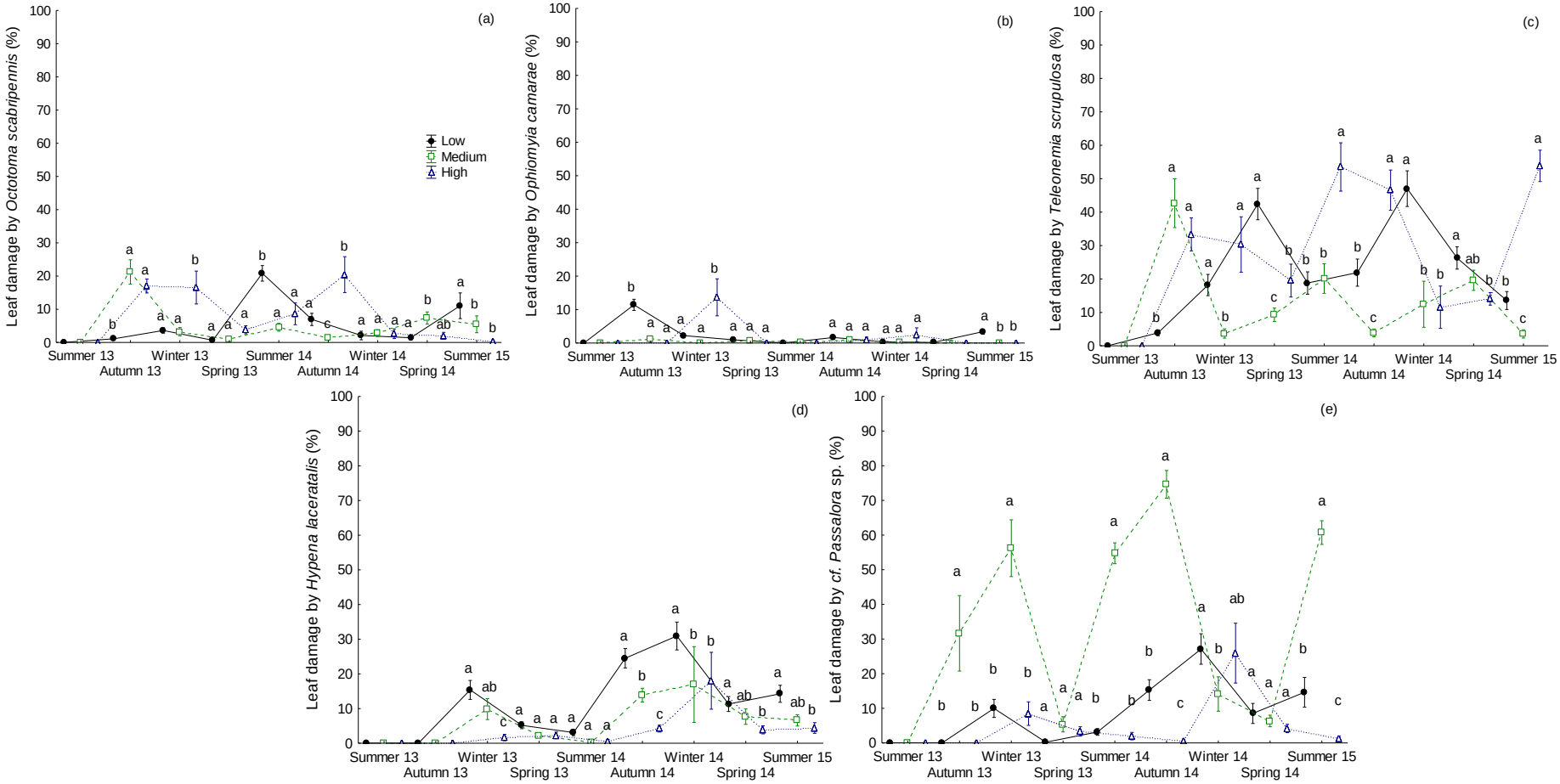

Figure 5.4 Percentage of leaves damaged by *Octotoma scabripennis* (a), *Ophiomyia camarae* (b), *Teleonemia scrupulosa* (c), *Hypena laceratalis* (d), and c*f. Passalora* sp. (e) compared between high (n = 4), medium (n = 6) and low altitude sites (n = 10) in Mpumalanga from summer 2013 to summer 2015 (mean ± S.E). Means ('low, medium, high') with different letters within the same season are significantly different using the Fisher LSD post-hoc tests ($P < 0.05$).

Leaf damage by *T. scrupulosa*, on the other hand, was mostly greater at high and low than medium altitudes in different seasons (Fig. 5.4.c). On the other hand, *cf. Passalora* sp. damage was greater at medium altitude than the rest in all seasons, except in spring 2013, winter 2014 and spring 2014 (Fig. 5.4.e)

Hypena laceratalis Walker (Lepidoptera: Noctuidae) percentage damage was also significantly affected by altitude, with greater activity at low altitude ($F_{(2, 57)} = 12.38$; $P < 0.01$) (Fig. 4.d); and it was also significantly affected by seasonal changes ($F_{(8, 456)} = 16.27$; $P < 0.01$) (Fig. 5.4.d).

5.4.2 Effects of light regime on plant growth and agents' performance

There were no significant differences in plant canopy aerial cover ($F_{(2, 17)} = 1.25$; $P = 0.30$) and plant height ($F_{(2, 16)} = 0.81$; $P = 0.46$) between plants growing under full sun, semi-shade and full shade; however seasonal changes existed, both in plant canopy cover ($F_{(8, 136)} = 22.86$; $P < 0.01$) and plant height ($F_{(8, 128)} = 16.64$; $P < 0.01$) (Fig. 5.5.a.b).

Stem length, on the other hand, was significantly greater in shade plants compared to plants growing under full sun and semi-shade environments ($F_{(2, 57)} = 6.21$; $P < 0.01$) (Fig. 5.5.c). There were also significant seasonal changes ($F_{(8, 456)} = 95.58$; $P < 0.01$) and interactions between season and light regime ($F_{(16, 456)} = 7.06$; $P < 0.01$) (Fig. 5.5.c); meaning that stem length differed between light regimes in some seasons and not others.

There were significant differences in the percentage of leaves and infructescences damaged by the suite of biocontrol agents present at the study sites between plants growing under full sun, semi-shade and full shade environments ($F_{(2, 57)} = 14.70$; $P < 0.01$), ($F_{(2, 57)} = 12.09$; $P < 0.01$), respectively. Percentage leaves and infructescences damaged were generally lower under full shade compared to full sun and semi-shade conditions (Fig. 5.6.a.c). There were also significant seasonal changes ($F_{(8, 456)} = 19.28$; $P < 0.01$), ($F_{(8, 456)} = 41.06$; $P < 0.01$) and interactions between season and light regime ($F_{(16, 456)} = 3.46$; $P < 0.01$), ($F_{(16, 456)} = 3.34$; $P < 0.01$), respectively (Fig. 5.6.a.c). These interactions meant that leaf and infructescence damage differed between treatments (light regimes) in some seasons and not others.

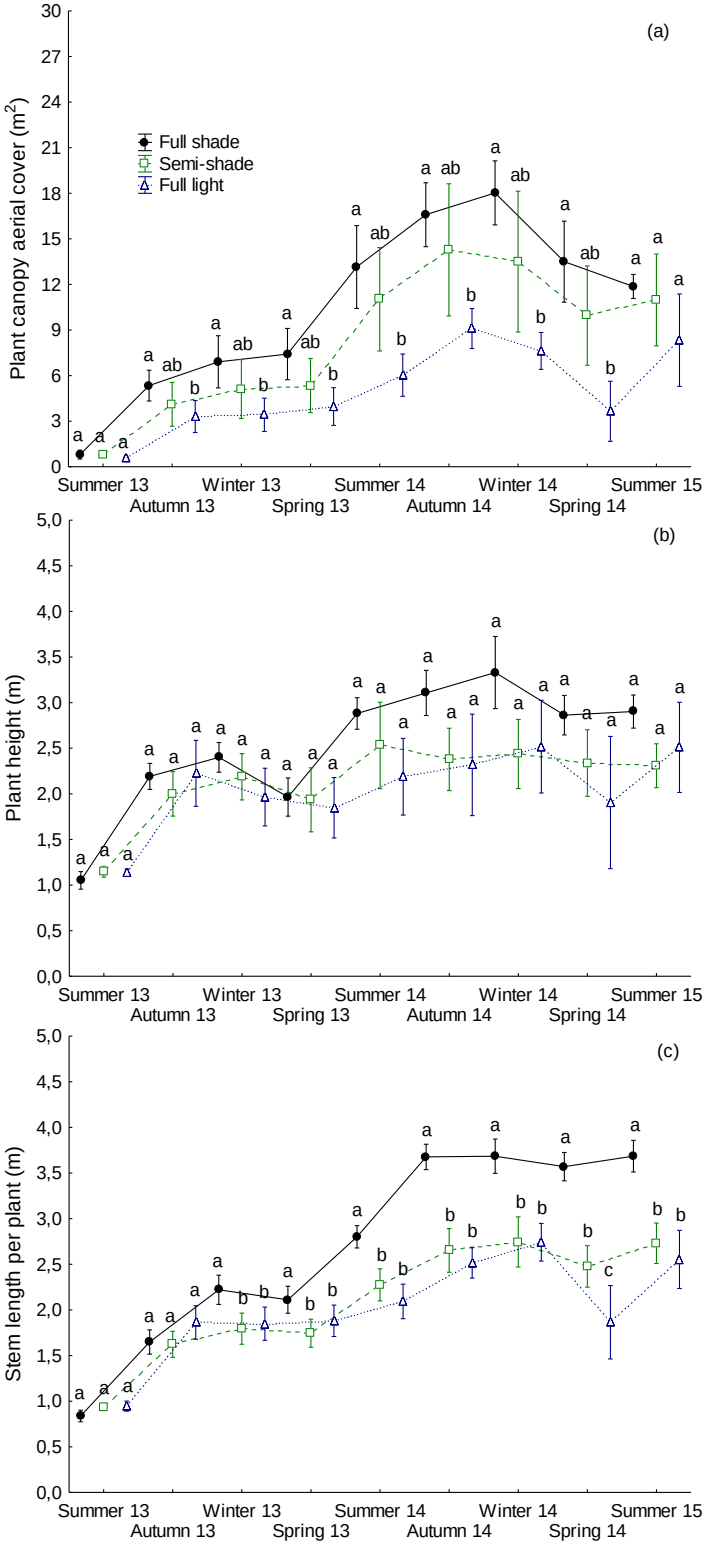

Figure 5.5 Plant canopy aerial cover (a), plant height (b) and stem length (c) compared between plants growing under full shade (n = 4), semi-shade (n = 8) and full sun (N= 8) conditions from summer 2013 to summer 2015 at sites in Mpumalanga (mean ± S.E). Means ('shade, semi-shade, full sun') with different letters within the same season are significantly different using the Fisher LSD post-hoc tests ($P < 0.05$).

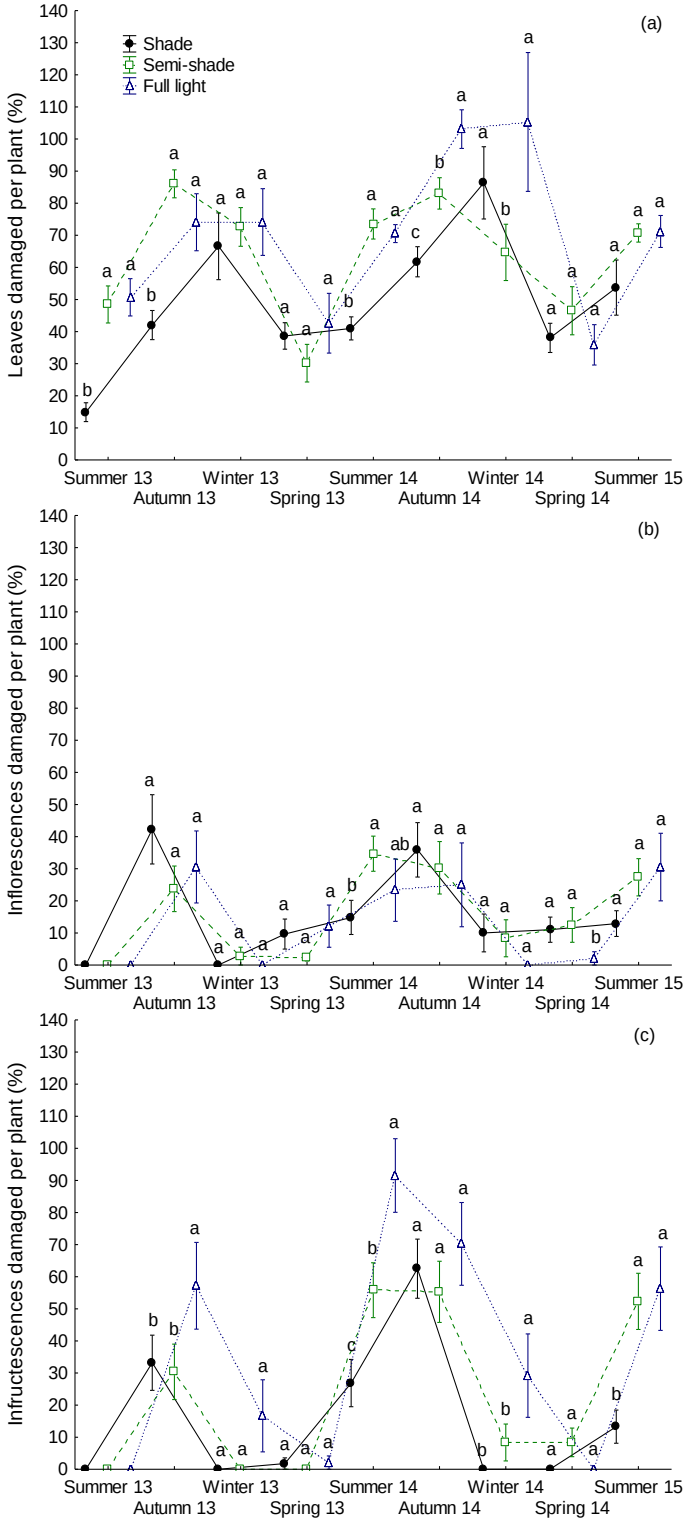

Figure 5.6 Percentage of leaves (a), inflorescences (b) and infructescences (c) damaged by the suite of biocontrol agents present at the study sites in Mpumalanga compared between plants growing under full shade (n = 4), semi-shade (n = 8) and full sun (N= 8) conditions from summer 2013 to summer 2015 (mean ± S.E). Means ('shade, semi-shade, full sun') with different letters within the same season are significantly different using the Fisher LSD post-hoc tests ($P < 0.05$).

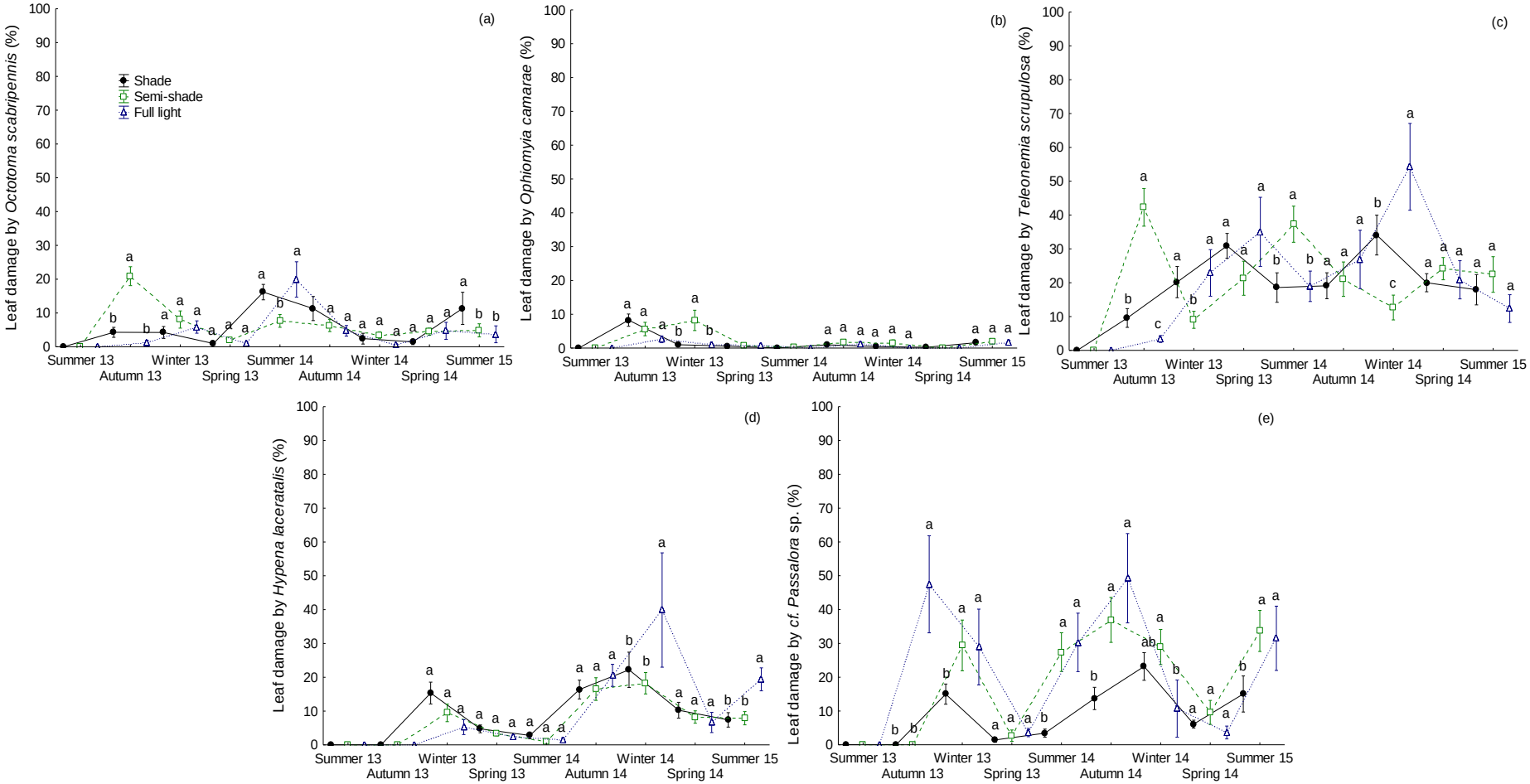

Figure 5.7 Percentage of leaves damaged by *Octotoma scabripennis* (a), *Ophiomyia camarae* (b), *Teleonemia scrupulosa* (c), *Hypena laceratalis* (d), and *cf. Passalora* sp. (e) compared between plants growing under full shade (n = 4), semi-shade (n = 8) and full sun (N= 8) conditions in Mpumalanga from summer 2013 to summer 2015 (mean ± S.E). Means ('full shade, semi-shade, full sun') with different letters within the same season are significantly different using the Fisher LSD post-hoc tests ($P < 0.05$).

The percentage of inflorescences damaged was not affected by shade ($F_{(2, 57)}$ = 0.14; P = 0.86), however seasonal changes were shown ($F_{(8, 456)}$ = 12.36; P < 0.01) (Fig. 5.6.b).

There were no significant differences in the percentage of leaves damaged by *O. scabripennis*, *O. camarae*, *T. scrupulosa* and *H. laceratalis* between full shade, semi-shade and full sun plants ($F_{(2, 57)}$ = 1.04; P = 0.35), ($F_{(2, 57)}$ = 2.58; P = 0.08), ($F_{(2, 57)}$ = 0.44; P = 0.64), ($F_{(2, 57)}$ = 1.39; P = 0.25), respectively (Fig. 5.7.a.b.c.d). There were, however, significant seasonal changes ($F_{(8, 456)}$ = 11.73; P < 0.01), ($F_{(8, 456)}$ = 8.81; P < 0.01), ($F_{(8, 456)}$ = 12.04; P < 0.01), ($F_{(8, 456)}$ = 25.97; P < 0.01) and interactions between season and light regime ($F_{(16, 456)}$ = 4.94; P < 0.01), ($F_{(16, 456)}$ = 2.65; P < 0.01), ($F_{(16, 456)}$ = 6.59; P < 0.01), ($F_{(16, 456)}$ = 2.09; P < 0.01), respectively (Fig. 5.7.a.b.c.d). These interactions meant that the performance of each of the abovementioned agents differed between treatments (light regimes) in some seasons and not others.

There were significant differences in the percentage of leaves damaged by *cf. Passalora* sp., with greater damage in plants under full sun and semi-shade conditions than on plants under shade conditions ($F_{(2, 57)}$ = 6.55; P < 0.01) (Fig. 5.7.e). There were also significant seasonal changes ($F_{(8, 456)}$ = 18.51; P < 0.01) and interactions between season and light regime ($F_{(16, 456)}$ = 5.71; P < 0.01) (Fig. 5.7.e).

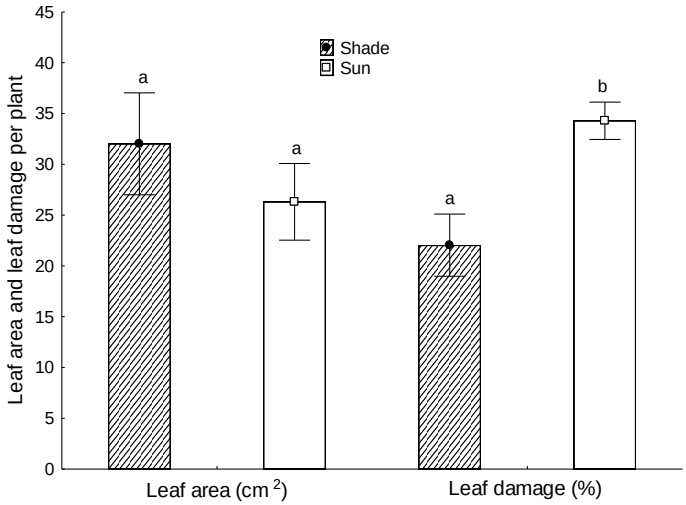

Figure 5.8 Leaf area and leaf damage (by biocontrol agents) compared between plants grown under shade and sun conditions (mean ±SE). Means with different letters within the same group (leaf area or leaf damage) are significantly different using paired t-tests (P < 0.05) (n = 6).

There was no significant difference in leaf area ($t_5 = 0.72$; $P = 0.49$) between shade and sun plants, but leaf damage by the suite of biocontrol agents was greater in shade compared to sun plants ($t_5 = 3.40$; $P = 0.01$) (Fig. 5.8).

Leaf area, nevertheless, was 17.8% slightly greater in shade compared to sun plants.

5.4.3 Effects of *Lantana camara* variety on plant growth and agents' performance

Plant canopy aerial cover, plant height and stem length were greater for the light-pink than the dark-pink *L. camara* variety ($F_{(1, 18)} = 14.24$; $P < 0.01$), ($F_{(1, 17)} = 6.48$; $P = 0.02$), ($F_{(1, 58)} = 21.51$; $P < 0.01$), respectively (Fig. 5.9.a.b.c). There were also significant seasonal differences ($F_{(8, 144)} = 47.00$; $P < 0.01$), ($F_{(8, 136)} = 25.57$; $P < 0.01$), ($F_{(8, 464)} = 117.30$; $P < 0.01$) in plant aerial cover, plant height, and stem length between light-pink and dark-pink varieties; and interactions between season and variety ($F_{(8, 144)} = 9.24$; $P < 0.01$), ($F_{(8, 136)} = 4.02$; $P < 0.01$), ($F_{(8, 464)} = 7.73$; $P < 0.01$), respectively
(Fig. 5.9.a.b.c).

While there were no significant differences in the percentage of leaves and inflorescences damaged by the suite of biocontrol agent present at the study sites between the dark-pink and the light-pink *L. camara* varieties ($F_{(1, 58)} = 3.76$; $P = 0.06$), ($F_{(1, 58)} = 0.40$; $P = 0.52$) (Fig. 5.10.a.b), there were significant seasonal changes ($F_{(8, 464)} = 17.23$; $P < 0.01$), ($F_{(8, 464)} = 12.81$; $P < 0.01$), respectively; and significant interactions between season and variety existed in the percentage of leaves damaged ($F_{(8, 464)} = 2.15$; $P = 0.02$) (Fig. 5.10.a.b). The post-hoc tests, however, revealed that the percentage of leaves damaged was greater in the dark-pink compared to the light-pink variety in autumn 2013, summer 2014 and autumn 2014 (Fig. 5.10.a).

The percentage of infructescences damaged by the suite of biocontrol agents was greater in the dark-pink compared to the light-pink variety in summer 2014 and summer 2015 ($F_{(1, 58)} = 6.69$; $P = 0.01$) (Fig. 5.10.c).

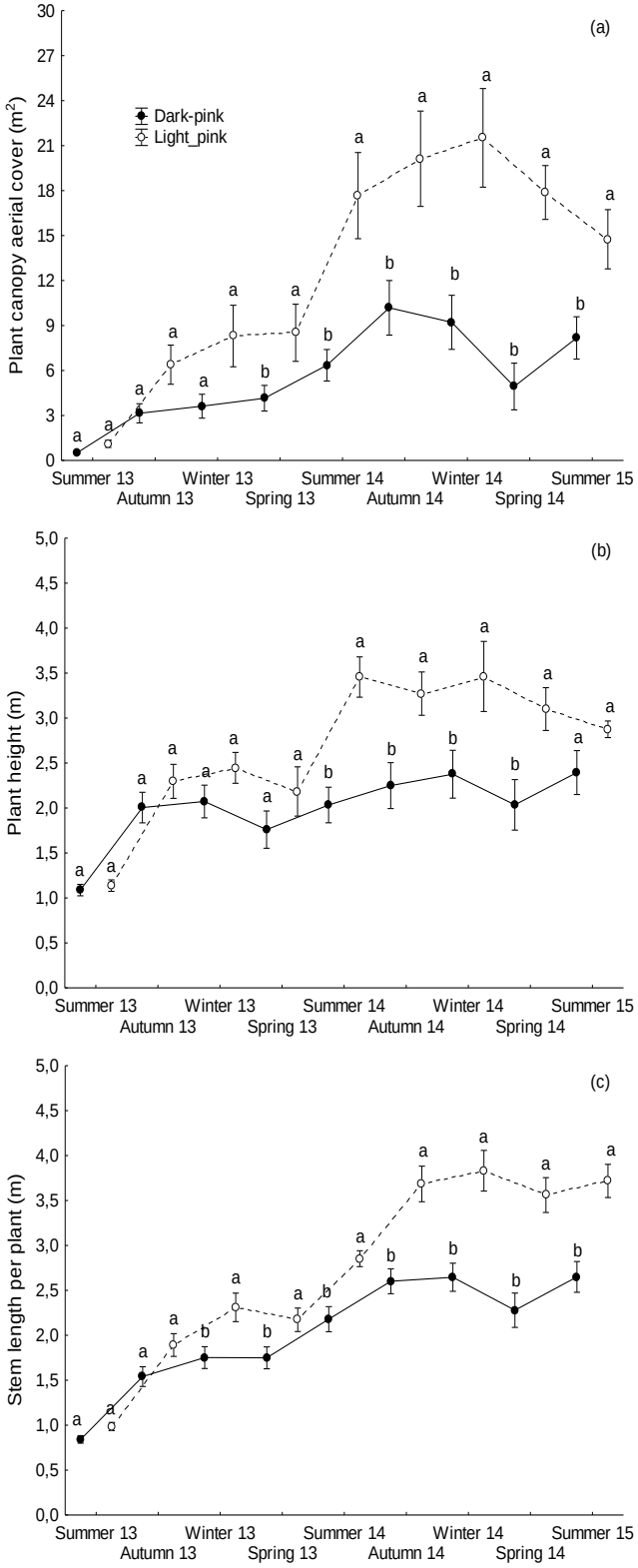

Figure 5.9 Plant canopy aerial cover (a), plant height (b) and stem length (c) compared between dark-pink (n = 11) and light-pink *L. camara* varieties (n = 8) from summer 2013 to summer 2015 at sites in Mpumalanga (mean ± S.E). Means ('dark-pink, light-pink') with different letters within the same season are significantly different using the Fisher LSD post-hoc tests ($P < 0.05$).

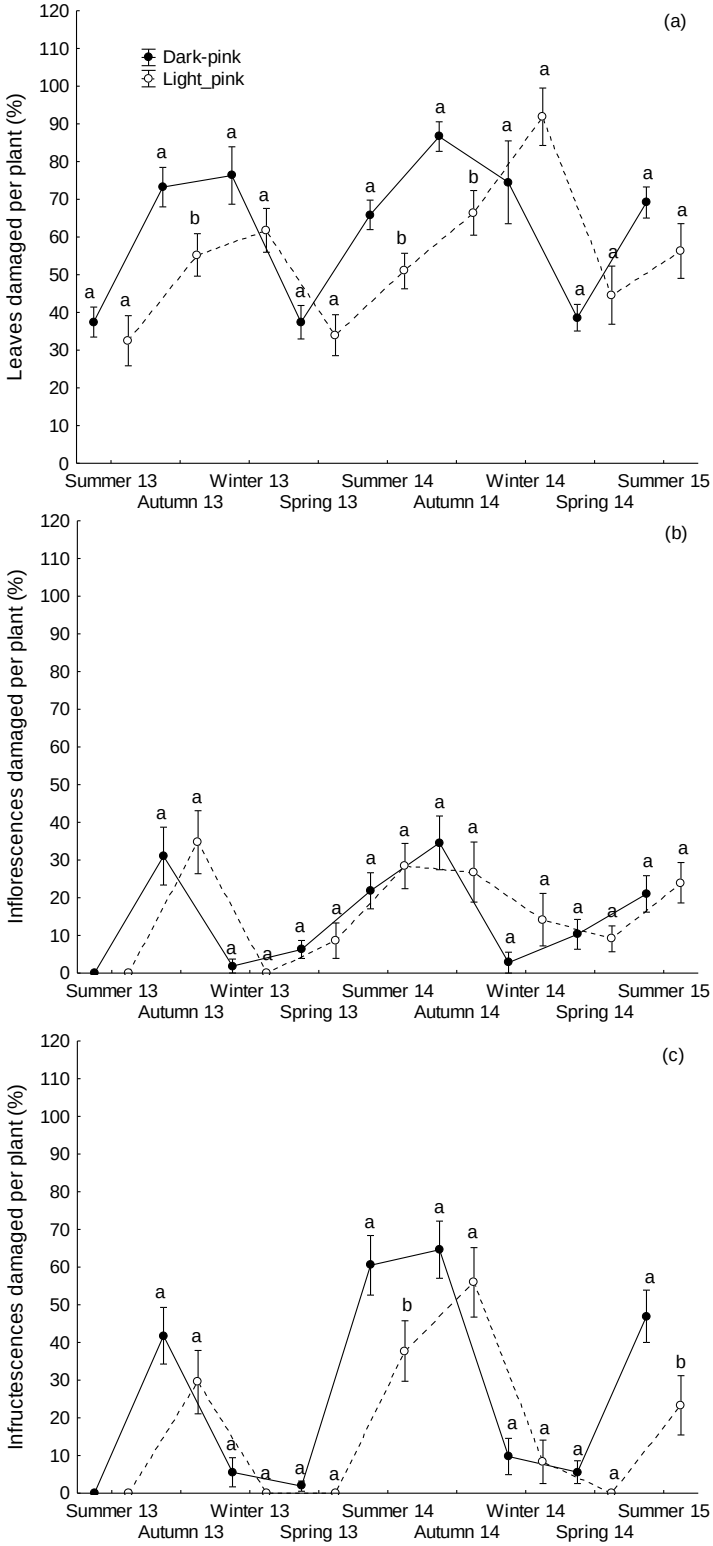

Figure 5.10 Percentage of leaves (a), inflorescences (b) and infructescences (c) damaged by the suite of biocontrol agents present at the study sites in Mpumalanga compared between dark-pink and light-pink *L. camara* varieties from summer 2013 to summer 2015 (mean ± S.E). Means ('dark-pink, light-pink') with different letters within the same season are significantly different using the Fisher LSD post-hoc tests ($P < 0.05$).

There were also significant seasonal changes in the percentage of infructescences damaged in both the dark-pink and light-pink *L. camara* varieties ($F_{(8, 464)} = 34.38$; $P < 0.01$) (Fig. 5.10.c).

Octotoma scabripennis Guèrin-Mèneville (Coleoptera: Chrysomelidae), *O. camarae* and *cf. Passalora* sp. were significantly affected by *L. camara* variety ($F_{(1, 58)} = 10.10$; $P < 0.01$), ($F_{(1, 58)} = 16.91$; $P < 0.01$), ($F_{(1, 58)} = 7.96$; $P < 0.01$), with significant seasonal changes ($F_{(8, 464)} = 10.54$; $P < 0.01$), ($F_{(8, 464)} = 16.24$; $P < 0.01$), ($F_{(8, 464)} = 16.08$; $P < 0.01$), and interactions between season and variety ($F_{(8, 464)} = 3.27$; $P < 0.01$), ($F_{(8, 464)} = 7.86$; $P < 0.01$), ($F_{(8, 464)} = 8.97$; $P < 0.01$), respectively (Fig. 5.11.a.b.e).

The percentage of leaves damaged by *O. scabripennis* was greater in the dark-pink than in the light-pink variety in autumn 2013 and summer 2015 (Fig. 11.a); whereas the percentage of leaves damaged by *O. camarae* was greater in the light-pink than in the dark-pink variety in autumn 2013 and winter 2013 (Fig. 5.11.b); and *cf. Passalora* sp. percentage damage was greater in the dark-pink than in the light-pink variety in all seasons, except in winter 2014, when it was greater on the light-pink variety (Fig. 5.11. e).

There were no significant differences in the percentage of leaves damaged by *T. scrupulosa* ($F_{(1, 58)} = 0.00$; $P = 0.95$) and *H. laceratalis* ($F_{(1, 58)} = 2.13$; $P = 0.14$) between the dark-pink and light-pink varieties (Fig. 5.11.c.d). There were significant seasonal changes in both agents ($F_{(8, 464)} = 9.66$; $P < 0.01$), ($F_{(8, 464)} = 22.93$; $P < 0.01$), respectively, and significant interactions between season and variety only in the percentage of leaves damaged by *T. scrupulosa* ($F_{(8, 464)} = 2.22$; $P = 0.02$) (Fig. 5.11.c.).

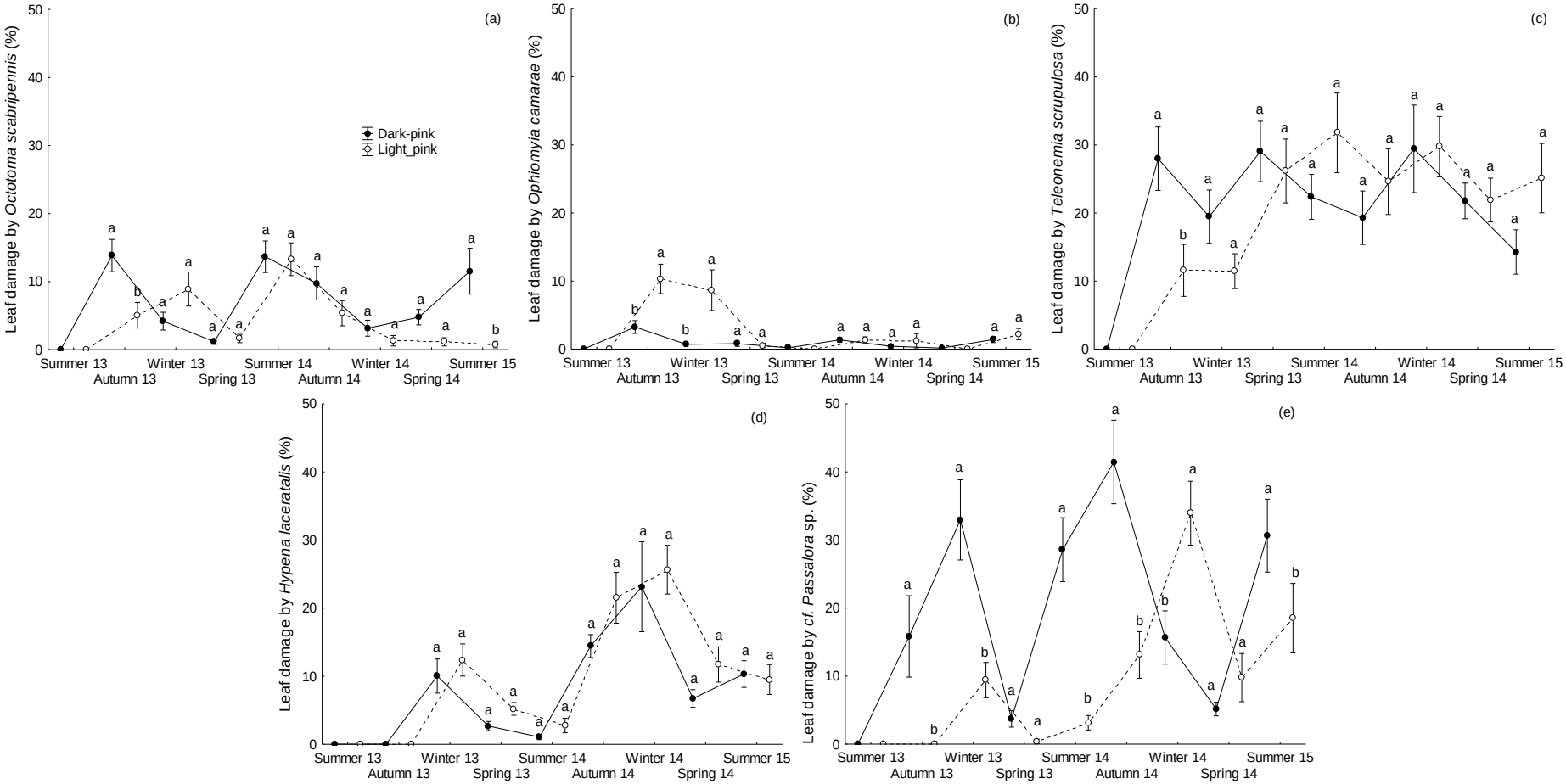

Figure 5.11 Percentage of leaves damaged by *Octotoma scabripennis* (a), *Ophiomyia camarae* (b), *Teleonemia scrupulosa* (c), *Hypena laceratalis* (d), and *cf. Passalora* sp. (e) compared between dark-pink and light-pink *L. camara* varieties in Mpumalanga from summer 2013 to summer 2015 (mean ± S.E). Means ('dark-pink, light-pink') with different letters within the same season are significantly different using the Fisher LSD post-hoc tests ($P < 0.05$).

5.4.4 Effect of altitude on *Aceria lantanae* performance

The upper altitudinal limit of *A. lantanae* between Sabie and Nelspruit was 780 m in 2015 and 950 m in 2016 (Fig. 5.12). In both years there was a strong negative relationship between *A. lantanae* abundance (intensity of flower galls per plant) and altitude, showing more galls at the lower altitude sites.

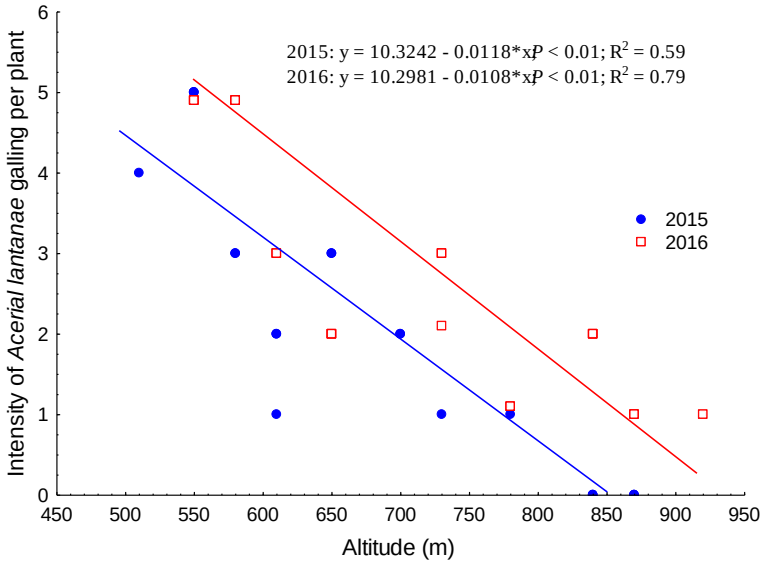

Figure 5.12 Relationship between altitude (m) and relative abundance of *A. lantanae* flower galls on the dark-pink *L. camara* variety along roadsides between Sabie and Nelspruit in Mpumalanga, showing different upper limits for 2015 and 2016.

5.5 Discussion

Lantana camara plants were about two to five times larger at low altitude sites than at medium or high altitudes. The unique microenvironment under which plants grew at low altitude sites, i.e. the presence of large indigenous *Acacia* spp., on one hand, and that of *Eucalyptus* spp. from plantations adjacent to these sites on the other, might have acted as a buffer protecting plants against frost damage during the cold winter months; whereas the open canopy cover around high and medium altitude sites exposed plants to severe frost damage, consequently temporarily stopping their growth.

The prevailing environmental conditions at different altitudes in this study did not have a significant effect on the biocontrol agents; instead, it appeared that some agents shifted their phenological peaks with altitude in search for resources, which enabled the suite of agents as a whole to maintain a strong presence at all three different altitudes throughout the seasons. For example, while *T. scrupulosa*

percentage damage peaked in summer and autumn at high altitude sites, it peaked in winter and spring at low altitude sites. The same trend was observed with *O. scabripennis* and *O. camarae*. As an exception to this rule, *H. laceratalis* and *cf. Passalora* sp. both displayed altitudinal preferences, with the former better adapted to low altitude and the latter to the specific environmental conditions that prevailed at the medium altitude study site, such as higher temperatures and relative humidity.

Aceria lantanae abundance was inversely proportional to altitude. At a national scale, *H. laceratalis* has been reported to be abundant mostly in summer (Baars and Neser, 1999; Day *et al*. 2003a); however it was more abundant toward the end of winter in the present study. This alternation in agent abundance from one season to the next at different altitudes stresses the importance of collecting agents adapted to different microhabitats to increase the chance of establishment in the new environment, but also it has highlighted the importance of introducing multiple agents rather than a single one in order to obtain a cumulative control effect on a difficult target weed, such as *L. camara*.

The multiple agent release method, also referred to as the 'cumulative stress model', relies on the ability of biocontrol agents to interact in concert and thus increase the control of a target weed (Rayamajhi *et al*. 2010; Turner *et al*. 2010). The alternative to the cumulative stress model is the 'lottery model', which also promotes multiple agent releases but with the hope of identifying the single, best performing agent to be focused on for further releases (Myers, 2008; McEvoy and Coombs, 2000). Identifying the single most performing agent in a 'lottery model' is a difficult undertaking. This study has shown the importance of multiple agent releases through the complementary effect at different altitudes and throughout the plant's growing season. Along with altitude, light regime also plays an important role in the plant-insect interaction.

The effect of light regime on plant growth and agent performance was generally variable, however there was an emerging trend showing that plant growth was greater under full shade conditions, whereas most biocontrol agents performed much better under semi-shade and full sun conditions. *Teleonemia scrupulosa* was found to perform well under full sun conditions, in a field-based

study, on all *L. camara* plant colour varieties (Heystek, 2006), and in a laboratory-based experiment, on the orange flowered variety (Mkasi, 2016). In another laboratory study, however, while the feeding rate by *Gargaphia decoris* Drake (Hemiptera: Tingidae) on *S. mauritianum* was greater in plants under shade, more feeding damage was reported on plants under full sun conditions (Cowie *et al*. 2016); but the high *G. decoris* herbivory on plants under shade could possibly have been because of low plant chemical defences. Full sun conditions promote photosynthesis and increased chemical defences against herbivory in plants (Roberts and Paul, 2006; Ruban, 2009), whereas shaded conditions create a cooler microhabitat which tends to be an ideal refuge for some phytophagous arthropods (Patrick and Olckers, 2014). In a field study in Mpumalanga, *G. decoris* was found to be more damaging on plants in the shade than in the sun (A. Sasa, pers. comm.; A. Urban, pers. comm.).

Physiological responses, such as increased plant growth (e.g. stem length or leaf area) are typical of light-deprived plants (Moore *et al*. 1995). In other words, in environments where light is limited, plants tend to produce larger leaves as a physiological coping mechanism. Furthermore, plants growing under low light conditions produce less carbohydrate, and consequently become less palatable for phytophagous animals to compensate for the low levels of defence chemicals (Elsayed, 2011). Since there are no data to suggest that shade plants in this study were unpalatable, the low performance of *H. laceratalis* under shade could simply mean that the prevailing cooler temperatures were not conducive for this agent. This implies that some *L. camara* biocontrol agents are less adapted to shaded conditions than others.

The high incidence of *cf. Passalora* sp., particularly at medium altitude sites, could be as a result of high relative humidity and temperature, comparatively to high and low altitude sites (Table 5.1), as these environmental parameters influence spore germination (Kallawicha et al. 2017).

The light-pink *L. camara* variety is reported to be the most common variety in South Africa (Simelane and Phenye, 2005). Results in the present study revealed that plant growth was greater in the light-pink than in the dark-pink *L. camara* variety; however the biocontrol agents were not affected by varietal

preferences here; with the exception of *cf. Passalora* sp. and *A. lantanae*, which showed an affinity for the dark-pink *L. camara* variety. Pathogens are extremely damaging and highly host specific, and so their impact on genetically diverse plants has been reported to be limited (Day and Urban, 2004).

In the present study the fungal leaf-spot pathogen *cf. Passalora* sp. showed an affinity specifically to the dark-pink *L. camara* variety. *Lantana camara* biocontrol agents perform better on some flower colour varieties than others depending on different geographic locations. For example, in Australia, *T. scrupulosa* was reported to prevail more on the red flower varieties than on the pink, white or orange varieties (Haseler, 1966; Radunz, 1971). Haseler (1966) also reported that *Neogalea sunia* Guenée (Lepidoptera: Noctuidae) had a preference for the white-pink variety, while *Salbia haemorrhoidalis* Guenée (Lepidoptera: Pyralidae) preferred the red variety. Conversely, in South Africa, most agents including *T. scrupulosa*, *O. camarae*, *Calycomyza lantanae* Frick (Diptera: Agromyzidae) display a preference for the pink flower *L. camara* varieties (Cilliers, 1987a; Cilliers and Neser, 1991; Simelane and Phenye, 2004).

In instances where two or more *L. camara* varieties co-occur on the same site, some biocontrol agents, such as *T. scrupulosa*, do not show preferences for either varieties (Baars and Heystek, 2003a), as is the case in the present study. There is, however, evidence suggesting that hybridization is one of the main factors responsible for the failure reported in the biocontrol of *L. camara* in many parts of the world (Sharma *et al*. 2005; Day and Zalucki, 2009). Not only does hybridization confer heightened resistance to phytophagous arthropods, it is also reported to increase the vegetative and reproductive growth vigour within *Lantana* sect. *Camara* (Sanders, 2006); and hence increasing the invasiveness of the weed (Maschinski *et al*. 2010).

In conclusion, the present study found that plant growth was affected by altitude, light regime and plant variety; however the impact of these biotic and abiotic environmental factors on the performance of the biocontrol agents was variable and generally insignificant. While the vegetative growth of *L. camara* was greater in plants in full shade, all these biocontrol agents showed a preference for plants growing under full sun and semi-shade conditions, and this trend could

be borne in mind when considering the introduction of additional biocontrol agents to benefit the forestry industry.

Chapter Six: General Discussion and Conclusions

6.1 Background

The aim of this chapter is to summarize the main findings of this study and discuss their relevance to the field of invasion biology in general and to biocontrol of invasive alien plants in particular, with the focus on *Lantana camara* L. (Verbenaceae), hereafter referred to as *L. camara*. This work contributes a considerable body of new knowledge on biocontrol of *L. camara*, but also raises important questions and recommendations for future studies.

An earlier study quantified the impact of an 'old' suite of biocontrol agents on the growth and reproduction of the pink flowering *L. camara* variety on the KwaZulu-Natal (KZN) coastal region, in South Africa (Cilliers, 1987b). The current study has, for the first time, quantified the combined impact of the 'old plus new' suite of biocontrol agents, on the vegetative and reproductive growth of *L. camara* in the field, in an inland region, through insecticidal exclusion experiments; and also determined the effect of some biotic and abiotic factors on plant growth and the performance of its biocontrol agents.

The main issues discussed in this chapter are as follows: (i) 'Working *for Water*' clearing operations, (ii) efficacy of carbofuran for insect exclusion, (ii) multiple agent release, (iv) micro-environmental effects on biocontrol, (v) phenotypic plasticity of agents' feeding phenology, and (vi) conclusions and recommendations for future studies.

6.2 '*Working for Water*' clearing operations

The invasive alien plant (IAP) clearing programme of '*Working for Water'* (WfW) in the Sabie River catchment, in Mpumalanga (South Africa) has successfully removed larger overstorey alien plants (e.g. *Eucalyptus* spp. and *Pinus* spp.), which dominated the landscape in 1996/7 during the pre-clearing period (Garner, 2006; Beater *et al*. 2008; Witkowski and Garner, 2008), but it has certainly not resulted in an overall reduction in IAP invasion intensity. The clearing of larger plants resulted in the emergence of a myriad of understorey alien plants, with *L. camara* being the most important of all, as a result of soil disturbance and an open canopy. Terrain disturbance associated with the clearing of IAPs can exacerbate their spread and densification (Witkowski and Wilson,

2001). Because of the shading of *Eucalyptus* spp., *L. camara* cover was very low (~1%) in 1996/7, but was dominant by 2005 comprising 26% of all IAPs in the study area. In the present study, *L. camara* invasion intensity increased by 13% from 2005 to 2012, implying that, although the invasion intensity of larger plants have significantly declined as a result of clearing operations, more effort is still needed to control the suite of understorey invasive trees and shrubs which continue to pose a threat to South Africa's water resources.

Biological invasion poses an imminent threat to the conservation of biodiversity and the functioning of ecosystems worldwide (Witkowski and Wilson, 2001; van Kleunen et al. 2015). The decline in global biodiversity and potential risk of homogenization of regional fauna and flora is one of the major negative impacts of biological invasion (Petersen *et al*., 2006). In addition to the loss of biodiversity; biological invasions, particularly that of IAPs, can result in significant loss of water resources (Le Maitre *et al*. 2002; Görgens and van Wilgen, 2004). Invasive alien plants are estimated to use about 7% of South Africa's water (Versveld *et al*. 1998). In a drought-prone country such as South Africa, the preservation of its scarce water resources is paramount, and proving even more important in recent years.

The *El Niño*-related drought that South Africa is currently experiencing, is said to be one of the most devastating in over 23 years (Vogel and van Zyl, 2016), with serious socio-economic, as well as environmental implications (Baudoin *et al*. 2017). These severe drought events only emphasize the importance of controlling IAPs that are reducing the already scarce water resources. It is within this context that the national WfW programme has been mandated to control the spread of IAPs, not only through clearing operations but using an integrated management strategy, including mechanical, chemical and biocontrol methods. Impacts of biocontrol on plants with a level of invasion that has reached regional proportions, such as *L. camara*, can only be quantified through the use of insecticidal biocontrol-agent-exclusion techniques in a field situation.

6.3 Carbofuran: insecticidal exclusion

The impact of *L. camara* biocontrol agents on plant growth and reproduction was difficult to assess, given problems with maintaining insecticidal exclusion plants insect-free over long time periods at these distant field sites. The analysis of carbofuran residues in exclusion plants (carbofuran-treated plants) showed that the insecticide persisted in these plants for only three weeks after application, while subsequent applications were made every two months. This, therefore, implies that in the interval between one insecticide application and the next, agents would have re-colonized their host. Interestingly, while the effect of carbofuran was short-lived in the *L. camara* experiment, it achieved a longer plant protection in a parallel experiment, on a different plant species, *Solanum mauritianum* Scop. (Solanaceae) (Sasa, 2018). Carbofuran applications to *S. mauritianum*, in the same study area and applied at the same dosage as in the *L. camara* experimental trials, successfully reduced both biocontrol agents, *Gargaphia decoris* Drake (Hemiptera: Tingidae) and *Anthonomus santacruzi* Hustache's (Coleoptera: Curculionidae) performance on exclusion plants (Sasa, 2018). This result suggests that carbofuran, although applied at the same rate and under similar micro-environmental conditions, responds differently to different plant species and/or biocontrol agent combinations.

It appears that the biodegradation of carbofuran is two times faster in *L. camara* than in *S. mauritianum*. In this study, carbofuran residues in *L. camara* peaked two weeks after the insecticide was applied and were undetectable by week four; whereas, in *S. mauritianum*, it only peaked at week four and disappeared after the eighth week (Sasa, 2018). This clearly suggests that carbofuran protection against herbivorous arthropods can be more effective (offering longer protection) on some plant species and agents than others. Little is known about the persistence of systemic insecticides, such as carbofuran, in plants; however, some noxious plants, including *L. camara*, have been reported to be highly resistant to a number of herbicides (Lebaron and Gressel, 1982; Sahid and Sugau, 1993), demonstrating their ability to biodegrade some applied chemicals.

6.4 Multiple agent release

Multiple agents on a single host plant, if not antagonistic in nature, can result in collectively exerting considerable pressure on the weed. Sometimes, however, a lottery approach is used, where several agents are released in the hope of eventually singling out the best among them (McEvoy and Coombs, 1999). On the other hand, competition among multiple agents released has been cited as one of the factors responsible for the failure or limited success in biocontrol (McEvoy, 2002). The phytophagous arthropods on *L. camara*, in its native range, are reported to co-exist, and even share common feeding guilds (e.g. on leaves and/or on flowers) (Winder and Harley, 1983; Palmer and Pullen, 1995). From the present study, it is concluded that the members of the suite of *L. camara* biocontrol agents complement one-other in maintaining pressure on the weed, although not to the extent of significantly reducing its growth and regeneration capacity in inland areas.

Lantana camara invasion intensity is still on the rise (Chapter Two), aided by its considerable regeneration capacity, supporting the propagule pressure hypothesis. As important as seedbank ecology is in biological invasions (Vivian-Smith and Panetta, 2009), our understanding of seedbank ecology of major weeds, such as *L. camara*, is still quite poor (Vivian-Smith *et al*. 2006; Vivian-Smith and Panetta, 2009). Seed-feeding agents capable of reducing seed production of their host by more than 95% have popularly been considered successful (Hoffmann and Moran, 1998; Sheppard *et al*. 2001; van Klinken and Flack, 2008); however any reduction in seedbank density and seedling recruitment, by any fraction, should nonetheless be considered important steps in the right direction in the fight against plant invasion (van Klinken *et al*. 2009). In the present study, the combined impact of all biocontrol agents resulted in non-significant reductions in the seedbank and seed production.

Seedbank germination rate was high in the laboratory (91 to 140 germinable seeds/m^2), but seedling emergence was very low under field conditions, with 0.8 to 1 seedling/m^2 (Chapter Three). According to a laboratory experiment by Vivian-Smith and Panetta (2009), environmental factors such as soil moisture, plant biotype and the depth of seed burial, all contribute to seedling emergence;

and they found that high soil moisture increases seedling emergence. Under field conditions, seedling emergence was estimated to be low (< 25%) (Vivian-Smith and Panetta, 2009). The present study sites are located in riparian zones, less than 15 m away from the river; and so, soil moisture should not have been a limiting factor for seedling emergence; which seems to corroborate reports that *L. camara* self-limits its own seed germination and seedling recruitment through light deprivation beneath its plant canopy, and also through the inhibitory effects of its own allelochemicals. In addition, the laboratory conditions allowed more light (unshaded) onto the seed germination trays, while in contrast in the field, shade cast from the overstorey riparian trees and adjacent tall plantation trees would have resulted in light limitation across much of the area of the study plots.

6.5 Micro-environmental effects on biocontrol

The success or lack thereof, of biocontrol is predicated upon biotic and abiotic factors, such as plant genotype, agent parasitism, climate, and altitude or elevation, as well as the biology and ecology of the host plant (Blossey, 1995; Gassmann and Schroeder, 1995; Broughton, 2000; Day *et al*. 2003a) and the biocontrol agents themselves. The performance of the suite of *L. camara* biocontrol agents in the present study was not affected by plant varietal differences, altitude or degrees of shading; with the exception of the leaf-spot pathogen, *cf. Passalora* sp. (Chupp) U. Braun & Crous var. *lantanae*, which was exceptionally abundant on the dark-pink-flowered *L. camara* variety at medium altitude sites but not at the other altitudinal ranges. Plant growth, on the other hand, was greater at low altitude sites, under shade conditions, while most agents performed well under full sun conditions.

It seems that the leaf-spot pathogen was favoured by micro-environmental conditions at the medium altitude sites, characterized by a fairly high relative humidity (medium altitude: 56%; though not as high as at high altitude: 65% RH) and a higher rainfall than at low altitude sites (Chapter Three). Many arthropods and pathogens increase their populations with increasing humidity levels (Roca and Lazzari, 1994; Broufas *et al*. 2009; Lu and Wu, 2011). It is plausible that the leaf pathogen in the present study flourished at medium altitude sites because of

the fairly high levels of relative humidity and rainfall, compared to the low altitude sites; nevertheless, this uncertainty should further be investigated in future studies. Dew point, relative humidity, rainfall, and temperature are all important requirements for spore germination (Kallawicha et al. 2017).

6.6 Phenotypic plasticity of biocontrol agents' feeding phenology

Some insect agents are able to shift their feeding peaks at different altitudes in different seasons, through phenological plasticity. The phenology of the pugnacious ant, *Anoplolepis custodiens* Smith (Hymenoptera: Formicidae) demonstrates plasticity in that foraging peaks at midday in winter, and in the early morning and late afternoon in summer, in response to preferred temperature (Steyn, 1954). During the period of the present study, *T. scrupulosa* shifted its feeding peak from autumn at medium attitude, to spring at low altitude, and to summer at high altitude. The shift in feeding peak resulted in this biocontrol agent maintaining its presence on *L. camara* throughout the entire study area and in all seasons. Like phenotypic plasticity, some *L. camara* biocontrol agents appear to have adapted their feeding behaviour to seasonal changes by shifting their peaks on an altitudinal gradient. This study suggests that *L. camara* biocontrol agents exhibit phenological plasticity, and hence are capable of changing and adapting their feeding habit at different altitudes, triggered by seasonal changes. It is also important to note that this apparent feeding phenological plasticity is driven by the seasonal-dependence of food quality for insect herbivores; for example, *T. scrupulosa* prefers to feed on younger leaves, which develop first, in early spring, at low altitudes, and later, as the temperature increases, in mid to late summer, at medium and high altitudes.

6.7 Conclusions and recommendations

While early clearing operations of IAPs were successful at removing large trees, such as *Eucalyptus* spp. and *Pinus* spp., which had invaded the riparian areas prior to the initial WfW clearing, the logging of these large woody trees opened up the canopy, thus promoting the invasion and densification of an array of understory IAP species, including *L. camara*. For effective evaluation of an IAP control

programme, such as WfW, maintaining good records (clearing and follow-up dates, agent releases, herbicide applications, etc.) for every site is an integral aspect, and should therefore be seriously improved. Data recording should be consistent across all affected regions countrywide, and the database made easily accessible. With the plethora of IAPs occurring in South Africa, the WfW management programme must be reflective of their ecological characteristic differences; and so tailor-made solutions, for at least the most problematic species, such as *L. camara*, must be considered.

The impact of the suite of biocontrol agents on *L. camara* plant growth and reproduction has been difficult to measure, given problems with maintaining exclusion plants agent-free through the insecticidal exclusion technique, when using carbofuran. Therefore, the effect of these agents, inland, which appears to be marginal overall, may have been slightly underestimated.

The study also established that *L. camara* control is influenced by abiotic factors such as environmental factors prevailing at different altitudes, i.e. temperature, rainfall, relative humidity; and shade. Plant growth was greater under shade conditions, whereas agent performance showed preferences for plants growing under semi-shade and full-sun conditions. These results, therefore, suggest that more agents are still needed, especially ones adapted to shaded or closed canopy micro-environments.

Residue analysis showed that carbofuran applied to soil broke down faster in *L. camara* than in *S. mauritianum*. The biochemical basis of the rapid breakdown of carbofuran in *L. camara* could be investigated. A more effective technique should be found to chemically exclude biocontrol agents from *L. camara*, before further impact studies are undertaken. For example, foliar chemical application could be combined with a soil applied systemic insecticide to maximize plant protection against insects (insect-exclusion); the frequency of application must be dictated by the degree of persistence of the insecticide used as well as the type of weed targeted; and finally, unintended negative effects of insecticides on the fauna must be thoroughly assessed prior to any applications.

The environmental impact of the insecticide on other organisms is unknown, and in the light of the difficulty in maintaining meaningful levels in the plant

tissue, this type of insecticidal exclusion must be carefully considered before being embarked upon.

Sampling should be done more frequently to provide more solid phenological data on plant growth and agent performance.

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