Impact of the biological control agent *Aceria lantanae* (Cook) (Acari: Trombidiformes: Eriophyidae) on the invasive weed *Lantana camara* L. (Verbenaceae) in South Africa

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DECLARATION

I declare that the work presented in this thesis is original and has not been submitted in any other form, for any degree or diploma, to this or any other University. This represents my own work and all sources used or quoted have been indicated and acknowledged in the text.

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ABSTRACT

This study was conducted to determine the establishment, dispersal, performance and impact of a recently introduced flower-galling mite, *Aceria lantanae* (Cook) (Acari: Trombiformes: Eriophyidae) on the inflorescence and seed production of the invasive *Lantana camara* L. (Verbenaceae) in Limpopo, Mpumalanga, Gauteng and KwaZulu-Natal provinces of South Africa. The climate-matching programme CLIMEX was used to predict the distribution range of the mite on the African continent. Furthermore, the influence of some climatic factors (i.e., elevation, temperature, rainfall and relative humidity) and the suitability of different *L. camara* varieties were also investigated. *Aceria lantanae* established and persisted for more than 12 months at 58.6% of the release sites in Limpopo, Mpumalanga, Gauteng and KwaZulu-Natal provinces. Continuous surveys also showed that the mite had dispersed widely throughout the geographic range of *L. camara* in South Africa and Swaziland, with the highest dispersal rate of 40.6 km per annum recorded between the inland area of Nkwene (Swaziland) and the coastal area of Ncotshane (KwaZulu-Natal). The performance of *A. lantanae* varied among sites, provinces and seasons, with the infestation levels ranging from 2.7% to 97% per site. Inflorescence and seed production declined significantly by up to 86% and 96%, respectively, on lantana stands that were infested with *A. lantanae* in KwaZulu-Natal compared to the control stands. The CLIMEX model predicted that the climatic conditions for *A. lantanae* would range from suitable to highly suitable within the distribution range of *L. camara* in southern Africa. Although not statistically significant, there was a slight decline in *A. lantanae* infestation levels, with increasing elevation and annual rainfall. Infestation levels were somewhat higher at sites receiving between 600 and 1000 mm of rainfall per year, and decreased slightly as the annual rainfall exceeds 1000 mm. This study also found that infestation levels of *A. lantanae* were neither related to temperature nor relative humidity. Mite infestations differed significantly amongst the 10 tested varieties of *L. camara*. Highly preferred varieties included 017 Orange Red, 021 White Pink and 018 Dark Pink, with infestations ranging from 50.4% to 61.2%. Those which were moderately attacked by *A. lantanae* included 163 Light Pink, 021 Total Pink, 165 Light Pink, 015 Yellow White, 021 Pink and 015 White Yellow varieties, with infestations ranging from 7.8% to 21.4%. Variety 010 Dark Pink was completely rejected

by the mite, with no infestations recorded during the study period. Furthermore, regression analysis showed that neither plant size nor inflorescence density influenced *A. lantanae* infestation levels. However, there was a significant increase in *A. lantanae* infestation on plants already infested by other lantana biocontrol agents. This study concluded that amongst all investigated parameters, varietal resistance was the major factor that influenced the sporadic establishments and overall performance of *A. lantanae* throughout the distribution range of *L. camara* in South Africa.

Keywords: *Aceria lantanae* establishment, dispersal rate, impact, seasonal performance, climate, lantana varieties

DEDICATION

I dedicate this research to my very first mentor, motivator and role model, Dr. R.N.N. Magoba for seeing potential in me. If it were not for his wise words of encouragement and motivations, I couldn't have stood and believed in myself this much. You are such a hero.

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CHAPTER ONE

GENERAL INTRODUCTION

1.1   Background to the study

Lantana camara L. (Verbenaceae), commonly known as lantana, is one of the most ecologically and economically harmful weeds of the tropical, subtropical and warm temperate regions of Africa, southern Asia, Australasia and the Pacific Islands (Urban *et al.* 2011). Consequently, the Natural Resource and Environmental Programme of the Department of Environmental Affairs regards *L. camara* as a priority weed in South Africa. As part of a long-term management strategy, biological control, which involves the selection and introduction of natural enemies to reduce and suppress a noxious weed, was initiated in South Africa in 1961 (Stirton 1977; Cilliers & Neser 1991; Baars & Neser 1999). Amongst 26 natural enemies that were released against *L. camara* in South Africa during the past five decades, one of the more recent is the flower-galling mite *Aceria lantanae* (Cook) (Acari: Trombidiformes: Eriophyidae) (Craemer & Neser 1990; Urban *et al*. 2003). Although *A. lantanae* has become widely established throughout the country since its initial release in 2007, its effectiveness in reducing the reproductive output of *L. camara* has not been quantified. The current study was therefore conducted to determine the establishment, performance and impact of *A. lantanae* on *L. camara* in densely invaded coastal and inland areas of the KwaZulu-Natal, Limpopo, Mpumalanga and Gauteng provinces of South Africa.

1.2   Biology, origin and invasiveness of *Lantana camara*

Lantana camara is a low, erect, prickly and vigorous shrub of tropical and subtropical Central and South American origin that bears strong woody and hairy quadratic stems that are covered with small spines (Fensham *et al.* 1994; Day *et al.* 2003). The leaves are arranged in opposite pairs, with crenate to serrate margins, and are wrinkled on the upper surface and rough on both sides (Stirton 1977; Gentle & Duggin 1997; Baars & Neser 1999). The plant has a very strong supportive

rooting system and can grow to a height of 1.2 - 4 m, or even to greater heights in the presence of other supporting vegetation (Gentle & Duggin 1997). Plants can grow individually, in clumps, or as dense thickets, and are capable of crowding out and outcompeting native vegetation (Day *et al*. 2003).

Pairs of inflorescences develop on the aerial buds of each branch, with each inflorescence consisting of about 10-40 clusters of flowers (Fensham *et al*. 1994; Gentle & Duggin 1997). These produce small hard fruitlets or berries that are initially shiny and greenish black coloured, but become greyish black and fleshy with a stone seed at maturity (Sastry & Kavathekar 1990; Day *et al*. 2003). Apart from asexual reproduction through suckering, sexual reproduction remains the primary means by which *L. camara* is propagated. Studies by Wijayabandara *et al.* (2011) showed that *L. camara* seeds are viable for at least 2-5 years in the soil. Seed ingestion by birds or other mammals promotes scarification (Khoshoo & Mahal 1967), which improves the plant's seed germination potential (Lonare *et al*. 2012). Anthropogenic activities or disturbances such as burning, slashing and clearing promote invasion through either seeds or vegetative propagules (Lee 2001; Lonare *et al*. 2012; Priyanka & Joshi 2013).

Over 40 hybrids of *L. camara* have been developed for ornamental purposes since its introduction to South Africa in the 19th century (Cilliers 1983; Graaff 1986; Cowling *et al*. 1997). Flower colour has been considered as the primary distinguishing feature for different *L. camara* varieties (Baars 2002; Heystek 2006; Heshula 2005, 2009). Corollary flower lobe colours that have been used for identification include Red, Pink, White/Pale Pink and Orange (Gentle & Duggin 1998; Thomas & Ellison 2000). Although flower colour is still globally utilized for distinguishing between the different forms of *L. camara*, some studies have strongly discouraged the use of flower colour as a primary identification tool, but have instead promoted the utilization of randomly amplified polymorphic DNA (RAPD) methods for improved accuracy (Scott *et al.* 1997; Scott 1998). Due to their morphological and genotypic differences, lantana cultivars differ in their degree of susceptibility to natural enemies (i.e., insect herbivores, nematodes and pathogens) and toxicity to livestock (Taylor 1989; Baars & Neser 1999; Simelane 2006a; Reinert *et al*. 2009; Urban *et al.* 2011).

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1.3   Climatic requirements and geographic distribution of *L. camara* in South Africa

The broad geographic distribution of *L. camara* is a reflection of its wide ecological tolerances and favourable genotypic traits that enable modifications in growth and development in response to environmental variability (Sharm *et al.* 2005). *Lantana camara* grows well in warm, moist subtropical and temperate areas, and it rarely occurs in areas where temperatures frequently fall to 5ºC (Cilliers & Neser 1991). Lantana does not appear to have an upper temperature or rainfall limit and is often found in tropical areas receiving 3000 mm of rainfall per year, provided that soils are sufficiently well drained (Day *et al.* 2003). In South Africa, *L. camara* has been found in areas with a mean annual surface temperature of greater than 12.5°C (Stirton 1977). Some varieties can withstand minor frosts, provided that these are infrequent (Graaff 1986). *Lantana camara* plants generally adapt and grow exceptionally well in diverse habitats with a variety of well-drained soil types (Gentle & Duggin 1997, 1998).

In South Africa, *L. camara* has invaded mountain slopes, watercourses, forests and roadsides, mostly in the eastern parts of the country, including the North West, Gauteng, Limpopo, Mpumalanga, KwaZulu-Natal and Eastern Cape provinces (Fig. 1.1). Invasion gradually becomes reduced from the southern coastal regions of the mid-Eastern Cape to the Western Cape Province (Fig. 1.1).
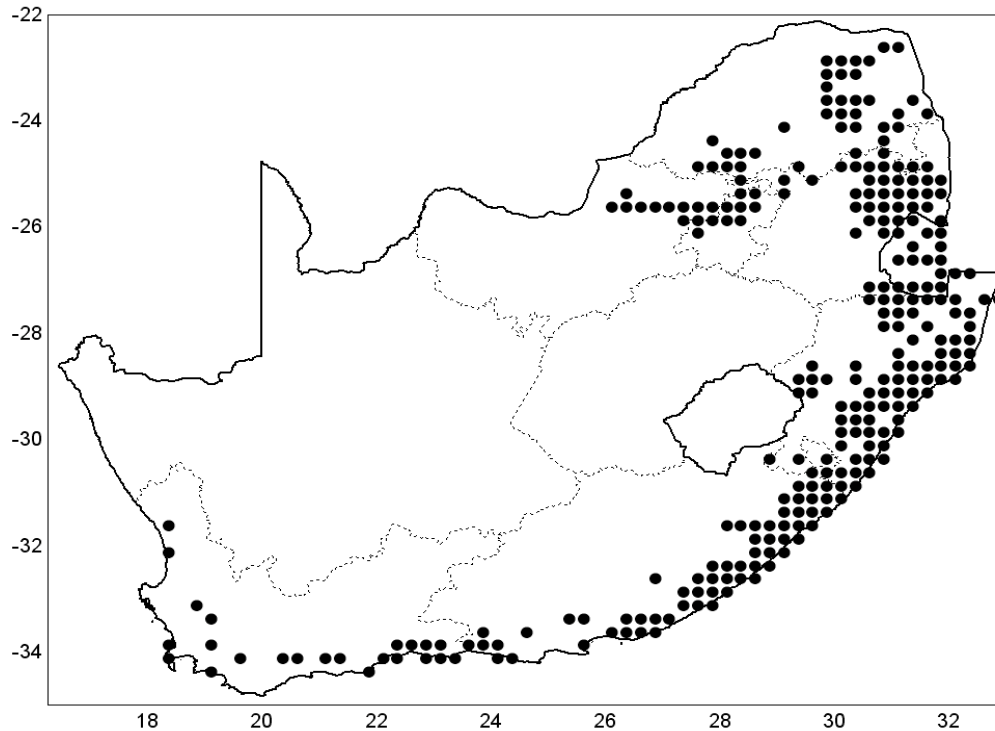
Fig. 1.1. Distribution of *Lantana camara* in South Africa (Henderson 2009). Data source: SAPIA database, Agricultural Research Council, Pretoria.

1.4   Harmful effects of *Lantana camara*

Since escaping from ornamental plantings in gardens, lantana has invaded natural ecosystems, where it transforms the indigenous vegetation into an impenetrable thicket that diminishes natural pasturage, reduces productivity of stock farming, obstructs access to water sources and plantations, threatens biodiversity and devalues the land (Lee 2001; Kumar *et al*. 2011; Urban *et al*. 2011). Lantana increases the risk of fire in dry rainforest by increasing fuel loads in these natural ecosystems (Humphries & Stanton 1992). Although some studies have argued that lantana improves soil fertility, the plant inhibits colonization by other plants, including native species, by releasing allelochemicals into the soil (Gentle & Duggin 1997; Kumar *et al*. 2011; Osunkoya & Perrett 2011). *Lantana camara* leaves, stems and roots contain a toxic compound known as lantadene (Wells & Stirton 1988; Pour *et al*. 2011), and exposure to this compound causes

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photosensitivity of the mucous membrane and loss of body weight in highly sensitive vertebrates such as pigs, cattle, goats, horses, deer and sheep. As a result of cytotoxicity, chronic damage to the liver, kidneys or gut could cause death in animals that ingest high quantities of this compound (Louw 1948; Pour *et al*. 2011).

1.5   Control of *Lantana camara*

In accordance with South Africa's alien plant legislations, namely the Conservation of Agricultural Resources Act (No 43 of 1983) (CARA) and National Environmental Management Act (No. 10 of 2004) (NEMBA), the presence of *L. camara* on properties is prohibited and the legislation enforces prompt eradication from invaded gardens and landscapes. Several conventional control techniques, including chemical and mechanical control continue to be practiced in South Africa. Mechanical control involves the utilization of human endeavour, implements or machinery to clear dense stands of the plant in a given ecosystem. Manipulation and destruction of lantana stands with the aid of machinery (e.g. bulldozers) and felling tools as well as other cultural practices such as veld burning, harrowing and disking is included under this management practice (Baars 2002). Several herbicides or chemical compounds are also utilized for the suppression of *L. camara* stands in South Africa (Grobler *et al.* 2000). For example, plants are cut at ground level and the cut stumps are painted with imazapyr (Chopper™ or Hatchet™) to prevent regrowth. Plants can also be sprayed with broad spectrum herbicides such as picloram (Access™ or Browser™) or fluroxypyr (Plenum™) (Grobler *et al.* 2000; Urban 2010).

Mechanical and chemical control strategies provide only temporary relief for *L. camara* infestations, as the plants immediately re-infest cleared areas through seedling recruitment and coppice regrowth from roots and stems that were untreated or insufficiently treated with herbicides (Cilliers & Neser 1991; Baars 2002). Continuous follow-up treatments are therefore essential to maintain *L. camara* densities below economic thresholds (Baars & Neser 1999; Day *et al.* 2003; Urban 2010). Chemicals can also have non-target effects on native plants and invertebrates, causing their demise in ecosystems that are regularly treated (Urban 2010). Utilization of

herbicides indirectly promotes invasion since the weed has competitive growth traits and allelopathic effects on slow-growing native species (Morton 1994; Lee 2001; Urban *et al*. 2010a, b; Priyanka & Joshi 2013). Due to the high cost of these conventional control strategies, biological control potentially remains the most sustainable and the best long-term control option.

1.5.1 Biological control of *Lantana camara*

In the context of invasive plant management, biological control is defined as a low environmental risk management practice that involves the utilization of introduced natural enemies (e.g. insects, pathogens and mites) to suppress or maintain the plant's densities at acceptable levels (Cory & Myers 2000). The global biological control programme against *L. camara* was initiated over a century ago, and over 41 biological control agents have been released in several countries worldwide. Of these, some 26 agent species have been released in South Africa since the early 1960s (Table 1.1) (Urban *et al*. 2011). The high numbers of agents that have been released against the weed are indicative of the difficulties that have been experienced in controlling it biologically. New agents have thus continually been sought to improve the success of biocontrol efforts.

With financial support from the Natural Resource and Environmental Programme (formerly, the *Working for Water* Programme) of the Department of Environmental Affairs since 1997, 17 candidate biological control agents were screened in quarantine in South Africa. Of these, seven were found to be acceptably host-specific and suitable for release. Among the most recently established agents is the lantana plant bug *Falconia intermedia* Distant (Hemiptera: Miridae) which was initially released in 2000 and flourished in several provinces, but is now confined to one site in the Eastern Cape (Heshula 2009; Heshula & Hill 2011). The lantana herringbone leaf miner *Ophiomyia camarae* Spencer (Diptera: Agromyzidae) was released in 2001 and is now common along the coast of KwaZulu-Natal, where it markedly suppresses the growth and reproduction of lantana, and has also dispersed naturally to 11 other African countries (Urban *et al*. 2010a, b). The lantana petiole weevil *Coelocephalapion camarae* Kissinger (Coleoptera: Brentidae), the lantana root beetle *Longitarsus bethae* Savini & Escalona (Chrysomelidae:

Altcinae) and the lantana flower gall mite *A. lantanae* were released in 2007 and represent the most recent agents to have been deployed. Although *L. bethae* and *C. camarae* are showing signs of successful establishment following their release, their low rates of spreading have made the release sites highly vulnerable to destruction by landowners (Baars 2002; D.O. Simelane pers. comm. 2014). On the other hand, *A. lantanae* is now well established, causing severe flower damage on susceptible lantana varieties at several sites in the coastal and Lowveld, Middleveld and Highveld regions of KZN, Mpumalanga, Limpopo and Gauteng Provinces in South Africa and in the neighboring country, Swaziland.

Table 1.1: Biocontrol agents released for the control of *Lantana camara* in South Africa (adapted from Julien & Griffiths 1998; Cilliers & Neser 1991; Baars & Neser 1999; Klein 2011; Urban *et al*. 2011).

Order: Family / Biocontrol Agent	Origin	Main release(s)	Feeding mode	Status	Damage inflicted
Acari: Tombidiformes					
Aceria lantanae (Cook)	Mexico	2007 2009 2012	Flower-galler	Established	Extensive, heavy galling in some coastal (KZN) and inland (LP) regions
Coleoptera: Cerambycidae					
Plagiohammus spinipennis (Thompson)	Mexico via Hawaii via Australia	1973	Stem-borer	Not established	-
Coleoptera: Chrysomelidae *Alagoasa parana* Samuelson	Brazil via Australia	1985	Leaf chewer	Not established	-
Longitarsus bethae Savini & Escalona	Mexico	2007 2012 2013	Root feeder	Established	Unknown or too early for post release.
Octotoma championi Baly	Costa Rica via Australia Central America via Australia	1978 1995	Leaf-miner	Establishment unconfirmed	Unknown
Octotoma scabripennis Guérin-Méneville	Mexico via Hawaii via Australia	1971 1974 1981	Leaf-miner	Established in moist, warm eastern range of lantana. Abundant in localized inland areas	Considerable defoliation, but localized
Uroplata girardi Pic	Paraguay via Hawaii via Australia	1974 1983 1984	Leaf-miner	Established, abundant in coastal regions. Present in low numbers in warm, moist inland areas	Extensive defoliation in coastal regions

* Insect species present in South Africa prior to deliberate introduction

Table 1.1 (Continued): Biocontrol agents released for the control of *Lantana camara* in South Africa (adapted from Julien & Griffiths 1998; Cilliers & Neser 1991; Baars & Neser 1999; Klein 2011; Urban *et al.* 2011).

Order: Family / Biocontrol Agent	Origin	Main release(s)	Feeding mode	Status	Damage inflicted
Uroplata lantanae Buzzi and Winder	Brazil via Australia	1984	Leaf miner	Not established	-
Uroplata fulvopustulata Baly	Costa Rica via Australia	1978	Leaf miner	Not established	-
Diptera: Agromyzidae					
Calycomyza lantanae (Frick)	Trinidad via Australia via Florida (USA)	1982 1989	Leaf-miner	Widely established in low numbers. Heavily parasitized	Slight damage inflicted
*Ophiomyia lantanae** (Froggatt)	Unknown	1961	Fruit/ seed-miner	Widely established and abundant, but heavily parasitized	Low impact on seed viability
Ophiomyia camarae Spencer	Florida (USA)	2000 2001	Leaf miner	Widely established	Moderate to considerable
Diptera: Tephritidae					
Eutreta xanthochaeta Aldrich	Mexico via Hawaii	1983	Stem/ shoot galler	Not established	-
Hemiptera: Miridae					
Falconia intermedia (Distant)	Jamaica	1999	Leaf sucker	Establishment in warm, moist areas	Moderate, greatly limited by distribution and population fluctuations

* Insect species present in South Africa prior to deliberate introduction

Table 1.1 (Continued): Biocontrol agents released for the control of *Lantana camara* in South Africa (adapted from Julien & Griffiths 1998; Cilliers & Neser 1991; Baars & Neser 1999; Klein 2011; Urban *et al*. 2011).

Order: Family / Biocontrol Agent	Origin	Main releases	Feeding mode	Status	Damage inflicted
Hemiptera: Tingidae					
Teleonema elata Drake	Brazil via Australia	1972	Leaf sucker	Not established	-
Teleonemia scrupulosa Stål	Mexico via Australia via Mauritius Florida (USA)	1961 1971 1984 1989	Leaf & flower sucker	Widely established in large numbers across the entire range of lantana; severe damage sporadic	Complete defoliation and abortion of flowers in subtropical regions
Leptobyrsa decora Drake	Colombia and Peru	1972	Leaf sucker	Not established	-
Homoptera: Ortheziidae *Orthezia insignis* Browne	Unknown	1961	Stem and foliage sucker	Widely established throughout invaded range	Unknown
Lepidoptera: Gracillariidae *Aristea onychote**	Unknown	?	Leaf miner	Widely established, present in low numbers, heavily parasitized	Unknown
Lepidoptera: Noctuidae *Hypena laceratalis** Walker	Kenya and Zimbabwe via Hawaii	1961	Leaf chewer	Widely established. Larvae are active during late summer and autumn and often parasitized	Moderate
Neogalia sunia (Guenée)	California (USA)	1962 1968	Leaf chewer	Not established	-

* Insect species present in South Africa prior to deliberate introduction

Table 1.1 (Continued): Biocontrol agents released for the control of *Lantana camara* in South Africa (adapted from Julien & Griffiths 1998; Cilliers & Neser 1991; Baars & Neser 1999; Klein 2011; Urban *et al.* 2011).

Order: Family / Biocontrol Agent	Origin	Main releases	Feeding mode	Status	Damage inflicted
Lepidoptera: Pterophoridae					
Lantanophaga pusillidactyla* (Walker)	Mexico via Hawaii	1961 1984	Flower, fruit and seed chewer	Widely established, but occurs in very low numbers	Unknown
Lepidoptera: Pyralidae					
Salbia haemorrhoidalis (Guenée)	Cuba via Hawaii	1962 1984	Leaf and flower chewer	Widely established in low numbers. Reared from native *Lippia* species	Unknown
Lepidoptera: Tortricidae					
Crocidosema lantana* Busck (Formerly: *Epinotia lantana*)	Hawaii	1961 1984	Flower-peduncle and shoot tip borer	Widely established in low numbers	Unknown
Mycopherellales: Mycopherellaceae					
Passalora lantanae (Chupp) U. Braun & Crous var *lantanae* (Formerly Mycovellosiella) [anamorphic fungus]	Florida (USA)	2002	Leaf spot pathogen	Not established	Unknown

* Insect species present in South Africa prior to deliberate introduction

1.5.2 Flower-galling mite, *Aceria lantanae*

Aceria lantanae is a microscopic beige and white coloured, worm-like mite with only two pairs of legs and an elongate, flexible abdomen that grows to about 0.1 to 0.15 mm (Fig. 1.2) (Flechtmann 1973; Oldfield 1996; Sabelis & Bruin 1996; Urban *et al.* 2001; Besaans 2012).

Fig. 1.2. Magnified view of the flower-galling mite, *A. lantanae* (C. Craemer & A. Hall). From Besaans (2012).

The flower-galling mite, *A. lantanae* (Fig. 1.2) completes its life cycle within a developing gall that is formed either on the inflorescences or buds of *L. camara* (Fig. 1.3). During dispersal, the mites vacate the galls by swarming to the gall surface (Urban *et al.* 2001; Besaans 2012) and are spread by wind currents and feeding vectors (birds and insects) (Sabelis & Bruin 1996; Amrine 2003; Smith *et al.* 2009; Craemer 2010). The herbivorous mites feed on and inject toxic saliva into the undifferentiated floral buds and leaf sheaths of *L. camara*. Infested *L. camara* tissues produce

microphyllous small leaflets that resemble 'witches brooms' (Fig. 1.3) instead of flowers and fruitlets (Cook 1909; Flechtmann 1973; Lindquist & Oldfield 1996; Urban *et al.* 2001, 2003; Mukwevho *et al.* 2014). Therefore, the induced flower galls generally cause a reduction in flowering, fruiting and seed production (Keifer & Denmark 1976; Cromroy 1984; Craemer 1993; Sabelis & Bruin 1996). This has the potential to greatly restrict seed dispersal and reduce the spread of *L. camara*.

Fig. 1.3. A mature flower gall comprised of microphyllus leaflets, which is a symptom of *Aceria lantanae* infestation on the inflorescence of its host plant, *Lantana camara*.

The galls are also thought to have an indirect impact on the biomass and growth rate of *L. camara* (Urban *et al.* 2001; Day & Urban 2004; Magoba 2013). Plant galls generally act as nutrient or metabolic sinks (Baars & Neser 1999, Urban *et al.* 2001), causing the plant to invest major

proportions of carbohydrates into gall formation rather than vegetative growth. The injection of saliva, which contains a hormone-mimicking chemical, by the mite could lead to further deformation of the infested plant (Craemer & Neser 1990; Craemer 2010; Smith *et al*. 2010). The stocks of *A. lantanae* that were released in South Africa were collected in Florida, USA, but the mite has also been recorded in Mexico, Cuba, and other countries in Central America (Flechtmann 1973; Keifer & Denmark 1976; Craemer 1993).

About 25–30% of gall-causing mites that are known across the globe fall within the genus *Aceria* (Smith *et al*. 2010). Although several of these comprise serious agricultural pests [e.g., *A. guerreronis* Keifer (Acari: Eriophyidae) on coconut], some have been utilized as biocontrol agents of invasive plants. These include *A. salsolae* DeLillo & Sobhian, *A. malherbae* Nuzzaci and *A. lantanae* which were released against *Salsola tragus* L. (Chenopodiaceae), *Convolvulus arvensis* L. (Convolvulaceae) and *L. camara*, respectively (Smith *et al*. 2009; Urban *et al*. 2011).

Following host-specificity testing in quarantine, permission to release *A. lantanae* against *L. camara* was granted in South Africa in 2007 (Urban *et al*. 2011; Magoba 2013). The mite has since been released, established and distributed throughout *L. camara*'s invasion range in South Africa. However, factors that may limit the establishment, spread and performance of *A. lantanae* have never been investigated in South Africa.

1.6 Purpose of the study

The main aim of this study was to determine the establishment, performance and impact of *A. lantanae* on *L. camara* in densely-invaded coastal and inland areas in the KwaZulu-Natal, Limpopo, Mpumalanga and Gauteng provinces of South Africa.

1.6.1 Specific objectives of the study

➢ To assess the establishment and dispersal rate of *A. lantanae* on *L. camara* in South Africa.
➢ To measure the impact of *A. lantanae* on the reproductive output of *L. camara*.
➢ To assess the effect of climate on the establishment and efficacy of *A. lantanae* on *L. camara* in South Africa.
➢ To determine the varietal preferences of *A. lantanae* under field conditions.

CHAPTER TWO

Establishment, spread and impact of the flower-galling mite *Aceria lantanae* (Cook) (Acari: Trombidiformes: Eriophyidae) on the inflorescences of *Lantana camara* L. (Verbenaceae) in South Africa

Abstract

The flower-galling mite, *Aceria lantanae* (Cook) (Acari: Trombidiformes: Eriophyidae), was released as a biocontrol agent of *Lantana camara* (Verbenaceae) in the Limpopo, Mpumalanga Gauteng and KwaZulu-Natal provinces of South Africa, between 2007 and 2012. Following the mite's release, flower galls were observed at a number of sites where it had not been released before. This study was therefore conducted to determine the establishment, spread and seasonal response of *A. lantanae* in Limpopo, Mpumalanga, Gauteng and KwaZulu-Natal provinces. The effect of *A. lantanae* on inflorescence and seed production of susceptible *L. camara* was determined in KwaZulu-Natal. Dispersal rates and distances were also measured to determine the ability of *A. lantanae* to disperse in South Africa and Swaziland. *Aceria lantanae* established at 58.6% of the release sites located in the four provinces. Surveys also indicated that the mite established and dispersed widely within the geographic range of *L. camara* in South Africa and Swaziland, with the highest dispersal rate of 40.6 km per annum recorded between the inland area of Nkwene (Swaziland) and coastal area of Ncotshane (KwaZulu-Natal). Mite infestation levels varied among sites, provinces and seasons, with inflorescence infestations ranging from 2.7% to 97%. Inflorescence and seed production declined significantly by up to 86% and 96%, respectively, on lantana stands that were infested with *A. lantanae* in KwaZulu-Natal compared with the control stands. Although *A. lantanae* has become established and has spread widely within the geographic range of lantana in South Africa, only a small proportion of the lantana varieties that are spread across the provinces appear to be susceptible to this mite.

Keywords: *Aceria lantanae*, lantana, agent establishment, impact, inflorescence and seed production, dispersal rate, weed biocontrol.

2.1. INTRODUCTION

Despite the initiation of a biological control programme more than a century ago and its adoption by several countries, lantana [*Lantana camara* L. (Verbenaceae)] is still rated amongst the worst invasive plants globally (Holm *et al.* 1977; Day *et al.* 2003). Lantana agent development by the Plant Protection Research Institute of the Agricultural Research Council (ARC-PPRI), South Africa, during the last 18 years, involved the evaluation of some 30 candidate agents in quarantine. Of these, seven were deemed suitable for release while 15 were rejected internally because of insufficient host specificity or an inability to breed sustainably on weedy hybrids of lantana (Baars & Neser 1999; Urban *et al.* 2011; Klein 2011). The remainder were shelved.

Amongst the diversity of biological control agents that have been deployed against weeds, seed-attacking agents that alter seed production and quality or cause deformation of inflorescences, thereby reducing further spread of the weed through seeds, have courted global interest (Crawley 1992; Hoffmann & Moran 1998; Urban *et al.* 2001; van Klinken *et al.* 2004; Besaans 2012). Seed-reducing herbivores reduce their host's reproductive potential by feeding directly on the reproductive tissues or ovipositing onto flowers or fruits with subsequent damage by their larvae (McKay *et al.* 2010). Leaf-feeding and gall-forming agents that reduce the growth rates of their target plants also indirectly inhibit their reproductive output (Olckers 2004; Simelane *et al.* 2011).

Eriophyid mites have been used successfully in various classical biological control programmes due to their ability to cause significant damage to plant parts, with subsequent suppression of growth and reproductive potential of the target plant (Rosenthal 1996; Mahr *et al.* 1999; Smith *et al.* 2010). More than 20 species of eriophyid mites have been screened for release against various invasive alien weeds globally (Rosenthal 1996; Smith *et al.* 2010). Mites that have been considered for biological control include those which attack inflorescences or seeds [e.g., *Aceria acroptiloni* Kovalev & Shevtchenko, *A. calathidis* (Gerber), *A. davidmansoni* sp. Nov [formerly misidentified as *A. genistae* (Nalepa)] (Xue *et al.* 2015), *A. grandis* (Nalepa), *A. lantanae* and *A. paniculatae* (Cotte)] and those causing blisters on the vegetative parts of their host plants [e.g., *A. prima* (Cotte), *A. brevisetosa* (Cotte), *A. centaureae* (Nalepa), *A. malherbae* and *A.*

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salsolae] (Boczek & Petanovic 1996; Smith *et al.* 2010; Urban *et al.* 2011; Paynter *et al.* 2012). Although establishment of several mite species (e.g., *Aceria davidmansoni* sp. nov., *A. lantanae*, *A. chondrillae* (Canestrini), *A. malherbae, Aculus hyperici* (Liro), *Cecidophyes rouhollahi* Craemer*, Floracarus perrepae* Knihinicki & Boczek and *Phyllocoptes fructiphilus* (Keifer) has been recorded, very little is known about their impact on weed population densities (Goolsby *et al.* 2006; Boughton & Pemberton 2011; Broughton *et al.* 2011).

The ability of a biological control agent to establish, spread and have a significant impact on the target weed is an important element that determines the success of a biological control programme (Boughton & Pemberton 2008, 2011; Paynter *et al.* 2012). This study was aimed at assessing the establishment, spread and density of *A. lantanae* across seasons in the Limpopo, Mpumalanga, Gauteng and KwaZulu-Natal provinces of South Africa. In addition, the impact of *A. lantanae* on the reproductive output of *L. camara* was assessed at five sites in KwaZulu-Natal.

2.2. MATERIALS AND METHODS

2.2.1. Collection and re-distribution of *Aceria lantanae*

Mass-releases of *A. lantanae* commenced in 2007, following the granting of the release permit by the Department of Agriculture, Forestry and Fisheries (DAFF) in the same year. Released inflorescence galls containing *A. lantanae* were collected from laboratory cultures at ARC-PPRI, Rietondale Campus (25°43.688'S 028°14.216'E). Infested inflorescences from three field sites [i.e., Lenyenye: Tzaneen (23°59.616'S, 030°15.126'E), Colbyn: Pretoria (25˚44.218'S, 028˚14.783'E) and Mkhuze: KwaZulu-Natal (27°36.143'S, 031°59.756'E)] with high establishment rates were also re-distributed to a number of lantana-invaded areas in Limpopo, Gauteng, Mpumalanga and KwaZulu-Natal provinces where establishment of *A. lantanae* had never been recorded (Table 2.1). Harvested galls were cable-tied onto the aerial buds of *A. lantanae*-free *L. camara* plants (irrespective of variety) to prevent them from falling off and ensure that infection of the inoculated plants was successful (Winder & Van Emden 1980; Urban *et al*. 2001).

2.2.2. Determination of establishment, distribution and impact of *Aceria lantanae*

The establishment of *A. lantanae* was assessed at 29 sites in Limpopo, Mpumalanga, Gauteng and KwaZulu-Natal where *A. lantana* had been released between 2007 and 2012 (Table 2.1), and these included landscapes such as riparian areas, roadsides and plantations. To determine establishment, lantana stands within each release site were thoroughly inspected for signs of *A. lantanae*, and the site was considered to have supported establishment if *A. lantanae*-infested inflorescences were found. To determine the mite's establishment and distribution pattern, a total of 102 sites were surveyed in the four provinces, with 12, 17, 23 and 50 sites in Gauteng, Limpopo, Mpumalanga and KwaZulu-Natal provinces, respectively. Surveys were conducted within a 2 km-radius from the exact release points. Depending on the density of *L. camara*, five to 14 lantana stands were thoroughly searched for *A. lantanae* galls (Urban *et al*. 2003; Balentine *et al*. 2009). As a result of abiotic factors and anthropogenic activities (e.g. site destruction), seasonal

assessments of *A. lantanae* establishment sites progressed for a full calendar year (2013-2014) at 14 of the 29 sites, with five, one, three and five sites in Limpopo, Mpumalanga, Gauteng and KwaZulu-Natal provinces, respectively. Preliminary data collected from eight sites in 2012 were used to determine changes in the performance of *A. lantanae* over time. At each site, ±10 *L. camara* plants or stands were randomly selected, and on these the percentage of inflorescences that were infested by *A. lantanae* (PII) was determined based on the total number of infested inflorescences in relation to the total number of inflorescences per plant or stand (*Formula a*). The sites where establishment was confirmed were mapped to show the current distribution of the mite in South Africa.

Formula a:

$$PII = \left(\frac{No. \ of \ inflorescence \ galls \ per \ plant}{Total \ no. \ of \ inflorescences \ per \ plant} \right) x \ 100$$

Table 2.1 Year(s) of release and number of releases involving *Aceria lantanae* in *Lantana camara*-invaded sites in Limpopo, Mpumalanga, Gauteng and KwaZulu-Natal provinces.

Province	Site name	Grid reference		Years of release	Number of releases
		S:	E:		
Limpopo	Bhuba Lodge	23°07.012'	030°08.041'	2009/12	2
	Westfalia Estates	23°44.827'	030°06.986'	2009	1
	Magoebaskloof	23°49.110'	030°03.706'	2009	1
	Tzaneen	23°50.954'	30°06.892'	2009	1
Mpumalanga	Montenears	25º20.817'	030°50.010'	2012/13	2
	Bundu Lodge	25º22.994'	030º00.216'	2009/12/13	3
	Sudwala Caves	25°28.523'	030°58.303'	2008	1
	Brondal	25°00.774'	30° 50.000'	2009	1
Gauteng	Rietondale	25°43.688'	028°14.216'	2008/13	1
KwaZulu-Natal	Ncotshane	27°21.261'	031°30.175'	2009/12	2
	Isikwe	28º22.927'	032º21.457'	2012	1
	Empangeni	28º46.951'	030º53.963'	2007/9	2
	Richards Bay	28°46.344'	032°07.581'	2009	1
	Vulindlela	28°53.361'	031°48.154'	2007	1
	Mtunzini DP	29º08.124'	031º33.323'	2007	1
	Eshowe	28°53.880'	031°28.888'	2007	1
	Amatikulu Village	29°03.087'	031°31.691'	2007	1
	New Germany	29°48.210'	030°53.433'	2012	1
	Shongweni	29º47.922'	030º45.841'	2012	1
	Tongaat	29º48.368'	030º43.975'	2012	1
	Hillcrest	29º46.591'	030º45.076'	2012	1
	Pietermaritzburg	29°33.218'	030°19.385'	2008	1
	Amanzimtoti	30°03.295'	030°51.316'	2008	1
	Umkomaas	30°12.121'	030°47.227'	2008	1
	Sappi Beamer	30º20.074'	030º31.819'	2012	1
	Port Shepstone	30º43.666'	030º20.454'	2012	1
	Port Edward	31º01.480'	030º06.309'	2009/12/13	3
	Amadida	31°01.485'	030°06.388'	2009	1
	Bizana	31º02.480'	030º06.309'	2009	1

Two-way analysis of variance (ANOVA) was used to compare infestation levels of *A. lantanae* between study sites within a province and between seasons. However, one-way ANOVA was used to compare infestation levels between seasons at Mpumalanga (Bundu Lodge) where only one study site was assessed. Student's *t*-test was used to determine whether there were significant differences in infestation levels between the years of assessment (2012 and 2013). These statistical tests and analysis were conducted with the aid of STATISTICA 6.0 software.

2.2.3. Dispersal rate of *Aceria lantanae* in South Africa

Scouting of *A. lantanae* was conducted at approximately 10 km intervals along the roadsides, watercourses and plantation areas beyond the mite's initial release localities, and these continued until the number of galled plants had dropped to zero on four successive observations (Paynter *et al.* 2012). Coordinates were recorded for each locality where symptoms of *A. lantanae* were observed. The distance between the release point and the point where *A. lantanae* was last found was measured using the Google Earth Ruler tool (http://www.google.com/earth/index.html; Google™ Earth (Ver. 7.1.2.2041, US Dept. of State Geographer, Landsat$^©$ 2014). The model of Paynter *et al.* (2012) was adopted to determine the dispersal rate (km/ year) for *A. lantanae* in the four aforementioned South Africa provinces and in Swaziland. *Formula b* was used to estimate the annual dispersal rate of *A. lantanae* at each of eight sites located in Swaziland, Limpopo, Mpumalanga and KwaZulu-Natal.

Formula b:

$$Dispersal\ rate\ (km/p.a.) = \left(\frac{distance\ dispersed\ (km)}{No.\ of\ months\ after\ release}\right) x\ 12\ months$$

The time taken by the mite to disperse over a certain distance was measured in months, and the dispersal rate was expressed in distance (km) dispersed per year.

2.2.4. Effect of *Aceria lantanae* on inflorescence and seed production

Five study sites were selected in KwaZulu-Natal province to determine the effect of *A. lantanae* on inflorescence and seed production of susceptible lantana plants. Because regular insecticide applications were required at frequent intervals, all study sites (i.e., Mkhuze, Mtubatuba, Empangeni, Port Edward and Amadida) were located in KwaZulu-Natal province, and near the researcher's residential area. At each site, two lantana stands, located at ±500 m apart, were selected for the experiment. On one of the two stands, the insecticidal chlorpyrifos was applied on a monthly basis at a dosage of 200 ml/litre of water in order to exclude *A. lantanae* from the plants. To allow infestation by *A. lantanae*, no insecticide was applied on the other stand. Twenty newly-developed aerial branches with a length of ±5 cm were tagged on both *A. lantanae*-infested and control stands (i.e. where chlorpyrifos was applied) for regular monitoring of inflorescence and seed production. To determine the impact of *A. lantanae* on inflorescence production, a minimum sample of 54 inflorescence heads (with or without galls) were randomly collected from *A. lantanae*-infested and *A. lantanae*-free stands, and a comparison of inflorescence density between the two treatments was conducted. To assess the impact of *A. lantanae* on seed production, the numbers of seeds per inflorescence were counted from both *A. lantanae*-infested and *A. lantanae*-free stands and this was conducted at three months after commencement of the trial. Student's *t*-test was used to determine whether the differences in seed production between *A. lantanae*-infested and insecticide-treated stands of *L. camara* were significant. Because the inflorescence production data failed to meet the assumptions of normality, even after log, $\log_{10}$ and square-root transformations, the mean numbers of inflorescences produced per branch were compared between *A. lantanae*-infested and insecticide-treated stands using non-parametric Mann-Whitney U-tests.

2.3. RESULTS

2.3.1. Establishment and infestation levels of *A. lantanae* at study sites in four provinces of South Africa

Establishment of *A. lantanae* varied from site to site and from province to province, and was often associated with certain lantana varieties, an aspect that will be investigated further (Chapter 4). Establishment of *A. lantanae* was recorded at 50%, 60%, 75% and 100% of release sites in KwaZulu-Natal, Limpopo, Mpumalanga and Gauteng provinces, respectively (Table 2.2). Overall, establishment of *A. lantanae* was achieved at 58.6% of 29 release sites, with the highest establishment (100%) in Gauteng, although only a single release site was involved in this province. Surveys conducted in 2012, 2013 and 2014 revealed that *A. lantanae* had become established and had dispersed widely throughout much of the geographical range of *L. camara* in South Africa (Fig. 2.1).

Table 2.2. Percentage establishment of *Aceria lantanae* at 29 sites located in Limpopo, Mpumalanga, Gauteng and KwaZulu-Natal provinces.

Province	No. of release sites	*A. lantanae* establishment sites	Percentage (%) establishment
Limpopo	4	2	50
Mpumalanga	4	3	75
Gauteng	1	1	100
KwaZulu-Natal	20	12	60
Total	**29**	**17**	**58.6**
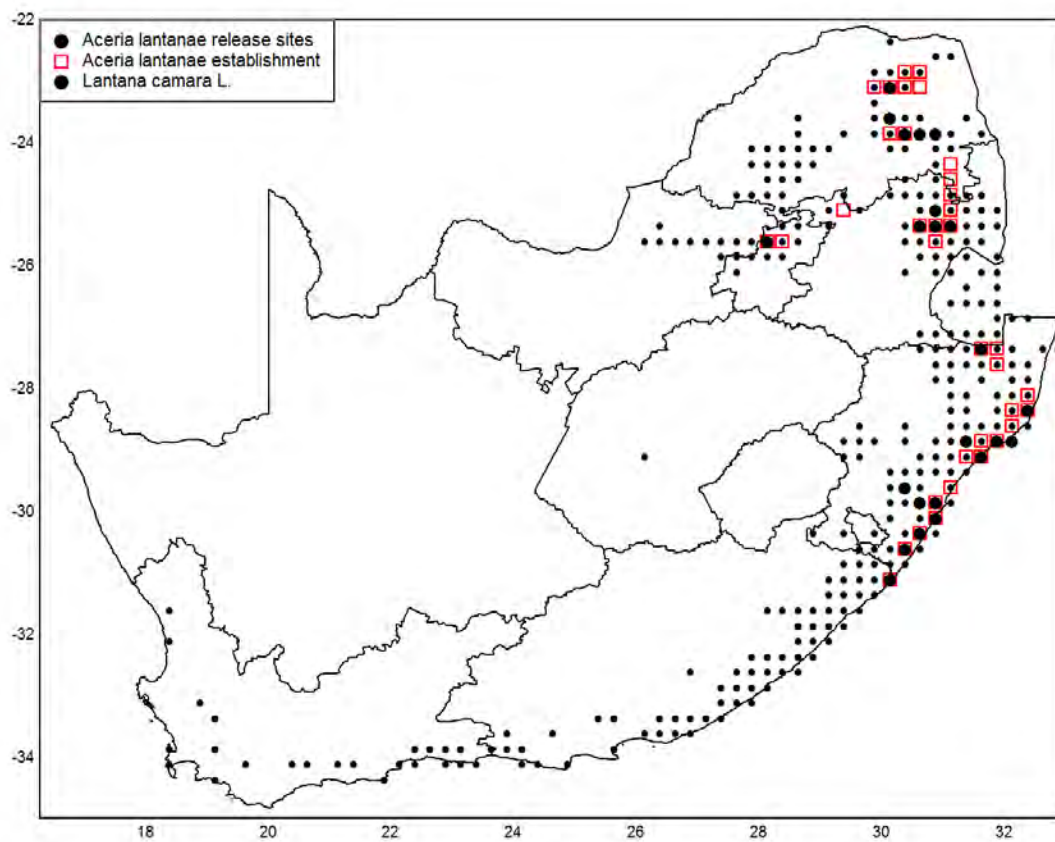
Fig. 2.1. Geographic distribution of *Lantana camara* (●) and localities where *Aceria lantanae* was initially released (●) and became established (□) in South Africa. Establishment and distribution of *A. lantanae* was recorded for Limpopo, Mpumalanga, Gauteng and KwaZulu-Natal provinces.

Mite infestation levels on susceptible lantana stands differed significantly between the different study sites, seasons and provinces (Fig. 2.2).
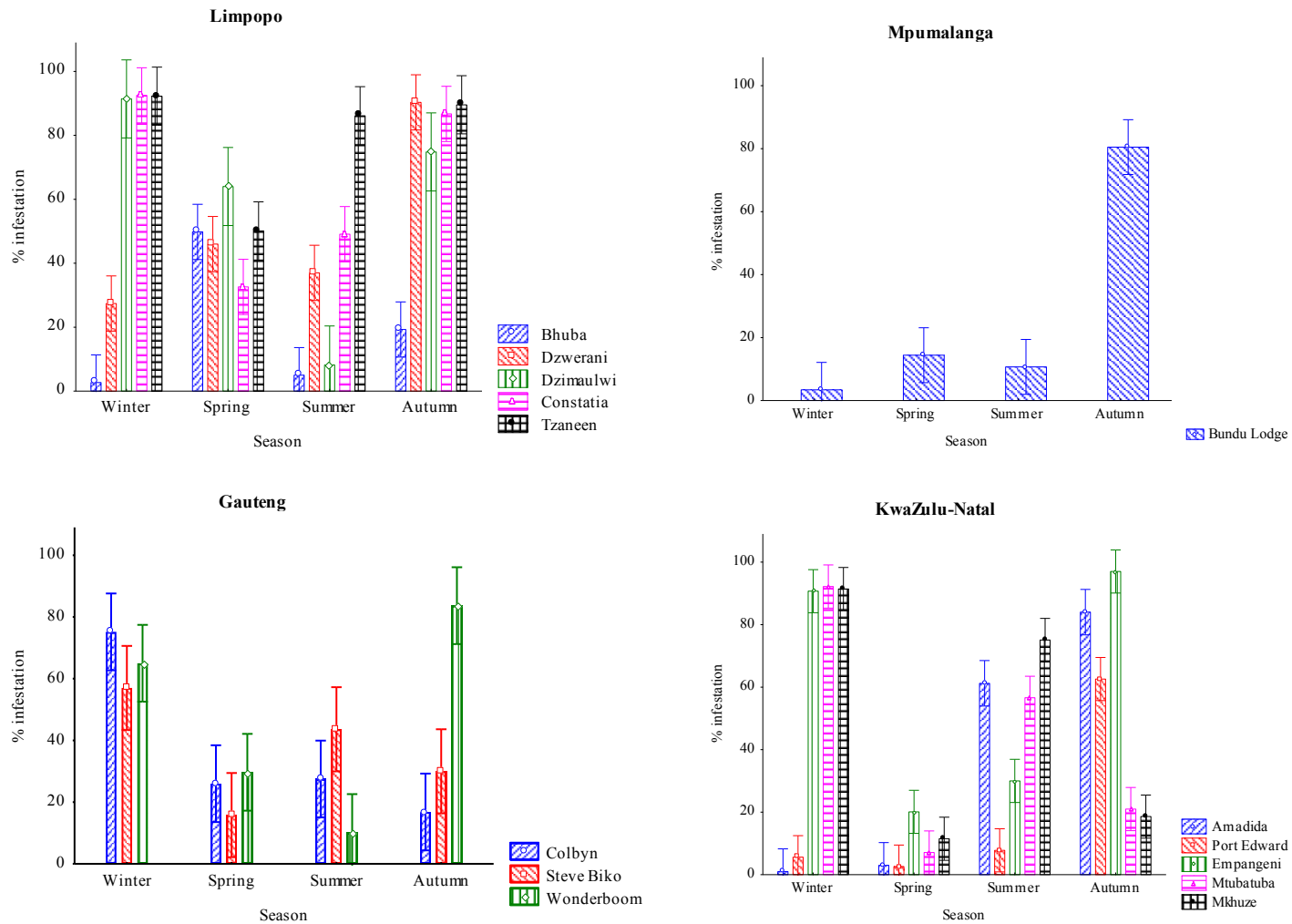
Fig. 2.2. Mean (±SE) percentage of inflorescences of *Lantana camara* that were infested by *Aceria lantanae* at five, one, three and five study sites in Limpopo, Mpumalanga, Gauteng and KwaZulu-Natal Provinces, respectively, in South Africa. Data were collected seasonally during a 12-month calendar year (2013-2014).

Infestation levels differed significantly between study sites in Limpopo ($F_{(4, 156)} = 102.24$, $P < 0.001$), Gauteng ($F_{(2, 56)} = 3.80$, $P = 0.03$) and KwaZulu-Natal ($F_{(4, 176)} = 72.05$, $P < 0.001$) provinces (Fig 2.2). Significant differences were also observed between sampling seasons in Limpopo ($F_{(3, 156)} = 49.68$, $P < 0.001$), Mpumalanga ($F_{(3, 36)} = 93.60$, $P < 0.001$), Gauteng ($F_{(3, 56)} = 26.78$, $P < 0.001$) and KwaZulu-Natal ($F_{(3, 176)} = 204.37$, $P < 0.001$) provinces (Fig 2.2). With the exception of the Bhuba Lodge site, infestations were significantly higher during autumn and winter at most of the sites in Limpopo. In Mpumalanga, where only one site (Bundu Lodge) was monitored, infestations of greater than 50% were only recorded in autumn and infestations were less than 20% during the other seasons. Peak infestation levels often occurred during autumn and winter at the study sites in Gauteng and KwaZulu-Natal provinces as well. Furthermore, the highest overall performance of *A. lantanae* in Limpopo, Gauteng and KwaZulu-Natal provinces was recorded at Lenyenye (range of 50.1 ± 10.2% to 92.2 ± 1.5%), Wonderboom (10.2 ± 3.1% to 83.7 ± 5.1%) and Empangeni (20.1 ± 2.9% to 97.0 ± 0.7%), respectively (Fig 2.2). The poorest performance in these three provinces was recorded at Bhuba (2.7 ± 1.4% to 49.8 ± 5.9%), Colbyn (26.0 ± 7.3% to 75.2 ± 9.6%), and Port Edward (2.6 ± 1.0% to 62.6 ± 7.3%), respectively (Fig 2.2).

Whilst there were significant increases in infestation levels of *A. lantanae* from 2012 to 2013 at four of the eight study sites in Limpopo, Mpumalanga, Gauteng and KwaZulu-Natal provinces (Table 2.3), significant decreases in infestations were recorded at two sites. At the remaining two sites, the infestation levels were similar during 2012 and 2013 and the differences were not significant (Table 2.3).

Table 2.3. Percentage (mean ± SE) infestation of *Aceria lantanae* at various study sites in Limpopo, Mpumalanga, Gauteng and KwaZulu-Natal provinces in 2012 and 2013.

| Province: Site name | Percentage infestation (Mean ±SE) | | Statistics | | |
	2012	2013	DF	T value	P value
LP: Tzaneen	84.8 ± 3.0	92.2 ± 1.6*	16	t = -2.23	**0.040**
LP: Tzaneen 2	60.6 ± 3.6	86.1 ± 3.5*	16	t = -5.05	**< 0.001**
MP: Bundu Lodge	14.4 ± 3.6	11.9 ± 4.0	16	t = -0.47	0.642
GP: Steve Biko	92.2 ± 2.3	57.0 ± 3.0**	8	t = 9.36	**< 0.001**
GP: Colbyn	66.5 ± 6.7	75.2 ± 9.6	10	t = -0.74	0.476
KZN: Amadida	83.4 ± 1.8	61.2 ± 4.3**	16	t = 4.79	**< 0.001**
KZN: Mkhuze	79.3 ± 3.5	91.4 ± 1.4*	18	t = -3.20	**0.008**
KZN: Mtubatuba	56.6 ± 6.2	77.7 ± 3.6*	18	t = -2.96	**0.008**

Province: LP = Limpopo, MP = Mpumalanga, GP =Gauteng, KZN = KwaZulu-Natal; * = significant increase and ** = significant decrease in infestation level during the sampling period; DF = degrees of freedom; statistically significant P-values are highlighted in bold.

2.3.2. Dispersal rate of *Aceria lantanae* in South Africa

Aceria lantanae dispersal distances were markedly higher in the inland Highveld area of Gauteng (Rietondale) and coastal areas of KwaZulu-Natal (Ncotshane and Port Edward) than in the Lowveld/Middleveld areas of Mpumalanga (Bundu Lodge) and Limpopo (Bhuba Lodge and Tzaneen). In the inland provinces, the dispersal rates varied between 6.0 km and 36.2 km per annum whereas dispersal rates of 36.1 to 40.6 km per annum were recorded in the KwaZulu-Natal coastal areas (Table 2.4). Overall, the dispersal rate of *A. lantanae* averaged at 25.84 ± 4.61 km/year, with dispersal rates of 36.6 ± 0.5, 36.2, 17.2 ± 5.2 and 11.3 ± 0 km/year recorded in KwaZulu-Natal, Gauteng, Limpopo and Mpumalanga, respectively. The dispersal rate between Ncotshane (KZN) and the central part of Swaziland (Nkwene) was 40.6 km/year, and was very similar to other dispersal rates recorded within KwaZulu-Natal Province (Table 2.4).

Table 2.4. Total distance dispersed (km) and dispersal rate (km/year) of *Aceria lantanae* from release sites located in Limpopo, Mpumalanga, Gauteng and KwaZulu-Natal provinces.

Area surveyed	Locality S:	E:	Source of mite[a]	First release[b]	Assessment[b]	Time post-release (Months)	Distance dispersed (Km)	Dispersal rate (Km/year)
Dzimaulwi[1]	22º47.880'	030º28.930'	Bhuba Lodge[1]	Feb-2009	Jun-2012	41	56.2	16.4
Constatia[1]	23º38.321'	030º39.031'	Tzaneen[1]	Feb-2009	Jun-2012	41	61.0	17.9
Acornhoek[2]	24º36.825'	031º02.329'	Bundu Lodge[2]	Jan-2009	Apr-2014	64	87.7	16.5
Marble Hall[1]	25°01.507'	029°21.306'	Rietondale[3]	Nov-2008	Oct-2012	48	144.8	36.2
Acer MP 5[2]	25º06.795'	031º03.689'	Bundu Lodge[2]	Jan-2009	Apr-2014	64	32.1	6.0
Nkwene[5]	26º46.556'	031º21.340'	Ncotshane[4]	Apr-2009	Nov-2010	20	67.6	40.6
Mkhuze[4]	27º36.143'	031º59.756'	Ncotshane[4]	Apr-2009	Oct-2010	19	58.7	37.1
N2 LP[4]	30º39.182'	030º29.863'	Port Edward[4]	Dec-2012	Apr-2014	17	51.1	36.1

Provinces/country in which *A. lantanae* was released or surveyed, [1]Limpopo, [2]Mpumalanga, [3]Gauteng and [4]KwaZulu-Natal; [5]Swaziland. [a]Release localities with their coordinates are recorded in Table 2.1. [b]Date (Month-Year).

2.3.3. Effect of *Aceria lantanae* on inflorescence and seed production

The numbers of inflorescences produced by plants in the insecticide-treated (control) stands were significantly higher than on plants in *A. lantanae*-infested stands, at all five sites. Inflorescence production varied between 18.8 and 27.9 inflorescences per plant in chlorpyrifos-treated lantana stands and between 3.7 and 5.9 inflorescences per plant in *A. lantanae*-infested stands (Fig. 2.3). Overall, inflorescence production declined by 72.9%, 75.1%, 77.1%, 77.2% and 86% at the Empangeni, Port Edward, Mtubatuba, Amadida and Mkuze study sites, respectively.
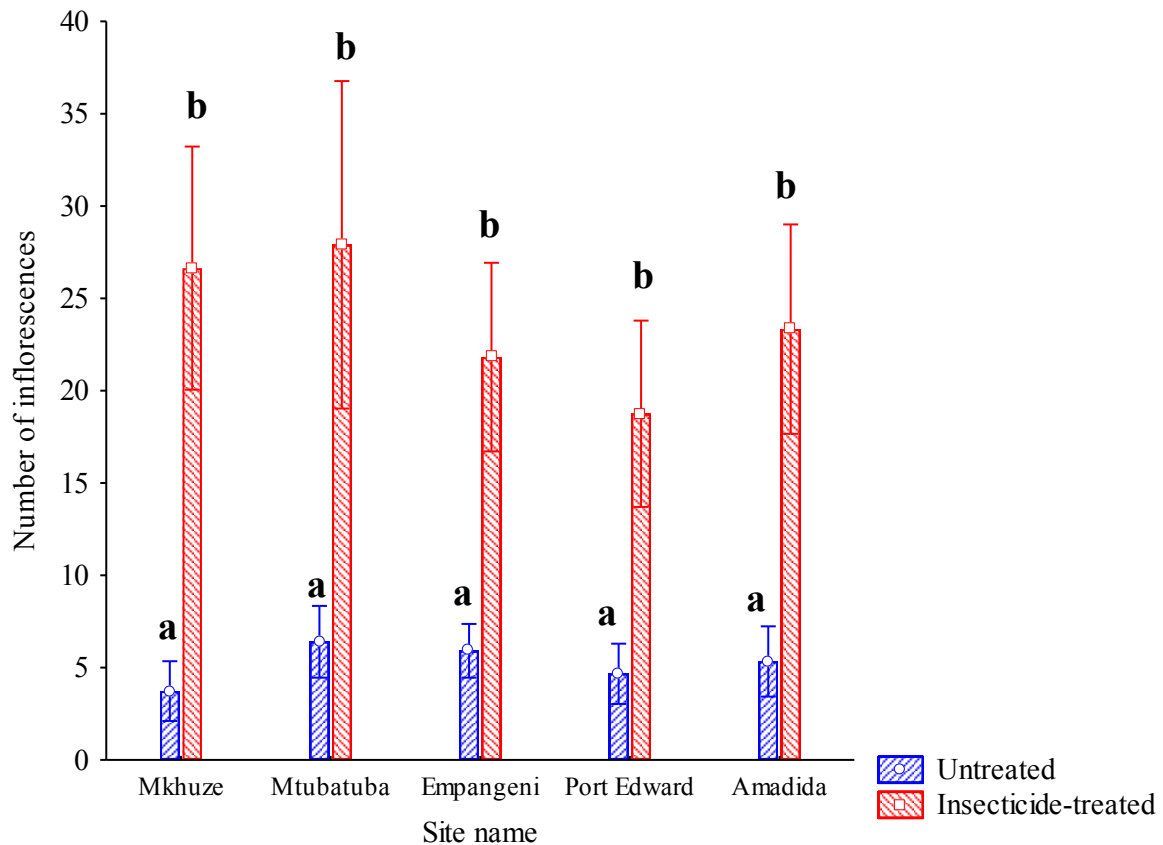
Fig. 2.3. Number of inflorescences (mean ± SE) produced by the *Aceria lantanae*-infested (untreated control) and insecticide-treated *L. camara* plants at five study sites in KwaZulu-Natal province. Means of the test pairs that are followed by different letters are significantly different (P < 0.05; Mann-Whitney U-tests).

Seed production by *A. lantanae*-infested lantana stands was significantly lower (P < 0.001) than that of the insecticide-treated stands at all five study sites in KwaZulu-Natal province (Table 2.5). The exclusion of *A. lantanae* by insecticidal application thus had a major impact on seed production, with substantial increases in mite-free plants. Overall, seed production declined by some 94% in mite-infested plants compared to mite-free plants, with only 1.02 seeds per inflorescence on infested inflorescences compared to 16.8 on the chlorpyrifos-treated plants (Table 2.5). Furthermore, seed production declined by 92%, 93%, 94%, 96% and 96% at the Mtubatuba, Amadida, Port Edward, Empangeni and Mkhuze study sites, respectively.

Table 2.5. Number of seeds (mean ± SE) produced by the insecticide-treated and *Aceria lantanae*-infested *L. camara* plants at five sites in KwaZulu-Natal province.

Site name	Mean (± SE) number of seeds produced		Statistics*		
	Treated	Untreated	DF	Test	P- value
Mkhuze	16.67 ± 0.63	0.67 ± 0.23	52	$t = 23.77$	**< 0.001**
Mtubatuba	16.37 ± 0.50	1.37 ± 0.34	58	$t = 24.81$	**< 0.001**
Empangeni	17.74 ± 0.65	0.80 ± 0.24	68	$t = 24.43$	**< 0.001**
Port Edward	16.76 ± 0.53	1.08 ± 0.26	72	$t = 26.57$	**< 0.001**
Amadida	16.33 ± 0.54	1.15 ± 0.29	64	$t = 24.68$	**< 0.001**
Overall samples	16.80 ± 0.46	1.02 ± 0.12	322	$t = 55.27$	**< 0.001**

* DF: Degrees of freedom; statistically significant P-values are highlighted in bold.

2.4.   DISCUSSION

Generally, the establishment of a biological control agent in its introduced range depends on the suitability of several biotic and abiotic factors, and these affect the development, longevity and fecundity of an agent (Crawley 1989; Byrne *et al*. 2002, 2003; May & Coetzee 2013). Results of the current study showed that the biological control agent *A. lantanae* established at 58.6% of the release sites in the Limpopo, Mpumalanga, Gauteng and KwaZulu-Natal provinces of South Africa. Where establishment on *L. camara* occurred, infestation levels varied from site to site and from province to province. Although *A. lantanae* caused significant reductions in inflorescence and seed production of susceptible lantana varieties at controlled experimental sites, overall establishment and infestation levels at the different release sites were sporadic and appeared to be variety-specific.

Because the native range (Florida, USA) of *A. lantanae* incorporates a humid subtropical climate (Duryea & Kampf 2007), it was assumed that the mite would become established under similar climatic conditions in South Africa, notably the humid coastal region of KwaZulu-Natal. However, the mite has become established over a wide range of climatic conditions in South Africa, including the dry inland regions of Limpopo, Gauteng, Mpumalanga and the humid coastal region of KwaZulu-Natal. The performance of *A. lantanae* varied from site to site within the same eco-climatic region, which contradicts the general assumption that the establishment and performance of a biological control agent is largely influenced by climatic variability in its introduced range (Cilliers & Neser 1991; McClay 1996; Impson *et al*. 1999; Broughton 2000).

It has been argued that the principal factor influencing lantana biocontrol is that the plant constitutes a hybrid species consisting of many genotypes, originating from two or more species of lantana in tropical America, and that it grows in a wide range of climatic areas, making it impossible for a single biological control agent to adapt to these varied conditions (Broughton 2000; Day & Neser 2000). Hybridization led to the development of over 40 varieties of lantana with different genetic make-ups, chemical composition, volatile compounds and physiological characteristics, which influence the ability of biological control agents to track these resources

(Graaff 1986; Raghu *et al*. 2005; van Klinken *et al*. 2009). Indeed, variability amongst lantana varieties has contributed towards the establishment failure or poor performance of some lantana agents (Broughton 2000; Baars & Heystek 2003; Simelane 2006a; Urban *et al*. 2011; Mpedi & Simelane 2014). It is very likely that the resistance of some lantana varieties is behind the sporadic establishment pattern of *A. lantanae* throughout the distribution range of lantana, as observed during the current study. The establishment pattern and performance of *A. lantanae* is consistent with that of the broom gall mite *Aceria davidmansoni*, which established at 32% and 50% of the release sites in Australia and New Zealand, respectively, where the occurrence of different broom (*Cytisus scoparius* L.) forms or unsuitable climatic conditions appeared to be the key factors affecting its establishment and performance (Sagliocco *et al.* 2011; Xue *et al.* 2015).

Parasitism and predation by native organisms often hinder the establishment, population build-up and general performance of biological control agents (van Klinken & Flack 2008; Byrne *et al*. 2011; Cakmak & Cobanoglu 2012; Egli & Olckers 2012; Sharratt & Olckers 2012; Kamburgil & Cakmak 2014). An unidentified predatory mite species was observed to attack *A. lantanae* on several occasions in quarantine, resulting in the demise of the mite culture (A. Urban, pers. comm. 2014). There is thus the possibility that predation of *A. lantanae* by the same or a similar mite species may be similarly severe under field conditions, resulting in poor establishment and performance of the mite at some sites in South Africa. Studies by Kamburgil & Cakmak (2014) also showed that heavy predation of the predatory mite *Cheletomimus bakeri* (Ehara) (Acari: Cheyletidae) reduced its efficiency as a biological control agent of the agricultural pest *Tetranychus cinnabarinus* Boisduval (Acari: Tetranychidae) in Turkey. Broughton (2000) reported that certain lantana biological control agents, namely *Octotoma scabripennis* Guérin-Méneville and *Uroplata giradi* Pic. (both Coleoptera: Chrysomelidae) were parasitized by generalist natural enemies in Australia and South Africa, thereby reducing their efficacy in these countries. Similarly, Sharratt & Olckers (2012) found that several native parasitoid species attacking the seed-feeding beetle *Acanthoscelides macrophthalmus* (Schaeffer) (Chrysomelidae: Bruchinae) were hampering the ability of the beetle to control *Leucaena leucocephala* (Lam.) de Wit (Fabaceae) in South Africa.

Studies have suggested that seed-feeding agents need to reduce seed production of their target weeds by some 95-99 % annually (*e.g.* Hoffmann & Moran 1998; Kriticos *et al.* 1999; van Klinken *et al.* 2008), so as to limit the number of viable seeds entering the seed bank and allow the proliferation of plant competitors to suppress seedling recruitment (Andersen 1989). Models by van Klinken *et al.* (2009) showed that even lower seed predation rates are able to aid in population regulation of the host plant, especially for poor seed dispersers. The current studies revealed that seed or inflorescence reduction in *A. lantanae*-infested stands exceeded 95% at some sites and experimental plots, which could be sufficient to regulate weed populations, provided that all varieties of lantana present are equally susceptible to the mite. Although population modeling and field observations suggest that 95% seed reduction by seed-feeding agents could be sufficient to regulate weed populations (Myers & Risling 2000; Sheppard *et al.* 2002; Buckley *et al.* 2005; van Klinken *et al.* 2009), only a small proportion of South African lantana varieties were susceptible to the mite while some were totally resistant (Urban *et al.* 2003), resulting in poor establishment and performance of the mite at some sites in South Africa. Whilst *A. lantanae* had a direct impact on flowering and seed production, the reduction in reproductive output of unsprayed lantana stands was likely due to the combined herbivore pressure of several lantana biocontrol agents, as these stands supported higher numbers of insect herbivores, and therefore did not attain full growth. Nonetheless, the results of this study are consistent with those of Waloff & Richards (1977) who found that seed production by unsprayed *C. scoparius* plants, over the weed's average 10-year life span, was reduced by 75% compared to that of the chemically-treated plants.

In South Africa, *A. lantanae* has dispersed widely within the distribution range of *L. camara*, with dispersal rates ranging from 6.0 to 40.6 km per annum. The dispersal rate of *A. lantanae* was 3 to 18-fold higher than that of the eriophyid mite *Aculus hyperici* which was introduced for the biological control of St John's wort, *Hypericum perforatum* L. (Hypericaceae) in south-eastern Australia (Mahr *et al.* 1999). However, the dispersal rate of *A. lantanae* appeared to be two times slower than that of *A. davidmansoni* (40.6 to 83.3 km per annum) which was released against Scotch broom in New Zealand (Paynter *et al.* 2012). The dispersal rate of *A. lantanae* in the inland areas of Limpopo and Mpumalanga was much lower than that along the coastal areas of KwaZulu-Natal province. These findings may be consistent with the study by Edwards *et al.* (1999), which

suggested that strong winds along the coastal areas enhance the dispersal capabilities of eriophyid mites. Because *A. lantanae* has dispersed widely, covering much of the distribution range of lantana in the Limpopo, Mpumalanga, KwaZulu-Natal, Gauteng and Eastern Cape provinces of South Africa (Magoba 2013), further releases or re-distribution of the mite are no longer advisable.

Although *A. lantanae* has established and spread widely within the distribution range of *L. camara* in South Africa, only a small proportion of the lantana varieties that occur in these provinces appeared to be susceptible to the mite. It is therefore likely that the establishment and performance of *A. lantanae* in South Africa is influenced by climatic unsuitability and/or varietal resistance and these have been investigated further in Chapters 3 and 4. However, other factors such as the recruitment of native parasitoids and predators by *A. lantanae* cannot be ruled out.

CHAPTER THREE

Effect of climatic conditions on the performance of the flower-galling mite *Aceria lantanae* on inflorescences of *Lantana camara* L. in South Africa

Abstract

Various factors can influence the population dynamics of biological control agents after their introduction into the new range, of which climate is fundamental. Therefore, a biological control agent whose native climatic range is similar to that of its introduced range has a greater chance of establishing. The flower galling mite *Aceria lantanae*, which is native to Florida (USA), was released as a biological control agent against *Lantana camara* in South Africa in 2007. This study was carried out to assess the effect of climatic conditions on the establishment and performance of *A. lantanae* in South Africa. The climate-matching programme CLIMEX was used to predict and broadly map areas in Africa that are climatically suitable for the performance of *A. lantanae.* Regression analysis was also used to determine the relationship between each of four key climatic factors (i.e., elevation, temperature, rainfall and relative humidity) and *A. lantanae* infestation levels at sites located in the Lowveld, Middleveld and Highveld regions of Limpopo, Mpumalanga, Gauteng and KwaZulu-Natal provinces in South Africa. The CLIMEX model predicted that the climatic conditions for *A. lantanae* would range from suitable to highly suitable within the distribution range of *L. camara* in southern Africa. Although not statistically significant, there was a slight decline in *A. lantanae* infestation levels with increasing elevation and annual rainfall. Infestation levels were somewhat higher at sites receiving between 600 and 1000 mm of rain per year, and decreased slightly as the annual rainfall exceeds 1000 mm. Infestation levels were slightly higher in the Lowveld and Middleveld than in the Highveld regions. This study also found that infestation levels of *A. lantanae* were not related to either temperature or relative humidity. Whilst the current distribution of *A. lantanae* in South Africa falls within that broadly predicted by CLIMEX, the levels of establishment and infestation varied considerably from site to site, and

these appear to be determined by the variety of *L. camara* present at the site, rather than local climatic conditions.

Keywords: *Aceria lantanae* performance, altitude, temperature, relative humidity, rainfall, weed biocontrol.

3.1. INTRODUCTION

Success of a biological control programme depends on the ability of biological control agents to establish and spread widely over the distribution range of the target weed (Boughton & Pemberton 2008, 2011; Paynter *et al*. 2012). Climatic conditions are among the most important factors that influence the establishment and interaction of biological control agents with their host plants (Cilliers & Neser 1991; Broughton 2000; McEvoy & Coombs 2001; Bale *et al.* 2002; Simelane & Phenye 2004). However, biotic factors such as release sizes and weed varietal resistance have also been reported to influence the establishment and performance of some biological control agents (Simelane & Phenye 2004; Simelane 2006a, b).

Broad climatic tolerances by invasive alien plants such as *L. camara* promote wider geographic distribution in both their native and new ranges (Alpert *et al*. 2000; Day *et al*. 2003). However, the distribution of specialised phytophagous insects, mites or pathogens may be widespread or confined to certain climatic regions, depending on their climatic requirements (Palmer & Pullen 1995). Biological control agents that are restricted to narrow native climatic ranges could struggle to establish or perform in their new range (Dhileepan *et al.* 2005). Therefore, the matching of climatic conditions between the agent's native range and its introduction range is crucial in predicting where the agent is likely to establish and become effective in controlling the target weed (McEvoy & Coombs 2001; Byrne *et al*. 2003; May & Coetzee 2013). The climate-matching programme CLIMEX is a widely used tool for modelling the potential distribution of an organism in its country of introduction by inferring the new geographical range based on eco-climatic characteristics of locality records from the native range (CLIMEX Version 3 software package).

Amongst various climatic factors, temperature plays a crucial role in the biological control agent's biology and developmental time, degree of interaction with or damage to its host plant species and ultimately its establishment in the introduced range (Crawley 1989; Byrne *et al*. 2002; Dhileepan *et al*. 2005, 2010; May & Coetzee 2013). Unsuitable temperature is one of the main climatic factors that contribute to the failure of biological control agent establishment (McClay & Hughes 1995; McClay 1996; McEvoy & Coombs 2001; Byrne *et al*. 2002; McClay & Hughes

2007). Cold stress in the Highveld regions of South Africa increased the mortality, decreased the fecundity, and prolonged the developmental and diapause periods of some introduced biocontrol agents (Cilliers & Hill 1996; McClay 1996; Byrne *et al*. 2002). Unfavourable temperatures retard the egg hatchability, developmental rate, survival and fecundity of biological control agents (Byrne *et al*. 2003; Simelane 2007; Smith 2014). Knowledge of critical thermal minimum (CT_{min}) and maximum (CT_{max}) temperatures, lethal temperatures (LT_{50}) and lethal humidity, together with climate matching, enhances predictions on the likelihood of agent establishment (Byrne *et al*. 2002, 2003; May & Coetzee 2013; Smith 2014). Unfortunately, thermal studies are often conducted after biological control agents have been released into their new range to account for possible constraints that are believed to have hampered their establishment or poor performance (Coetzee *et al*. 2007). Release of ineffective agents is not only a waste of resources but also poses an unnecessary risk to non-target plant species (McEvoy & Coombs 2001; May & Coetzee 2013).

Altitude, rainfall and atmospheric humidity have also been reported to influence the establishment and population growth of some biological control agents (Moran *et al*. 1987; Hill *et al*. 1993; Norris *et al*. 2002; Simelane & Phenye 2004; Simelane 2007; Nohisham *et al*. 2013). For example, establishment of the lantana herringbone leaf-miner *Ophiomyia camarae* Spencer (Diptera: Agromyzidae) was restricted to elevations below 900m in South Africa (Simelane & Phenye 2004). Rain during, or immediately after, an agent's release could displace vulnerable stages of some agent species, thereby reducing their chances of establishment (Crawley 1987; Moran *et al*. 1987; Moran & Hoffmann 1987; Weisser *et al*. 1997; Norris *et al*. 2002). For example, heavy rains destroyed cochineal insect agents of *Opuntia* spp. weeds (Cactaceae) by dislodging the immobile females and nymphs from their host (Moran *et al*. 1987; Moran & Hoffmann 1987). Although rainfall has been implicated as a reason for the failure of several biological control programmes, pre-release studies rarely include the effect of rainfall during the selection of potential biological control agents (Moran *et al*. 1987; Norris *et al*. 2002). Atmospheric humidity is also known to influence the physiology, development and reproduction of biological control agents (Simelane 2007; Nohisham *et al*. 2013). After prolonged dehydration, egg hatch and larval development are retarded while high humidity often promotes infections of agents by parasitic pathogens (Nohisham *et al*. 2013).

The current study is thus aimed at evaluating the effect of key climatic factors (elevation, temperature, rainfall and relative humidity) on the performance of the flower-galling mite *A. lantanae* at various field sites in the Limpopo, Mpumalanga, Gauteng and KwaZulu-Natal provinces of South Africa.

3.2. MATERIALS AND METHODS

3.2.1. Study sites

The study was conducted at a total of 46 sites (17 of those being release sites) located near 24 towns in Limpopo, Mpumalanga, Gauteng and KwaZulu-Natal, South Africa. The performance of *A. lantanae* [percentage of inflorescences that were infested by *A. lantanae* (PII)] was recorded from a minimum of 10 individual lantana bushes, once per season at each study site during 2013 and 2014 (see Chapter 2). Meteorological data for these study sites was supplied by the Institute of Soil, Climate and Water of the Agricultural Research Council (ARC-ISCW), and some data were obtained from the CLIMEX 3.0 software (Sutherst & Maywald 1985, 2005; Sutherst *et al*. 2007).

3.2.2. Predicting the potential distribution of *A. lantanae* in Africa

Climatic parameters for 17 different release study sites in South Africa (Table 3.1) and the original collection site (Miami, Florida, USA) were obtained from the CLIMEX 3.0 software (Sutherst & Maywald 2005; Sutherst *et al*. 2007). Long-term monthly meteorological data [i.e., temperatures (T_x: maximum, T: average and T_n: minimum), rainfall, and relative humidity (RH_x: maximum and RH_n: minimum)] pertaining to these sites were summarized (Table 3.1) and matched with the collection site in Miami to predict the distribution range of *A. lantanae* in Africa. CLIMEX was then used to map areas in Africa that are climatically suitable for *A. lantanae.* In addition, altitude was recorded for the 17 release sites in South Africa and the collection site in the native region (Table 3.1).

Table 3.1. Mean monthly meteorological data at 17 selected study (release) sites in South Africa and at the original *A. lantanae* collection site in Miami, Florida (USA).

Study sites	Elevation (m)	Temperatures (°C)			Relative Humidity %		Rainfall (mm)
		T_x	T	T_n	RH_n	RH_x	
Miami (Florida, USA)	72	28.35	24.45	20.24	61.71	79.73	27.69
Wesfalia/ Magoebaskloof (LP)[a]	817/ 825	13.92	20.28	26.63	49.92	76.21	14.67
Brondal (MP)[a]	1252	10.04	16.7	23.35	50.38	78.46	17.25
Richards Bay (KZN)[a]	35	16.33	21.28	26.24	59.1	81.9	21
Pietermaritzburg (KZN)[a]	1028	10.28	16.56	22.86	53.37	81.42	17
Hillcrest/ Tongaat (KZN)[a]	598/ 577	14.08	19.28	24.47	58.63	83	18.25
Umkomaas/ Amanzimtoti (KZN)[a]	112/ 96	15.74	19.93	24.12	64.02	84.73	20.38
Sappi Beamer (KZN)[a]	414	20.05	25.62	31.19	52.85	74.83	26.29
Bhubha (LP)[b]	679	14.51	20.9	27.29	48.54	74.1	15.02
Bundu (MP)[b]	743	13.5	20.35	27.21	49.23	77.62	13.56
Mtubatuba (KZN)[b]	80	16.87	21.76	26.66	60.15	83.48	22.04
Wonderboom (GP)[b]	1263	10.75	17.84	24.93	43.87	70.87	12.98
Constantia (LP)[b]	439	15.42	21.91	28.38	48.71	74.54	11.52
Tzaneen (LP)[b]	678	13.92	20.28	26.63	49.92	76.21	14.67

[a]*Aceria lantanae* was not observed for more than a year after its introduction.
[b]*Aceria lantanae* persisted for more than a year (12 months) after its introduction.
State: USA= United States of America. Provinces, LP= Limpopo; MP= Mpumalanga; GP= Gauteng; KZN= KwaZulu-Natal.

3.2.3. Effect of altitude on the performance of *A. lantanae*

Altitude was recorded at each of the 46 study sites, and these were further categorized into three ecological regions (Loffler & Loffler 2005; Magagula 2010) namely, the Lowveld, Middleveld and Highveld. Localities within the altitude ranges of <400 m, 401- 900 m and 901- 1800 m were classified as Lowveld, Middleveld and Highveld regions, respectively. Mite infestation levels (PII) were recorded at each of the study sites during the peak infestation period for *A. lantanae*. Linear regression was used to determine the relationship between elevation and infestation levels. One-way analysis of variance (ANOVA) was used to determine whether there were significant differences in establishment rates of *A. lantanae* between the three different ecological regions of South Africa.

3.2.4. Effect of temperature, relative humidity and rainfall on the performance of *A. lantanae*

Climatic parameters, including mean monthly temperatures [T_x, T and T_n], rainfall and relative humidity [RH_x and RH_n], were supplied by the ARC-ISCW. Linear regression was also used to determine the relationship between each of these six climatic parameters and the infestation levels of *A. lantanae* at each of the 46 study sites. Monthly temperature and relative humidity data were averaged into the different seasons to synchronize them with the target plant's flowering periods whilst annual rainfall data were used in the analysis.

3.3. RESULTS

3.3.1. Predicting the potential distribution of *A. lantanae* in Africa

The model projections coincide with the current distribution of *L. camara* in South Africa (see Fig. 1.1). The CLIMEX model predicted that climatic conditions in southern Africa will range from suitable to highly suitable for *A. lantanae* establishment. Interestingly, the sub-Saharan African region, particularly, the western and central African regions, appear to be more suitable for *A. lantanae* performance than the southern African regions (Fig. 3.1).
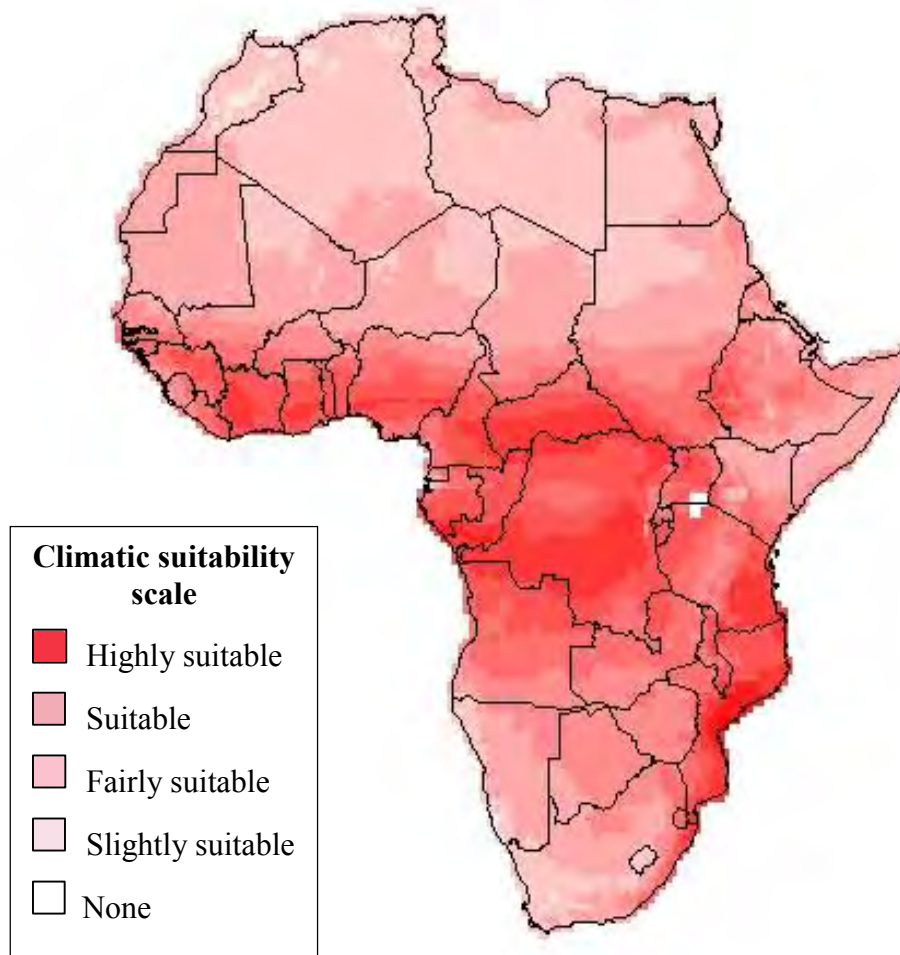
Fig. 3.1 The predicted distribution of *Aceria lantanae* in Africa using CLIMEX.

3.3.2. Relationship between altitude and performance of *A. lantanae*

Aceria lantanae has become established over a wide range of altitudes, ranging from 32m to 1,397m above sea level. There was a negative relationship between *A. lantanae* infestation levels and elevation, although this was weak and not statistically significant (Fig. 3.2). Infestation levels in each of the three ecological regions (i.e. Lowveld, Middleveld and Highveld) varied from zero to at least 83.7%. Although not statistically significant ($F_{(2, 43)}$ = 0.5681; P = 0.5708), mean infestation levels were somewhat higher in the Lowveld and Middleveld regions than in the Highveld region (Table 3.2), suggesting some effect of altitude.
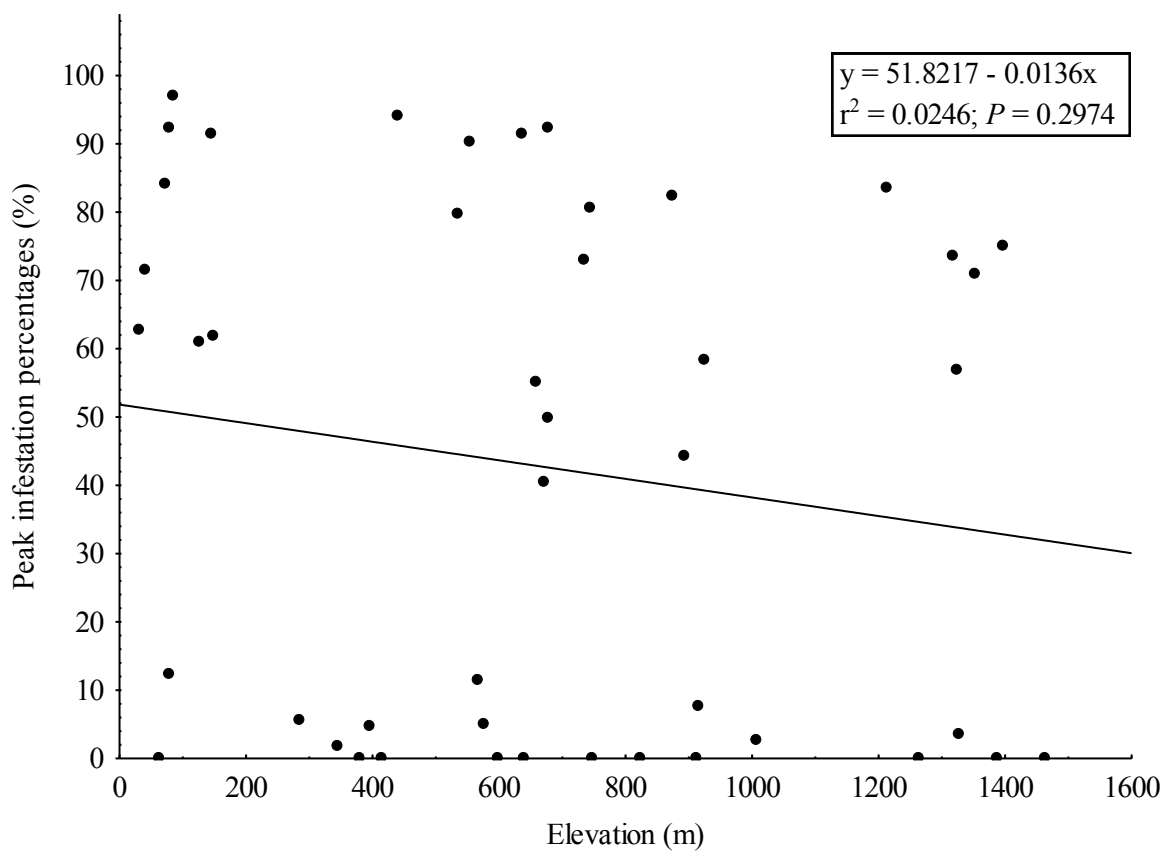
Fig. 3.2 Relationship between the peak infestation percentages of *Aceria lantanae* and elevation.

Table 3.2. Infestation percentages of *A. lantanae* in three ecological zones (Lowveld, Middleveld and Highveld) in Limpopo, Mpumalanga, Gauteng and KwaZulu-Natal provinces.

EcoZone	Elevation (m)	Number of study sites	Infestation percentage (%)	
			Range	Mean (±SE)[b]
Lowveld	1-400	14	0 – 97.0	46.2 ± 10.6
Middleveld	401-900	19	0 – 94.0	46.8 ± 08.8
Highveld	901-1800	13	0 – 83.7	33.3 ± 09.9
Overall	1-1800	46	0 – 97.0	42.8 ± 05.6

[b]Infestation levels between the ecological regions did not differ significantly ($P > 0.05$).

3.3.3. Relationship between temperatures and performance of *A. lantanae*

There was no significant relationship ($P > 0.05$) between infestation levels of *A. lantanae* and any of the three temperature criteria [i.e. maximum (T_x), average (T) or minimum (T_n)] (Fig. 3.3). Infestation percentages that varied from zero to 90% were recorded over a wide range of temperatures with high infestation levels (i.e. above 60%) recorded at both low (±5.10°C) and high (±30.05°C) temperatures. Despite this trend, mild to high temperatures (24°C to 30°C) appeared to be more favourable for the mite (Fig. 3.3 a-c), which coincides with the temperature range at the original collection site (Miami, USA) (see Table 3.1).
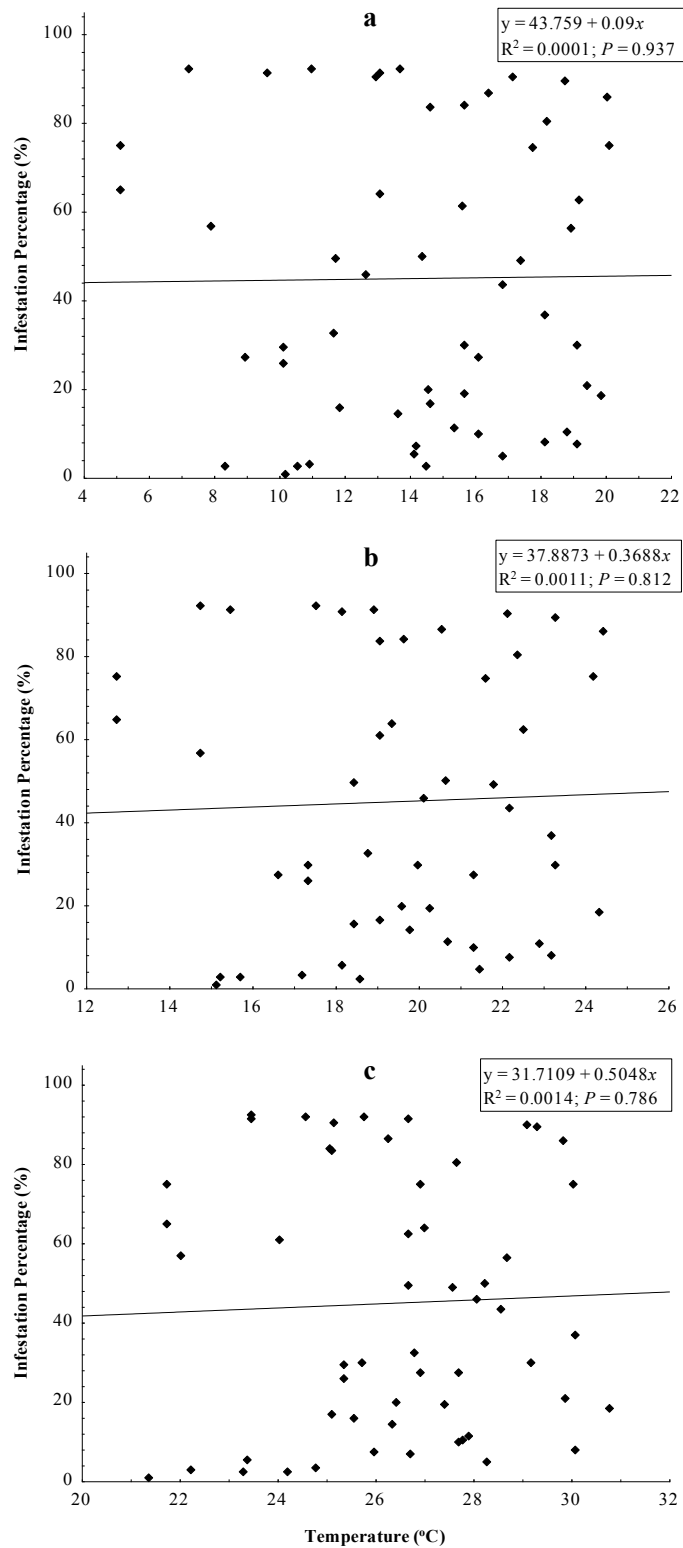
Fig. 3.3. Relationship between *Aceria lantanae* infestation percentages and temperature [minimum-T_n (a), average -T (b) and maximum -T_x (c)].

3.3.4. Relationship between rainfall and performance of *A. lantanae*

There was a negative relationship between *A. lantanae* infestation levels and the amount of rainfall received per year (Fig. 3.4), although this was also weak and not statistically significant ($P > 0.05$). Infestation percentages that ranged from zero to higher than 90% were recorded at study sites that received between 689.4 mm and 1323.9 mm of rain per annum. Despite the lack of a significant relationship, very high infestation levels (above 90%) were recorded in areas receiving between 600 and 1000 mm of annual rainfall (Fig. 3.4). Whilst this might have been caused by factors other than rainfall, no *A. lantanae* infestations were recorded at two sites where the annual rainfall received was greater than 1323.9 mm.
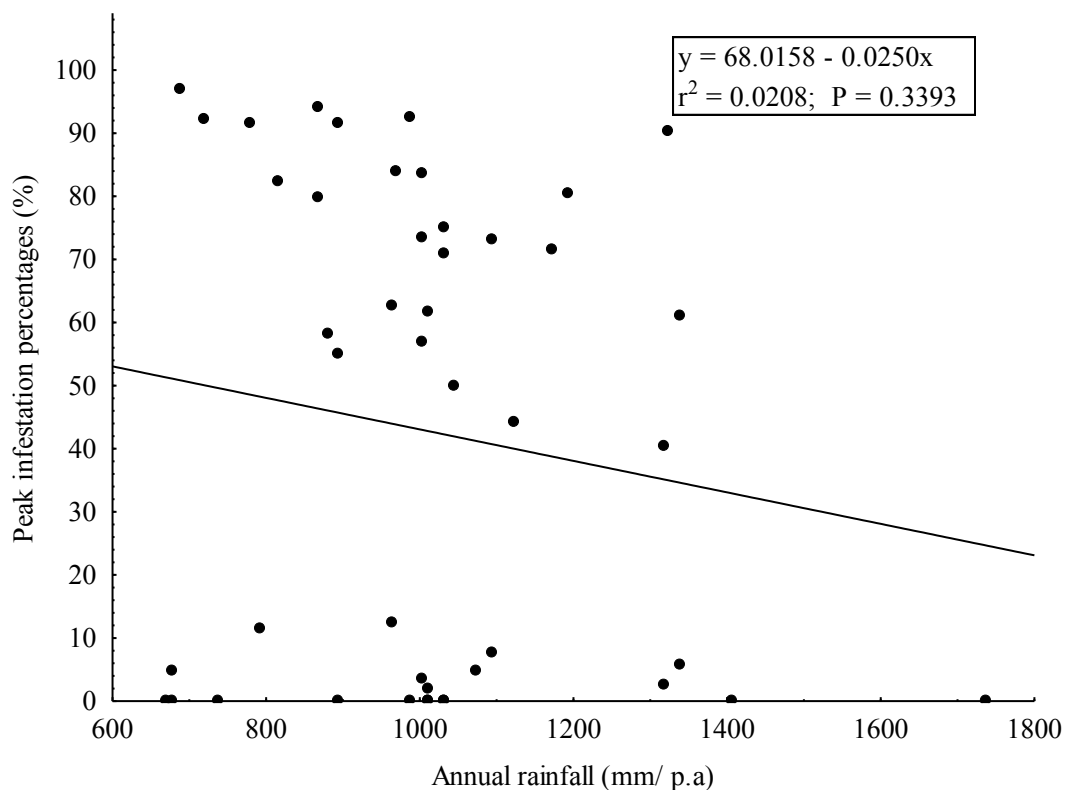
Fig. 3.4. Relationship between *Aceria lantanae* infestation percentages on *Lantana camara* plants and the annual rainfall (mm/ p.a.), at sites in Limpopo, Mpumalanga, Gauteng and KwaZulu-Natal.

3.3.5. Relationship between relative humidity and performance of *A. lantanae*

Although there were positive relationships between *A. lantanae* infestation levels and both minimum (RH_n) and maximum (RH_x) relative humidity (Fig. 3.5), these were similarly weak and not statistically significant ($P > 0.05$). Infestations ranging from 75% to 95% were recorded at relative humidity levels ranging from 23% to 95% (Fig 3.5a, b).
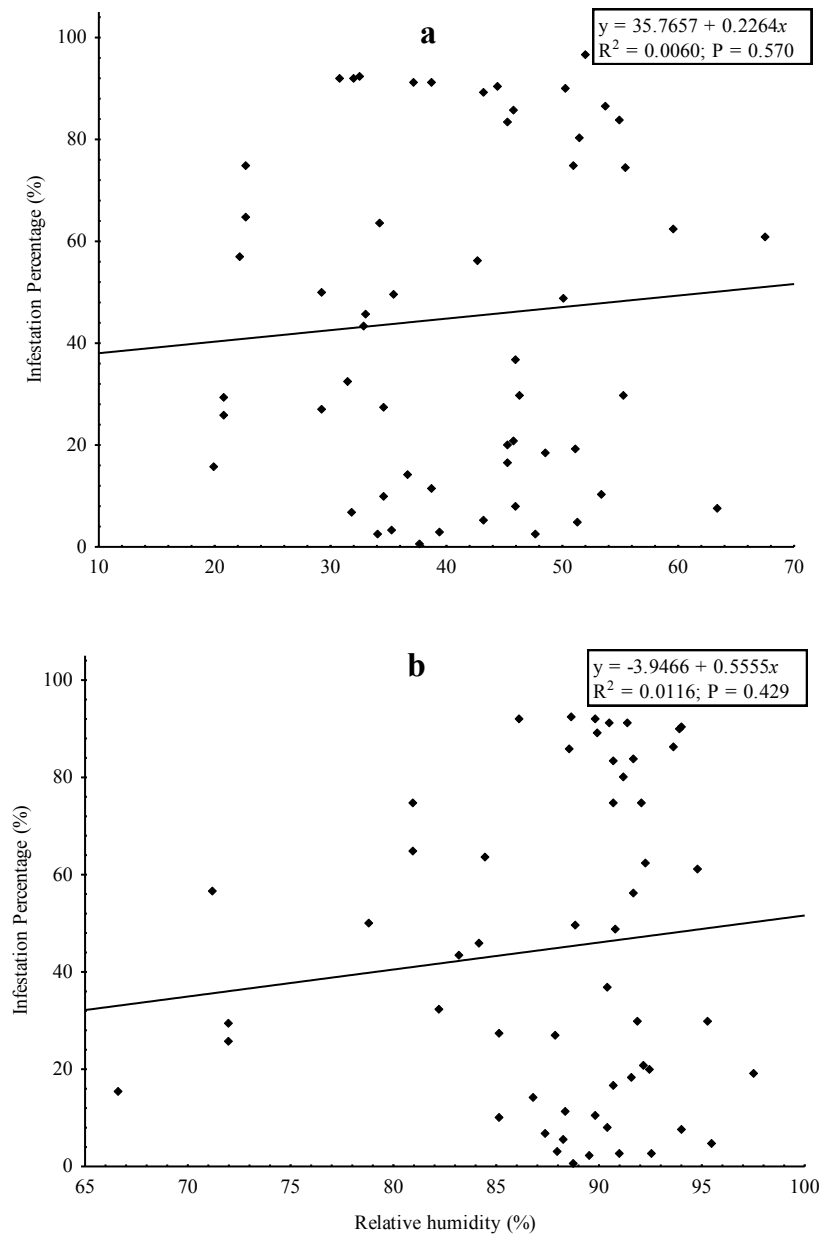
Fig. 3.5. Relationship between *Aceria lantanae* infestation percentages on *Lantana camara* plants and relative humidity [a: minimum (RH_n) and b: maximum (RH_x)], at sites in Limpopo, Mpumalanga, Gauteng and KwaZulu-Natal.

3.4. DISCUSSION

In weed biological control programmes, establishment failures or poor performance of biological control agents are often associated with climatic incompatibility (Winder *et al.* 1984; Neser & Cilliers 1990; Cilliers & Neser 1991; McClay 1996; Baars & Neser 1999; Broughton 2000; Day & Neser 2000; Simelane & Phenye 2004). However, the current study found no significant relationship between the performance of *A. lantanae* and any of the climatic factors that were assessed. This supports the CLIMEX prediction that the flower-galling mite is likely to establish widely throughout the distribution range of *L. camara* in southern Africa.

The current distribution of *A. lantanae* in South Africa coincides roughly with that of *L. camara*. Interestingly, the sub-Saharan tropical region and the humid east coast of South Africa appear to be more suitable than the inland regions of South Africa, and yet the mite has established widely within a broad spectrum of ecological regions in this country. In fact, *A. lantanae* is one of the few lantana biological control agents to have established widely in the Lowveld, Middleveld and Highveld regions, while the majority of lantana agents are largely confined along the humid east coast of the country (Urban *et al.* 2011). Welton & Swenson (1962) also observed that eriophyid mites displayed broad geographic tolerance, presumably because of their protection within the gall tissues. The somewhat narrower distribution range predicted by CLIMEX is presumably due to the limited meteorological data (based on the single collection site) from the native range of *A. lantanae*.

The lack of significant relationships between the performance of *A. lantanae* and any of the climatic factors is in contrast to the majority of lantana biocontrol agents which are largely confined to the warm and humid Lowveld regions of South Africa (Urban *et al.* 2011). For example, the herringbone leaf miner, *Ophiomyia camarae* Spencer (Diptera: Agromyzidae) was restricted to lower altitudes not exceeding 900m (Simelane & Phenye 2004) while the root-feeding flea beetle *Longitarsus bethae* Savini & Escalona failed to establish in the high altitude areas of Gauteng and North West provinces (D.O. Simelane, pers. comm. 2015). In other biological control systems, climatic factors also influenced the population dynamics of biological control agents. For example, parasitism levels of the agricultural pest *Plutella xylostella* L. (Lepidoptera: Plutellidae) were positively related to average weekly temperatures (Nofemela 2010, 2013). Survival of the gorse spider mite *Tetranychus lintearius* (Dufour), a

biological control agent of gorse (*Ulex europaeus* L.: Fabaceae), was significantly greater in indoor experimental plots than in outdoor plots that were exposed to direct rainfall (Hill *et al.* 1993, 2000). The cochineal insect *Dactylopius opuntiae* (Cockerell) and thrips *Sericothrips staphylinus* Haliday (Thysanoptera: Thripidae) are often washed off their hosts during heavy rainfall, thereby reducing their population densities (Moran & Hoffmann 1987; Norris *et al.* 2002). To prevent desiccation and increase hatchability, eggs of the tortoise beetle *Gratiana spadicea* (Klug) (Coleoptera: Chrysomelidae), root-feeding flea beetle *L. bethae* and bamboo borer *Dinoderus minutus* Fabricius must be maintained at high relative humidity levels during their incubation periods (Woods & Singer 2001; Byrne *et al.* 2002; Simelane 2007).

The lack of climatic influence on the establishment and performance of *A. lantanae* is not surprising given its sporadic pattern of establishment throughout the invaded ecological regions of South Africa. Within the same ecological region, establishment rates varied considerably, suggesting that factors other than climate might be affecting the performance of the mite. It therefore seems probable that the particular variety of *L. camara* is the key factor that influences the establishment and performance of *A. lantanae* in South Africa. *Aceria lantanae* reduced the flower production of *L. camara* by zero to 96% under quarantine conditions, depending on the lantana variety involved (Mpedi & Urban 2003; Urban *et al.* 2004), and this is consistent with the infestation levels recorded at various sites in South Africa (Chapter 2). The intractability of *L. camara* to biological control is because it comprises an array of alloploids, which provides the genetic capability not only for hybrid growth and reproductive vigour, but also the production of a broad spectrum of defensive allelochemicals that confer a degree of resistance to most biocontrol agents (Radunz 1971; Taylor 1989; Urban *et al.* 2003; Simelane 2006a; Urban *et al.* 2011). The influence of varietal resistance on the performance of *A. lantanae* was thus investigated separately (see Chapter 4).

CHAPTER FOUR

Varietal preferences of *Aceria lantanae* (Cook), a biological control agent for *Lantana camara* L.

Abstract

Compatibility between the flower-galling mite *Aceria lantanae* and the different varieties of its target weed, *Lantana camara*, are believed to influence its establishment and effectiveness as a biocontrol agent in South Africa. Field trials were conducted to measure the susceptibility of the more common lantana varieties to *A. lantanae*. Mite infestations differed significantly amongst the 10 selected varieties. Highly preferred lantana varieties included 017 Orange Red, 021 White Pink and 018 Dark Pink, with infestations ranging from 50.4% to 61.2%. Those which were moderately attacked by *A. lantanae* included 163 Light Pink, 021 Total Pink, 165 Light Pink, 015 Yellow White, 021 Pink and 015 White Yellow varieties, with infestations ranging from 7.8% to 21.4%. Variety 010 Dark Pink was completely rejected by the mite, with no infestations recorded during the study period. Furthermore, regression analysis showed that neither plant size nor inflorescence density affected *A. lantanae* infestation levels. However, there was a significant increase in *A. lantanae* infestation, with infestation by other lantana biocontrol agents. These data support the contention that varietal resistance and not climatic factors is the major determinant of the efficacy of *A. lantanae*. Variable susceptibility to the mite by the different varieties explains the sporadic establishment and varying infestation levels of *A. lantanae* observed throughout the distribution range of *L. camara* in South Africa.

Keywords: *Aceria lantanae*, lantana varieties, inflorescence production, varietal resistance, weed biocontrol.

4.1. INTRODUCTION

Generally, substantial intraspecific variation in chemistry and architecture occurs within plant species, and variability in these traits could create a certain degree of susceptibility or resistance to herbivores. Hybridization of *Lantana camara* L. (Verbenaceae) performed during the development of new genotypes with colourful inflorescences for horticultural purposes, involved the mixing of genetic traits that improved the plant's aggressiveness to extreme environmental conditions (Oosthuizen 1964; Stirton 1977; Gentle & Duggin 1997). As a result, over 40 hybrids of *L. camara* with similar morphological characteristics, but differing visibly in inflorescence colour, spininess, and hairiness of the stems or leaves, were developed in South Africa (Smith & Smith 1982; Spies & Stirton 1982; Graaff 1986). Consequently, lantana genotypes occurring in South Africa and other countries where hybridization was practiced do not match genetically with any of the *L. camara* genotypes that occur naturally in the native range in tropical and subtropical South and Central America (Howard 1969; Swarbrick *et al.* 1998; Day & Neser 2000).

Hybridization also alters the chemical composition, genetic diversity, and physiological and morphological traits of plants, which form their baseline defensive structures against natural enemies (Smith & Smith 1982; Cilliers 1983; Neser & Cilliers 1990; Cilliers & Neser 1991; Day & Neser 2000). These traits in the newly-developed varieties of *L. camara* have often affected the establishment, development and fecundity of introduced natural enemies that are associated with the parental genotype in the native range (Cilliers 1983; Cilliers & Neser 1991; Baars & Neser 1999). Thus varietal resistance has been widely identified as a key factor affecting the establishment of biological control agents on *L. camara* in various countries (Radunz 1971; Taylor 1989; Baars & Neser 1999; Broughton 2000; Urban *et al.* 2004; Baars & Heystek 2003; Simelane 2006a, b; Baars & Hill 2010). For example, pre-release studies conducted in quarantine found that performance of the root-feeding flea beetle *Longitarsus bethae* Savini & Escalona (Chrysomelidae: Altcinae), leaf-feeding tortoise beetle *Charidotis pygmaea* Klug (Chrysomelidae) and sap-sucking bug *Falconia intermedia* Distant (Miridae) varied among *L. camara* varieties (Williams 2004; Simelane 2006a; Heystek

2006). Although their poor performance in the field may not be attributed to varietal resistance alone, it is likely that this may have contributed to the demise of *F. intermedia* and the low spreading rate of *L. bethae* in South Africa (Heshula 2009; Heshula & Hill 2011, 2012; D.O. Simelane pers. comm. 2015). However, field studies have revealed that several lantana biological control agents such as the leaf-feeding beetle *Octotoma scabripennis* Guër. (Chrysomelidae), leaf-mining fly *Calycomyza lantanae* Frick (Agromyzidae), seed-feeding fly, *Ophiomyia lantanae* Froggatt (Agromyzidae) and leaf-sucking bug *Teleonemia scrupulosa* Stål (Tingidae) are not variety-specific in both South Africa and Australia (Cilliers 1987; Day *et al*. 2003). Furthermore, varietal resistance makes screening of imported natural enemies impractical in quarantine, as the insect cultures might be lost before the host suitability studies are completed (Mpedi & Simelane 2014).

The establishment and sustainability of introduced biocontrol agents on all common lantana varieties is essential for the success of any classical biocontrol programme against the weed (Neser & Cilliers 1990; Cilliers & Neser 1991; Palmer *et al*. 2010). The establishment and performance of *A. lantanae* differed considerably among the release sites located within the same climatic and environmental conditions (Chapter 2), suggesting that some varieties of *L. camara* may be unsuitable for the mite. The current study was therefore conducted to measure the degree of susceptibility of different South African lantana varieties to the flower galling mite, *A. lantanae*. The effect of plant-related factors (i.e., plant size, inflorescence density and degree of infestation by other biocontrol agents) on the level of infestation by *A. lantanae* was also investigated.

4.2. MATERIALS AND METHODS

4.2.1. Collection, propagation and maintenance of plants

The 10 *L. camara* varieties used in the study (Table 4.1) were propagated through cuttings, and the original stocks were collected from nine densely invaded regions in Limpopo, Mpumalanga, Gauteng and KwaZulu-Natal provinces of South Africa. As shown in Table 4.1., selection of these varieties was primarily driven by their morphological characteristics (leaf/stem spininess and hairiness) and the colour of the corollary lobe (i.e., light pink, dark pink, white, orange-red and Hawaii red inflorescences) (Smith & Smith 1982; Baars 2002; Heystek 2006; Heshula & Hill 2011). Actively-developing young shoots of 5 - 15 cm in length were collected from the different parental *L. camara* plants at each site. Except for the apical pair, all leaves were removed and the cut area was immediately dipped into a root growth hormone (Dip and Grow®). Cuttings were then planted into peat cylinders and transported to the ARC-PPRI, Rietondale, where they were cultured on a warm mist-bed until root growth was initiated. After 4 - 6 weeks, the cuttings were transplanted into individual 10-litre pots containing a standardized soil mixture with the ratio of 4: 4: 2 for sand: compost: loam, respectively, plus 16kg vermiculite in every 100 kg of soil mix to enhance its water holding capacity.

Table 4.1. Distinguishing features (morphological and inflorescence colour) among the *L. camara* varieties and the localities from which they were collected.

L. camara varieties	Code	Distinguishing morphological features	Flower colour (young)[a]	Flower colour (mature)[b]	Area/ Province (Grid reference)
Total Pink	021 TP	Leaves broad, rough, hairy; Shoot and Stem hairy with large multiple spines.	Yellow[1]; Violet[2]	Dark Pink[1,2]	Hazyview/ Mpumalanga (25º44.509'S, 030º58.790'E)
Dark Pink	010 DP	Leaves small, tough, hairy; Shoot and Stem hairy with small scattered spines; Stem colour maroon and green with small scattered spines.	Dark yellow[1]; Dark pink[2]	Orange[1]; Dark Pink[2]	Colbyn/ Gauteng (25º44.218'S, 028º14.783'E)
Dark Pink	018 DP	Leaves small, tough, hairy; Shoot and Stem hairy with small scattered spines.	Yellow[1]; Light pink[2] with white ring.	Dark Pink[1,2]	Rietondale/ Gauteng (25°43.688'S, 028°14.216'E)
White Pink	021 P	Leaves broad, rough; Shoot and Stem slightly hairy, heavy spines closely packed on four corners.	Yellow[1]; Pink-white[2]	Light Pink[1,2]	Umhlanga/ KwaZulu-Natal (29º42.747'S, 031º03.176'E)
Light Pink	021 WP	Leaves small, light coloured, slightly hairy; Shoot and Stem slightly hairy, small multiple spines.	White[1] ; Violet-Light pink[2]	Light Pink[1,2]	Dzwerani/ Limpopo (23º02.777'S, 030º24.628'E)

Superscript letters represent the stage of maturity of the inflorescence (i.e. [a]Young flower and [b]Mature flower); Numbers represent the inflorescence parts (i.e., [1]flower throat and [2]lobe/ corolla).

Table 4.1 (Continued): Distinguishing features (morphological and inflorescence colour) among the *L. camara* varieties and the localities from which they were collected.

L. camara varieties	Code	Distinguishing morphological features	Flower colour (young)[a]	Flower colour (mature)[b]	Area/ Province (Grid reference)
Light Pink	163 LP	Leaves broad, slightly hairy; Shoot and Stem slightly hairy, small multiple spines.	Yellow[1]; Violet-Light pink[2]	Light Pink[1,2]	Rietondale/ Gauteng (25°43.993'S, 28°13.976'E)
Light Pink	165 LP	Leaves broad, slightly hairy; Shoot and Stem slightly hairy with small sparse spines.	White[1]; Violet-Light pink[2]	Light Pink[1,2]	Ncotshane/ KwaZulu-Natal (27°21.261'S, 031°30.175'E)
Orange Red	015 OR	Leaves small, tough, very hairy; Shoot and Stem very hairy with small scattered spines.	Yellow[1,2]	Orange-Red[1,2]	New Germany/ KwaZulu-Natal (29° 48.210'S, 030°53.433'E)
Hawaii Red	017 OR	Leaves small, tough, very hairy; Shoot and Stem very hairy with small scattered spines.	Orange[1]; Yellow[2]	Orange Red[1,2]	Constantia/ Limpopo (23°38.321'S, 030°39.031'E)
White yellow	015 WY	Leaves small, fleshy, shiny; Shoot and Stem slightly hairy with few small scattered spines.	Yellow[1]; White[2]	White[1,2]	N3 White/ KwaZulu-Natal (29°44.490'S, 030°37.595'E)

Superscript letters represent the stage of maturity of the inflorescence (i.e. [a]Young flower and [b]Mature flower); Numbers represent the inflorescence parts (i.e., [1]flower throat and [2]lobe/ corolla).

4.2.2. Experimental design

The varietal suitability trial was conducted in an open field at the ARC-PPRI, Rietondale, South Africa (25°45.369'S, 28°13.186'E). The 10 lantana varieties (Table 4.1) were planted, and each variety was replicated five times. Well established potted plants were transplanted at the experimental site which was 324m^2 in size, and each plant was grown at an inter- and intra-row spacing of 3m by 3m. Each *L. camara* variety replicate was grown diagonally from the adjacent replicate, appearing only once in each of the five rows and in five of the 10 columns (Fig. 4.1).

A	B	C	D	E	F	G^a	H	I	J
J	A	B	C	D	E	F	G^a	H	I
I	J	A	B	C	D	E	F	G	H
H	I	J	A	B	C	D	E	F	G
G^a	H	I	J	A	B	C	D	E	F

Fig. 4.1. Schematic diagram presenting the experimental layout of *L. camara* varieties planted in a field plot at the ARC- PPRI, Rietondale, Pretoria. A= Orange Red (015 OR), B= White Pink (021 WP), C= Dark Pink (018 DP), D= Yellow White (015 WY), E= Hawaii Red (017 OR), F= Pink (021 P), G= Dark Pink (010 DP); H= Total Pink (021 TP), I= Light Pink (163 LP) and J= Light Pink (165 LP). [a]Plants that died during the trial.

Eight weeks after planting, fresh flower galls containing *A. lantanae* were collected from three different sites, namely Tzaneen (23°59.616'S, 030°15.126'E) in Limpopo, Pretoria (25°44.218'S, 028°14.783'E) in Gauteng and Mkhuze (27°36.143'S, 031°59.756'E) in KwaZulu-Natal, and were

placed on each of the 50 experimental plants. Galls were cable-tied onto the aerial buds of each *L. camara* plant to reduce the risks of infested galls falling off, which would have reduced the chances of the mites reaching the inflorescences (Winder & Van Emden 1980). Twenty weeks after planting (in April 2015), the number of galled inflorescences per plant were recorded, which coincided with the peak infestation period for *A. lantanae* in Gauteng (Chapter 2). Infestation percentage was calculated using *formula a* (see Chapter 2). Plant height, canopy diameter, inflorescence density (i.e., number of flower heads per plant) and abundance of other lantana biocontrol agents were also recorded on each of the 47 experimental plants that survived to the end of the trial (Fig. 4.1). A five-point rating scale (Table 4.2) was used to measure the abundance and activity of other lantana biocontrol agents on each plant.

Table 4.2. A five-point rating scale for measuring the level of abundance of lantana herbivores and their activity on the plants.

Abundance level	Rating scale	Description of each scale
None	0	No agent or feeding damage on the plant.
Rare	1	Very few herbivores with minor feeding damage of less than 10%.
Occasional	2	Few herbivores and moderate feeding damage of 10% to 30% on the plant.
Frequent	3	Herbivores were frequently found with feeding damage of 31% to 60% on the plant.
Abundant	4	Herbivores were very common with feeding damage of over 60% on the plant.

4.2.3. Data analysis

Since the data did not meet the assumptions of normality even after multiple transformations, a non-parametric Kruskal-Wallis One-way Analysis of Variance was used to test for significant differences in infestation levels between the different varieties of *L. camara*. Linear regression was used to determine the relationships between the percentage of inflorescences galled by *A. lantanae* and plant-related factors other than varieties, which included plant size (height and canopy) and inflorescence density. A Mann-Whitney U-test was carried out to determine whether the percentage of inflorescences that were galled differed significantly between the two most common categories of biocontrol agent abundance (i.e. rating scales 1 and 2). Data analysis was conducted with the aid of STATISTICA 6 software.

4.3.   RESULTS

4.3.1.   Host preferences of *A. lantanae* in relation to *L. camara* varieties

In the multiple-choice trials, only one of 10 lantana varieties (10%) displayed total resistance to attack by the flower-galling mite *A. lantanae* (Fig. 4.2). Nevertheless, infestation levels varied significantly between the varieties ($H_{(9, 47)} = 34.33171$; $P < 0.001$) screened during this trial. High infestations ranging from 50% to 62% were recorded on three varieties, namely 017 Orange Red, 021 White Pink and 018 Dark Pink (Fig. 4.2). Moderate infestations of 12% to 25% were recorded on four varieties, including 015 Orange Red, 021 Pink, 165 Light Pink, and 015 Yellow White. Low infestations of less than 12% were recorded on two varieties (163 Light Pink and 021 Total Pink), while one variety (010 Dark Pink) was totally rejected by *A. lantanae*, with no flower galls formed on any of the plants (Fig. 4.2).
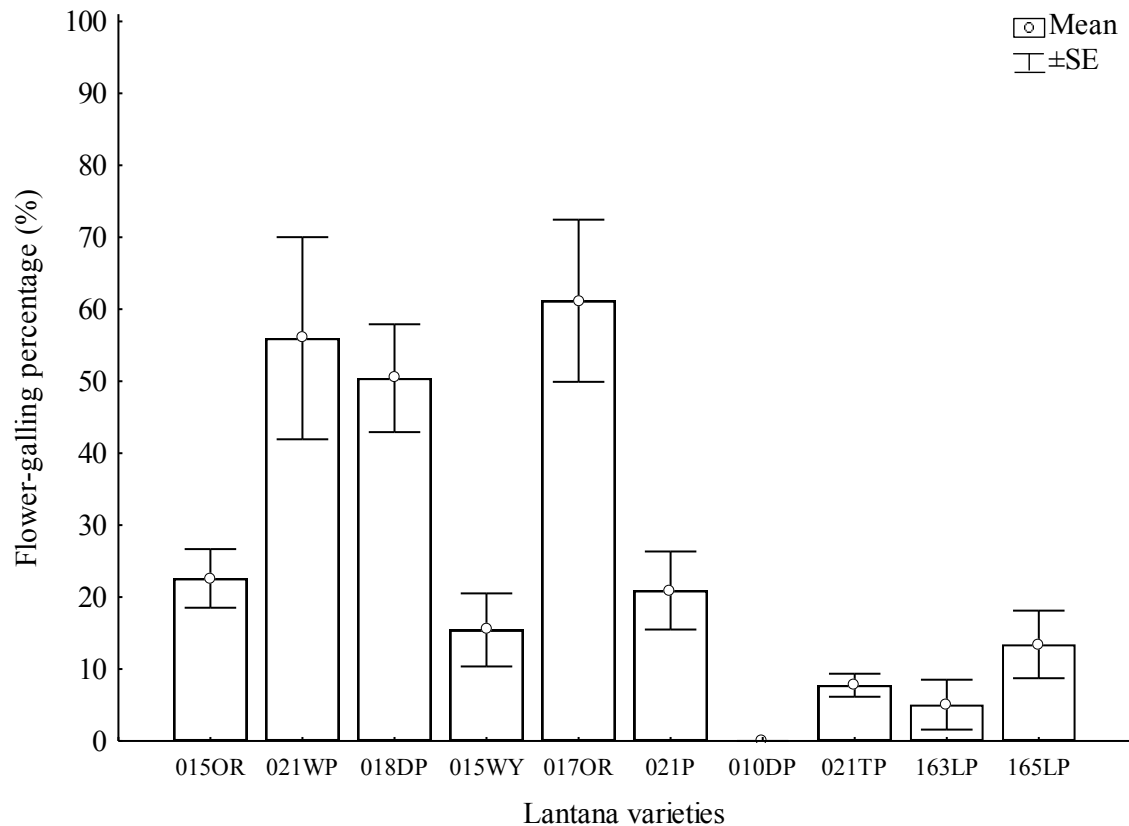
Fig. 4.2. Infestation percentages (mean ± SE) of *Aceria lantanae* on 10 different South African varieties of *Lantana camara*. Varietal descriptions are provided in Table 4.1.

4.3.2. Relationship between plant size and *A. lantanae* infestations

Linear regression showed no relationship between *A. lantanae* infestation levels and either plant height or canopy diameter ($P > 0.05$) (Fig 4.3 A and B). The infestation levels varied from 0 to 100% and this was influenced by neither plant height nor canopy diameter.
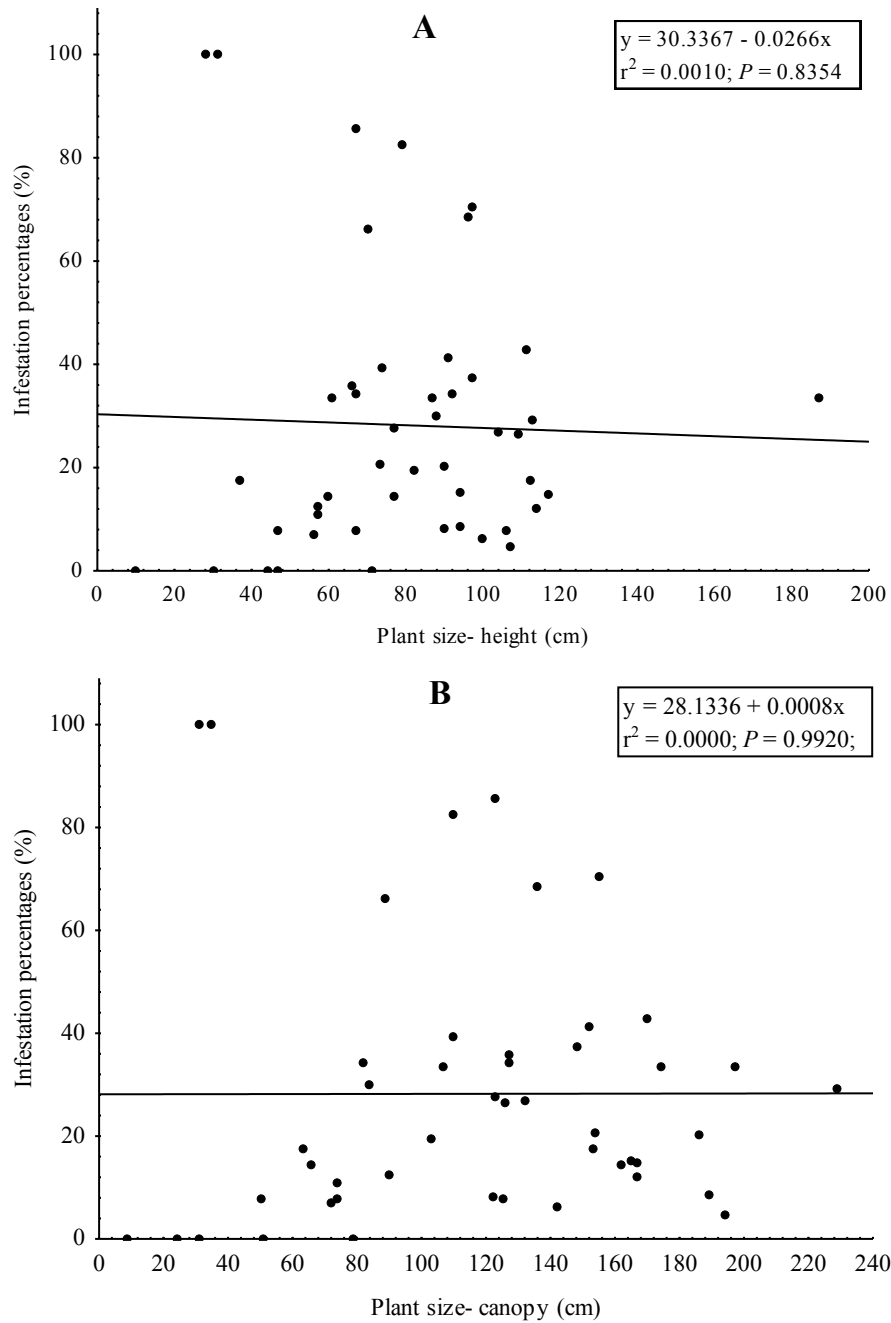
Fig. 4.3. Relationship between *Aceria lantanae* infestations and plant sizes (A= plant height; B= canopy diameter).

4.3.3. Relationship between inflorescence production and *A. lantanae* infestations

Although there was a positive relationship between *A. lantanae* infestation levels and the number of inflorescences produced per individual plant (Fig. 3.4), this was weak and not significant (P > 0.05). There was thus no indication that the mite populations were significantly influenced by food availability.
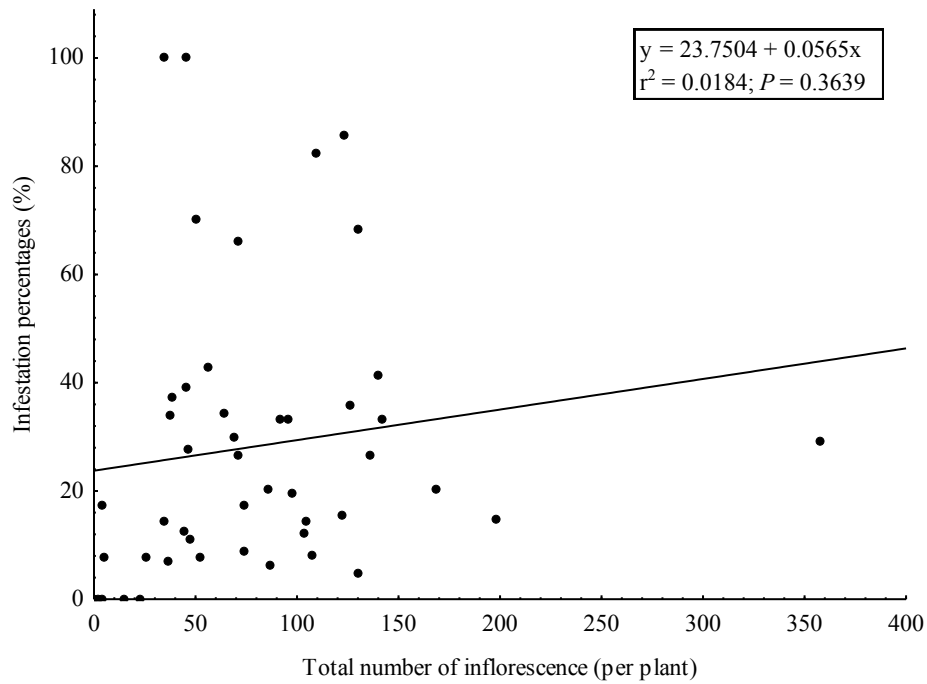
Fig. 4.4. Relationship between *Aceria lantanae* infestation levels and the numbers of inflorescences produced per plant.

4.3.4. Influence of other biocontrol agents on *A. lantanae* abundance

An increase in *A. lantanae* infestation was often associated with an increase in the activity of other lantana biocontrol agents (Fig. 4.6). *Aceria lantanae* infestation percentages were significantly lower on plants with low activity by other biocontrol agents than on those with moderate activity ($P < 0.05$). Mean *A. lantanae* infestations were recorded at 24.3%, 47.5% and 70.4% for lantana plants displaying activity by other biocontrol agents in the categories 1 (rare and minor damage), 2 (occasional and moderate damage) and 3 (frequent and higher damage), respectively.
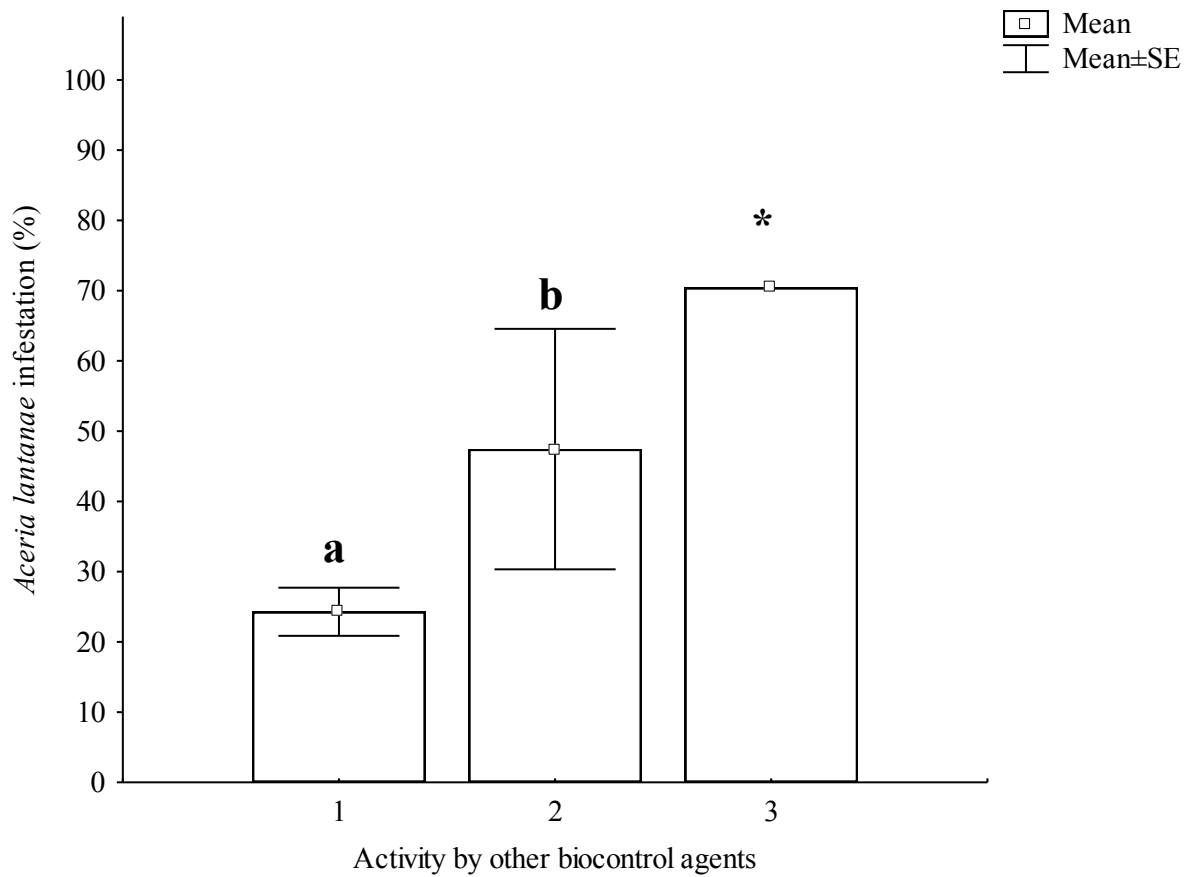
Fig. 4.5. Infestation percentages (mean ± SE) of *Aceria lantanae* at three different activity levels of other biocontrol agents of *L. camara*. *Absence of an error bar is due to only one sample; thus no statistical comparison. Bars followed by different letters are significantly different (P < 0.05, Mann-Whitney U-test).

4.4.   DISCUSSION

The suitability of different genotypes or varieties of a host plant for a biological control agent will influence its success in the country of introduction. Given that neither plant size nor inflorescence density had any influence on levels of infestation by *A. lantanae*, the present study has verified that the mite's performance is significantly influenced by the variety of *L. camara* rather than food availability. These results are consistent with those of previous studies which showed that the performance of other lantana biocontrol agents such as *T. scrupulosa*, *Neogalia sunia* (Guenée), *F. intermedia* and *L. bethae* were also influenced by the weed's variety under both laboratory and field conditions (Neser & Cilliers 1990; Cilliers & Neser, 1991; Baars 2002; Heystek 2006; Rienert *et al*. 2006; Simelane 2006; Heshula 2009; Reinert *et al*. 2009; Heshula & Hill 2011).

During earlier laboratory studies, *A. lantanae* displayed varying performance on different South African and Australian varieties of lantana (Urban *et al.* 2004; Mpedi & Urban 2010). In the current outdoor study, *A. lantanae* failed to establish on one of the 10 lantana varieties, while six other varieties displayed a high degree of resistance to the mite, with less than 30% of inflorescences infested per plant. Given that seed-feeding agents need to reduce seed production of the target weed by 95-99% annually to limit the number of viable seeds entering the seed bank (*e.g.* Hoffmann & Moran 1998; Kriticos *et al.* 1999; Van Klinken *et al.* 2009) and allow the proliferation of plant competitors to suppress seedling recruitment (Andersen 1989), the recorded mite infestations on nine of the 10 selected lantana varieties appeared to be insufficient to regulate *L. camara* populations.

During field exploration for natural enemies of lantana, varietal preferences by several biological control agents have been observed in the native range, with different varieties displaying different levels of resistance to certain herbivore species (Day & Neser 2000; Goolsby *et al.* 2006). Therefore, the collection of biocontrol agents from different lantana varieties in the native range could increase the genetic diversity of these agents and therefore improve their chances of establishment and impact on the different lantana varieties in South Africa (Baars & Neser 1999).

For example, importation of a new strain of *Uroplata girardi* Pic (Coleoptera: Chrysomelidae) resulted in its establishment in South Africa following failure of the previous strain to do so (Harley & Kassulke 1971; Cilliers & Neser 1991). The strain of *A. lantanae* that was released in South Africa was collected from Florida (USA), and complementing this with other strains from different lantana varieties from Central and South America could increase the number of lantana varieties that are likely to be attacked in South Africa. Furthermore, the lack of any relationship between inflorescence density and infestation by *A. lantanae* suggests that the mite is not food-limited, and that there is still a niche for additional biotypes of *A. lantanae* or other seed-attacking agents in the lantana biocontrol system.

The infestation level of the mite *A. lantanae* was significantly linked with the abundance of other established lantana biocontrol agents. It could be speculated that *A. lantanae* and the other biocontrol agents that attacked the experimental plants may have been collected from genetically related or similar varieties of *L. camara* in the native range. Studies by Day & Neser (2000) and Day & Urban (2004) demonstrated that the greater proportion of insect species that were introduced for biocontrol in South Africa, Hawaii and Australia were collected from *Lantana urticifolia* Miller and *Lantana tiliifolia* Chamisso in Mexico. However, a molecular study needs to be undertaken to determine the extent of similarity between highly susceptible lantana varieties in South Africa and *L. urticifolia* and *L. tiliifolia*, from which most of the agents were collected.

Studies by Heshula & Hill (2011) have shown that the development of resistant traits in some lantana varieties can be induced by herbivore activity, thereby affecting the population density of the following generations. Morphological and physiological changes in plants can increase the developmental time and mortality of herbivores while reducing their fecundity (Radunz 1971; Taylor 1989; Heshula & Hill 2012). Similarly, there was a significant increase in trichome density in some *L. camara* varieties after prolonged stress induced by heavy feeding of the sap-sucking mirid, *Falconia intermedia* Distant (Hemiptera: Miridae) (Heshula & Hill 2011). Population crashes of *F. intermedia* were further observed during both laboratory and field trials after impressive establishments and population build-ups of the mirid in previous generations (Heshula 2005, 2009; Heshula & Hill 2011, 2012). However, it remains to be verified whether *A. lantanae*

induces morphological and physiological changes in *L. camara* that could in turn inhibit or augment its performance.

Given that neither climatic factors (Chapter 3) nor non-varietal (i.e. size or inflorescence density) plant-related factors (Chapter 4) had any influence on infestation by *A. lantanae*, these data support the contention that varietal resistance largely determines the susceptibility of *L. camara* to *A. lantanae*. This explains the sporadic establishment and varying levels of infestations by the mite that were observed throughout the distribution range of *L. camara* in South Africa (Chapter 2).

CHAPTER FIVE

GENERAL DISCUSSION AND CONCLUSIONS

The ability of a biological control agent to establish, spread and have a significant impact on the target weed is an important element that determines the success of a biological control programme (Broughton 2000; Day & Neser 2000; Boughton & Pemberton 2011). Following its release into the country in 2007, the flower gall-forming mite *A. lantanae* has established at various sites throughout the distribution range of its host *L. camara*. The current study was therefore conducted to determine the performance and impact of *A. lantanae* on *L. camara* in KwaZulu-Natal, Limpopo, Mpumalanga and Gauteng provinces of South Africa, where infestations of *L. camara* are particularly severe. The influence of key climatic factors (i.e., elevation, temperature, rainfall and relative humidity) and the variety of *L. camara* on establishment and infestation levels of *A. lantanae* were also investigated in the study.

5.1. Establishment pattern and rate of spread of *A. lantanae*

Despite having spread widely throughout the distribution range of its host *L. camara*, the mite *A. lantanae* remains characterised by patchy establishment and inflorescence infestations in the different ecological regions of South Africa (Chapter 2). The establishment of the mite varied among sites and regions with no clear evidence that habitat characteristics at each site might have affected its establishment and development. The findings of the current study are consistent with those relating to the gall-forming mite *A. malherbae*, a biological control agent of bindweed *Convolvulus arvensis* L. (Convolvulaceae). The establishment and distribution of *A. malherbae* was also reported to be patchy after its release, between 1992 and 1995, in Montana State, USA (McClay *et al.* 1999). Given the evidence that *A. lantanae* is variety-influenced (Chapter 4), and that *L. camara* varieties have a very patchy spatial distribution throughout the geographic range of the weed in South Africa (Heystek 2006; Urban *et al.* 2011), the current distribution pattern of the mite is not surprising. Although the number of release sites and the number of individuals released at the sites are known to influence the likelihood of establishment and the proliferation of biocontrol agents on a regional basis (Grevstad 1999; Broughton 2000; Day & Neser 2000; Shea

& Possingham 2000; Simelane & Phenye 2004), this is unlikely to have been the case for *A. lantanae*. Release efforts were consistent between the sites, with several lantana branches that were placed at each site containing massive and fresh *A. lantanae*-infested galls (P. Mpedi, pers. comm. 2014). A number of eriophyid mite species have been used as biocontrol agents worldwide, with variable success, ranging from having a negligible to a heavy impact on their respective hosts (Appendix 1).

Whilst the map generated by CLIMEX indicated that tropical and subtropical parts of Africa would be suitable for the establishment and development of *A. lantanae* (Chapter 3), other factors within the predicted range determined the prevalence and distribution of the mite in South Africa (Chapter 2). A major limitation in the use of CLIMEX as a predictor of the potential new range of a biocontrol agent is the assumption that climate is the main, if not the only, determinant of the species' distribution. Climate may not be essential or relevant for some species as their survival may be limited to a subset of all possible conditions that directly affect their fitness (Sutherst *et al.* 2000; Hulme 2003). Kearney (2006) defined the observed distribution of a species as an environmental volume or niche where the species actually occurs and which is usually smaller than its fundamental or potential distribution. This implies that the observed geographical distribution of a species is a reflection of a subset of its fundamental or potential distribution, which is limited by biotic interactions such as host genotype and the presence or absence of predators.

The high rate of spread (over 40 km per year) of *A. lantanae* can be described as long-distance dispersal and such events may occur during periods of negligible population increase and have little relationship to population size (Muller-Landau *et al.* 2003). Because the establishment of agents with long-distance dispersal capabilities is rarely influenced by population size, such biocontrol agents could spread in small numbers through wind, other organisms or human-aided transport (Fagan *et al.* 2002). The discovery of a population of *A. lantanae* in Marble Hall (Limpopo), which is believed to have dispersed for some 140 km from either its rearing site in Pretoria or release site near Mbombela, is an indication that its spread was random and may have been facilitated by some of the afore-mentioned mechanisms. This could partly explain the patchy distributions of *A. lantanae*, and other eriophyid mite species (e.g., Ishihara *et al.* 2007; Lake *et*

al. 2014). Fagan *et al.* 2002 also argued that good dispersers might incur a trade-off by lacking the ability to suppress pest populations locally. Thus control agents that are able to spread quickly throughout the region of pest infestation might have only a negligible impact locally, whereas species that are able to enforce substantial control locally might be unable to provide regional control. It remains to be seen whether *A. lantanae* infestations, ranging from nil to 100%, that were recorded in the various sites and experimental plots, will increase or be sustained over time.

5.2. Impact of *A. lantanae* on inflorescence and seed production of *L. camara*

Among the dozens of biocontrol agents released against *L. camara* in South Africa, only two are attacking the reproductive organs of the weed directly, and these are the gall mite *A. lantanae* and the fruit-attacking fly *Ophiomyia lantanae* Frogratt (Diptera: Agromyzidae) (Urban *et al.* 2011). Whilst the fly *O. lantanae* has a negligible effect on the production and viability of seeds (Broughton 1999; Vivian-Smith *et al.* 2006), the gall mite *A. lantanae* is highly damaging on susceptible *L. camara* varieties (Chapters 2 and 4). In a chemical exclusion experiment, *A. lantanae,* acting in concert with other established agents, substantially reduced seed production of susceptible *L. camara* varieties by 92 - 96% (Chapter 2). Considering that *L. camara* spreads through seeds, it is imperative that more seed- or fruit-attacking agents be introduced to complement *A. lantanae*. Because a large proportion of *L. camara* varieties display some resistance to the mite *A. lantanae*, the introduction of new strains of *A. lantanae*, collected from different genotypes of *L. camara* in the native range, might improve the establishment rate and impact of the mite in South Africa. Indeed, there is evidence that inflorescence-feeding herbivores can limit the seed production, seedling recruitment, plant density and maternal fitness of the host plant (Louda & Potvin 1995; Hoffmann & Moran 1998).

5.3. Response of *A. lantanae* to climatic conditions

Despite its patchy infestations on *L. camara* inflorescences (Chapter 2), *A. lantanae* established widely throughout the main ecological regions of the country (Chapter 3). Whilst the long-term weather (i.e., temperature, humidity and rainfall) records collected over a year showed that none of these influenced the establishment and performance of *A. lantanae* (Chapter 3), the general population trends of the mite were associated with seasonal climatic changes and plant growth cycles, and the peak infestation periods varied from region to region. Generally, warm summer/autumn weather was favourable for plant recovery from extensive leaf abscission in winter and also for mite dispersal, which resulted in rapid increases in infestation. *Aceria lantanae* activity and movement appeared to be negatively affected by chilly winter conditions, causing substantial population declines at the majority of study sites (Chapter 2), and thereby reducing the infestation levels of the mite.

Studies by McEvoy & Coombs (2001) showed that climate prevented the establishment of at least half of introduced insect biocontrol agents, while that of Smith *et al*. (2010) showed that a third of introduced eriophyid mite species failed to establish due to climatic factors. However, similar to that of *A. lantanae*, the establishments of *Floracarus perrepae* Knihinicki & Boczek (Acariformes: Eriophyidae) on the climbing fern *Lygodium microphyllum* (Cav.) R. Br (Polypodiales: Lygodiaceae) in Florida (Lake *et al*. 2014) and *A. davidmansoni* (previously misidentified as *A. genistae*) on Scotch broom *Cytisus scoparius* (L.) in Australia and New Zealand (Sagliocco *et al*. 2011) were also patchy, and were not affected by climatic factors. In the current study, the relationship between annual rainfall and the mite's infestation percentages was not significant, although *A. lantanae* infestations were not recorded at two study sites with annual rainfall greater than 1,324 mm. Nasareen & Ramani (2015) also reported a decline in the populations of the gall-forming mites, *Aceria pongamiae* Keifer and *A. doctersi* (Nalepa) (Acari: Eriophyidae) in India following high rainfall in June. Due to insufficient samples in the current study, further investigation on the effect of rainfall on the ability of *A. lantanae* to colonize a site seems warranted.

Besides the host genotype (Chapter 4), several other factors, such as microclimate, habitat composition, and recruited natural enemies may have influenced the establishment success of *A. lantanae* (Day & Urban 2004). Clark *et al.* (2001) also found that the habitat, particularly the size and continuity of knapweed (*Centaurea maculosa* L.; Asteraceae) sites, was consistently associated with the presence or absence of two knapweed biological control agents within their introduced range. Further research to determine the habitat characteristics that promote mite dispersal from one site to another, such as wind speed, direction, distance and time between established patches might improve our understanding of the factors that determine the establishment patterns of *A. lantanae*.

5.4. Varietal preferences of *A. lantanae*

This study has provided sufficient evidence that *L. camara* genotypes influence the establishment and proliferation of the flower-galling mite *A. lantanae*. In addition to predators and adverse abiotic conditions, varietal resistance was listed as one of the major factors that reduce the ability of eriophyid mites to control populations of their target weed (Smith *et al.* 2010). For example, *Aceria chondrillae* (Canestrini), a biological control agent for rush skeleton weed (*Chondrilla juncea* L.; Asteraceae), preferentially fed and developed on selected biotypes of the weed (Campanella *et al.* 2009). While the mite *F. perrepae* readily induced galls on its native host, *Lygodium microphyllum* (Cav.) R. Br. (Schizaeaceae), in Australia, it exhibited a diminished ability to induce galls on the fern biotypes that were introduced into Florida (Freeman *et al.* 2005; Ozman & Goolsby 2005). A study by McIntyre & Whitham (2003) also showed that plant genotype and hybridization influenced the spatial distribution and population dynamics of the poplar bud gall mite *Aceria parapopuli* (Keifer).

Given that *L. camara* genotypes occurring in South Africa and other countries where hybridization was practised do not match genetically with those that occur naturally in the weed's native range (Howard 1969; Swarbrick *et al.* 1998; Day & Neser 2000), it was expected that strain-specific biocontrol agents such as *A. lantanae* would colonize and establish on a proportion of *L. camara* varieties, as was demonstrated in the current study (Chapter 4). Varietal resistance has been regarded as a key factor affecting the establishment and performance of several biological

control agents of *L. camara* in South Africa (Baars & Neser 1999; Baars & Heystek 2003; Urban *et al.* 2004; Simelane 2006b; Baars & Hill 2010) and Australia (Radunz 1971; Taylor 1989; Broughton 2000). The ineffectiveness of biocontrol agents on some varieties has been one of the motivations for using a suite of agents for the biocontrol of *L. camara* (Baars & Neser, 1999; Broughton 2000; Day & Urban 2004). Likewise, the introduction of various strains of *A. lantanae* collected from different genotypes of *L. camara* in the native range will go a long way towards improving the establishment rate and the impact of the mite in South Africa and elsewhere in the world.

5.5. Conclusion

Despite the patchy infestations of *A. lantanae* observed throughout the distribution range of *L. camara* in South Africa, the mite has established and spread widely, and this is consistent with the broad geographic distribution predicted by the CLIMEX model (Chapter 3). Whilst the current study found no relationship between any of the climatic factors and the performance *A. lantanae* (Chapter 3), the mite's performance was significantly influenced by the genotype of *L. camara*, with over 50% of the flower heads destroyed in only 30% of the varieties tested (Chapter 4). This explains the patchy establishment and varying levels of infestations by the mite that were observed throughout the distribution range of *L. camara* in Limpopo, Mpumalanga, Gauteng and KwaZulu-Natal provinces (Chapter 2). Therefore, the introduction of additional strains of *A. lantanae* that target varieties of *L. camara* that displayed resistance to the current strain should enhance the impact of the mite in South Africa.

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APPENDICES

Appendix 1: List of gall mite species (Acari: Eriophyidae) that were released as biological control agents of invasive weeds around the world, with an assessment of the outcomes of the releases.

Weed name	Agent[a]	Country[b]	Year	Establishment	Impact	Limitation factor	References
Calystegia sepium (L.) R. Br. (Convolvulaceae)	*Aceria malherbae* Nuzzaci	USA	1993	Unknown	Unknown	None	Rosenthal 1983; Smith *et al*. 2010
Chondrilla juncea L. (Asteraceae)	*Aceria chondrillae* (Canestrini)	USA	1977	Established	Variable	Predation; Climate	Milan *et al*. 2006
		Canada	1993	Established	Slight	None	Milan *et al*. 2006
		Australia	1971; 1985	Established	Variable/ Slight	None	Cullen 2012
		Argentina	1989	Established, high	Unknown	None	Smith *et al*. 2010; Cullen 2012
Chrysanthemoides monilifera (L.) Norl. (Asteraceae)	*Aceria* sp.	Australia	2008	Limited	Too early for evaluation	Possibly predation; Climate	Adair *et al*. 2012; Morley *et al*. 2012
Cirsium arvense (L.) Scop. (Asteraceae)	*Aceria anthocoptes* (Nalepa)	Canada	Unknown	Established	Unknown	None	Walter & Latonas 2012
		USA	Unknown	Established	Slight	None	Cripps *et al*. 2011
Convolvulus arvensis L. (Convolvulaceae)	*Aceria malherbae* Nuzzaci	USA	1989	Established	Variable	Climate	Rosenthal 1995
		Canada	1989	Established	Unknown	Climate	McClay *et al*. 1999

[a]Introduced biological control agents.

[b]Country of agent's introduction.

Appendix 1 (Continued): List of gall mites (Acari: Eriophyidae) that were released as biological control agents of invasive weeds around the world, with an assessment of the outcomes of the releases.

Weed name	Agent[a]	Country[b]	Year	Establishment	Impact	Limitation factor	References
Convolvulus arvensis L. (Convolvulaceae)	*Aceria malherbae* Nuzzaci	Mexico	2004	Not established	Not established	None	Rodríguez *et al.* 2008
		RSA	1994	Not established	Compromised	Land use	Klein 2011
Cytisus scoparius (L.) Link (Fabaceae)	*Aceria davidmansoni* sp. nov. [Previously misidentified as *Aceria genistae* (Nalepa)]	Canada	Unknown	Moderate	Slight	None	Andreas *et al.* 2011
		USA	Unknown	Variable	Variable	Possibly predation	Smith *et al.* 2010
		Australia	2008	Established	Too early for evaluation	None	Sagliocco *et al.* 2011 (Xue *et al.* 2015)
		New Zealand	2007	Established	Too early for evaluation	None	Paynter *et al.* 2012; Sagliocco *et al.* 2011
Lantana camara L. sens. lat. (Verbenaceae)	*Aceria lantanae* (Cook)	Australia	2012	Unknown	Unknown	None	Day 2012
		RSA	2007	Variable	Heavy	Variety	Mukwevho *et al.* 2014
		USA	1976	Variable	Unknown	None	Denmark & Keifer 1991
Rhaponticum repens (L.) Hidalgo (Asteraceae)	*Aceria acroptiloni* Shevchenko & Kovalev	Uzbekistan	1973	Established	Heavy	None	Smith *et al.* 2010
		Ukraine	1997	Variable	Heavy	None	Kovalev 1973

[a]Introduced biological control agents.
[b]Country of agent's introduction.

Appendix 1 (Continued): List of gall mites (Acari: Eriophyidae) that were released as biological control agents of invasive weeds around the world, with an assessment of the outcomes of the releases.

Weed name	Agent[a]	Country[b]	Year	Establishment	Impact	Limitation Factor	References
Ulex europaeus L. (Fabaceae)	*Aceria davidmansoni* sp. nov.	New Zealand	2007	High	Slight	None	Paynter *et al.* 2012; Xue *et al.* 2015

[a]Introduced biological control agents.
[b]Country of agent's introduction.