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Interspecific interactions between *Uroplata girardi* (Coleoptera: Chrysomelidae) and *Ophiomyia camarae* (Diptera: Agromyzidae), and their impact on a shared host *Lantana camara* (Verbenaceae) in South Africa

By

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Submitted in fulfillment of the requirements for the degree:

Masters of Science in Entomology

In the

Faculty of Natural and Agricultural Science

Department of Zoology and Entomology

University of Pretoria

2009

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Abstract

In biological control of weeds, several agent species may be released to attack the target weed with the aim of causing cumulative stress, leading to overall control of the weed. However, this approach may result in competition among phytophagous insects and has been found to contribute to the failure of agents to control the target weed. To test whether competition is the factor limiting biocontrol success of *Lantana camara* L. (Verbenaceae) in South Africa this study was conducted. Influence of herbivory and interaction by two leaf-mining agents *Uroplata girardi* Pic (Coleoptera: Chrysomelidae) and *Ophiomyia camarae* Spencer (Diptera: Agromyzidae) on survival and performance of lantana were investigated using semi-field trials, laboratory experiments and field surveys. Caged lantana under the semi-field conditions were inoculated with *U. girardi*, *O. camarae* and with both agents combined to measure impact on its performance and to determine population growth of the biocontrol agents. Female choice for each agent species between infested and uninfested plants was determined in laboratory experiments where caged potted lantana plants were initially inoculated with each species separately, at the later stage the other species and a new plant were introduced to the same cage. To validate the incidence of competition between the agents, five sites in KwaZulu-Natal were surveyed.

The impact of *U. girardi* was greater in the absence of *O. camarae* whereas their combination appeared to be less their individual effect on lantana. Therefore, there was no synergistic effect between the two agents. *Ophiomyia camarae* was able to avoid competition by avoiding infested leaves for oviposition while *U. girardi* laid its eggs indiscriminately and when both utilize the same plant, population growth of the beetle was adversely affected while *O. camarae* was not. In the field, *O. camarae* populations increased rapidly from December to May while those of *U. girardi* were at a minimum during the same period. As a superior competitor, *O. camarae* reproduces prolifically and is likely to exert substantial herbivore pressure on its host in the field. While, *U. girardi* population is likely to recover when the fly population declines, it is likely to avoid being displaced completely as previously documented. This study emphasizes the

significance of conducting interaction studies on insects, especially those sharing the same niche prior to release. Such studies will avoid the negative impact of competition between agents but will also limit the number of agents used, thus reducing the risk of non-target effects.

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Declaration

I, **Vuyokazi Maceduma April** declare that the thesis/dissertation, which I hereby submit for the degree **Master of Science in Entomology** at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

Signature: ..……..

Date: …….

Acknowledgements

I would like to express my sincere gratitude and appreciation to:

My supervisors, Drs. M.P. Robertson and D.O. Simelane for their positive attitudes, constructive criticism and guidance throughout this study. Thank you for the sound advices and allowing me to err as part of my learning, I learnt a lot from you!

- Agricultural Research Council – Professional Development Programme Fund and University of Pretoria Postgraduate Bursary for financial assistance.
- All the Working for Water funded projects for assisting financially for the construction of my experimental cages.
- The Plant Protection Research Institute (PPRI) for the use of their facilities at Rietondale.
- Ms. Glenda Swart, Weather South Africa for providing and allowing me to use the weather data for Pretoria and KwaZulu-Natal.
- The staff of Phahla Metrorail Station in Emanzimtoti for allowing us to sample in their property and made sure that we are safe from the train (though it was bit scary to cross the railway line, I admit).
- Dr. Alan Urban, for his constructive comments and all the advice he offered from the beginning to the final part of this project, thank you very much.

I wouldn't achieve my dreams and wishes if it wasn't for a support, love and assurance from:-

- Prof. Sam Waladde, University of Fort Hare, you paved the way and I followed.
- My parents, Nelson "K" and maYolie; my siblings, Lovey "Ntosh", Yang' inkosi and Mihle "Maisa" for all the love, emotional and financial support and encouraging me to pursue my dreams.

- Ayanda Nongogo (a.k.a Chomie), Lulama “Lulu” Madire (a.k.a. mamaL) and Sicelo Nofemela, thank you very much for tolerating my craziness, for your patience, support and landing a hand during my proposal writing, sampling and the reassurance during writing-up
- To the lovely former and current staff of ARC-PPRI Weeds Division (too many to mention) in Pretoria, you guys are the best. Thank you for all the help, advice, consultation, lending your equipments, baking carrot cakes and making me laugh when the going gets tough, especially you Frits Heystek☺.
- To my adopted family the Nongogo’s. Mama, thanks for your encouraging words and prayers that made me strong when I couldn’t see light at the end of the tunnel. U Thixo wozuko akusikelele, Nokwanda!
- To my adopted sisters Lusanda and Leletu Susela, your words of encouragement kept me strong. Thanks for allowing me to vent my anger and frustrations and passing the Kleenex whenever I cried.
- To everyone who played a role in my well being, achievements and gave me unconditional love and support; thank you so much, God bless!
- Rod Baxter and Thulie Wistebaar, your amity kept me afloat and thanks for reading through my first drafts.

To the Creator of life and its beauty, Lord Almighty God for His grace and guidance from the beginning until the end of this study, thank you Lord!

This thesis is dedicated to my father, N.K. April; it’s yours Ceduma and to my two friends who passed away in 2008, Nomvuyiso Joy Matokazi and my childhood friend Ntombekhaya Ntoza Dlali. I will always love you and may you both rests in peace!

Disclaimer

Chapter Two and Three in this thesis are being prepared for publication in two different scientific journals. As a result overlap in content may occur throughout the thesis to secure publishable entities.

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Chapter One

General Introduction

It is common for a plant to be simultaneously attacked by a complex of phytophagous insects (Dorn *et al.*, 2003). In weed biological control systems, there is substantial evidence that interactions between herbivorous insects could have a detrimental outcome on the biological control programme (Masters *et al.*, 1992; Gonzalez-Megias & Gomez, 2003). For example, one insect can influence the effectiveness of the other in controlling their shared host plant, especially if the agents feed on the same plant part or share the same feeding niche (Denoth *et al.*, 2002; Crowe & Bourchier, 2006). Consequently, attempts in biological control of weeds have emphasized that the number of biocontrol agents that utilize the same plant part be minimized while increasing those that feed on different parts of the plant (Olckers *et al.*, 2002; Denoth *et al.*, 2002; Simelane, 2006).

Classical biological control of plants has aimed to reduce the reproductive success and distribution of targeted species, and to allow the native plants to outcompete and displace these weeds (Hajek, 2004). Williams (2005) and Simelane (2005) reported that using biological control proved to be cost effective and environmentally friendly compared to mechanical and chemical control. For example, *Azolla filiculoides* Lamarck (Azollaceae), an invader of aquatic ecosystems was successfully controlled by a frond-feeding weevil *Stenopelmus rufinasus* Gyllenhal (Coleoptera: Curculionidae) in South Africa (McConnachie *et al.*, 2003). The weevil reduced the azolla population to the point where it was no longer considered as a problem, thus resulting in considerable savings as alternative control measures were not required (McConnachie *et al.,* 2003). The use of insects and pathogens as biological control agents for alien invasive weeds has been advocated, but both failures and success have been reported (Day & Neser, 2000; Thomas & Ellison, 2000). Although biocontrol is self-sustaining, not all targeted weeds are controlled successfully (McFadyen, 1998;

Zalucki *et al.*, 2007), and as a result additional biocontrol agents are often released (Julien & Griffiths, 1998).

McEvoy & Coombs (1999) reported that once the agent becomes widely established in a new country it cannot be eradicated, making it essential that all possible consequences are effectively considered beforehand. For example, successful biocontrol agents may disperse far beyond the original target area. This occurred with *Ophiomyia lantanae* (Froggatt) (Diptera: Agromyzidae); a lantana seed fly in Southern Asia (Ooi, 1987). Additionally, *Zygogramma bicolorata* Pallister (Coleoptera: Chrysomelidae) released in India for biocontrol of *Parthenium hysterophorus* L. (Asteraceae) in 1984 attacked the sunflower (*Helianthus annuus*), however the damage was considered minor (McFadyen, 1998). The famous non-target effects of *Rhinocyllus conicus* (Frölich) (Coleoptera: Curculionidae) released against *Carduus nutans* L. (Asteraceae) in North America was found to be using native *Cirsium* spp. (Louda *et al.*, 2003). These cases indicate some of the risks associated with biological control. As a result, the impact of the agent in its new range should be considered.

The use of multiple agents has become a contentious issue (Louda, *et al.*, 2005; McEvoy & Coombs, 1999). It has been argued that, when more than two species are released on one host species, negative interactions among the agents may occur, rendering one of the species either displaced or ineffective in controlling the target weed (Masters *et al.*, 1992; Rhagu & Dhileepan, 2005). While research on interaction among insect herbivores that utilize the same plant part has been well documented, recent studies have also shown that spatially separated insects can reduce the quality and quantity of food resources of one species, indirectly affecting the performance of the other (Masters *et al.*, 1992; Woodburn, 1996; Blossey &Hunt-Joshi, 2003; Hunt-Joshi & Blossey, 2005; Simelane, 2006). When the plants are attacked simultaneously by a complex of herbivorous species, the outcome of the interaction could be that some species may utilize specific niches in which they eventually become particularly competent (Dorn *et al.*, 2003),

resulting in the displacement of an inferior species by a competitively superior one. For example, the interspecific competition between *Urophora affinis* Fravenfeld (Diptera: Tephritidae) and *Larinus minutus* Gyllenhal (Coleoptera: Curculionidae) biocontrol agents released against *Centaurea stobe* L. (Asteraceae) in Canada, where *U. affinis* an inferior biocontrol agent based on the target weed mortality, was a superior competitor when both agents were combined (Crowe & Bourchier, 2006). Thus interaction within and among herbivorous insect species have also been important in shaping the host ranges of some species, and that some species may maximize their fitness by developing means of monopolizing a particular host plant, hence excluding other species (Huffaker & Messenger, 1976). Competition among insects is frequently documented in situations where there has been some biological disturbance, and mostly among introduced insects that feed on introduced plants (Huffaker & Messenger, 1976, Louda *et al.*, 1997, Crowe & Bourchier, 2006). Despite the possibility of negative interactions, the conventional wisdom in biological control of weeds has been the careful selection and release of several biocontrol agents against the target weed, in anticipation that the combined damage of agents would result in cumulative stress on the plant (Harris, 1985), thereby improving the chances of successful control of the weed (Rhagu & Dhileepan, 2005).

Indeed, there is substantial evidence that enhanced weed biocontrol could be obtained from the combined forces of natural enemies (McEvoy, 1985; Hoffmann, 1998). However, the establishment of 12 intentionally introduced biocontrol agents (Table 1) against a noxious alien invader; *Lantana camara* L. (Verbenaceae) (Figure 1), in South Africa has not enhanced the level of biocontrol of the weed (Baars & Neser, 1999; Day & Neser, 2000; Simelane, 2006).

Figure 1: *Lantana camara* infestation in the field (© ARC-PPRI Weeds Research Division).

Biological control of *Lantana camara*

The weed *L. camara,* commonly known as lantana, is a thicket-forming shrub that has spread from gardens into pastures (Figure 2), woodlands and forests, where it competes for resources and reduces the productivity of pastures and forest plantations (Day, 2003). It is a threat to biodiversity in several countries such as Australia, India, Hawaii, Uganda and South Africa (Thomas & Ellison, 2000; Broughton, 2000; Day & Neser, 2000) as it out-competes native vegetation and it is a fire hazard. *Lantana* is a tropical and subtropical fast growing multi-branched shrub (Figure 1) about 2-4 m tall that originates from Central and South America (Day & Neser, 2000, Broughton, 2000, Simelane, 2002, Day *et al.*, 2003). In the mid 16th and 17th centuries, lantana species were imported into Europe from America (Broughton, 2000). In Europe hundreds of cultivars were created from the stock and the new stock was transported back to America and into Australia, India and Africa in the mid 19th century as an ornamental and hedge plant

(Broughton, 2000). Many of these varieties escaped confined cultivation and became weeds, and were often referred to as *L. camara* (Broughton, 2000). Lantana varieties have been classified according to flower colour; presence and absence of thorns and growth habit (Heystek, 2006). They also differ in their susceptibility to insect attack and chemical substances (Broughton, 2000; Day & Neser, 2000).

Lantana has become naturalized in more than 50 countries and is declared a weed in these areas. Since its introduction in the 1850's; lantana has became naturalized in most provinces in South Africa (Fig. 2) (Stirton, 1977). It is one of the most invasive alien species in South Africa and it is a declared Category 1 weed under regulation 15 by the Conservation of Agricultural Resources Act 43 of 1983. It is recognised as one of the world's 10 worst weeds (Simelane, 2005), and received one of the highest scores in a prioritization for alien invasive species management in South Africa (Robertson *et al.*, 2003).

Lantana camara forms thickets and grows vigorously, which has resulted in it being an important invader of forests, riverbanks, grasslands, open and semi-open plant communities. It has invaded about 2.2 million hectares of forest plantations, watercourses and savannas (Simelane & Phenye, 2005). It is an aggressive pioneer and tends to crowd out indigenous flora and is a serious invader of disturbed ecosystems. Lantana invasion suppresses natural vegetation, thereby reducing the carrying capacity of pastures and increasing surface runoff by decreasing the soil capacity to absorb heavy rain, which can lead to flooding (Heshula, 2005). Furthermore, lantana leaves and fruits contain a number of chemicals that are toxic to animals and when consumed could lead to mortality (Baars *et al.*, 2003). The ability of lantana to invade fields and pastures where livestock feed increases the risk of the animals being exposed to its damaging chemicals and increases the chances that livestock will feed on it.

Control measures such as chemical and mechanical control methods have been implemented against lantana with varying success. Chemical control using herbicides is effective but very costly. The use of such control methods produces only temporary relief as seedlings and coppice growth from stems and roots rapidly re-infest cleared areas (Cilliers & Neser, 1991, Henderson, 2001). Mechanical control by means of chopping and felling is also efficient; however, it is labour intensive and expensive. The use of these two control methods (chemical and mechanical) is limited by the fact that lantana stands are impenetrable, thorny and in the midst of desired indigenous vegetation; hence, both control methods have limited success against lantana. Another control method that has been receiving international recognition is biological control using various insect natural enemies. Biological control entails importation, host specificity testing and release of insect agents to the area in which the weed invades. This kind of control procedure is time consuming and expensive; but, it is the best option in the quest to control lantana. It is environmentally safe, long term, cost effective and agents may be self-sustaining following establishment.

Biological control of lantana was initiated in the 1960's in South Africa (Baars, 2003). Five natural enemies were initially introduced; three lepidopterans *Hypena laceratalis* Walker, *Neogalia sunia* Guenèe (Noctuidae) and *Salbia haemorrhoidalis* Guenèe (Pyralidae). A fruit feeding fly *Ophiomyia lantanae* (Diptera: Agromyzidae) and a tingid *Teleonemia scrupulosa* Stål (Hemiptera: Tingidae) (Oosthuizen, 1964). Other biological control agents were also released on lantana including two Hispinae leaf mining beetles *Octotoma scabripennis* Guérin- Méneville and *Uroplata girardi* Pic (Coleoptera: Chrysomelidae) and a leaf mining fly *Calcomyza lantanae* Frick (Diptera: Agromyzidae). The leaf feeding moth *S. haemorrhoidalis* had established after release but its range and impact is still unknown (Oosthuizen, 1964). The fifth species released was *T. scrupulosa*, which is widely established and abundant. It is considered as the most damaging of all agents released on lantana; causing periodic defoliation of lantana and a decrease in seed production. The tingid populations are at their

peak in mid-summer and consequently cause most damage during this time (Cilliers, 1987a).

The two leaf mining beetles, *O. scabripennis* and *U. girardi* were released in the 1970's in selected sites in KwaZulu-Natal. Both have established with *U. girardi* reaching high populations (Cilliers, 1987b). At their peak, both species cause defoliation of the plant (Cilliers, 1987b). Release of the other agents and their present status are summarized in Table 1. Despite the release of large numbers of agents and establishment of several; the agents have failed to effectively control the spread of lantana and the success of biological control on lantana has been limited. This has mainly been attributed to factors such as genetic diversity of lantana and climatic conditions (Baars & Heystek, 2003; Williams, 2005).

The diverse climatic conditions in the distribution range of lantana in South Africa have been cited as another factor for the failure of natural enemies to establish (Day & Neser, 2000). Lantana is capable of surviving adverse conditions such as drought and very cold temperature causing the plant leaves to dry up and fall off. Due to this, natural enemies introduced from tropical regions to temperate climates have a small chance of establishing and the moisture or rainfall becomes a barrier (Baars & Neser, 1999, Baars *et al.*, 2003). Furthermore, extensive leaf abscission during winter may leave leaf-feeding agents with no food or shelter leading to starvation and increased non-establishment and mortality. Williams (2003) found that the lack of an overwintering mechanism such as adult or pupal diapause by introduced insects contributed to non-establishment of the agents. This may result in agents not causing substantial amount of damage to the plant since they would be spending time recovering and building up their population density.

Biological control agents released against lantana have provided insufficient control to reduce its weedy status. However, some successes have been achieved; but were mostly not quantified. Since it has been acknowledged that success in controlling lantana depends on releasing a suite of natural enemies (Cilliers, 1987a; Baars & Neser, 1999; Day & Neser, 2000, Baars *et al.*, 2003)

another biological control agent *Ophiomyia camarae* Spencer (Diptera: Agromyzidae) was imported and released in South Africa in 2001 (Simelane, 2002). Release of *O. camarae* was aimed at complementing the existing biocontrol agent's guild as it was found that the agent would contribute substantially to the biocontrol of lantana (Simelane, 2002).

In South Africa alone, more than 23 biological control agents have been introduced and others have been recently released (Table 1). Accordingly, biological control of lantana has relied upon the introduction of numerous species of natural enemies in many countries (Perkins & Swezey, 1924; Oosthuizen, 1964; Cilliers, 1983). It was always assumed that the introduction of multiple agents would confer the following advantages: it would extend the attack to; (i) more biotypes of the weed; (ii) over a wider geographic range; (iii) under more varied climatic conditions, and (iv) increase the overall impact on the target (Cilliers & Neser, 1991; Baars & Neser, 1999; Day *et al.*, 2003). However, concerns have been expressed about the risks of multiple-species introductions (Denoth *et al.*, 2002). Despite multi-species introduction of biocontrol agents against *L. camara* in South Africa, biocontrol of the weed on a broad-scale is ineffective. There is a possibility that the ineffectiveness of some of the introduced biocontrol agents may be associated with competitive interaction among the agents. For example, recent field surveys suggest a steady decline of the hispid beetle *U. girardi* populations following the introduction and population explosion of the herringbone leaf-miner *O. camarae* along the KwaZulu-Natal coastal region (Heystek 2006). Since its release in 1974, *U. girardi* has always been the most abundant and damaging agent in the coastal regions of KwaZulu-Natal (Cilliers 1987b, Baars & Heystek, 2003). Approximately three years after the release of *O. camarae* in 2001, a steady decline in the population density of *U. girardi* was observed (Heystek, 2006) (Figure 3 and 4). It is hypothesized that the attack by the fly *O. camarae* reduces resource availability for the beetle *U. girardi*, which could eventually cause the displacement of the latter. To test this

assumption, the current study was conducted. Specifically, the study was conducted to answer the following questions:

1. Do semi-field data support the suggestion that there is interspecific interaction between *O. camarae* and *U. girardi*?

2. Do field data validate the existence of interspecific competition between *O. camarae* and *U. girardi* in KwaZulu-Natal?

3. Can females of one agent detect and avoid plants that are infested by the colonies of the other agent?

4. Is the combined damage of both agents (*U. girardi* and *O. camarae*) greater than either species would cause alone?

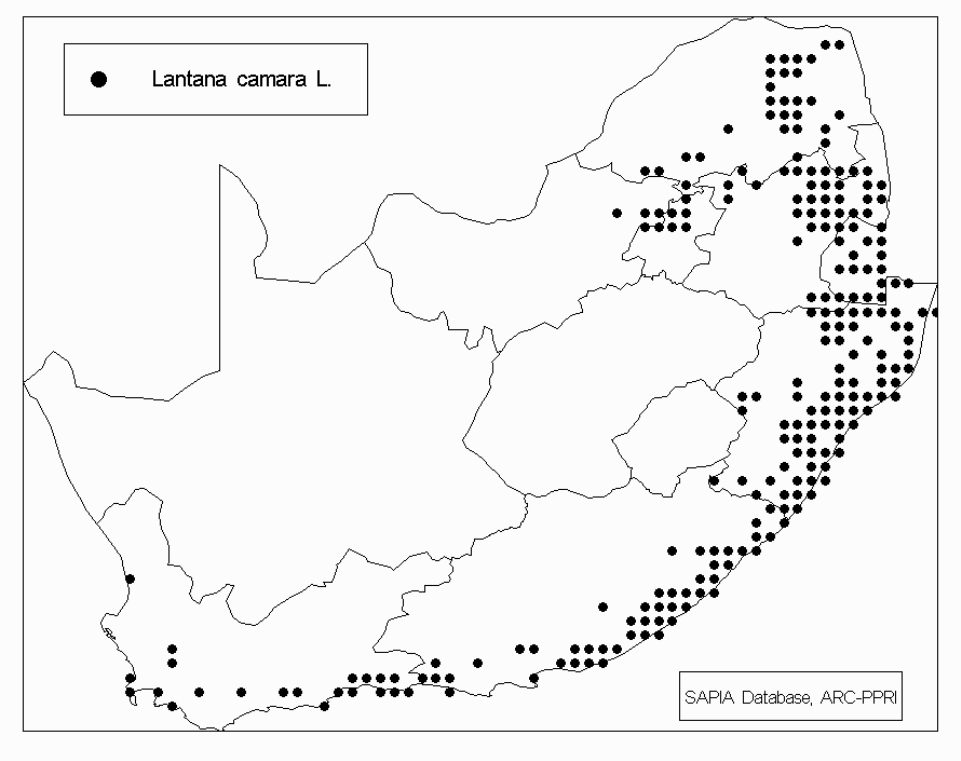

Figure 2: Recorded localities of *Lantana camara* in South Africa. (Henderson, L. 2001)

Table 1: Insect species introduced and released for biological control of *Lantana camara* in South Africa.

Insect species	Order: Family	Mode of attack	Release date	Reference
Alagoasa parana Samuelson	**Coleoptera** Chrysomelidae; Alticinae	Leaf chewer	1985*	Williams, 2003
Alagoasa extrema Jacoby	**Coleoptera** Chrysomelidae; Alticinae	Leaf chewer	rejected	Williams, 2005
Autoplusia illustrate Guenée	**Lepidoptera** Noctuidae	Leaf chewer	1981*	Baars *et al.*, 1999
Calycomyza lantanae Frick	**Diptera** Agromyzidae	Leaf miner	1982/9	Williams, 2003
Coelocephalapion camarae Kissinger	**Coleoptera** Curculionidae: Brentidae	Leaf- pentiole galler	2008	Heystek, 2008
Epinotia lantana Busck	**Lepidoptera** Tortricidae	Flower-peduncle and shoot-tip borer	1984***	Baars *et al.*, 1999
Eutreta xanthocheata Aldrich	**Diptera** Tephritidae	Stem galler	1983*	Williams, 2003
Falconia intermedia Distant	**Heteroptera** Miridae	Leaf sucker	1999	Baars *et al.*, 2003
Hypena laceratalis Walker	**Lepidoptera** Noctuidae	Leaf chewer	1961***	Williams, 2003
Lantanophaga pusillidactyla Walker	**Lepidoptera** Pterophoridae	Flower, fruit & seed chewer	1984	Baars, 2003
Leptobyrsa decora Drake	**Hemiptera** Tigidae	Leaf sucker	1972*	Williams, 2003

Longitarsus bethae Escolona & Salvini	**Coleoptera** Chrysomelidae: Alticinae	Root feeder	2008	Simelane, 2008
Neogalea sunia Guenée	**Lepidoptera** Noctuidae	Leaf chewer	1962-69*	Williams, 2003
Octotoma championi Baly	**Coleoptera** Chrysomelidae: Hispinae	Leaf miner	1978*	Williams, 2003
Octotoma scabripennis Guérin-Méneville	**Coleoptera** Chrysomelidae: Hispinae	Leaf miner	1974/5	Cilliers, 1983
Ophiomyia camarae Spencer	**Diptera** Agromyzidae	Leaf miner	2001	Simelane, 2002
Ophiomyia lantanae Froggatt	**Diptera** Agromyzidae	Fruit miner	1961***	Baars *et al.*, 1999
Plagiohammus spinipennis Thomson	**Coleoptera** Cerambycidae	Stem borer	1973*	Williams, 2003
Salbia haemorrhoidalis Guenée	**Lepidoptera** Pyralidae	Leaf feeder	1962	Baars, 2003
Teleonemia elata Drake	**Hemiptera** Tingidae	Flower & leaf sucker	1972*	Williams, 2003
Teleonemia scrupulosa Stål	**Hemiptera** Tingidae	Flower & leaf sucker	1961-89	Cilliers, 1983
Uroplata fulvopustulata Baly	**Coleoptera** Chrysomelidae: Hispinae	Leaf miner	1978*	Day & Neser, 2000
Uroplata girardi Pic	**Coleoptera** Chrysomelidae: Hispinae	Leaf miner	1974/5	Cilliers, 1983

* Insect species that have not established

*** Insect species already present in South Africa prior to deliberate introduction

Table 2: Modified definitions of categories used to measure insect abundance during a field survey of the biological control agents attacking *L. camara* in South Africa as identified by Baars (2003).

Parameter	Categories	V[a]	Definitions of categories
Insect abundance	None	0	No life stages present (0%)
	Rare	1	Very few individuals encountered, and patchy in distribution at the site sampled (20-30%)
	Occasional	2	Individuals present on all plants; most life stages encountered regularly (31 -40%)
	Frequently	3	Individuals present on all plants; most life stages regularly encountered with an even distribution over site sampled (41-50%)
	Abundant	4	Plants with large numbers of larval mines and individuals on most of the shoots on each plant; even distribution of individuals in large numbers (+ 50%)

[a] Values assigned to the categories.

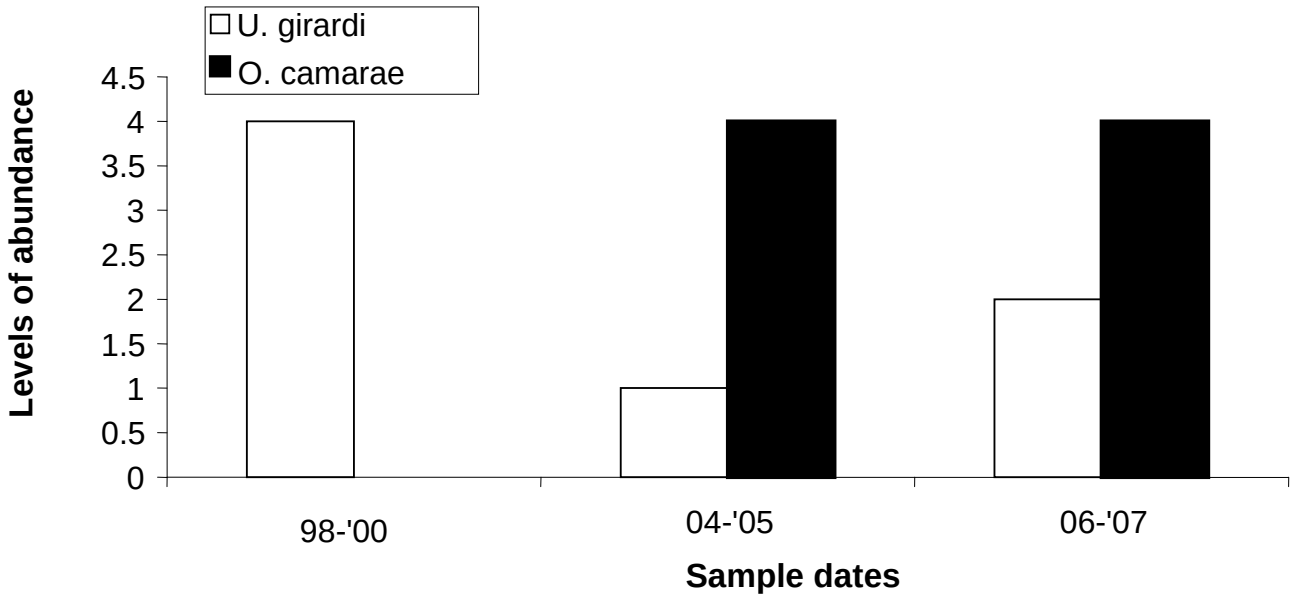

Figure 3: Population density of *Uroplata girardi* and *Ophiomyia camarae* between 1998 and 2007 in Emanzimtoti, KwaZulu-Natal. Level of abundance was recorded using the scale presented in Table 2 (Baars 1998, Heystek, 2004, April, 2006 unpubl. data) and used to enumerate % of agents' population growth in the field in Chapter 3.

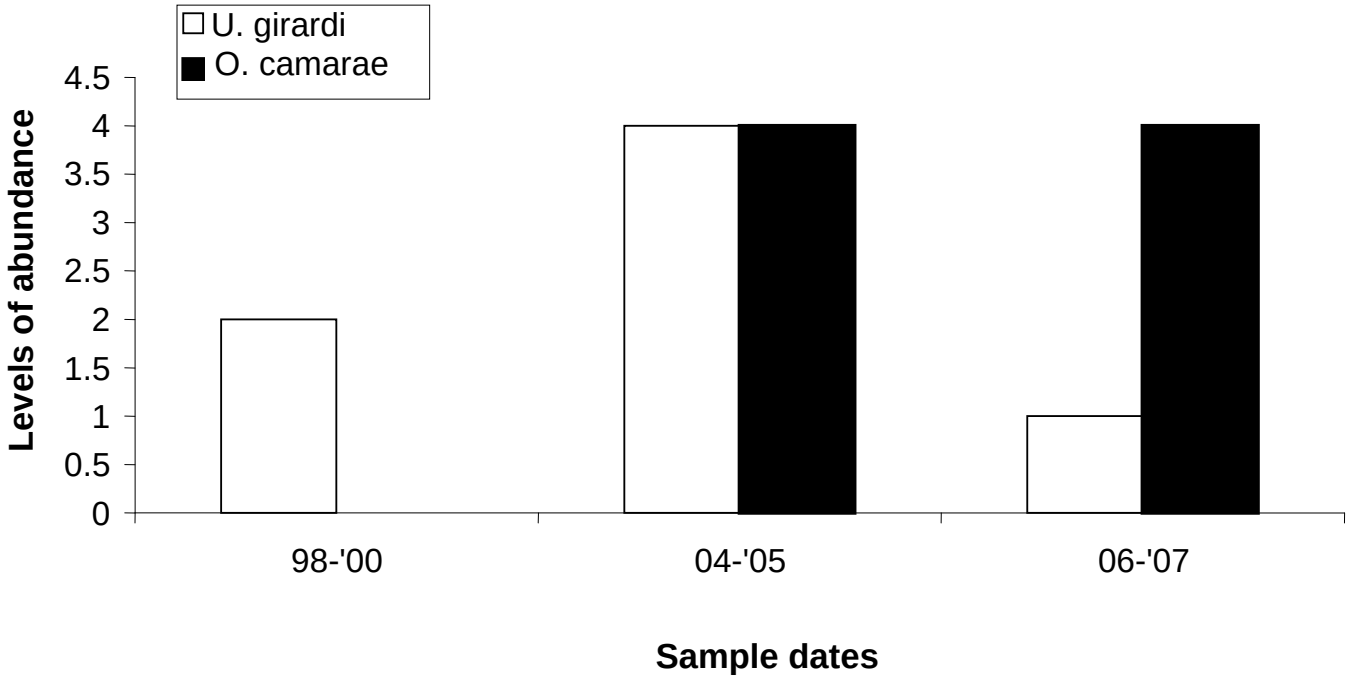

Figure 4: Population density of *Uroplata girardi* and *Ophiomyia camarae* between 1998 and 2007 in Richards Bay. Level of abundance was recorded using the scale presented in Table 2 (Baars, 1998, Heystek, 2004, April, 2006 unpubl. data) and used to enumerate % of agents' population growth in the field in Chapter 3.

Thesis Outline

Chapter One introduces the thesis and gives background on the study. Chapter Two investigates the impact of *Uroplata girardi* and *Ophiomyia camarae* singly and when combined on growth and reproductive capacity of *Lantana camara* and compares this to insect-free controls. Chapter Three investigates the use of two species sharing the same host plant as biocontrol agents, the possibility of, competitive interactions among the agents through their population growth in exclusion semi-field trials and in the field where both agents co-occur. The chapter further investigates oviposition preference of the females between the infested and uninfested leaves of lantana in the laboratory. Chapter Four is a general discussion of all the results obtained in this study with emphasis on interaction between biocontrol agents, their combined effect versus individual effect on the target weed and factors that may hinder their success in controlling the plant and recommendations.

References

Baars, J-R. 2003. Geographic range, impact and parasitism of Lepidopteran species associated with the invasive weed *Lantana camara* in South Africa. *Biological control* **28:** 293-301.

Baars, J-R. & Heystek, F. 2003. Geographical range and impact of five biocontrol agents established on *Lantana camara* in South Africa. *BioControl* **48:** 743-759.

Baars, J-R., Urban, A.J. & Hill, M.P. 2003. Biology, host range and risk assessment supporting releases in Africa of *Falconia intermedia* (Heteroptera: Miridae) a new biocontrol agent for *Lantana camara*. Biological Control **28:** 282-292.

Baars, J-R. & Neser, S. 1999. Past and present initiatives on the biological control of *Lantana camara* (Verbenaceae) in South Africa. In: Olckers, T. and Hill, M.P. (eds.), *Biological control of weeds in South Africa* (1990-1998), *African Entomology Memoir* **1:** 21-33.

Blossey, B. & Hunt-Joshi, T.R. 2003. Belowground herbivory by insects: influence on plants and aboveground herbivores. *Annual Reviews in Entomology* **48:** 521-547.

Broughton, S. 2000. Review and Evaluation of Lantana Biocontrol programs. Biological Control **17:** 272-286.

Cilliers, C.J. 1987a. Notes on the biology of the established insect natural enemies of *Lantana camara* L. (Verbenaceae) and their seasonal history in South Africa. *Journal of the Entomological Society of Southern Africa* **50**: 1-13.

Cilliers, C.J. 1987b. The evaluation of three insect natural enemies for the biological control of the weed *Lantana camara* L., *Journal of the Entomological Society of Southern Africa* **50:** 15-34.

Cilliers, C.J. 1983. The weed, *Lantana camara* L., and the insect natural enemies imported for its biological control into South Africa. J*ournal of the Entomological Society of Southern Africa* **46:** 131-138.

Cilliers, C.J. & Neser, S. 1991. Biological control of *Lantana camara* (Verbenaceae) in South Africa. *Agriculture, Ecosystems and Environment* **37:** 57-75.

Crowe, M.L. & Bourchier, R.S. 2006. Interspecific interactions between the gall-fly *Urophora affins* Frfld. (Diptera: Tephritidae) and the weevil *Larinus minutus* Gyll. (Coleoptera: Curculionidae), two biological control agents released against spotted knapweed, *Centaurea stobe* L. spp. *micranthos*. *Biocontrol Science and Technology* **16:** 417-430.

Day, M.D. 2003. *Lantana camara:* Weed of national significance. CRC Weed management guide.

Day, M.D. & Neser, S. 2000. Factors Influencing the Biological Control of Lantana camara in Australia and South Africa. In: Neal R. Spencer (ed.). *Proceedings of the X International Symposium on Biological Control of weeds* (897-908), Montana State University, Bozeman, Montana, USA.

Day, M.D, Wiley, C.J., Playford, J. & Zalucki, M.P. 2003. *Lantana camara:* Current Management Status and Future Prospects. Canberra. *ACIAR Monograph* 102.

Denoth, M., Frid, L. & Myers, J.H. 2002. Multiple agents in biological control: improving the odds? *Biological Control* **24:** 20-30.

Dorn, B., Mattiacci, L., Bellotti, A.C. & Dorn, S. 2003. Effects of mixed species infestation on the cassava mealybug and its encyrtid parasitoid. *Biological Control* **27:** 1-10.

Gonzalez-Megias, A. & Gómez, J.M. 2003. Consequences of removing a keystone herbivore for the abundance and diversity of arthropods associated with a cruciferous shrub. *Ecological Entomology* **28:** 299-308.

Hajek, A.E. 2004. Classical biological control. *Natural Enemies: An introduction to Biological Control*. Cambridge University press, 39-56.

Harris, P. 1985. Biological control of weeds: bureaucrats, botanists, beekeepers, and other bottlenecks. In: Delfosse, E.S. (Ed.) *Proceedings of the VI International Symposium on Biological control of Weeds*. Agriculture Canada. 3-12. Ottawa.

Heshula, U-N.L.P. 2005. Establishment and impact of the sap-sucking mirid, *Falconia intermedia* (Distant) (Hemiptera: Miridae) on *Lantana camara* (Verbenaceae) varieties in the Eastern Cape Province, South Africa. Rhodes University, South Africa.

Heystek, F. 2008. Adding pressure on lantana. *Plant Protection News* **74:** 12.

Heystek, F. 2006 Laboratory and field host utilization by established biological control agents of *Lantana camara* L., in South Africa. MSc Thesis, Rhodes University, South Africa.

Henderson, L. 2001. Alien Weeds and Invasive Plants. *Plant Protection Research Institute Handbook* **12:** 300.

Hoffmann, J.H. 1998. Interactions between three weevil species in the biocontrol of *Sesbania punicea* (Fabaceae): the role of simulation models in evaluation. *Agriculture, Ecosystems and Environment* **32:** 77-87.

Huffaker, C.B. & Messenger, P.S. 1976. Interaction among insects. In: Huffaker C.B. & Messenger, P.S (Eds) Theory and Practice of Biological Control 265-271. Academic Press, New York.

Hunt-Joshi, T.R. & Blossey, B. 2005. Interactions of root and leaf herbivores on purple loosestrife (*Lythrum salicaria*). *Oecologia* **142:** 554-563.

Julien, M.H, & Griffiths, M.W. 1998. Biological control of weeds: A world catalogue of agents and their target weeds. 4th Edition. CABI Publishing, UK.

Louda, S.M., Rand, T.A., Russell, F.L. & Arnett, A.E. 2005. Assessment of ecological risks in weed biocontrol: Input from retrospective ecological analyses. *Biological Control* **35:** 253-264.

Louda, S.M., Pemberton, R.W., Johnson, M.T. & Follett, P.A. 2003. Nontarget Effects- the Achilles' heel of Biological Control? Retrospective analyses to reduce risk associated with biocontrol introductions. *Annual Reviews of Entomology* **48:** 365-396.

Louda, S. M., Kendall, D., Connor, J. & Simberloff, D. 1997. Ecological effects of an insect introduced for biological control of weeds. *Science* **277:** 1088-1090.

Masters, G.J. & Brown, V.K. 1992. Plant-mediated interaction between two spatially separated insects. *Ecology* **6:** 175-79.

McConnachie, A.J., De Wit, M.P., Hill, M.P. & Byrne, M.J. 2003. Economic evaluation of the successful biological control of *Azolla filiculoides* in South Africa. *Biological Control* **28:** 25-32.

McEvoy, P.B. 1985. Depression in ragwort (*Senecio jacobaeae*) abundance following introduction of *Tyria jacobaeae* and *Longitarsus jacobaeae* on the central coast of Oregon. In: Delfosse, E. S. (Ed), Proceedings VI

International Symposium of Biological Control of Weeds, 19-25 August 1984, Vancouver, Canada. *Agriculture Canada*. 57-64.

McEvoy, P.B. & Coombs, E.M. 1999. Why things bite back: Unintended consequences of biological control of weeds. In: Follett, P. A., Duan, J. J. (eds.), *Nontarget effects of Biological Control*. Kluwer Academic Publishers, Boston, 176-194.

McFayden, R.E. 1998. Biological control of weeds. *Annual Reviews of Entomology* **43:** 369-393.

Olckers, T., Medal, J.C. & Gandolfo, D.E. 2002. Insect herbivores associated with species of *Solanum* (Solanaceae) in northeastern Argentina and southeastern Paraguay, with reference to biological control of weeds in South Africa and the United States of America. *Florida Entomologist*. **85:** 254-260.

Ooi, P.A.C. 1987. A fortuitous biological control of lantana in Malaysia. *PANS* **33:** 234-235.

Oosthuizen, M.J. 1964. The biological control of *Lantana camara* L., in Natal. *Journal of the Entomological Society of Southern Africa* **21:** 3-16.

Perkins, R.C.L. & Swezey, O.H. 1924. The introduction into Hawaii of insects that attack lantana. *Hawaiian Sugar Planters' Association Series Bulletin* **16:**1-83.

Rhagu, S. & Dhileepan, K. 2005. The value of simulating herbivory in selecting effective weed biological control agents. *Biological Control* **34:** 265-273.

Robertson, M.P., Villet, M.H., Palmer, A.R., Fairbanks, D.H.K., Henderson, L., Higgins, S., Hoffmann, J.H., Le Maitre, D.M., Riggs, I., Shackleton, C.M. & Zimmermann, H.G. 2003. A proposed prioritization system for the

management of invasive alien plants in South Africa. *South African Journal of Science*. **99:** 37-43.

Simelane, D.O. 2008. Attacking the root of the lantana problem. *Plant Protection News* **74:** 11-12.

Simelane, D.O. 2006. Effect of herbivory by *Teleonemia scrupulosa* on the performance of *Longitarsus bethae* on their shared host, *Lantana camara. Biological Control* **39:** 385-391.

Simelane, D.O. 2005. Biological control of *Lantana camara* in South Africa: targeting a different niche with a root-feeding agent, *Longitarsus* sp. *BioControl* **50:** 375-387.

Simelane, D.O. 2002. Biology and host range of *Ophiomyia camarae*, a biological control agent for *Lantana camara* in South Africa. *BioControl* **47:** 575-585.

Simelane, D.O. & Phenye, M.S. 2005. Suppression of growth and reproductive capacity of the weed *Lantana camara* (Verbenaceae) by *Ophiomyia camarae* (Diptera: Agromyzidae) and *Teleonemia scrupulosa* (Heteroptera: Tingidae). *Biocontrol Science and Technology* **15:** 153-163.

Stirton, C.H. 1977. Some thoughts on the polyploidy complex Lantana camara L. (Verbenaceae). In: A.A. Balkema (ed) *Proceedings of the Second National Weeds Conference of South Africa*. 321-344. Cape Town.

Thomas, S.E. & Ellison, C.A. 2000. A century of classical biological control of Lantana camara: Can Pathogens make a significant difference? In: Neal R. Spencer (Ed.) *Proceedings of the X International Symposium on Biological Control of weeds* (97-104), Montana State University, Bozeman, Montana, USA.

Williams, H.E. 2003. The suitability of *Alagoasa extrema* Jacoby (Coleoptera: Chrysomelidae: Alticinae), as a biological control agent for *Lantana camara* L. in South Africa. MSc dissertation, Rhodes University.

Williams, H.E. 2005. The potential impact of the trimorphic flea beetle, *Alagoasa extrema*, on a selected weedy variety of *Lantana camara*. *BioControl* **50:** 647-655.

Woodburn, T.L. 1996. Interspecific competition between *Rhinocyllus conicus* and *Urophora solstitialis*, two biocontrol agents released in Australia against *Carduus nutans*. In V. C. Moran and J. H. Hoffmann (Ed.) Proceedings of the IX International Symposium on Biological Control of Weeds. University of Cape Town, South Africa. 409-441.

Zalucki, M.P., Day, M.D. & Playford, J. 2007. Will biological control of *Lantana camara* ever succeeds? Patterns, processes & prospects. *Biological Control* **42:** 251-261.

Chapter Two

Impact of *Uroplata girardi* Pic (Coleoptera: Chrysomelidae) and *Ophiomyia camarae* Spencer (Diptera: Agromyzidae) on their shared host, *Lantana camara* L. (Verbenaceae)

Abstract

The influence of herbivory by two introduced phytophagous insects on the survival and performance of *Lantana camara* L. (Verbenaceae), a perennial invasive weed was studied in replicated semi-field plots. Caged plants under the semi-field conditions were inoculated with *Uroplata girardi* Pic (Coleoptera: Chrysomelidae) and *Ophiomyia camarae* Spencer (Diptera: Agromyzidae) to measure the impact of these insects on plant performance. Feeding by *U. girardi* reduced the leaf density, fruit, and flower biomass by 59, 28 and 24% respectively. Whereas, *O. camarae* had a moderate effect on leaf density, attack by the herring-bone leafminer reduced fruit, flower and root biomass by 35, 61 and 81%, respectively. The impact of feeding damage by the two agents (*U. girardi* and *O. camarae* combined) was significantly greater than that caused by *O. camarae* alone; but less than that caused by *U. girardi* alone. Combined feeding damage by both agents caused a reduction in leaf density, fruit and flower biomass by 17, 19 and 40%, respectively. At the end of the trial, there was no significant effect on stem diameter, plant height and canopy width in all the treatments. Overall, attack by *O. camarae* caused a greater reduction in root growth and reproductive output of the host (*L. camara*) than did *U. girardi* at the same population density, but also a greater production of above-ground vegetative growth. The effect of the two biocontrol agents was not simply additive, since their combined impact was intermediate between their individual impacts, which is probably the result of interference competition.

Keywords: Biological control, competition, interference, resource allocation, interaction

Introduction

Most plant species have acquired guilds of natural enemies that exert some degree of regulation on their population (Rayamajhi *et al.*, 2006). Insects and fungi are two of the most common groups of organisms that play a major role in ecosystem functioning, and are therefore used in classical biological control of pest plants and insects (Hajek, 2004). The impact of herbivores on plants is often associated with a significant control of reproduction and distribution of the host (Thomas & Reid, 2007). Likewise, removing insect herbivores from plants often increases the plant population size or fitness (Keane & Crawley, 2002). In classical biological control of weeds, either single or multiple biological control agents can be released against an invasive alien plant. However, the release of multiple versus single biocontrol agents against a target weed has received increased attention in recent literature (McEvoy & Coombs, 1999; Pearson & Callaway, 2003; Louda *et al.*, 2005), with both positive (Hoffmann & Moran, 1999; Anderson *et al.*, 2000) and negative (Louda *et al.*, 1997; Crowe & Bourchier, 2006; Seastedt *et al.*, 2007) outcomes being reported.

Lantana camara (tickberry shrub) is endemic to South and Central America, and has been a target for biological control in South Africa since the 1960's (Chapter 1) serves as a good case study to assess the effect of multiple-releases in biological control. The release of a few biocontrol agents against lantana did not effectively control this weed in the 1970's (Cilliers & Neser, 1991; Baars & Neser, 1999). Since the 1970's, more than 20 biocontrol agents have been introduced and released against lantana in South Africa (Day & Neser, 2000) to supplement the existing ones (Chapter 1). The rationale of releasing a suite of biocontrol agents was aimed at increasing cumulative stress on the plant, thereby enhancing the biological control of the weed (Chapter 1). A South American leaf mining hispine beetle (*Uroplata girardi*) was among the biocontrol agents released more than three decades ago (Cilliers, 1987b), and has since been reported to be causing significant impact on lantana, particularly along the coastal regions of KwaZulu-Natal (Baars & Heystek, 2003). As biocontrol agents

remained largely ineffective in the inland regions, there was a need to release more agents to curb lantana infestations in these regions (Baars & Neser, 1999). Consequently, the sap-sucking mirid (*Falconia intermedia*) and a leaf-mining fly (*Ophiomyia camarae*) were released into South Africa in 1999 and 2001, respectively (Simelane, 2002; Baars *et al.*, 2003). While *F. intermedia* is reported to have established at only a few release sites in the Eastern Cape (F. Heystek, pers. comm.), *O. camarae* is fully established and has become abundant along the eastern coastal regions of the country. However, *O. camarae* appears to be less adapted to high altitude areas which are mainly located inland (Simelane & Phenye, 2004). Both of the leafminers, *U. girardi* and *O. camarae*, occur along the eastern coastal region, and prefer the same lantana variety (Heystek, 2006), suggesting that they may compete for the resource. Heystek (2006) observed that populations of *U. girardi* appeared to decline when populations of *O. camarae* were increasing in the same region, indicating a possible negative interaction between the two agents (Heystek, 2006; Chapter 3). Although the impact of *O. camarae* alone on lantana variety 009 Light Pink has been measured under semi-field conditions (Simelane & Phenye, 2005), there are no comparable data on the impact of *U. girardi* alone or the combined impact of both agents together. This study therefore investigates the impact of single and joint attack by *U. girardi* and *O. camarae* on growth and reproduction of lantana.

Materials and methods

Semi-field experiments were carried out in an open field at Rietondale Research Centre (25˚43′36.7″ S; 23˚14′03.3″ E) in Pretoria, Gauteng, South Africa. Gauteng province is situated in the highlands (highveld) and experiences a variation in temperature, ranging from 16.1˚C to 30.3˚C in summer with a relative humidity of 55% to 85% in the morning, 24% to 63% in the afternoon and 32% to 78% in the evening. In winter the temperature ranges between 4.3˚C and 21˚C. The annual rainfall average is 630.2 mm (Weather SA, 2008). Experiments were conducted over a period of six months during the summer season, from November 2006 until the end of May 2007.

***Organisms used in the study*:**

Plants used in the study were propagated from *L. camara* cuttings of variety 021 Dark Pink cut from a mother plant in Kuswag road, Emanzimtoti 30˚02′08″S; 30˚53′42″E. Variety 021 appears to be the most common variety in KwaZulu-Natal, and it seems to be the most preferred by both *U. girardi* and *O. camarae* (Simelane, 2005; Heystek, unpublished data). Forty eight shoot cuttings of 7 cm length each were planted in a peat soil crate and put on a warm bed inside a glasshouse to induce rooting. Each rooted cutting was initially planted into a plastic nursery bag of 4.74L (150 x 125 x 300 mm), with a soil mix containing two parts compost, two parts vermiculite, and one part sand before being transplanted into the experimental site. Thirty lantana plants of equal size (approximately 25 leaves per plant) were then transplanted individually into pits (30 cm x 30 cm) that were dug 3 m apart in the field at the experimental site.

Uroplata girardi and *Ophiomyia camarae* adults used to start cultures were obtained from selected sites in KwaZulu-Natal (Table 1). *U. girardi* was collected either as live adults or as pupae. Leaves infested with *U. girardi* and *O. camarae* pupae were collected and put into 3-L perforated containers in the lab in order to collect newly emerged adults. Moist paper towels were placed on the leaves to retain moisture, thus preventing desiccation of pupae. To mass-rear the agents, about 30 unsexed newly emerged adults of both agents were confined with potted lantana plants in cages (55 cm x 55cm x 95 cm) in the glasshouse. To extend the duration of adult survival of *O. camarae*, plants were sprayed with water at least twice per day and one thin stripe of honey was applied in each cage for the flies to feed on. Plants were watered three times a week. For both agents, leaves containing pupae were harvested. First generations of both agents were used in the experiments.

Experimental design

The thirty lantana plants that were transplanted individually at the experimental site were allowed to grow in the field for five weeks and irrigated three times a week. Twenty dome-shaped cages (1.8 m x 1.8 m x 1.8 m), made of Psylla

screen mesh with a sleeve in the centre of the floor, were erected over the plants. Access to the cages was through a zip fastener. The experimental design consisted of two controls and three treatments arranged in a randomized block design. The first control comprised of five uncaged plants (uncaged C) that were exposed to any biotic and abiotic environmental conditions. The second control comprised of five caged plants (one plant per cage) and contained no insects (caged C). The first treatment comprised of five caged plants infested with 30 unsexed *U. girardi* adults per cage at the beginning of the experiment (Uro). The second treatment comprised of five caged plants infested with 30 unsexed *O. camarae* adults per cage at the beginning of the experiment (Oph). The third treatment comprised of five caged plants infested with 30 unsexed adults of each of the two (*U. girardi* and *O. camarae*) insect species per cage (Uro + Oph).

Data collection and analysis

To determine the impact of the biocontrol agents in each treatment, several plant growth parameters were measured namely: leaf density, stem diameter, plant length and canopy size. Leaf density was determined by counting the number of leaves per plant in each treatment. Stem diameter was determined by measuring the base of the stem with a vernier calliper. Plant length was determined by measuring the plant height from the base to the tip of the plant. The canopy size (length and width) were measured by a measuring tape. All the growth parameters were measured and recorded at the start of the experiment (i.e. when plants were inoculated with agents).

Based on the initial and final measure of each of the parameters of the control (caged) and treatment plants, percentage of growth loss and biomass was calculated. With the exception of the above- and below-ground plant biomass, all growth parameters were measured on a monthly basis. The above- and below-ground plant biomass was only measured at the start and end of the experiment. To measure biomass, five plants were excavated at the start of the trials and five plants for each control and treatment were excavated at the end of the trial. Roots were removed and washed to remove soil, and the above ground material

(i.e. flowers, fruits, leaves, and branches) was separated. All the plant material was chopped, put into labelled paper bags, oven-dried at 72°C for 48 hours and weighed (Simelane & Phenye, 2005).

One-way analysis of variance (ANOVA) was performed to examine differences in the number of leaves, stem diameter, plant height, plant width and plant biomass among treatments, and the means were separated by Fisher's least significant difference (LSD) test (Statistica v. 6.1, 2004). To stabilize the variance, the data were square root-transformed before being subjected to parametric statistical analysis, but untransformed data are presented.

Results

Effects of single and joint attack by *U. girardi* and *O. camarae* on plant performance:

Leaf density

Single and joint attack by *U. girardi* and *O. camarae* had a significant ($F = 6.12$; $df = 4,134$; $p < 0.05$) impact on leaf density of lantana during the six-month period. The *U. girardi* treatment showed a significantly reduced number of leaves per plant compared to the *O. camarae* treatment and when both the agents were present. The caged control had a significantly higher number of leaves compared to the uncaged control and the other treatments (Fig. 1). Between December 2006 and March 2007, single and joint attack by both insect species caused significant defoliation, reducing leaf density by 17 to 59% (Fig. 2). Although *U. girardi*-attacked plants continued to be suppressed throughout the study, *O. camarae*-attacked plants and those jointly attacked by both agents appeared to recover soon after March 2007. By May 2007, leaves on plants attacked by *U. girardi* had been reduced by 59% while those attacked by *O. camarae* and jointly attacked by both agents had been slightly reduced by 10 and 17.3%, respectively. *Teleonemia scrupulosa* Stål (Heteroptera: Tingidae) was the only established agent that colonized and caused visible feeding damage on the uncaged plants. By May 2007, most of the uncaged plants had been severely

stunted by this agent, causing almost 50% reductions in leaf density on these plants.

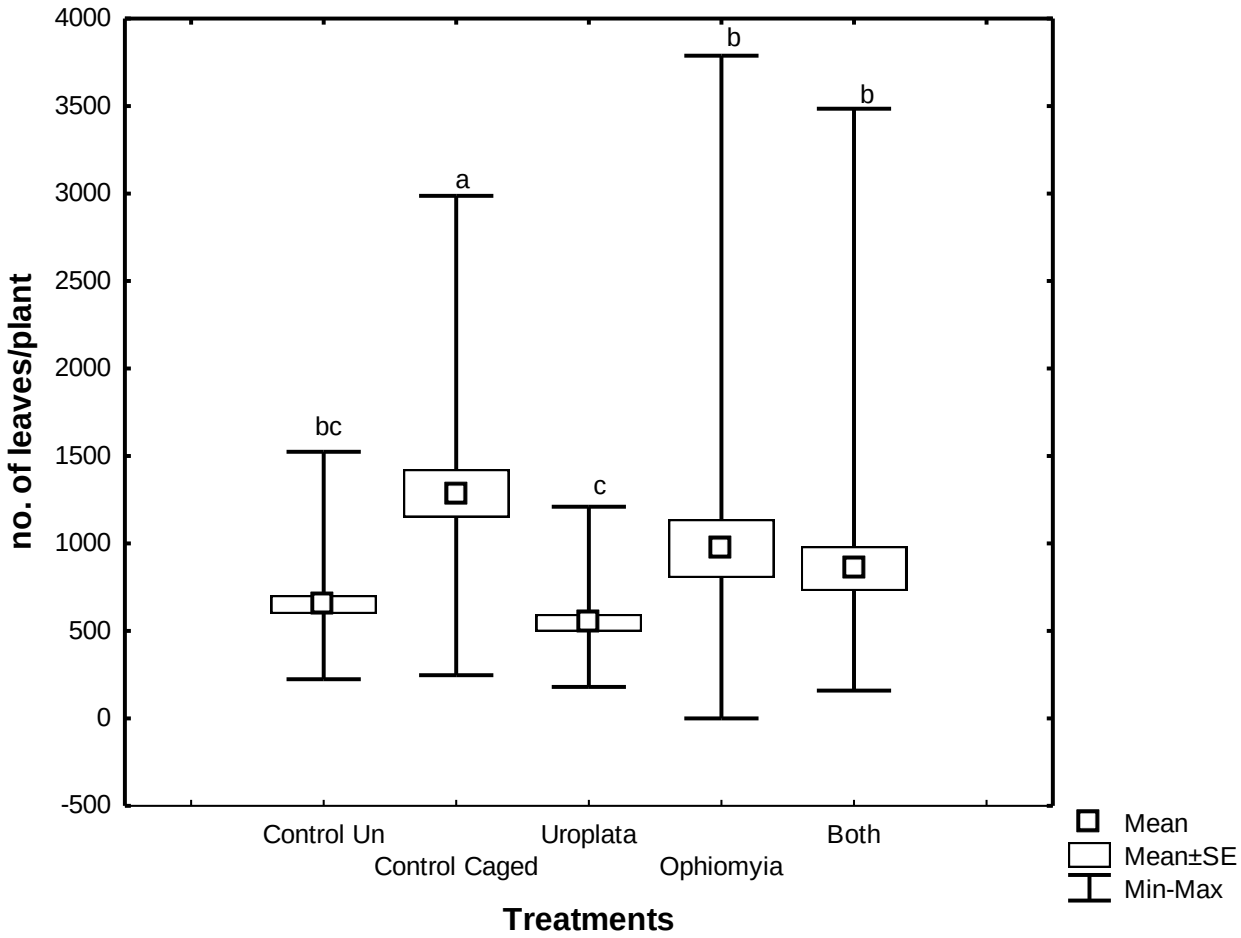

Figure 1: Impact of *Uroplata girardi* and *Ophiomyia camarae*, singly and combined, on leaf density (Mean±SE) of *Lantana camara* (i.e. mean number of attached leaves per plant during the 6-month period of the trial). Means compared with one-way ANOVA: treatments with the same letter(s) are not significantly different ($p > 0.05$; Fisher LSD).

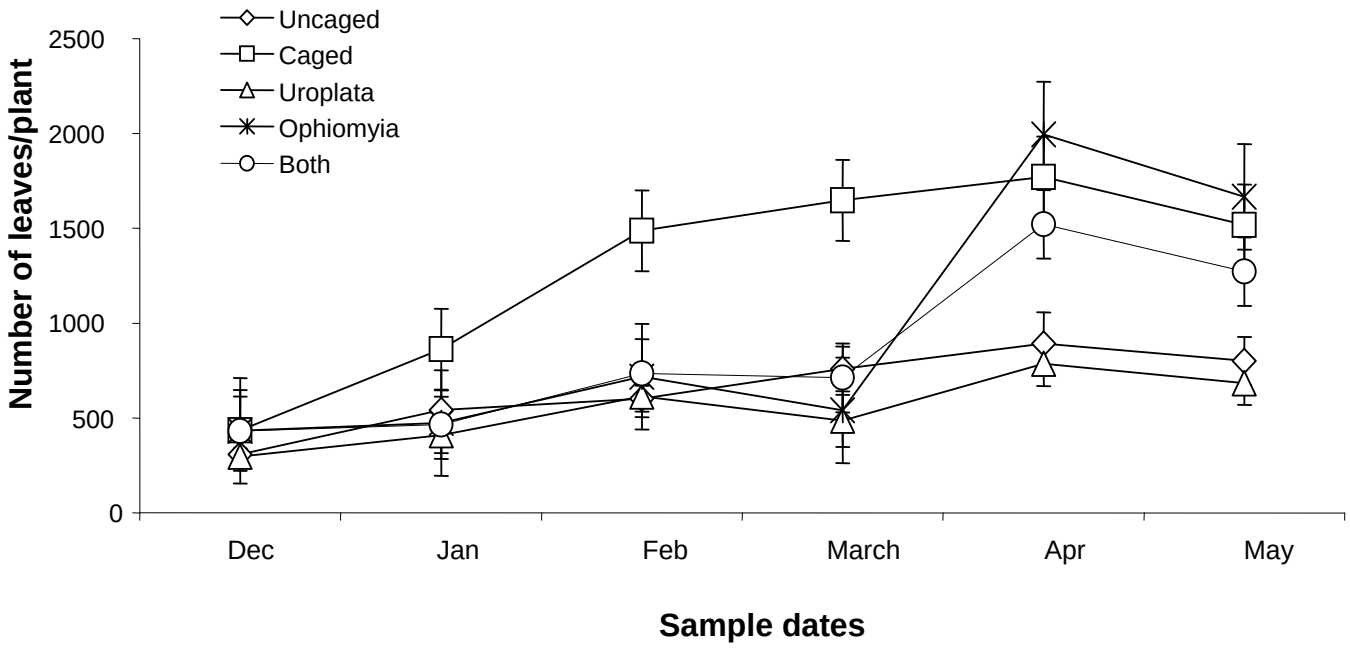

Figure 2: Mean±SE number of *L. camara* leaves per plant counted between December 2006 and May 2007 in each of the three treatments and two controls.

Stem diameter

Although stems of plants that were jointly attacked by both agents were slightly thicker than those of other treatments, these stems did not differ significantly from those of other treatments and controls at the end of the trial ($F = 0.767$; $df = 4$; $p = 0.547$) (Fig. 3). By the end of the experiment, stem diameter for the uncaged control and *O. camarae* inoculated plants had increased by 7%. There was no significant difference between the stem diameter of the *U. girardi*-inoculated plants and those of the control. However, jointly attacked plants showed a 10% increase in stem diameter.

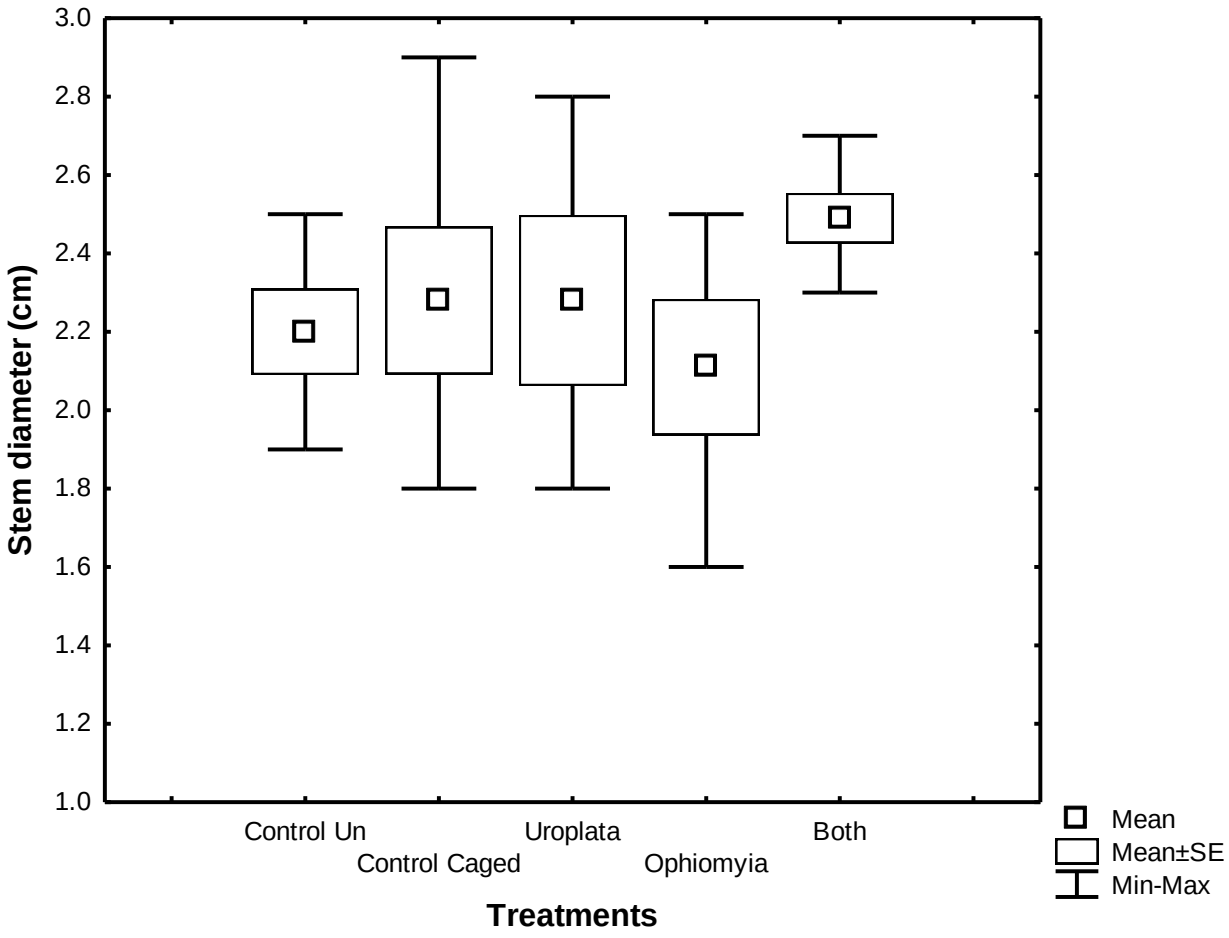

Figure 3: Impact of *Uroplata girardi* and *Ophiomyia camarae*, singly and combined, on basal stem diameter (Mean±SE) of *Lantana camara* at the end of the 6-month trial.

Plant height

Due to heavy attack by *T. scrupulosa*, uncaged control plants were markedly stunted, and were therefore significantly shorter than the other treatments and caged control plants ($F = 3.904$; $df = 4,20$; $p = 0.017$). However, the caged control plants did not differ significantly from other treatment plants (Fig. 4). By the end of the experiment, caged control plants had grown 50% taller than the uncaged control plants while *U. girardi*-attacked, *O. camarae*-attacked and those attacked by both agents had not been significantly affected (Fig. 5).

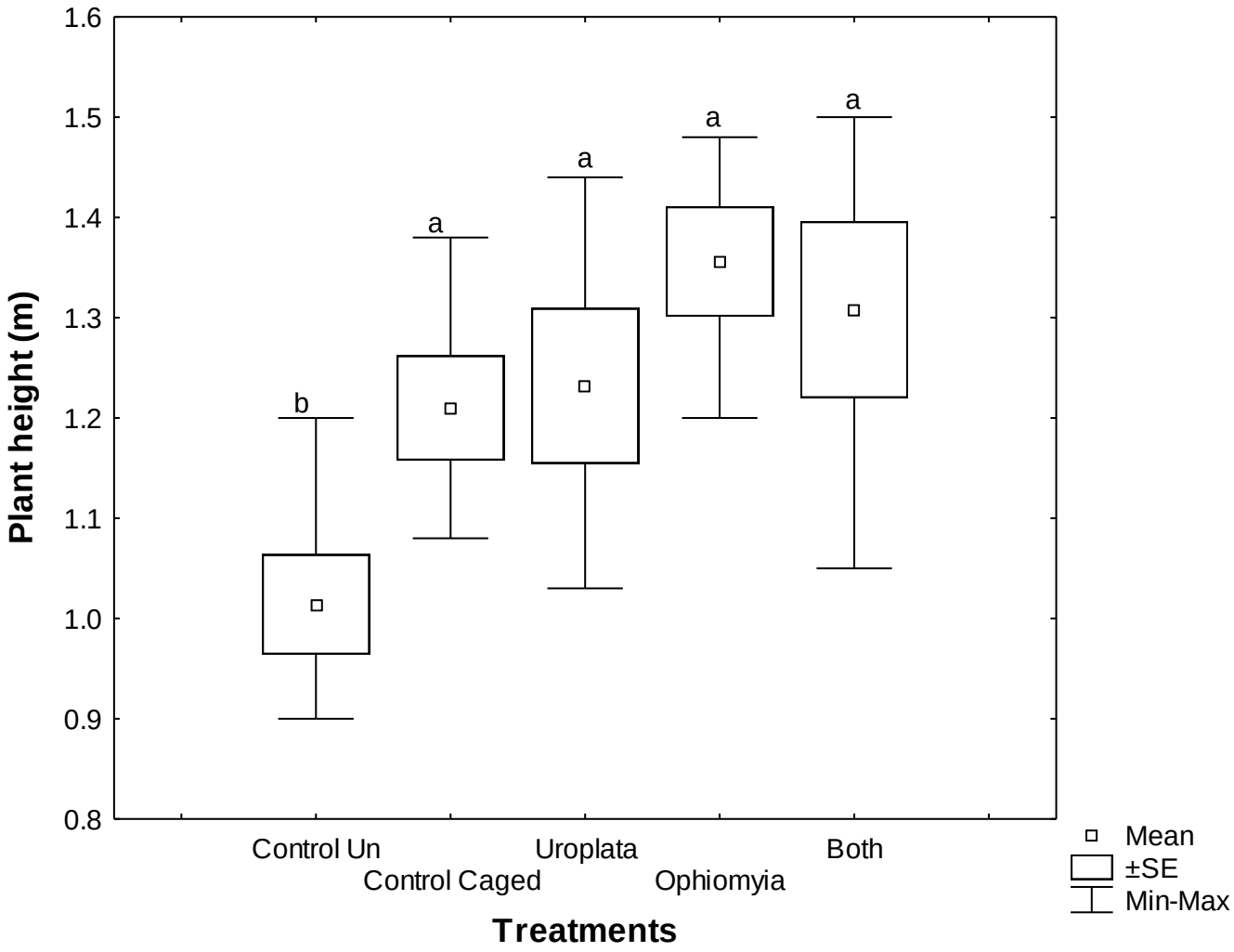

Figure 4: Impact of *Uroplata girardi* and *Ophiomyia camarae*, singly and combined, on plant height (Mean±SE) of *Lantana camara* at the end of the 6-month trial. Means compared with one-way ANOVA: treatments with the same letter are not significantly different ($p > 0.05$; Fisher LSD).

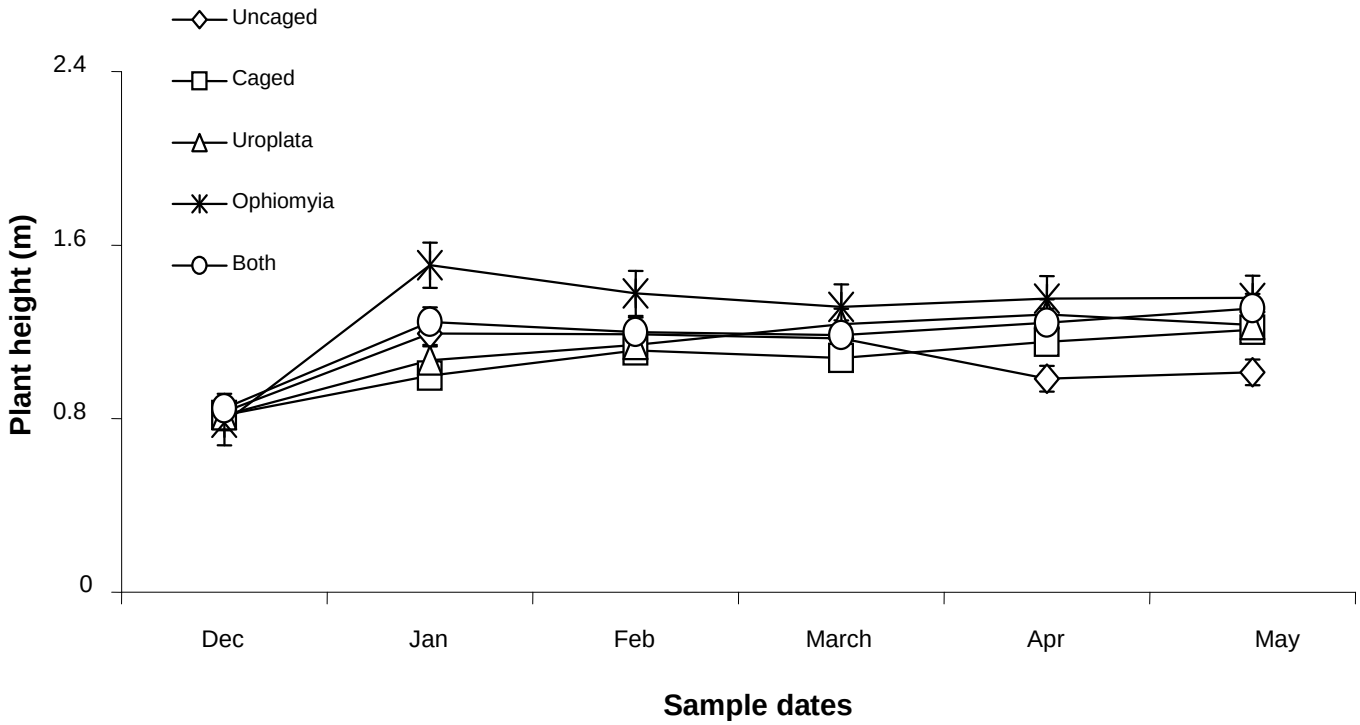

Figure 5: Plant height (Mean±SE) during December 2006-May 2007 in *U. girardi*, *O. camarae* treatments and controls.

Canopy size (Plant width)

By the end of experiment, there was no significant difference on the canopy size of the plant among the treatments (F = 1.2; df = 4; p = 0.4) (Fig. 6 and 7). Despite being significantly shorter than other treatments (see Fig. 5), the canopy size of uncaged control plants did not differ significantly from those of other treatments by end of the trials.

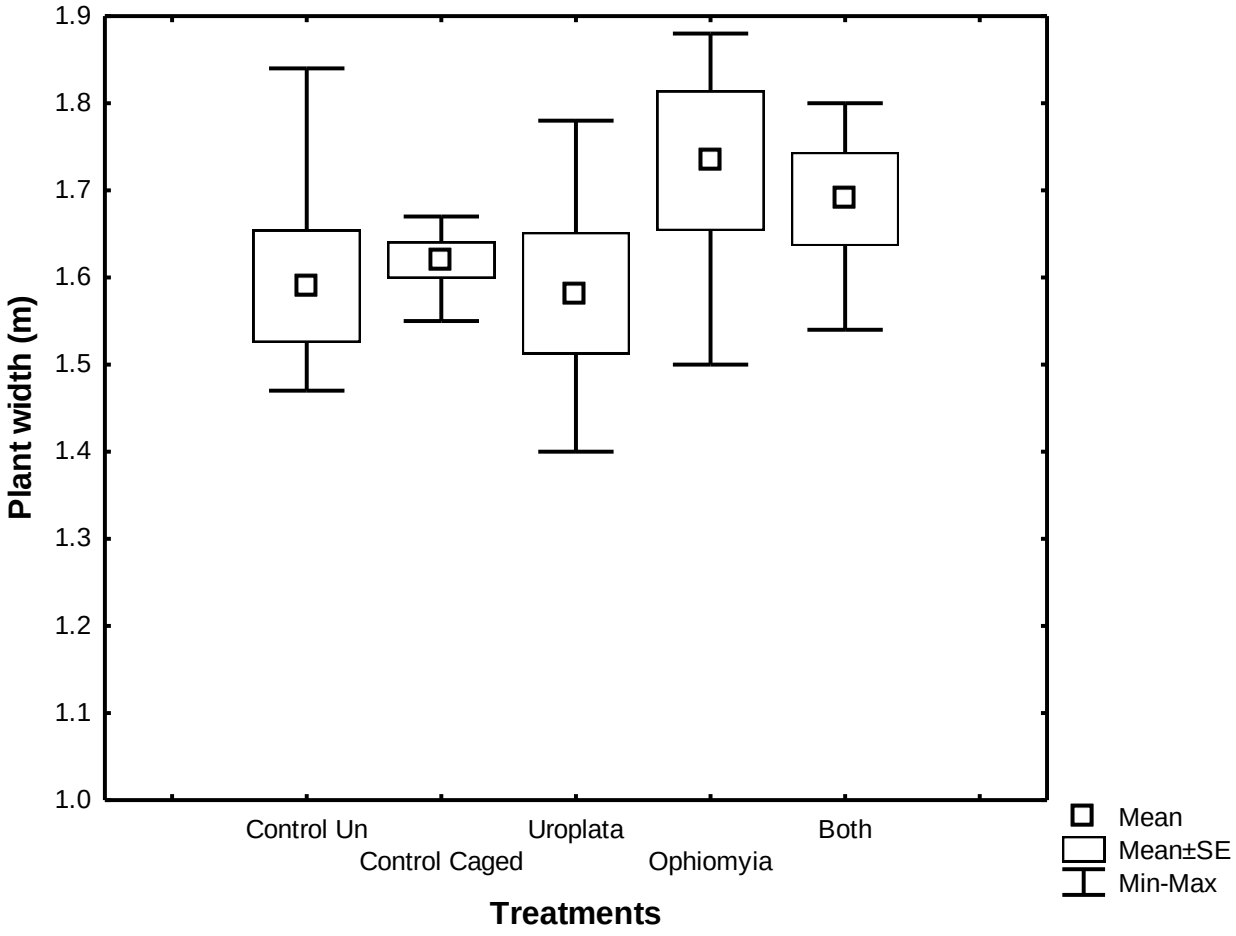

Figure 6: Impact of *Uroplata girardi* and *Ophiomyia camarae*, singly and combined, on canopy width (Mean±SE) of *Lantana camara* at the end of the 6-month trial.

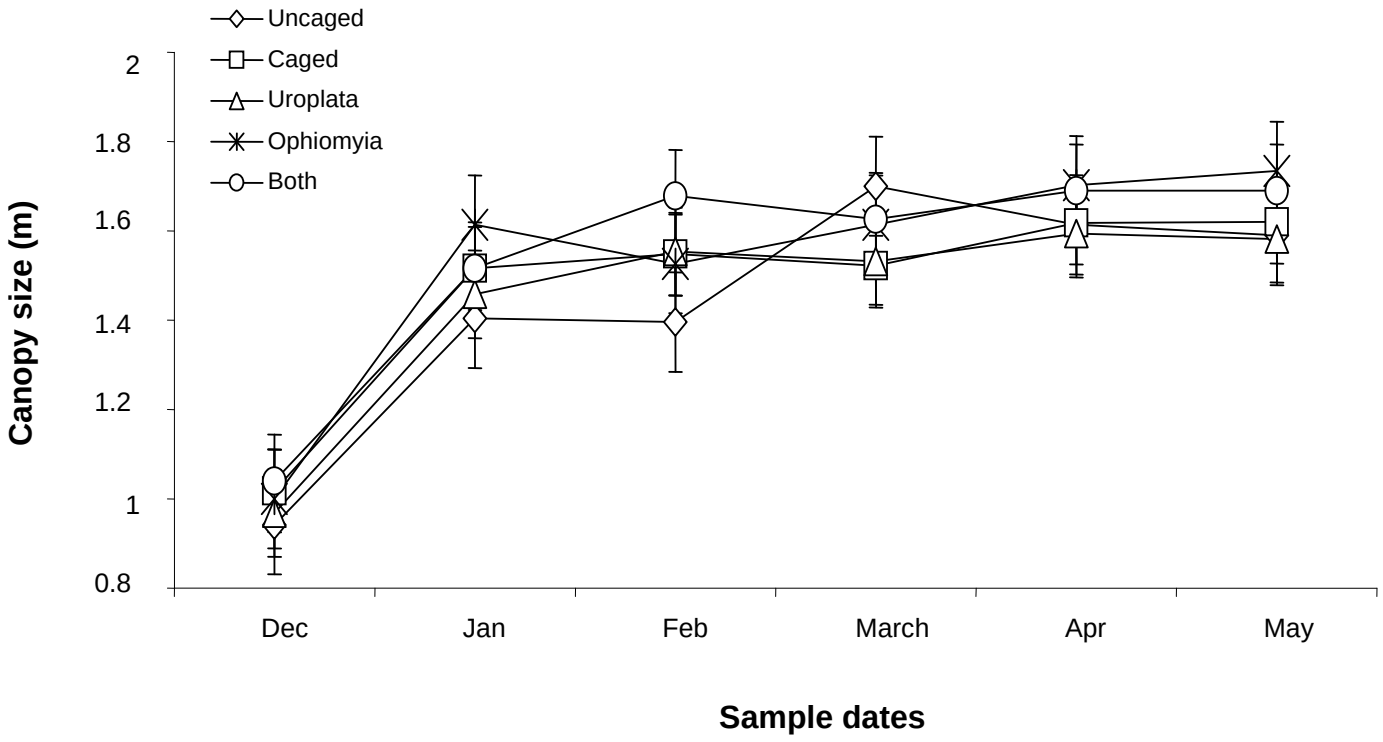

Figure 7: Plant width (Mean±SE) from December 2006 to May 2007 in *U. girardi*, *O. camarae* treatments and controls.

Above- and below-ground dry plant biomass

Joint and single attack by *U. girardi* and *O. camarae* had a significant impact on the biomass of lantana reproductive ($F = 9$; $df = 10$; $p < 0.001$) (Fig. 8) and the vegetative parts ($F = 7$; $df = 10$; $p < 0.001$) (Fig. 9). On the plants that were jointly attacked by *U. girardi* and *O. camarae*, fruit and flower biomass were reduced by 19 and 40%, respectively. For plants attacked separately by *U. girardi* and *O. camarae*, flower biomass was reduced by 24 and 61%, respectively, while fruit biomass was reduced by 28 and 35%, respectively. Due to heavy attack by *T. scrupulosa,* there were neither flowers nor fruits produced by uncaged control plants. Above-ground biomass for *U. girardi* treatments increased by 9% whilst that of *O. camarae* and both agents combined increased by 86 and 70%, respectively. For the uncaged control, there was a 36% decrease in above-ground biomass (Fig. 9). There was a significant difference in root biomass

between treatments and controls (F = 3.3; df = 5; p < 0.05) (Fig. 10). On *O. camarae*-attacked plants, a significant reduction in root biomass (81%) was recorded compared to 3 and 15% reductions recorded on jointly- and *U. girardi*-attacked plants, respectively. Root biomass of uncaged plants increased markedly by 88% compared to the caged control.

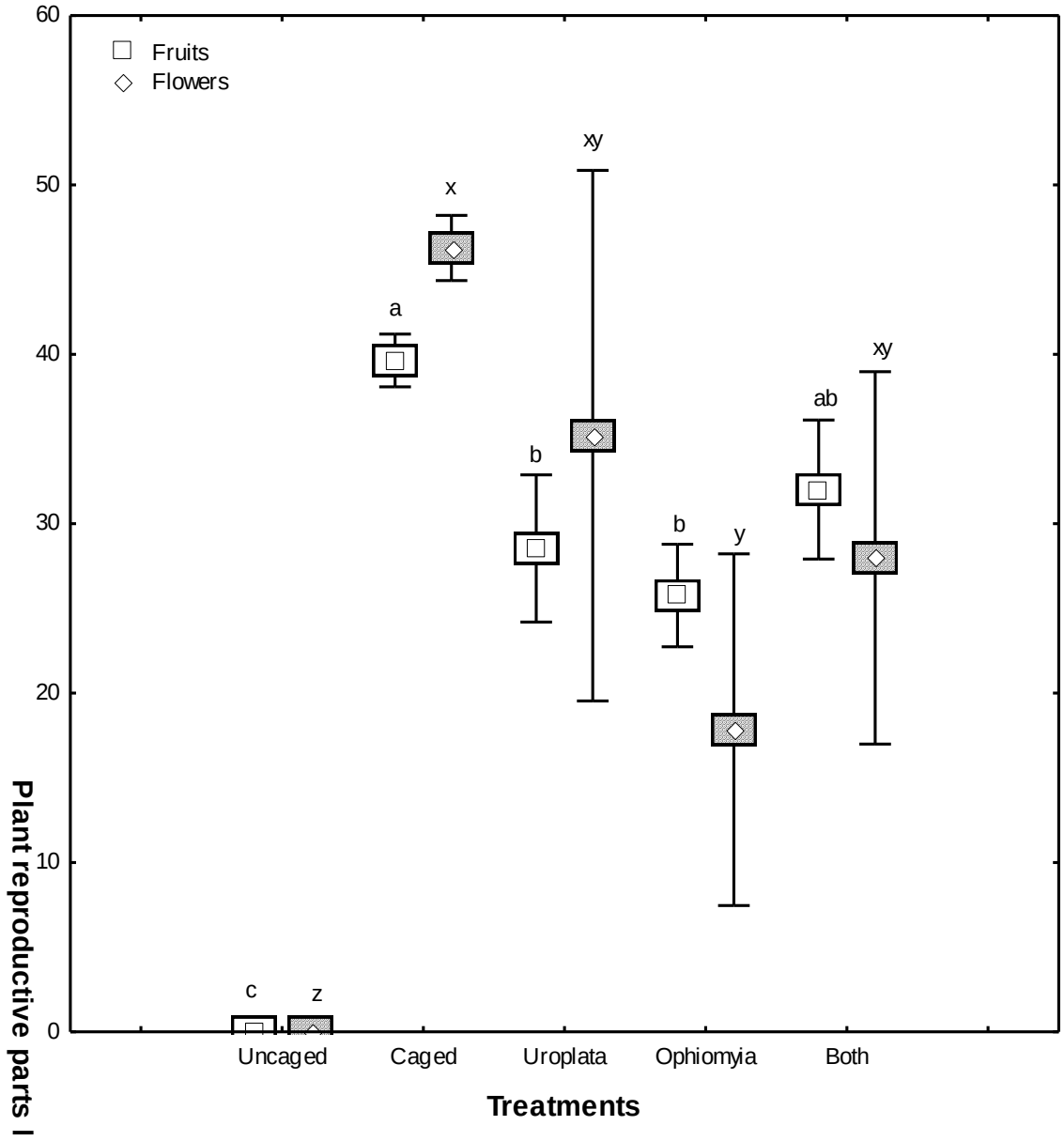

Figure 8: Reproduction parts dry biomass (Mean±SE) by May 2007 in *U. girardi*, *O. camarae* treatments and controls. Means followed by the same letter(s) are

not significantly different (p > 0.05; Fisher LSD). For fruit biomass letters a, b and c are used whereas letters x, y and z are used for flower biomass.

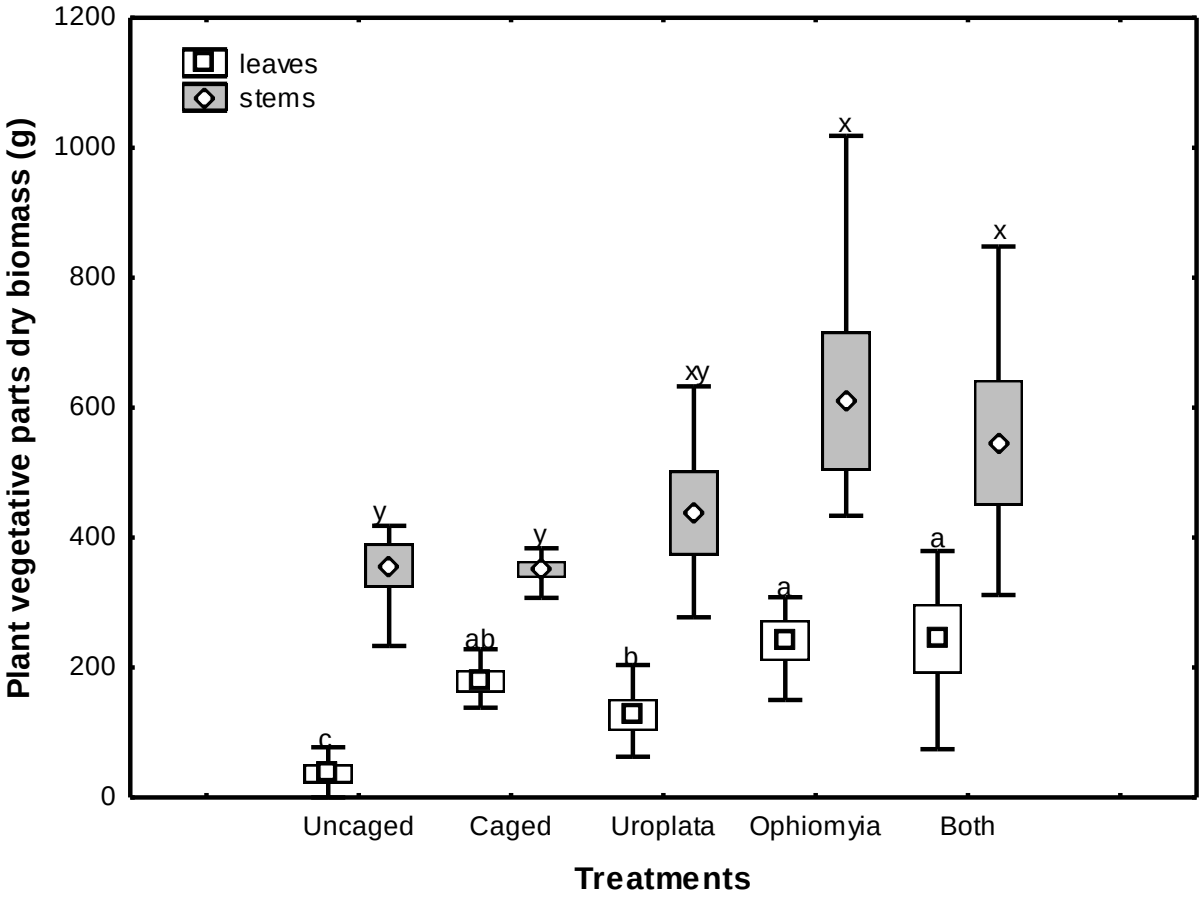

Figure 9: Above-ground dry biomass (Mean±SE) by May 2007 in *U. girardi*, *O. camarae* treatments and controls. Mean those followed by the same letter(s) are not significantly different (p > 0.05; Fisher LSD). For leaf biomass letters a, b and c are used whereas letters x, y and z are used for stem biomass.

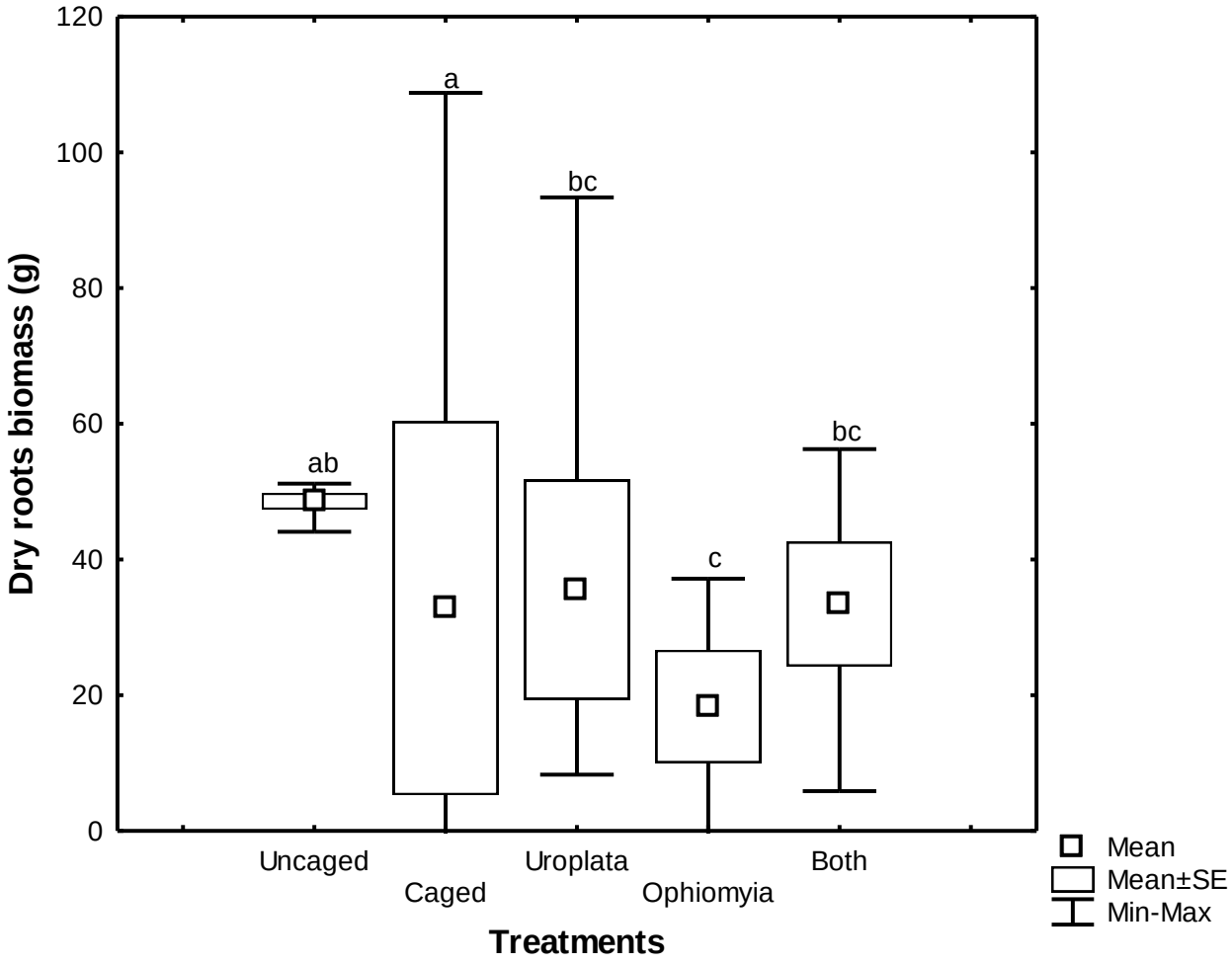

Figure 10: Plant dry root biomass (Mean±SE) during December 2006 to May 2007 in *U. girardi*, *O. camarae* treatments and controls. Means followed by the same letter(s) are not significantly different ($p > 0.05$; Fisher LSD).

Discussion

The results suggest that *U. girardi* significantly lowered leaf density by 59% compared to 10% by *O. camarae*-attacked plants and 17.3% by both agents combined (Fig 1). It is likely that the damage was exacerbated by the fact *U. girardi* adults and larvae feed on the same plant while *O. camarae* only feeds as a larva (Cilliers, 1987b). Because the adults of *U. girardi* are also long-lived, this may have caused an exponential increase of the population of this beetle, thereby intensifying damage and suppressing plant growth. In contrast, *O. camarae*, adults are short-lived and only form oviposition punctures which do not seem to cause much damage to the leaves. Although adults of *O. camarae* are highly prolific, the damage is only caused during larval feeding. The larvae of both insect species interrupt water and nutrient flow within the leaf and thus reduce the plant photosynthetic ability (Cilliers, 1987b; Simelane, 2002). The leaves of *U. girardi*-attacked plants dry-up while those attacked by *O. camarae* turn yellow and fall off (Cilliers, 1987a; Simelane & Phenye, 2005). However, the damage caused by *U. girardi* in the semi-field trials was substantially higher than those observed under natural field condition along the KwaZulu-Natal coastal regions. The cages used in the present study may have excluded the parasitoids of *U. girardi,* causing exponential increase in the populations of this beetle and thus exacerbating damage to the plants. Baars & Heystek (2003) reared five parasitoid species from the field-collected *U. girardi* pupae, and these parasitoids may be keeping the field populations of this beetle at low levels. Although Urban & Phenye (2005) reported the incidence of parasitism on *O. camarae* in KwaZulu-Natal, this seems to be low and does not appear to be significantly reducing the populations of the leafminer. The level of leaf damage caused by *O. camarae* in the current study was similar to that observed in the field, and it is therefore hoped that the impact caused on various plant parameters could eventually lead to a reduction in the weed population density.

However, the impact of both agents confined together was similar to the level of damage caused by *O. camarae* alone (Fig. 1). Crowe & Bourchier (2006) found

that the overall attack on knapweed (*Centaurea stobe*) by *Urophora affinis* (gall-fly) and *Larinus minutus* (weevil) was not as high as it could be if *L. minutus* was attacking on its own. This was also displayed by *U. girardi* which was able to defoliate large numbers of leaves when confined alone and yet it could not do so when confined with *O. camarae* in a single cage, suggesting that one or both agents may be exhibiting some form of interaction which could be antagonistic.

Both *U. girardi* and *O. camarae*, either individually and combined, caused no significant effect on overall plant stem diameter, plant height and canopy size (Fig. 3, 4 and 6). Previous studies (e.g. Dhileepan *et al.*, 2000; Simelane & Phenye, 2005; Kaplan & Denno, 2007) have demonstrated that defoliation may have an immediate negative effect on plant growth, reproduction and fitness, especially if maintained for a long period of time. However, lantana plants in the cages did not show any sign of stress compared to the uncaged plants. The uncaged control plants were significantly affected through loss of leaves caused by *T. scrupulosa* and environmental effects (sun, cold, rain and wind exposure), resulting in stunted growth and reduced plant reproductive ability (Fig. 4, 8 and 9). Simelane & Phenye (2005) also reported that *T. scrupulosa* was widespread and caused significant effects on growth of the exposed plants colonized and attacked by this tingid. In addition, other environmental factors may have played a role in reduction of these parameters in uncaged plants. According to Raghu *et al.* (2006) plants experience damage from a wide variety of environmental factors (abiotic and biotic) and exhibit a diversity of responses that can be broadly classified into susceptibility and resistance. Therefore, exclusion trials might limit other factors that inflict damage on the plant such as exposure to wind, sun, dryness, raindrop intensity and frost. During the trials, the uncaged control plants were more susceptible to a variety of environmental factors that could have hampered their growth.

Uroplata girardi and *O. camarae* attack, either individually or combined, had a significant effect on the reproductive capacity of the plant. *O. camarae* caused a

significant impact on the flower production (Fig. 8). Simelane & Phenye (2005) reported up to 100% reduction in flower production of the plants that were inoculated with different densities (10 and 20 pairs) of *O. camarae.* Decline in flower production directly impacts on plant reproductive capacity, causing a reduction in the spread of the target weed (Hendrix, 1988). Hoffmann & Moran (1991) and Briese (1996) also demonstrated that agents that affect plant reproductive capacity also hinder their spread and re-colonization of cleared areas. Since the release of *O. camarae* along the KZN coastal regions, the plant vigour of lantana has been adversely affected, and this may be causing a significant reduction in flower and fruit production and subsequent spread of the weed (Knoll, 2006; Simelane pers. comm.). There are several other examples of weed biocontrol agents whose effect on vegetative parts indirectly cause reduction in flowering and fruiting (Hoffmann & Moran, 1999; Adair, 2005).

Attack by *U. girardi* had a significant impact on plant growth, especially the leaves. Feeding by *U. girardi* is indicated by feeding scars, and these can result in defoliation, especially when the beetle numbers are high. Heavy foliar feeding has been shown to reduce the density of plants. For example, Dhileepan *et al.*, (2000) reported that foliar feeding by *Zygogramma bicolorata* (Coleoptera: Chrysomelidae) on *Parthenium hysterophorus* (Asteraceae) reduced the plant biomass by 55-89%, resulting in reduced plant density. Impact of foliar feeders on biocontrol of weeds has been linked to reduction in weed competitiveness; influencing its population growth and allowing other desirable plants to compete for space (Briese, 1996; Dhileepan *et al.*, 2000).

Furthermore, attack by *U. girardi* and *O. camarae* had a negative impact on root biomass of lantana (Fig. 10), particularly in plants attacked by *O. camarae* only. Reduction in root biomass may be due to compensation response at the expense of vegetative and reproductive parts reduced as a result of insect attack (Kaplan *et al.*, 2008). Water stress and nutrient availability are well documented as factors that affect the preference and performance of leaf feeders. Plant root condition has been indirectly implicated in the population dynamics of the above-ground

herbivores (Huberty & Denno, 2004). Kaplan *et al.* (2008) reported that roots provide the ideal storage site for resources used in above ground defence. For example, in case of the plant attack by above ground herbivores, more nutrition to sustain the plant is extracted from root system, limiting the quality and quantity of below ground tissues (Blossey & Hunt-Joshi, 2003) and decreasing the photosynthetic capacity of the plant (Bezemer *et al.*, 2003). *O. camarae* feeding activity would have reduced water and nutrient transportation to the leaves (Simelane, 2002), this may have resulted in the depletion of storage reserves thereby reducing the root biomass.

The effect of feeding damage on leaf density caused by the two agents combined was similar to that caused by *O. camarae* alone (Fig. 1). Although the combined attack by the agents had a significant effect on roots of lantana compared to the controls, their effect was similar to that caused by *U. girardi* alone but less than that caused by *O. camarae* alone (Fig. 10). The results suggest that the joint attack by both *U. girardi* and *O. camarae* was neither additive nor synergistic. It is likely that the resource availability and resource quality of one insect species was altered by the other, delaying establishment and the population build-up of both species (Chapter 3) in the cages. This is very likely to occur, especially if the agents are not spatially separated but rather share the same niche. However, field studies (Chapter 3) indicated that the two agents are spatially separated along the coastal regions of KwaZulu-Natal, and this could mitigate the effect of negative interaction between the two agents. Blossey (1995) found that environmental factors could also separate two agents in the field, and this could somewhat mitigate the competitive interaction between the two species. For example, *U. girardi* prefers lantana in open sunny areas (Day & Neser, 2000) whereas *O. camarae* often prefers plants growing under shade (Simelane & Phenye, 2004). In a situation where resource availability is unlimited, interaction between the agents that are neither spatially nor temporally separated is less likely to be antagonistic but competitive interaction may prevail when the food resource begins to diminish.

The damage caused by *T. scrupulosa* on uncaged control plants was quite severe, and this seems to suggest that the combination of the three agents (*U. girardi, O. camarae* and *T. scrupulosa*) might enhance the biological control of lantana. Although it is very damaging, *T. scrupulosa* is known to be sporadic in the field (Baars & Neser, 1999). It is also unlikely that the joint impact by the three agents would be synergistic in the wild, unless they are temporally separated.

Environmental factors play a major role in decreasing the impact of herbivory on lantana, particularly the low temperatures (Cilliers, 1987a; Day & Neser, 2000). In May there was a decrease in leaf density, and this may be attributed to frost and drought during the winter season (Fig 2). On the Highveld (e.g. Gauteng) and other inland areas, leaves are lost due to frost and the plants often recover during the spring (Sept-Nov) (Fig. 2). Due to plant dormancy during winter and spring, a lag is created between the plant recuperation and agent attack (Baars, 2003). Both *U. girardi* and *O. camarae* attack showed a significant effect on plant (leaves, reproductive, vegetative capacity and roots). However, in favourable conditions the plants are able to compensate, thus the agents might have a periodic effect on lantana (Baars & Heystek, 2003). Even though both *U. girardi* and *O. camarae* attack have an effect on individual plant fitness, their impact on lantana populations needs to be quantified.

Based on the results reported here, it can be concluded that *U. girardi* and *O. camarae* damage caused on lantana was neither synergistic nor additive. Although the release of biocontrol agents against lantana may have played a role in limiting population density of the weed, their impact is not sufficient to bring this shrub under control. However, the results reported here found that cumulative stress by *U. girardi* and *O. camarae* on lantana did not materialize; proposing that the impact on the target weed could be generated with a different combination of insects feeding on plant parts in addition to those feeding on the leaves. Furthermore, interaction studies between the released agents and those

proposed for release should be integrated within the pre-released studies so as to predict the effects of interaction between the agents in the field.

References

Adair, R.J. 2005. The biology of *Dasinuera dielsi* Rübsaamen (Diptera: Cecidomydae) in relation to the biological control of *Acacia cyclops* (Mimosaceae) in South Africa. *Australian Journal of Entomology* **44:** 446-456.

Anderson, G.L., Delfosse, E.S., Spencer, N.R., Prosser, C.W. & Richard, R.D. 2000. Biological control of leafy spurge: an emerging success story. In: Spencer, N.R. (Ed.) *Proceedings of the X International Symposium on Biological control of Weeds,* Montana State University, Bozeman.15-25. Montana, USA.

Baars, J-R. 2003. Biological control initiatives against *Lantana camara* L. (Verbenaceae) in South Africa: An assessment of the present status of the programme, and an evaluation of *Coelocephalapion camarae* Kissinger (Coleoptera: Brentidae) and *Falconia intermedia* Distant (Heteroptera: Miridae), two new candidate natural enemies for release on the weed. PhD Thesis. Rhodes University, South Africa.

Baars, J-R. & Heystek, F. 2003. Geographical range and impact of five biocontrol agents established on *Lantana camara* in South Africa. *BioControl* **48:** 743-759.

Baars, J-R., Urban, A.J. & Hill, M.P. 2003. Biology, host range and risk assessment supporting releases in Africa of *Falconia intermedia* (Heteroptera: Miridae) a new biocontrol agent for *Lantana camara*. *Biological Control* **28:** 282-292.

Baars, J-R. & Neser, S. 1999. Past and present initiatives on the biological control of *Lantana camara* (Verbenaceae) in South Africa. *African Entomology Memoir* **1:** 21-33.

Bezemer, T.M., Wagenaar, R., Van Dam, N.M. & Wäckers, F.L. 2003. Interaction between above- and below-ground insect herbivores as mediated by the plant defence system. *Oikos* **101:** 555-562.

Blossey, B. 1995. A comparison of various approaches for evaluating potential biological control agents using insects on *Lythrum salicaria*. *Biological Control* **5:** 113-122.

Blossey, B. & Hunt-Joshi, T.R. 2003. Belowground herbivory by insects: influence on plants and aboveground herbivores. *Annual Reviews in Entomology* **48**: 521-547.

Briese, D.T. 1996. Potential impact of the stem-boring weevil *Lixus cardui* on the growth and reproductive capacity of *Onopordum* thistles. *Biocontrol Science and Technology* **6:** 251-261.

Cilliers, C.J. 1987a. Notes on the biology of the established insect natural enemies of *Lantana camara* L. (Verbenaceae) and their seasonal history in South Africa. *Journal of the Entomological Society of Southern Africa* **50**: 1-13.

Cilliers, C.J. 1987b. The evaluation of three insect natural enemies for the biological control of the weed *Lantana camara* L., *Journal of the Entomological Society of Southern Africa* **50**: 15-34.

Cilliers, C.J. & Neser, S. 1991. Biological control of *Lantana camara* (Verbenaceae) in South Africa. *Agriculture, Ecosystems and Environment* **37**: 57-75.

Crowe, M.L. & Bourchier, R.S. 2006. Interspecific interactions between the gall-fly *Urophora affins* Frfld. (Diptera: Tephritidae) and the weevil *Larinus minutus* Gyll. (Coleoptera: Curculionidae), two biological control agents

released against spotted knapweed, *Centaurea stobe* L. spp. *micranthos*. *Biocontrol Science and Technology* **16**: 417-430.

Day, M.D. & Neser, S. 2000. Factors influencing the biocontrol of *Lantana camara* in Australia and South Africa. In: N.R. Spencer (ed.) *Proceedings of the X International Symposium on Biological Control of Weeds,* Montana State University, Bozeman. 897-908. Montana, USA.

Dhileepan, K., Setter, S.D. & McFadyen, R.E. 2000. Response of the weed *Parthenium hysterophorus* (Asteraceae) to defoliation by the introduced biocontrol agent *Zygogramma bicolorata* (Coleoptera: Chrysomelidae). *Biological Control* **19:** 9-16.

Hajek, A.E. 2004. Classical biological control. *Natural Enemies: An introduction to Biological Control*. 39-56. Cambridge University press.

Heystek, F. 2006. Laboratory and field host utilization by established biological control agents of *Lantana camara* L., in South Africa. *MSc Thesis*. Rhodes University, South Africa.

Hendrix, S.D. 1988. Herbivory and its impact on plant reproduction. In: Lovett-Doust, J & Lovett-Doust, L. (Eds.) Plant Reproductive Ecology: Patterns and Strategies. 246-262. Oxford Publishers.

Hoffmann, J.H. & Moran, V.C. 1991. Biological control of *Sesbania punicea* (Fabaceae) in South Africa. *Agriculture, Ecosystems and Environment* **37:** 157-173.

Hoffmann, J.H. & Moran, V.C. 1999. A review of the agents and factors that contributed to the successful biological control of *Sesbania* punicea (Cav.) Benth. (Papilonaceae) in South Africa. *African Entomology Memoir* **1***:* 75-79.

Huberty, A.F. & Denno, R.F. 2004. Plant water stress and its consequences for herbivorous insects: a new synthesis. Ecology **85**: 1383-1398.

Kaplan, I., Halitschlke, R., Kessler, A., Rehill, B.J., Sardanelli, S. & Denno, R.F. 2008. Physiological integration of roots and shoots in plant defence strategies links above and below ground herbivory. *Ecology Letters* **11**: 841-851.

Kaplan, I. & Denno, R.F. 2007. Interspecific interaction in phytophagous insects revisited: a quantitative assessment of competition theory. *Ecology Letters* **10:** 977-994.

Keane, R.M. & Crawley, M.J. 2002. Exotic plant invasions and the enemy release hypothesis. *Trends in Ecology and Evolution* **17**: 164-170.

Knoll, C. 2006. Biocontrol agent for *Lantana camara*, one of the ten worst Weeds in the world. *Environmental Management* **2:** 30-31.

Louda, S. M., Kendall, D., Connor, J. & Simberloff, D. 1997. Ecological effects of an insect introduced for the biological control of weeds. *Science* **277**: 1088-1090.

Louda, S.M., Rand, T.A., Russell, F.L. & Arnett, A.E. 2005. Assessment of ecological risks in weed biocontrol: input from retrospective ecological analyses. *Biological Control* **35**: 253-264.

McEvoy, P.B. & Coombs, E.M. 1999. Why things bite back: Unintended consequences of biological control of weeds. In: Follett, P. A. & Duan, J. J. (eds.) *Nontarget effects of Biological Control*. 176-194. Kluwer Academic Publishers, Boston.

Pearson, D. E. & Callaway, R.M. 2003. Indirect effects of host-specific biological control agents. *Trends in Ecology and Evolution* **18**: 456-461.

Raghu, S., Wilson, J.R & Dhileepan, K. 2006. Refining the process of agent selection through understanding plant demography and plant response to herbivory. *Australian Journal of Entomology* **45**: 308-316.

Rayamajhi, M.B., Van, T.K., Pratt, P.D. & Center, T.D. 2006. Interactive association between *Puccinia psidii* and *Oxyops vitiosa*, two introduced natural enemies of *Melaleuca quinquenervia* in Florida. *Biological Control* **37**: 56-67.

Seastedt, T.R., Knochel, D.G., Garmoe, M. & Shosky, S.A. 2007. Interactions and effects of multiple biological control insects on diffuse and spotted knapweed in the Front Range of Colorado. *Biological Control* **42**: 345-354.

Simelane, D.O. 2002. Biology and host range of *Ophiomyia camarae*, a biological control agent for *Lantana camara* in South Africa. *BioControl* **47**: 575-85.

Simelane, D.O. & Phenye, M.S. 2004. Preliminary assessment of release and establishment of lantana herringbone leaf miner, *Ophiomyia camarae* (Diptera: Agromyzidae), in South Africa. In: Cullen, J.M., Briese, D.T., Kriticos, D.J., Lonsdale, W.M., Morin, L. & Scott, J.K. (Eds.) *Proceedings of the XI International Symposium on Biological Control of Weeds*. CSIRO Entomology. 447-450. Canberra, Australia.

Simelane, D.O., & Phenye, M.S. 2005. Suppression of growth and reproductive capacity of the weed *Lantana camara* (Verbenaceae) by *Ophiomyia camarae* (Diptera: Agromyzidae) and *Teleonemia scrupulosa* (Heteroptera: Tingidae). *BioControl Science and Technology* **15**: 153-163.

Statistica Enterprise System, Technology. 2004. Statistica 6.1 for Windows, Tulsa Oklahoma.

Thomas, M.B. & Reid, A.M. 2007 Are exotic natural enemies an effective way of controlling invasive plants? *Trends in Ecology and Evolution* **18**: 447-451.

Urban, A.J. & Phenye, M.S. 2005. Impact of *Ophiomyia camarae* (Diptera: Agromyzidae) on *Lantana camara* (Verbenaceae): prediction and realization. In Villet, M.H. (Ed) *15th Entomological Congress of the Entomological Society of South Africa*, Grahamstown. 84. Entomological Society of Southern Africa, Pretoria.

Chapter Three

Interaction between *Uroplata girardi* Pic (Coleoptera: Chrysomelidae) and *Ophiomyia camarae* Spencer (Diptera: Agromyzidae) on their shared host *Lantana camara* L. (Verbenaceae)

Abstract

Competition is an important factor influencing the performance and fitness of phytophagous insects. To test the effects of competition on the survival and fitness of *Uroplata girardi* and *Ophiomyia camarae* population growth, two leaf-mining biocontrol agents released against *Lantana camara*, oviposition trials were carried out. Female oviposition preference of each insect species was determined in laboratory to measure its ability to differentiate between infested and uninfested leaves. Semi-field trials were conducted to determine the population growth of each insect species, either in isolation or when both agents are sharing the same plant. Field surveys were conducted to validate the occurrence of interspecific interaction between the two agents in KwaZulu-Natal. Results showed that *O. camarae* females were able avoid *U. girardi*-infested leaves for oviposition while *U. girardi* laid their eggs indiscriminately on both *O. camarae*-infested and uninfested leaves. When both agents were allowed to utilize the same plant, the population growth of *U. girardi* was adversely affected while that of *O. camarae* was unaffected. In the field *O. camarae* populations increased rapidly from December to May while those of *U. girardi* were kept at a minimum during the same period. As a superior competitor, *O. camarae* is likely to exert substantial herbivore pressure on the weed by regulating its density in the field. Because of the decline of *O. camarae* populations between May and November, *U. girardi* is likely to recover, thus avoiding being displaced completely by *O. camarae*. The study emphasizes the importance of conducting interaction studies on insect agents prior to their release into the environment.

Keywords: Interspecific interaction, population growth, oviposition preference, *Uroplata girardi*, *Ophiomyia camarae*, biocontrol

Introduction

Multiple releases of agents has become a contentious issue in weed biological control worldwide, with results ranging from failure to success in controlling the target weed species (Julien & Griffiths, 1998, Denoth *et al.*, 2002). Although there are several cases where the success in biological control of an alien invasive weed was attributed to the combined effect of two or more biocontrol agents (Hoffmann & Moran, 1999; Anderson *et al.,* 2000), some weeds have been successfully controlled by one species of a biological control agent (Denoth *et al.,* 2002; Crowe & Bourchier, 2006). The argument that using multiple agents may actually reduce the likelihood of success through the process of competitive exclusion (Ehler & Hall, 1982) is largely based on common logic rather than empirical evidence found in weed biocontrol literature. However, it cannot be disputed that the multiple release of agents has been used as a lottery which is based on the assumption that the more agents introduced, the more likely it is that the most effective agent will be amongst them (Myers, 1982).

Biological control of *Lantana camara* L. (Verbenaceae), a vigorous South American invasive shrub, was initiated in South Africa in the 1960s (Chapter 1). Since the implementation of a classical biocontrol programme, multiple agent species have been released, aiming at imposing cumulative stress on the plant (Cilliers, 1983; Denoth *et al.*, 2002). It was assumed that the combined effect of the agents would increase plant damage and result in better control (Cilliers & Neser, 1991; Baars & Neser, 1999; Day & Neser, 2000; Simelane & Phenye, 2005). In South Africa, 12 biocontrol agents released against *L. camara* are fully established and eight of these utilize the same niche (i.e. plant leaves) for feeding and development (Baars & Neser, 1999; Baars; 2003). To weaken the plant while minimizing the likelihood of interspecific competition among agents, different niches of the weed should be targeted (Harris, 1985; Julien & Griffiths,

1998; Hajek, 2004). However, it is also argued that plants are common resource budget; and herbivores that are not spatially separated but sharing the same plant would automatically compete as one species would lower the availability of the resource for the other species (McEvoy, 2002). Based on this theory, the practice of releasing multiple biocontrol agents in a weed biocontrol system has been widely challenged (McEvoy & Coombs, 1999; Denoth *et al.,* 2002; Crowe & Bourchier, 2006).

The leaf mining hispine beetle *Uroplata girardi* (Cilliers, 1987) and the herringbone leaf mining fly *Ophiomyia camarae* (Simelane, 2002) are among the suite of biocontrol agents released against lantana in South Africa. Although both agents (*U. girardi* and *O. camarae*) occur along the eastern coastal regions and cause visible damage to lantana, the combined effect of these agents on the target weed is yet to be determined. *U. girardi,* released in the 1970s, was found to be very abundant at some KwaZulu-Natal (KZN) sites in 1999, and this translates to over 50% of larva-infested leaves per plant (Baars & Heystek, 2003). Since its release into South Africa in 2001, *O. camarae* has become abundant and widely distributed, particularly along the coastal regions of KZN (Simelane & Phenye, 2003). Heystek (2006) observed that the populations of *U. girardi* appeared to be declining while those of *O. camarae* were increasing in the same region, indicating the possibility of a negative interaction between the two agents. In fact, both *O. camarae* and *U. girardi* were found to be abundant (over 50% infested leaves/ plant) in Richards Bay during 2004-5. During 2006-7, *U. girardi* was very rare (±20% infested leaves per plant) while *O. camarae* continued to be abundant (over 50% infested leaves/ plant) (Heystek, 2006, Chapter 1). Since both species attack the leaves of their shared host and thus utilize the same resource, some form of interaction is expected but the nature of this interaction is not yet known. This study aims to (i) determine the female oviposition preference of each insect species to measure its ability to differentiate between infested (poor quality) and uninfested (good

quality) leaves in the laboratory, (ii) determine the effect of co-infestation of the host by the two biocontrol agents on their population growth under semi-field conditions and (iii) to determine whether the co-existence of the two agents in the field results in the population decline of *U. girardi* over time.

Study organisms

The leaf-feeding beetle *Uroplata girardi* originated from Brazil, and was introduced into South Africa in the early 1970's (Cilliers, 1987). The larvae of *U. girardi* form mines in the plant leaves. The adults are reddish brown in colour, and the elytra are relatively darker than the legs and abdomen (Cilliers, 1987). Adults feed on the upper leaf surface and scarify areas of the leaf tip, causing it to curl and provide shelter for the insect (Day et al., 2003). The female inserts the egg into the upper surface of the leaf and covers it with black material excreted by collateral glands. Eggs are laid singly often next to a feeding scar (Cilliers, 1987). The larvae excavate a large chamber in which frass is deposited and from which smaller larval feeding tunnels radiate (Cilliers, 1987). Larvae complete their development after about 20 days and pupate within the leaf for 8-14 days (Cilliers, 1987). *U. girardi*'s life cycle takes about 31-52 days, with approximately three generations per year (Cilliers, 1987; Day et al., 2003).

Ophiomyia camarae is a leaf-mining fly that is widespread in central and southern America. It was screened as a potential biocontrol agent for lantana from 1998, and was eventually released into South Africa in 2001 (Simelane, 2002). The life history of the leafminer was described by Simelane (2002). The adults are about 1.5 to 2.0mm in length, with the wing length of about 1.4 to 1.7mm. The body is black with a brilliantly shinning abdomen and dark red compound eyes. Generally, the adults do not feed, but they can consume water and plant nectar. Females insert their eggs singly into the leaf tissue in the lateral vein, on the underside of the leaf. The larvae tunnel along the leaf vein, forming a herringbone-shaped mine in the leaves. Pupation occurs in the leaf and the

development time from egg to adult is about 30 days (Simelane, 2002). The female adult lives for about 21 days, completing more than ten generations a year under laboratory conditions.

Materials and methods

i) *Oviposition preference of one of the two agents (U. girardi* and *O. camarae*) *on plant leaves infested by the other agent:*

A laboratory study was carried out in ARC Rietondale, Pretoria, South Africa, to determine the oviposition preference of *U. girardi* and *O. camarae* on infested and uninfested lantana leaves. The study was conducted over a period of two months (i.e., from 01 September 2008 until the end of October 2008). Twelve rooted cuttings that were initially planted into plastic nursery bags (Chapter 2) were transferred into 10 litre pots for use in this study. A paired-choice test was used to measure the effect of plant damage (infested or uninfested) by one insect species on oviposition preference of the other species. Six potted lantana plants with ±150 leaves were individually housed in gauze-covered cages (55 x 55 x 95 cm). Three separately caged potted lantana plants were inoculated with 30 unsexed *U. girardi* adults (Uro) and another three were inoculated with 15 pairs of *O. camarae* adults (Oph). The two insect species were left in their respective cages until larval damage on the leaves was visible. When larval damage was observed, adults were removed from the cages and a clean (uninfested) potted lantana plant (Control) was placed next to the infested one. Thirty unsexed *U. girardi* adults were confined for 12 days in a cage (55 x 55 x 95 cm) containing the two (*O. camarae*- and uninfested) plants, and females were expected to make oviposition choices based on the infestation status of the leaf. Similarly, 30 unsexed adults of *O. camarae* were confined for the same period in a cage containing *U. girardi*-infested and uninfested (control) plants. At the end of the trial, leaves of both plants were inspected for larval mines of each of the two species. The oviposition preference of each species was evaluated by comparing the number of leaves containing larval damage of both species against those with only one species.

ii) Population growth of U. girardi and O. camarae under semi-field conditions:

Experiments aimed at assessing the population growth of *U. girardi* and *O. camarae* were carried out under semi-field conditions in an open field owned by Agricultural Research Council (ARC) at Rietondale during a six-month period. The design of this experiment is similar to that described in Chapter 2. Three treatments were replicated five times and arranged in a randomized block design. The first treatment consisted of five caged plants infested with 30 unsexed *U. girardi* adults (Uro). The second treatment consisted of five caged plants infested with 30 unsexed *O. camarae* adults (Oph). The third treatment consisted of five caged plants infested with 30 unsexed adults of both insect species (*U. girardi* and *O. camarae*, Uro + Oph). The population growth was determined by counting the total number of infested leaves per plant every month during a six-month period. In each treatment newly infested leaves with mines were counted and recorded. The number of larval mines of each species in the cage where both species were initially released was compared with those recorded in the treatment in which the same species was released individually.

iii) Population dynamics of the two agents (U. girardi and O. camarae) at selected KwaZulu-Natal sites:

To establish evidence of competitive interaction between the two insect species in the field, their population dynamics were monitored every two months at five selected sites in KwaZulu-Natal (Table 1) over a one-year period. Three sites; Phahla, Kuswag and Scottburgh were located on the south coast of KwaZulu-Natal (KZN). The two other sites; Tongaat and Richards Bay were on the north coast of the province. KwaZulu-Natal is one of the coastal provinces of South Africa and is characterized by warm moist climate throughout the year. The average temperature on the south coast ranges between 18˚C to 28.4˚C with a relative humidity of 59% to 86% in the morning, 56% to 79% in the afternoon and 73% to 91% in the evening. The temperatures during winter are mild with averages ranging between 12.6˚C and 23.8˚C. The average annual rainfall is

1143.4 mm. On the north coast the average minimum and maximum summer temperatures range between 19.8 ˚C to 30.8˚C with relative humidity of 80% to 88% in the mornings, 58% to 77% in the afternoon and 83% to 91% in the evenings. Winter average temperatures are between 11.2˚C to 24.5˚C and the average annual rainfall is 1144.4 mm (WeatherSA, 2006/7).

Two sites, Phahla station and Kuswag road were adjacent to each other and were approximately 200 meters apart. All the sites were located on the coast of KZN province which is characterized by warm and moist climate throughout the year. To calculate the percentage infestation of each insect species, the number of larva-infested leaves was divided by the total number of leaves on a branch of approximately 110cm in length with approximately 100 leaves. At each site, ten branches were sampled from each plant on which the number of larva-infested leaves by each insect species was determined per branch. The same procedure was repeated on at least three plants per site.

Table 1: Sites in KwaZulu-Natal where *U. girardi* and *O. camarae* populations were monitored.

Site	Location	Coordinates
Kuswag road	Amanzimtoti*[a]	30°02´08˜S; 30°53´42˜E
Phahla Metro rail station	Amanzimtoti[a]	30°02´28˜S; 30°53´23˜E
	Amanzimtoti*	29°33´36˜S; 31°07´29˜E
	Scottburgh[a]	30°16´11˜S; 30°45´13˜E
	Tongaat*[a]	28°44´25˜S; 31°55´88˜E
	Empangeni*	28°44´27˜S; 31°55´58˜E
	Richards Bay*[a]	28°46´08˜S; 32°04´50˜E

* - sites where the agents were collected for starting a culture.

[a] - sites visited for sampling.

Data analyses

Descriptive statistics were used to quantify the abundance of each insect species. Student's *t*-tests were used to compare the percentage of larval mines of a species formed on previously infested leaves against those formed on uninfested (clean) leaves and Mann-Whitney *U*-tests were used to compare the total number of leaves with larval damage in the individual species treatments to those in the combined species treatment (Statistica v. 6.1, 2004). Three-way analysis (ANOVA) was performed to determine the infestation levels between sites, sampling dates, and biocontrol agents. To stabilize the variance, data were initially transformed by arcsine of the square root before being subjected to t-tests and three-way ANOVA.

Results

i) *Oviposition preference of U. girardi and O. camarae on infested versus uninfested leaves:*

Based on larval mine counts, *O. camarae* females showed a stronger oviposition preference for uninfested lantana leaves to those already infested with *U. girardi* (t = 3.89; p = 0.02) (Fig. 1). Of the total number of mines formed by *O. camarae* larvae, 76% (108) were formed on uninfested leaves compared to 24% (28) formed on leaves already infested with *U. girardi*. In contrast, *U. girardi* females appeared to have laid their eggs indiscriminately, resulting in similar numbers of larval mines formed on both *O. camarae*-infested and uninfested leaves (t = 0.38; p = 0.72). Of the total number of mines formed by *U. girardi* larvae, 52.7% (36) were formed on uninfested leaves versus 47.3% (25) formed on leaves already infested with *O. camarae*.

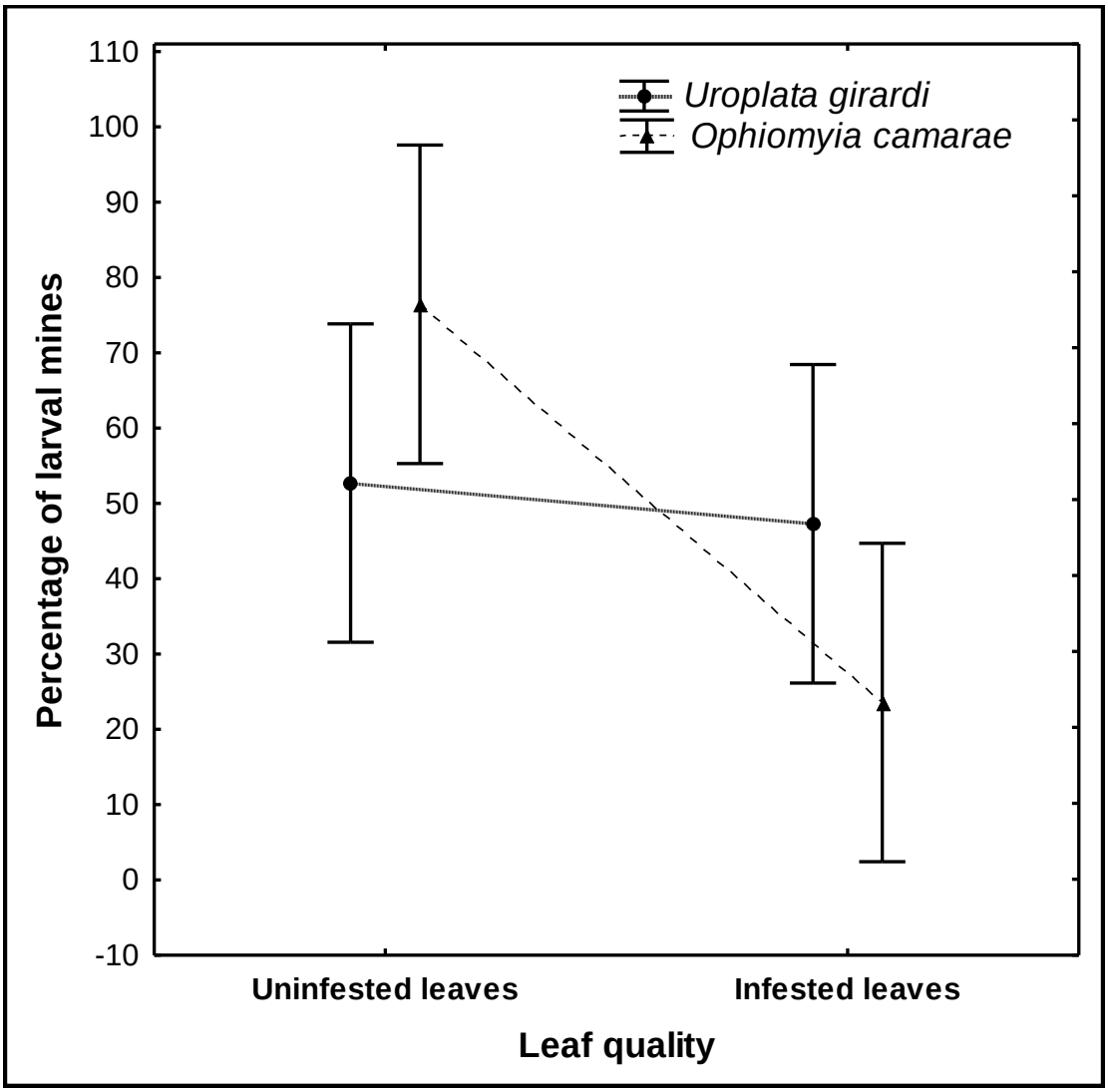

Figure 1. Percentage of eggs laid based on larval mines formed by either *Uroplata girardi* or *Ophiomyia camarae* on either infested or uninfested (clean) leaves. (■) = The percentage of eggs laid by *U. girardi* on leaves that were either uninfested (clean) or previously infested with *O. camarae*. (▲) = The percentage of eggs laid by *O. camarae* on leaves that were either uninfested (clean) or previously infested with *U. girardi*.

ii) *Population growth in the semi-field trials:*

There was no significant difference in number of leaves with *U. girardi* larval mines between singly confined and that in combined treatments (*U* = 13 and p = 0.42) during a six-month period. Similarly, there was no significant difference in

the number of leaves with *O. camarae* larval mines between singly confined and that in combined treatments (U = 12 and p = 0.33) during the same period. In the single species treatments, the number of larval mines of both *U. girardi* and *O. camarae* increased through the sampling period but showed different patterns (Fig. 2). The number of *U. girardi* mines increased from December until it reached a peak in February, and then declined gradually until the termination of the trial in May. On the other hand, the number of *O. camarae* mines increased rapidly from December until reaching a peak in April, and then declined rapidly thereafter (Fig. 2).

In the combined treatments, *U. girardi* appears to be affected by *O. camarae* existence (Fig. 3); because the number of leaves for the former was lower than the single treatment (Fig. 2). In December (first sampling date), the combined treatment plants had no *U. girardi* larval mines while there were a few *O. camarae* larval mines (Fig. 3) in the same treatment. The number of larval mines for both agents increased gradually from January to February, reaching 65.6 ± 31.6 for *U. girardi* versus 80.2 ± 25.0 for *O. camarae*. However, the number of larval mines for *U. girardi* began to decline after February, reaching 24.2 ± 13.2, 7.6 ± 3.7 and 1.4 ± 0.7 in March, April and May, respectively. In contrast, the number of *O. camarae* larval mines kept on increasing, reaching 149.8 ± 90.5 in March and eventually reaching a peak of 746.2 ± 562.3 in April. In May, however, a sharp decline in *O. camarae* population was observed, with 14.2 ± 5.0 larval mines recorded per plant. Although the population increases of *O. camarae* started a bit earlier in the single species treatment, the trend was similar to that observed in the combined species treatment. In both treatments (single and combined treatments), a decline in the *U. girardi* population started as early as March while that of *O. camarae* was observed in May.

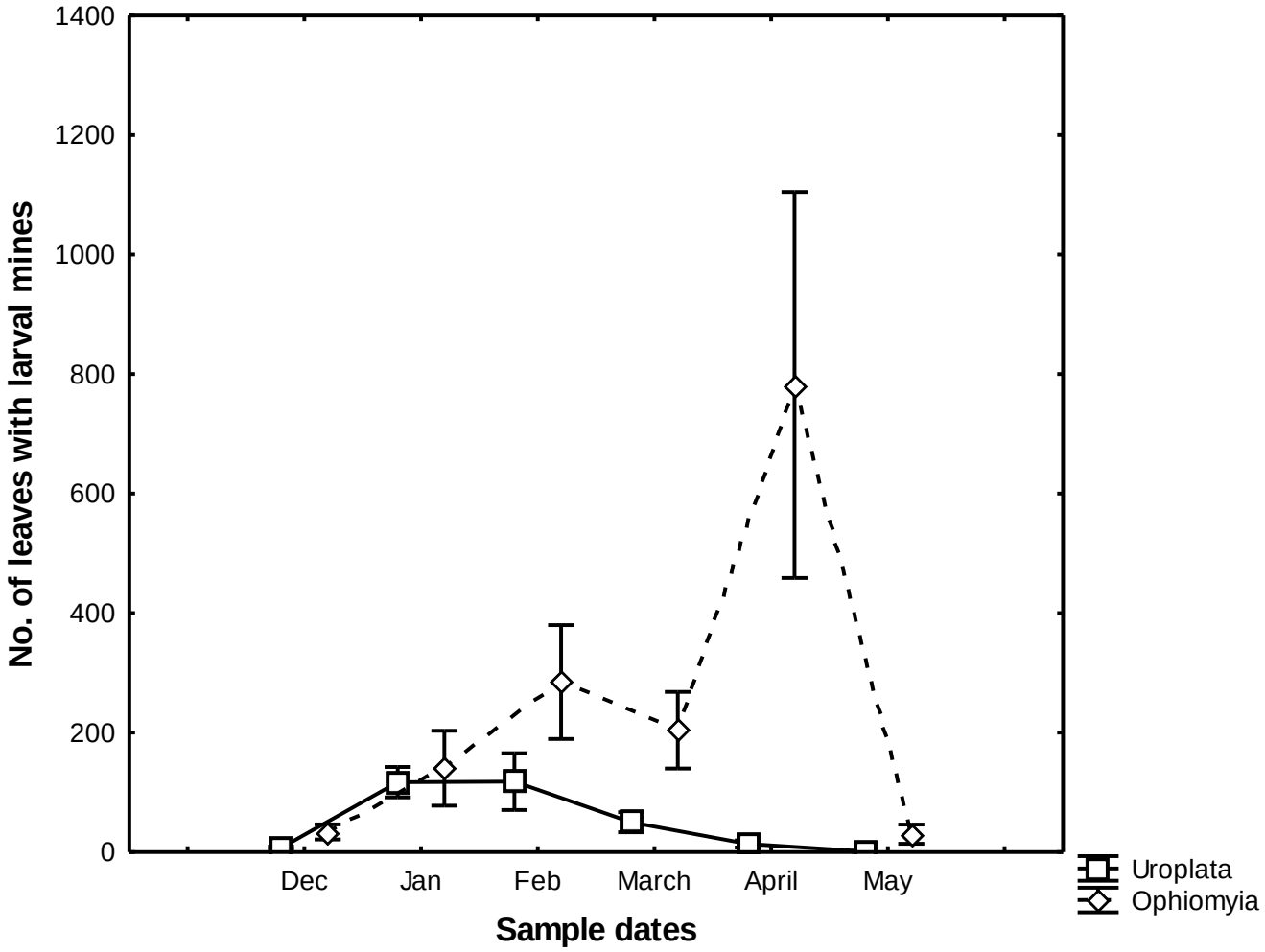

Figure 2: The number of leaves infested by *U. girardi* and *O. camarae* when each agent was confined singly on *L. camara* plants from December 2006 to May 2007.

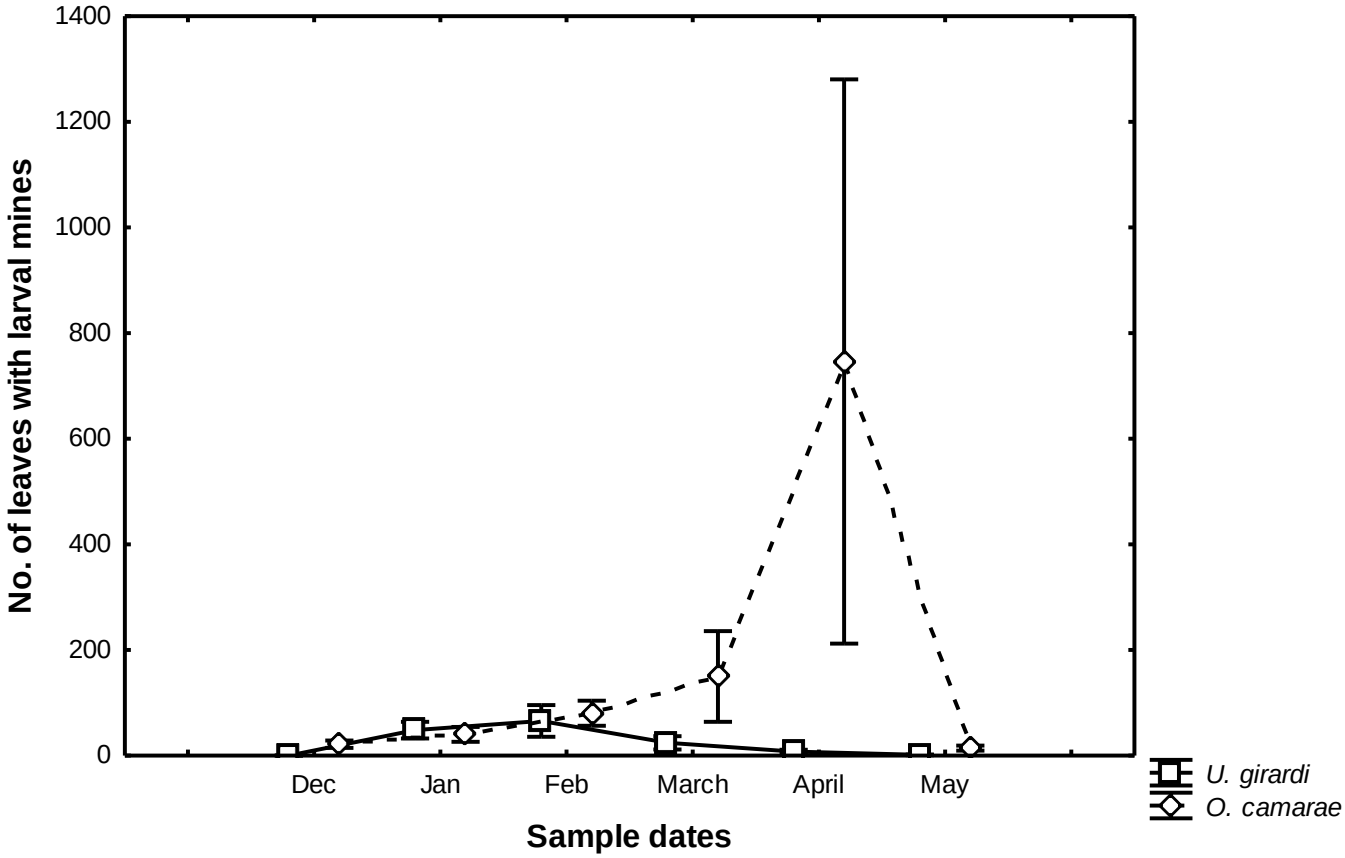

Figure 3: The number of leaves infested by *U. girardi* and *O. camarae* when both agents were confined together on *L. camara* plants from December 2006 to May 2007.

iii) *Population growth of U. girardi and O. camarae in the field.*

There was a considerable variation in the number of leaves with larval mines of *U. girardi* and *O. camarae* between the sites (Fig. 4). Leaves with *Uroplata girardi* larval mines varied from site to site and showed the highest abundance at Phahla Station in Emanzimtoti; whereas at Kuswag road and Tongaat the agent persisted in low numbers and was rare in Scottburgh and Richards Bay. The percentage of infestation by *U. girardi* varied between the sites ranging from 2.1% in Kuswag (leaves = 7643; infested = 161), 23% Phahla (leaves = 7927; infested = 1789), 2.3 % Tongaat (leaves = 7014; infested = 161), 1% Scottburgh

(leaves = 7011; infested = 40) and 1% Richards Bay (leaves = 6299; infested = 38).

There was also variation in the number of leaves with *O. camarae* larval mines between the sites. Apart from Tongaat and Phahla Station where the *O. camarae* population was low, the rest of the sites had high levels of larval infestation. In addition, the percentage of leaf infestation also differed across sites, with 26% at Kuswag (leaves = 7643; infested = 1978), 17% at Phahla (leaves = 7927; infested = 1360), 16% at Tongaat (leaves = 7014; infested = 1125), 29% at Scottburgh (leaves = 7011; infested = 2020) and 29% at Richards Bay (leaves = 6299; infested = 1849).

When data were pooled for all sites, there were many more *O. camarae* larval mines than *U. girardi* larval mines over the sampling period (Fig. 5). With the exception of Phahla station site, the peak in *O. camarae* populations at four sites was often associated with sharp declines in *U. girardi* populations though the time of these peaks varied from site to site during the sampling period.

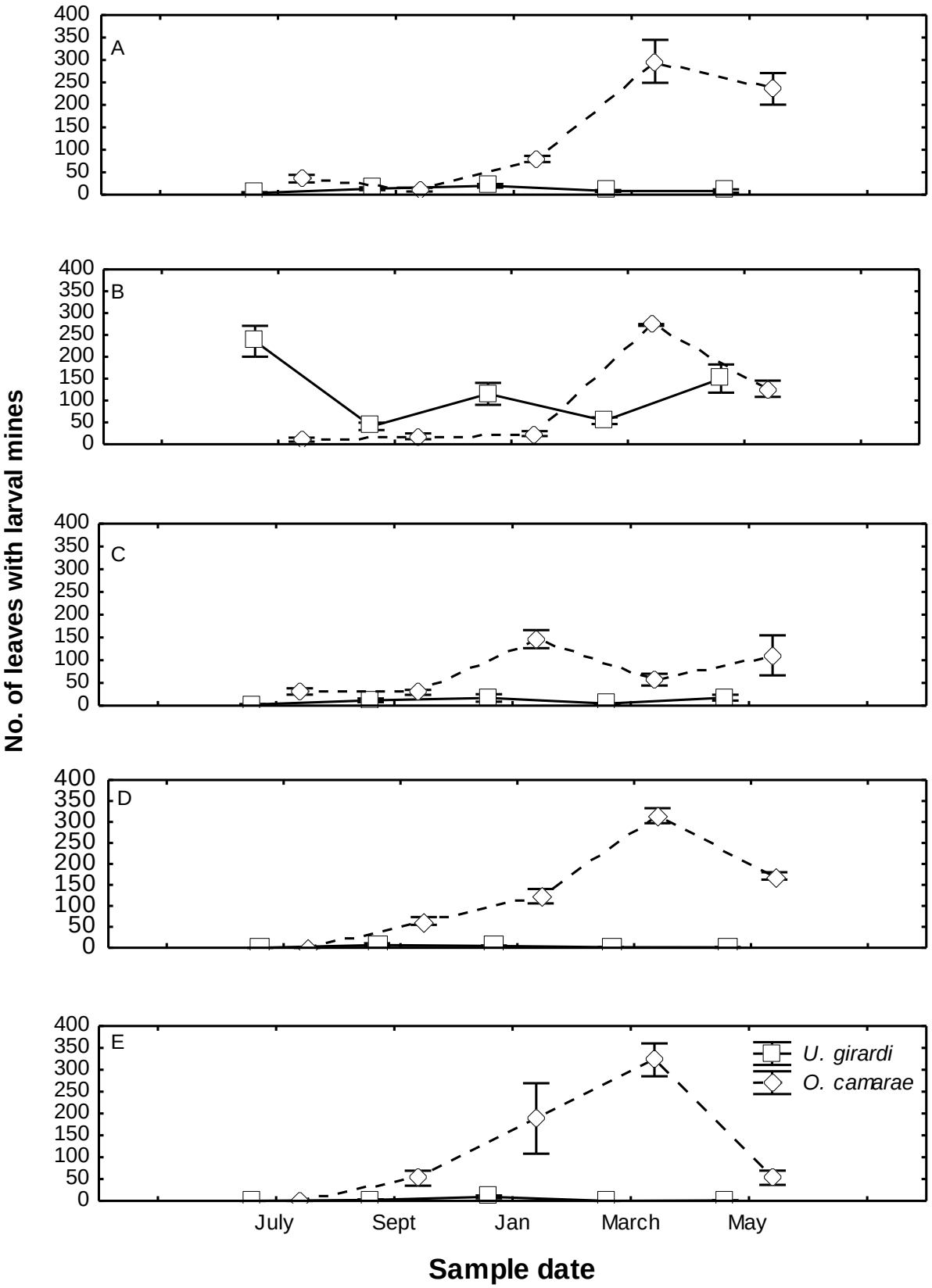
A
B
C
D
E
400
350
300
250
200
150
100
50
0
No. of leaves with larval mines
U. girardi
O. camarae
July
Sept
Jan
March
May
Sample date

Figure 4: Combined number of leaves with larval mines of *U. girardi* and *O. camarae* at field sites in KwaZulu-Natal (A) Kuswag road, (B) Phahla Station, (C) Tongaat, (D) Scottburgh and (E) Richards Bay.

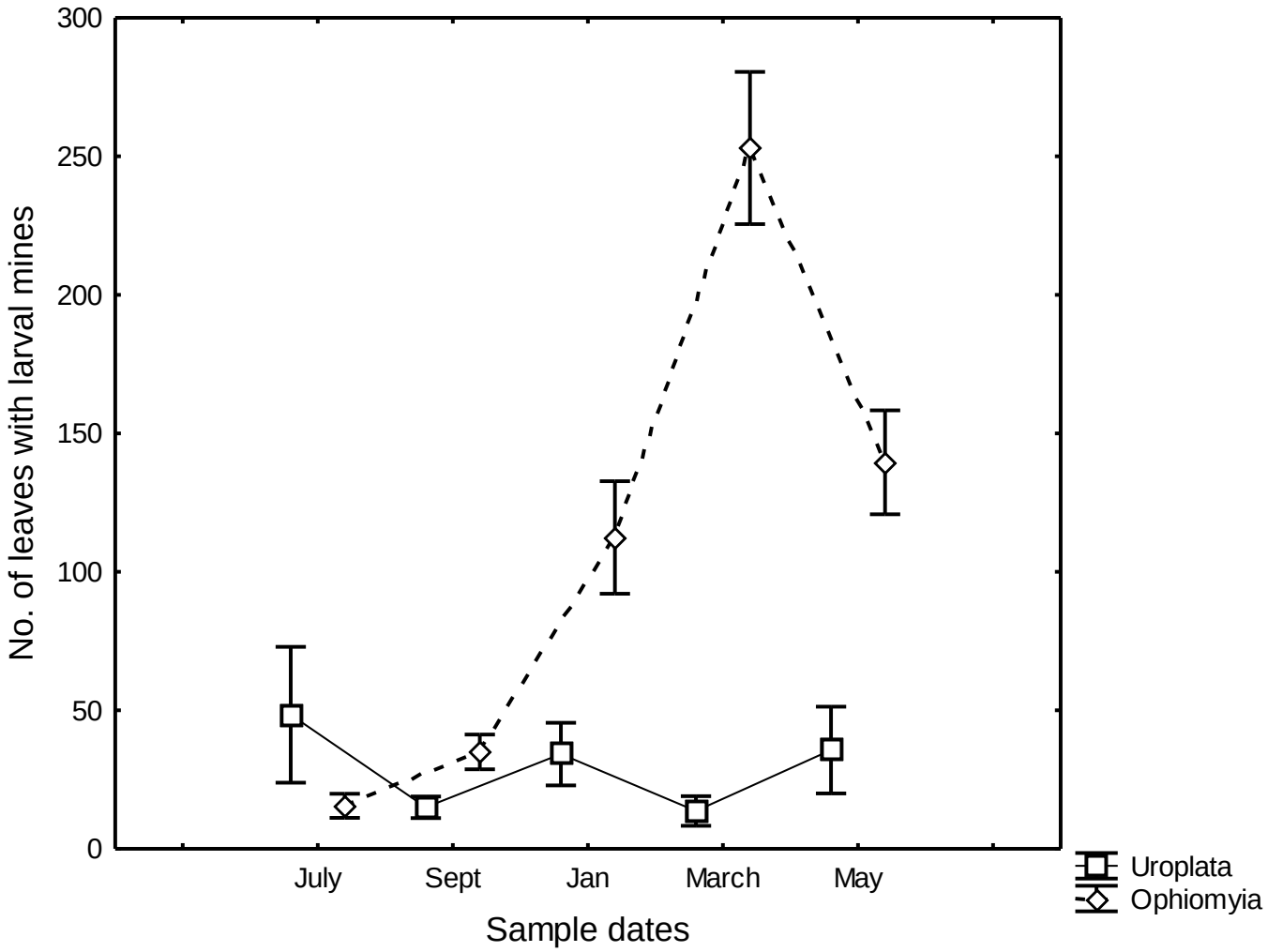

Figure 5: Number of leaves with *U. girardi* and *O. camarae* larval mines (Mean±SE) from July 2006 to May 2007 combined for all field sites in KwaZulu-Natal.

Discussion

Interspecific competition theory predicts that two species engage in a reciprocal struggle for food resources, and these interactions are more intense between species that share the same feeding niche (Schoener, 1974; Schoener, 1983). Similarly, it is expected for multiple release of weed biological control agents to result in competitive interaction, especially among agents that feed on the same plant part. However, because of the preference-performance principle, a theory that predicts a strong selection pressure on adults to oviposit on plants or plant parts of high nutritional quality so that offspring fitness can be maximized (Jaenike, 1978; Leather, 1994), some insect species could circumvent the negative effects of competition, and this may contribute to their competitive superiority. The results of this study suggest that *O. camarae* females are able to detect the quality of leaves, and they can therefore select uninfested leaves over those infested with *U. girardi* for oviposition (Fig. 1). In contrast, *Uroplata girardi,* appears to be unable detect the quality of leaves, and utilizes both the *O. camarae* infested and uninfested leaves equally for egg laying (Fig. 1). The population explosion and success of *O. camarae* in the field in recent years could be attributed to its ability to select good quality substrate for oviposition. The failure of *U. girardi* to detect good quality leaves could hamper the survival of its offspring, rendering it ineffective in controlling the weed. This could be true if the *U. girardi* female oviposits on leaves already infested with *O. camarae* as these leaves may abscise during the pupal stage of the latter and during egg or larval stage of the former. This is highly likely to increase the mortality of *U. girardi*, hence a decline in population of this beetle in the field. This could possibly explain the dwindling of populations of this beetle in the recent years following the release and establishment of its apparently superior competitor, *O. camarae*.

Under semi-field conditions, the study suggests that *U. girardi* population growth was impeded by *O. camarae'*s presence (Fig. 3), with larval mines peaking at approximately 50 when confined with *O. camarae* compared to approximately 100 when confined alone (Fig. 2). Although initial establishment of both agents

was delayed in the combined treatment, the population of *O. camarae* increased dramatically from March to April. In the single treatment, initial establishment of *O. camarae* was quicker but the population also peaked in April. Low population levels of *U. girardi* in the combined treatment is not surprising as the laboratory study has shown that the beetle's failure to detect good quality (uninfested) leaves for oviposition may hinder the survival of its offspring and therefore result in poor population growth of this beetle. *O. camarae* has a shorter generation time than *U. girardi*, resulting in a higher intrinsic rate of increase and higher abundance in the field. For successful biological control, agents with high intrinsic rates of increase should be selected for release as they likely to increase rapidly and sustain herbivore pressure on the target weed (Hajek, 2004).

Uroplata girardi and *O. camarae* population density varied between the sites surveyed in KwaZulu-Natal although the pattern of growth in four of the five sites was very similar (Fig. 4). *Ophiomyia camarae* populations persisted in high numbers in four of the sites (Fig. 4A, C, D and E) except at Phahla station where both agent's populations were different (Fig. 4B). At Phahla station, *U. girardi* abundance was initially high, but began to decline from March while that of *O. camarae* increased. By May, the *U. girardi* population had increased again while that of *O. camarae* had not recovered. This site had abundant food resources and had a substantial number of uninfested leaves per plant in comparison to the others. This could mean that both agents were not yet in short supply of food resources, and therefore this may have limited the negative effects of competition on *U. girardi* populations. It is speculated that the effect of competitive interaction could prevail once the food supply begins to diminish. It is also interesting to note that the growth patterns of insect populations in four (Fig. 4A, C, D and E) of the five sites resemble those of a mixed species treatment (Fig. 4) under semi-field conditions. This is another clear demonstration that interspecific competition between the two agents may be exhibiting its negative effects on *U. girardi*, causing the population decline of this beetle in recent years.

Although competition between the two agents appears to be impacting negatively on *U. girardi*, this beetle is not expected to be permanently displaced by *O. camarae*. Over the past four years, it has been observed that *O. camarae* populations decline from June and then resume population growth from November to April, and this may allow the recovery and population build-up of *U. girardi* during this period (Simelane, pers. comm.). The field study at four KZN sites show that *O. camarae* populations begin to decline in May and its abundance remains low until the summer. The environmental resistance which inhibit the populations of *O. camarae* during this period could allow the populations of *U. girardi* to recover somewhat, thus mitigating the negative effects of competition on this beetle (Schoener, 1974). The negative effects of competition between the two agents could be mitigated by the fact they are spatially separated by environmental conditions within the vicinity in which the host invades. It has been frequently observed that *O. camarae* prefers to attack lantana growing under shade (Simelane & Phenye, 2004) while *U. girardi* prefers to attack those plants in the open. However, *O. camarae* eventually spreads everywhere as the food supply in the shades diminishes.

This study highlights the need to conduct interaction studies on herbivorous insects utilizing the same host plant, particularly those sharing the same niche, before they can be released into the environment. This could limit the chances of introducing agents that would compete, and thereby reducing the effectiveness of one of them in controlling the target weed.

References

Anderson, G.L., Delfosse, E.S., Spencer, N.R., Prosser, C.W. & Richard, R.D. 2000. Biological control of leafy spurge: an emerging success story. In: Spencer, N.R. (Ed.) *Proceedings of the X International Symposium on Biological control of Weeds,* Montana State University, Bozeman.15-25. Montana, USA.

Baars, J-R. 2003. Geographic range, impact and parasitism of Lepidopteran species associated with the invasive weed *Lantana camara* in South Africa. *Biological control* **28**: 293-301.

Baars, J-R. & Heystek, F. 2003. Geographical range and impact of five biocontrol agents established on *Lantana camara* in South Africa. *BioControl* **48**: 743-759.

Baars, J-R. & Neser, S. 1999. Past and present initiatives on the biological control of *Lantana camara* (Verbenaceae) in South Africa. *African Entomology Memoir* **1:** 21-33

Cilliers, C.J. 1987. The evaluation of three insect natural enemies for the biological control of the weed *Lantana camara* L., *Journal of the Entomological Society of Southern Africa* **50**: 15-34.

Cilliers, C.J. 1983. The weed, *Lantana camara* L., and the insect natural enemies imported for its biological control into South Africa. J*ournal of the Entomological Society of Southern Africa* **46**: 131-138.

Cilliers, C.J. & Neser, S. 1991. Biological control of *Lantana camara* (Verbenaceae) in South Africa. *Agriculture, Ecosystems and Environment* **37**: 57-75.

Crowe, M.L. & Bourchier, R.S. 2006. Interspecific interactions between the gall-fly *Urophora affins* Frfld. (Diptera: Tephritidae) and the weevil *Larinus*

minutus Gyll. (Coleoptera: Curculionidae), two biological control agents released against spotted knapweed, *Centaurea stobe* L. spp. *micranthos*. *Biocontrol Science and Technology* **16**: 417-430.

Day, M.D., Wiley, C.J., Playford, J. & Zalucki, M.P. 2003. *Lantana camara:* Current Management Status and Future Prospects. *ACIAR Monograph.* 102.

Denoth, M., Frid, L. & Myers, J.H. 2002. Multiple agents in biological control: improving the odds? *Biological Control* **24**: 20-30.

Ehler, L.E & Hall, R.W. 1982. Evidence of competitive exclusion of introduced natural enemies in Biological control. *Environmental Entomology* **11**: 1-4.

Hajek, A.E. 2004. Biology and ecology of agents used for biological control of weeds. *Natural Enemies: An introduction to Biological Control*. 217-231. Cambridge University press.

Harris, P. 1985. Biological control of weeds: bureaucrats, botanists, beekeepers, and other bottlenecks. In: Delfosse, E.S. (Ed.) *Proceedings of the VI International Symposium on Biological control of Weeds*. Agriculture Canada. 3-12. Canadian Govt. Printing Office, Ottawa.

Heystek, F. 2006. Laboratory and field host utilization by established biological control agents of *Lantana camara* L., in South Africa. *MSc Thesis*. Rhodes University, South Africa.

Hoffmann, J.H. & Moran, V.C. 1999. A review of the agents and factors that contributed to the successful biological control of *Sesbania* punicea (Cav.) Benth. (Papilonaceae) in South Africa. *African Entomology Memoir* **1***:* 75-79.

Jaenike, J. 1978. On optimal oviposition behaviour in phytophagous insects.

Theoretical Population Biology **264**: 701-707.

Julien, M.H, & Griffiths, M.W. 1998. Biological control of weeds: A world catalogue of agents and their target weeds. 4th Edition. CABI Publishing, UK.

Leather, S.R. 1994. Insect growth and reproductive rates. In: Leather, S.R., Watt, A.D., Mills, N.J. & Walters, KFA (eds) Individuals, Populations and Patterns in Ecology. 35-43.

McEvoy, P.B. 2002. Insect-plant interactions on a planet of weeds. *Entomologia Experimentalis et Applicata* **104**: 165-179.

McEvoy, P.B. & Coombs, E.M. 1999. Why things bite back: Unintended consequences of biological control of weeds. In: Follett, P. A. & Duan, J. J. (eds.) *Nontarget effects of Biological Control*. 176-194. Kluwer Academic Publishers, Boston.

Myers, J.H. 1985. How many insects species are necessary for successful biological control of weeds? In: Delfosse, E.S. (Ed.) *Proceedings of the VI International Symposium on Biological control of Weeds*. Agriculture Canada. Canadian Govt. Printing Office, Ottawa.

Schoener, T.W. 1974. Resource partitioning in ecological communities. *Science* **184**: 27-39.

Schoener, T.W. 1983. Field experiments on interspecific competition. *American Naturalist* **122**, 240-285.

Simelane, D.O. 2002. Biology and host range of *Ophiomyia camarae*, a biological control agent for *Lantana camara* in South Africa. *BioControl* **47**: 575-85.

Simelane, D.O., & Phenye, M.S. 2005. Suppression of growth and reproductive capacity of the weed *Lantana camara* (Verbenaceae) by *Ophiomyia camarae* (Diptera: Agromyzidae) and *Teleonemia scrupulosa* (Heteroptera: Tingidae). *BioControl Science and Technology* **15**: 153-163.

Simelane, D.O. & Phenye, M.S. 2004. Preliminary assessment of release and establishment of lantana herringbone leaf miner, *Ophiomyia camarae* (Diptera: Agromyzidae), in South Africa. In: Cullen, J.M., Briese, D.T., Kriticos, D.J., Lonsdale, W.M., Morin, L. & Scott, J.K. (Eds.) *Proceedings of the XI International Symposium on Biological Control of Weeds*. CSIRO Entomology. 447-450. Canberra, Australia.

Statistica Enterprise System, Technology. 2004. Statistica 6.1 for Windows, Tulsa Oklahoma.

CHAPTER FOUR

General Discussion

The impact of *Uroplata girardi* feeding had a greater effect on lantana foliage than *O. camarae* and when both agents were combined. In this study *U. girardi* was able to defoliate a large number of leaves when confined alone and yet it did not do so when confined with *O. camarae* in a single cage, suggesting that one or both agents may be exhibiting some form of interaction which could be antagonistic. However, the damage caused by *U. girardi* in the semi-field trials was substantially higher than that observed under natural field conditions along the KwaZulu-Natal coastal regions. The level of leaf damage caused by *O. camarae* in the semi-field trials was similar to that observed in the field. Furthermore, both agents did not have a significant impact on overall plant stem diameter, plant length and canopy size. Simelane & Phenye (2005) demonstrated that defoliation may have an immediate negative effect on plant growth, reproduction and fitness, especially if maintained for a long period of time However, plants infested and uninfested in the cages of the semi-field trials did not show any reduction in size and reproduction capacity compared to the uncaged plants which were significantly affected through loss of leaves. *Teleonemia scrupulosa* a widespread lantana biocontrol agent was found to cause significant effects on growth of the exposed plants (uncaged) colonized and attacked by this tingid. In addition, exposure to factors such as frost, sun, wind and rain may have played a role in reduction of these parameters in uncaged plants.

The reproductive capacity of lantana was significantly affected by *U. girardi* and *O. camarae* attack, either individually or combined by 28, 61 and 19 %, respectively. *Ophiomyia camarae* attack had a significant impact on flower production and a decline in flower production directly impacts on plant reproductive capacity, causing a reduction in the spread of the target weed

(Hendrix, 1988; Hoffmann & Moran, 1991). Since the release of *O. camarae* along the KwaZulu-Natal coastal regions, the plant vigour of lantana has been adversely affected, and this may be causing a significant reduction in flower and fruit production and subsequent spread of the weed (Simelane pers. comm.).

Furthermore, *O. camarae* attack had a negative impact on root biomass of lantana. Roots provide the ideal storage site for resources used in above ground defence and that reduction in root biomass may be due to a compensation response at the expense of vegetative and reproductive parts reduced as a result of insect attack (Kaplan *et al.*, 2008). Plant root condition has been indirectly implicated in the population dynamics of the above-ground herbivores (Huberty & Denno, 2004). Therefore, *O. camarae* feeding reduces water and nutrient transportation to the leaves (Simelane, 2002) which appear to have resulted in reduced root biomass and a depletion of storage reserves.

Leaf feeders on lantana are the most highly represented guild, but their effect appears to have been constrained. Ability of lantana to morphologically change its leaf texture, stems and flower colour in response to attack by herbivores has resulted in most biocontrol agents released against lantana showing varietal preferences in the field (see Baars & Heystek, 2003; Heystek, 2006). The Dark Pink flowering lantana (Variety 021) has been shown to be the most preferred variety in the field not only due to its abundance but also due to its broad leaves. *Uroplata girardi* and *O. camarae* appear to develop successfully on this variety in the field as observed by Heystek (2006). In the semi-field trials, this variety sustained the feeding of *U. girardi* adults and larval mining. However, the varietal preferences of *U. girardi* and O*. camarae* could have a negative effect on biocontrol of lantana as a weed in South Africa because this limits the effect of the agent to other varieties. For example, in Gauteng, orange flowering lantana is very abundant and not likely to be attacked by the agents in the field other than in the quarantine (pers. observations). When mass rearing *U. girardi* and *O. camarae* for cultures; red, pink, light pink, orange, yellow and white flowering

lantana plants were used. *U. girardi* only fed on plants with red and orange flowers but selected other plants for egg laying, this could be because these two varieties had small hairy leaves compared to the others. In contrast, *O. camarae* did not show a preference for any variety in the laboratory. It attacked almost any shape of leaf including the smallest on the plant. Due to this personal observation, the varietal preference by the agents could be linked with a geographic area and unfavourable environmental factors the plants are exposed to in the field, as a result hinder the agents' impact (Day & Neser, 2000; Baars & Heystek, 2003). Even though we expected that the vigour of both agents when combined would cause higher defoliation; and lead to reduced growth and biomass (Briese, 1996). Feeding damage by both agents combined was similar to that caused by *O. camarae* alone. However, combined attack by the agents had a significant effect on roots of lantana compared to the controls but similar to that caused by *U. girardi* alone and less than that caused by *O. camarae* alone. As a result, the combined attack by both agents seems to be hampered as compared to when each agent species is in isolation. The combined attack by the agents was neither additive nor synergistic in controlling lantana. It is likely that the resource availability and resource quality of one insect species was altered by the other.

The field studies (Chapter 3) indicated that the two agents may prefer different environments along the coastal regions of KwaZulu-Natal. *Ophiomyia camarae* has been found persisting in high numbers at a site where lantana is growing in shade (Simelane & Phenye, 2004). Furthermore, the Kuswag site in the field had a high density of *Ophiomyia camarae,* as most lantana plants sampled were under or among large trees, compared to the exposed lantana in Phahla station. However, both agents do co-exist at both sites. In classical biological control, agents with a high intrinsic rate of increase are often selected for weed control since they are likely to regulate their number to that of the host density exerting substantial herbivory pressure on the plant (Baars, 2003). Oviposition behaviour of female insects plays a role in ensuring that the host plant species selected will

maximize larval growth and survival (Leather, 1994). In the laboratory *O. camarae* females appear to be able to detect the quality of leaves, and they can therefore select uninfested leaves over those infested with *U. girardi* for oviposition. In contrast, *U. girardi,* appears to be unable to detect the quality of leaves, and utilizes both the *O. camarae* infested and uninfested leaves equally for egg laying. When *U. girardi* females oviposit on leaves already infested with *O. camarae* this may result in leaf abscission during the pupal stage of *U. girardi*. This is highly likely to increase the mortality of *U. girardi*, hence a decline in populations of this beetle in the field. This could possibly explain the dwindling of populations of this beetle in the recent years following the release and establishment of its apparently superior competitor, *O. camarae.* Therefore, the population explosion and success of *O. camarae* in the field in recent years could be attributed to its ability to select good quality substrate for oviposition. The failure of *U. girardi* to detect good quality leaves could hamper the survival of its offspring, thus rendering it ineffective in controlling lantana when *O. camarae* is present.

Furthermore, under semi-field conditions *U. girardi* population growth was impeded by *O. camarae'*s presence. Although initial population growth of both agents was delayed in the combined treatment, the population of *O. camarae* increased dramatically from March to April. The low population levels of *U. girardi* in the combined treatment is not surprising as the laboratory study has shown that *U. girardi's* failure to detect good quality (uninfested) leaves for oviposition may hinder the survival of its offspring and therefore result in poor population growth of *U. girardi*. In the field, *U. girardi* and *O. camarae* population densities varied between the sites. The population density of *O. camarae* was higher at four of the five surveyed field sites, while that of *U. girardi* was high at one site. Variation in population growth between the sites may be due to abundant food resources and substantial number of uninfested leaves per plant at Phahla compared to other sites. This could mean that both agents were not yet in short supply of food resources, and therefore this may have limited the

negative effects of competition on *U. girardi* populations. It is speculated that the effect of competitive interaction could prevail once the food supply begins to diminish.

It is also interesting to note that the growth patterns of insect populations at four (Fig. 4 A, C, D and E) of the five sites resemble those of a mixed species treatment under semi-field conditions (Fig. 3). This suggests that interspecific competition between the two agents may be exhibiting its negative effects on *U. girardi*, causing the population decline of this beetle in recent years. Furthermore, *O. camarae* has a shorter generation time (±30 days) than *U. girardi* (±41.5 days), resulting in a higher intrinsic rate of increase and higher abundance in the field. Successful biocontrol agents with high intrinsic rates of increase should be selected for release as they are likely to increase rapidly and sustain herbivore pressure on the target weed (Hajek, 2004).

Specialized herbivores likely to be used in biological control programmes are adapted to select high quality food, with ovipositing females showing a strong preference for high quality plant modules (Leather, 1994). This ovipositing preference is linked in many species to larval survival (Price, 1999). The ability of *Ophiomyia camarae* to select uninfested leaves for oviposition more frequently than *U. girardi* infested ones may increase larval survival. In contrast *U. girardi* showed no apparent preference or discrimination between the used and unused leaves. Herbivore species with a strong preference for high quality modules can become abundant locally when the carrying capacity is high, often after disturbance when young plants establish (Price, 1999). When female phytophagous insects are highly selective on vigorous growing plants and such resources are in short supply, competition for ovipositing sites is likely to become important and may occur even at low herbivore density (Price, 1991).

Although competition between the two agents appears to be impacting negatively on *U. girardi*, it is not expected to be permanently displaced by *O. camarae*. Over

the past four years, it has been observed that *O. camarae* populations decline from June (winter) and then resume population growth from November to April, and this may allow the recovery and population build-up of *U. girardi* during this period (Simelane, pers. comm.). In the field study at four KwaZulu-Natal sites show that *O. camarae* populations begin to decline in May (late-autumn) and its abundance remains low until the summer (Fig. 5). The temperature could allow population build up of *U. girardi* around the coastal areas, thus mitigating the negative effects of competition on *U. girardi* (Schoener, 1974). The negative effects of competition between the two agents could be mitigated by the fact that they are spatially separated by environmental conditions at a local scale in which the host invades. It has been frequently observed that *O. camarae* prefers to attack lantana growing under shade (Simelane & Phenye, 2004) while *U. girardi* prefers to attack those plants in the open. However, *O. camarae* eventually spreads everywhere as the food supply in the shades diminishes. This study highlights the need to conduct interaction studies on herbivorous insects utilizing the same host plant, particularly those sharing the same niche, before they can be released into the environment. This could limit the chances of introducing agents that would compete, and thereby reducing the effectiveness each other in controlling the target weed. However, further research needs to be done to determine the potential impact and interaction between *O. camarae, U. girardi* and the recently released agents of *L. camara,* the root feeding flea beetle *Longitarsus bethae* (Coleoptera: Chrysomelidae) and leaf-petiole gall forming weevil *Coelocephalapion camarae* (Coleoptera: Curculionidae) and also determine the geographic distribution of these agents in the country in order to give an indication of where they are likely to co-occur and compete in South Africa.

Based on this study, it can be concluded that *U. girardi* and *O. camarae* damage caused to lantana was neither synergistic nor additive. Although the release of biocontrol agents against lantana may have played a role in limiting population density of the weed, their impact is not sufficient to bring this shrub under control.

However, the results reported here found that cumulative stress by *U. girardi* and *O. camarae* on lantana was insufficient. It was found that the damage caused by *T. scrupulosa* on uncaged control plants was quite severe, and this seems to suggest that the combination of the three agents (*U. girardi, O. camarae* and *T. scrupulosa*) might enhance the biological control of lantana. Although it is very damaging, *T. scrupulosa* is known to be sporadic in the field (Baars & Neser, 1999). It is also unlikely that the joint impact by the three agents would be synergistic in the wild, unless they are temporally separated. Interaction studies between the released agents and those proposed for release should be integrated within the pre-released studies. Such studies would enable predictions to be made about the effects of interaction between the agents in the field. This is important since there are risks associated with introducing an exotic species and unnecessary introductions should be avoided (Gerber *et al*., 2008). It is also recommended that the number of agents utilizing the same niche should be kept to a minimum while rather targeting those that use different niches (Olckers *et al.*, 2002; Simelane, 2006).

REFERENCES

Baars, J-R. 2003. Geographic range, impact and parasitism of Lepidopteran species associated with the invasive weed *Lantana camara* in South Africa. *Biological control* **28**: 293-301.

Baars, J-R. & Neser, S. 1999. Past and present initiatives on the biological control of *Lantana camara* (Verbenaceae) in South Africa. *African Entomology Memoir* **1:** 21-33.

Briese, D.T. 1996. Potential impact of the stem-boring weevil *Lixus cardui* on the growth and reproductive capacity of *Onopordum* thistles. *Biocontrol Science and Technology* **6:** 251-261.

Day, M.D. & Neser, S. 2000. Factors Influencing the Biological Control of Lantana camara in Australia and South Africa. In: Neal R. Spencer (ed.). *Proceedings of the X International Symposium on Biological Control of weeds*, Montana State University, Bozeman, USA. 897-908.

Gerber, E., Hinz, H.L. & Blossey, B. 2008. Pre-release impact assessment of two stem-boring weevils proposed as biological control agents for *A. petiolata*. *Biological Control* **45:** 360-367.

Hajek, A.E. 2004. Agents used for biological control of weeds. *Natural Enemies: An introduction to Biological Control*. Cambridge University press. 220-238.

Hendrix, S.D. 1988. Herbivory and its impact on plant reproduction. In: Lovett-Doust, J & Lovett-Doust, L. (Eds.) Plant Reproductive Ecology: Patterns and Strategies. 246-262. Oxford Publishers.

Hoffmann, J.H. & Moran, V.C. 1991. Biological control of *Sesbania punicea* (Fabaceae) in South Africa. *Agriculture, Ecosystems and Environment* **37:** 157-173.

Huberty, A.F. & Denno, R.F. 2004. Plant water stress and its consequences for herbivorous insects: a new synthesis. Ecology **85**: 1383-1398.

Kaplan, I., Halitschlke, R., Kessler, A., Rehill, B.J., Sardanelli, S. & Denno, R.F. 2008. Physiological integration of roots and shoots in plant defence strategies links above and below ground herbivory. *Ecology Letters* **11**: 841-851.

Leather, S.R. 1994. Insect growth and reproductive rates. In: Leather, S.R., Watt, A.D., Mills, N.J. & Walters, KFA (eds) *Individuals, Populations and Patterns in Ecology*. 35-43.

Olckers, T., Medal, J.C. & Gandolfo, D.E. 2002. Insect herbivores associated with species of *Solanum* (Solanaceae) in northeastern Argentina and southeastern Paraguay, with reference to biological control of weeds in South Africa and the United States of America. *Florida Entomologist* **85:** 254-260.

Price, P.W. 1999. Host plant resource quality, Insect herbivores and Biocontrol. In: Neal R. Spencer (ed) *Proceedings of the X International Symposium on Biological Control of Weeds*. Montana State University, Bozeman, Montana, USA. 583-590.

Price, P.W. 1991. The plant vigor hypothesis and herbivore attack. *Oikos* **62:** 244-251.

Schoener, T.W. 1974. Resource partitioning in ecological communities. *Science* **184**: 27-39.

Simelane, D.O. 2006. Effect of herbivory by *Teleonemia scrupulosa* on the performance of *Longitarsus bethae* on their shared host, *Lantana camara. Biological Control* **39:** 385-391.

Simelane, D.O. 2002. Biology and host range of *Ophiomyia camarae*, a biological control agent for *Lantana camara* in South Africa. *BioControl* **47**: 575-85.

Simelane, D.O., & Phenye, M.S. 2005. Suppression of growth and reproductive capacity of the weed *Lantana camara* (Verbenaceae) by *Ophiomyia camarae* (Diptera: Agromyzidae) and *Teleonemia scrupulosa* (Heteroptera: Tingidae). *BioControl Science and Technology* **15**: 153-163.

Simelane, D.O. & Phenye, M.S. 2004. Preliminary assessment of release and establishment of lantana herringbone leaf miner, *Ophiomyia camarae* (Diptera: Agromyzidae), in South Africa. In: Cullen, J.M., Briese, D.T., Kriticos, D.J., Lonsdale, W.M., Morin, L. & Scott, J.K. (Eds.) *Proceedings of the XI International Symposium on Biological Control of Weeds*. CSIRO Entomology. 447-450. Canberra, Australia.