Laboratory and field host utilization by established biological control agents of
***Lantana camara* L. in South Africa**.

THESIS

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ABSTRACT

Varieties of *Lantana camara* (lantana) have been introduced into many countries of the world as ornamental plants and have become invasive weeds in many countries including South Africa. In South Africa, it mostly invades the sub-tropical eastern and northern range. Mechanical and chemical control options are expensive and ineffective. A biocontrol programme was initiated in South Africa in 1961. To date, 22 insect species, and a fungus have been introduced, of these 10, and the fungus have established. Three indigenous lepidopteran species and an exotic generalist pest mealybug are also associated with the weed.

The variable success of some of the agents released on *L. camara* worldwide has been ascribed to a few factors. One important aspect is the large range of varieties encountered in the field. It is therefore essential to be able to predict the possible establishment and impact of agents on many varieties. Laboratory trials on five of the established agents showed clear varietal preferences. In the field, most of the biocontrol agents had limited geographic ranges, linked to altitudinal conditions, as higher populations were recorded at low lying warm summer rainfall areas. A pink and orange flower corolla lobe and throat colour combination and plants with few to medium leaf hairs were most abundant in South Africa. Most of the agent species had individual preferences towards different flower colour combinations, as the agents built up different population levels on varieties in the field, within the suitable geographic region for the insect species. Eight agents preferred smooth leaved varieties, while three preferred hairy leaves, and three had no specific preference to leaf hairiness. Varietal preferences thus did play a significant role in agent populations and accompanied impact achieved in the field.

New candidate agents need to be proven specific under quarantine conditions and the results extrapolated to predict specificity in the field, while avoiding potential non-target effects. Many authors have questioned the validity of laboratory host specificity trials. The conventional wisdom is that insects portray a far wider host range in the laboratory than what they would do in the field. In other words, laboratory studies measure the

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physiological host range of an agent and are conservative and usually don't reflect the ecological host range of agents in the field. To avoid unnecessary rejections of biocontrol agents, this study has made a retrospective study of the host specificity of agents established in the field. Their laboratory and field host ranges were compared and it was found that virtually all the agents reflect similar or less non-target effects in the field than predicted during multiple choice trials. Of the 14 agents, only one introduced species, *Teleonemia scrupulosa*, and the indigenous species, *Hypena laceratalis* and *Aristea onychote* were able to sustain populations on non-target species in the field in the absence of *L. camara*. Insect populations on non-target species were much reduced compared to that on *L. camara*. Furthermore non-target effects were only recorded on plant species closely related to the target weed. The multiple choice trials therefore predict field non-target effects accurately. Predictions of non-target effects of candidate agents can therefore be accurately predicted by laboratory studies, in terms of species likely to be affected and to what extent. One field that need further study though is the impact of non-target effects, especially on *Lippia* species by *L. camara* biocontrol agents.

ACKNOWLEDGEMENTS

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General Introduction

1.1 Background on lantana

Lantana camara L. (Verbenaceae), commonly called lantana, is considered one of the world's worst weeds (Cilliers & Neser 1991; Swarbrick *et al*. 1995; Baars & Neser 1999). It flowers prolifically and produces thousands of berries throughout the year, which are eaten, and spread by birds. Hundreds of manmade cultivars or varieties were spread from South and Central America around the world by collectors, including South Africa (Swarbrick *et al.*1995; Baars & Neser 1999). It has naturalised in the warm and wet temperate and sub-tropical regions of South Africa (Figure1.1). It forms dense impenetrable thickets, and through alelopathy prevents regeneration of indigenous vegetation (Gentle & Duggin 1997). It reduces pasture, and biodiversity. It is also poisonous to livestock, and an estimated R 1 728 900 (in 1996 Rands) is lost annually through cattle being poisoned by lantana (Kellerman *et al.* 1996).
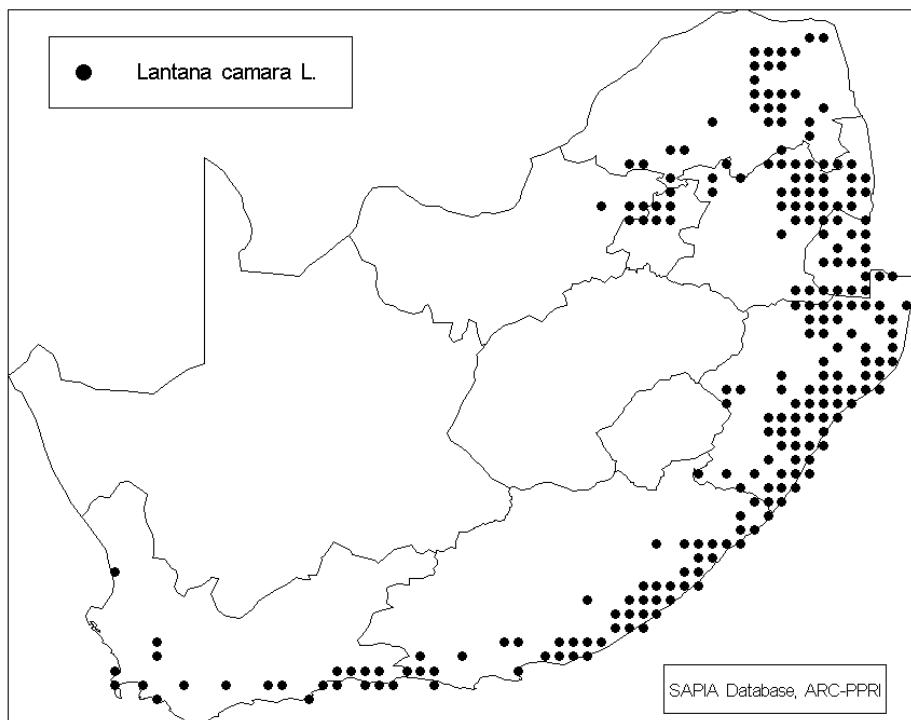
Figure 1.1 Recorded localities of *Lantana camara* in South Africa, as on Southern African Plant Invaders Atlas (SAPIA) Database (Henderson 2001).

1.2 Control options

Lantana is a declared weed in South Africa, and landowners, by law have to control it (The Conservation of Agricultural Resources Act, Act no. 43 of 1983). The control options include chemical, mechanical, or biological control, or a combination of these, all of which, have cost implications.

Chemicals that are registered for use in South Africa include glyphosate, imazapyr, picloram and tebuthiuron as either one or more of; foliar spray, cut stump treatment, and coppice growth applications (Grobler *et al.* 2000). These are however expensive, and follow up treatments are continually required for coppice growth, and seedling regeneration. Non-target damage to indigenous plants during the application of chemicals, as well as mechanical damage by trampling during access to infestations also retards the rehabilitation of infested areas.

Mechanical control is very labour intensive, and thus expensive. Often, the weed is burnt during winter, but enough fuel for a hot fire is rarely acquired, and plants are not killed, and re-sprout (Mr. J. Buckle, A. Sephton, & M. Snijders 2002 - All involved in *Working For Water*, weed control, Personal communication). The hazard of uncontrolled fires makes this method a risky option in mountainous and densely vegetated areas.

A lantana biocontrol programme was initiated in 1902, with introductions of insect natural enemies from Mexico into Hawaii (Perkins & Swezey 1924). By 1998, 36 agent species were introduced against lantana of which 23 had established worldwide (Julien & Griffiths 1998). Some control has been achieved, particularly on small islands (Waterhouse & Norris 1987; Denton *et al.* 1991; Swarbrick *et al.* 1995; Muniappan *et al.* 1996).

The South African lantana biocontrol project was initiated in 1961 (Oosthuizen 1964). To date, 22 insect species, and a fungus have been introduced, of these 10, and the fungus have established (Table1.1) (Anonymous 1999). Three of these lantana agents, the lantana lace bug *Teleonemia scrupulosa* Stål (Tingidae) and the two leaf-mining beetles,

Octotoma scabripennis Guérin-Méneville (Chrysomelidae) and *Uroplata girardi* Pic (Chrysomelidae), are considered to stress plant growth and reproduction significantly in South Africa (Cilliers 1987b; Baars & Heystek 2003a). The same agents are also considered most damaging in other countries (Harley *et al.* 1979; Swarbrick *et al.* 1995; Muniappan *et al.* 1996). Their contributions remain limited, as these agents do not sustain high population levels throughout the growing season, and the two leaf-mining beetles are restricted to specific climatic regions in South Africa, and thus cause only localized damage to lantana (Cilliers 1987a; b; Baars & Neser 1999; Baars & Heystek 2003a). Other agents, such as *Calycomyza lantanae* (Frick) (Argomyzidae), *Epinotia lantana* (Busck) (Tortricidae) and *Salbia haemorrhoidalis* Guenée (Pyralidae), have acquired native parasitoids, which further reduce their biocontrol potential (Baars & Neser 1999). Recent introductions of the herringbone leaf miner, *Ophiomyia camarae* (Froggat) (Agromyzidae) (Simelane 2003), and the fungus, *Mycovellosiella lantanae* (Chupp) Deighton var. *lantanae* (Mycosphaerellaceae) (den Breëyen 2003; den Breëyen & Morris 2003) has established, but the impact on the weed is unknown. Further candidates to be released into South Africa, when approval is obtained from the governing bodies, are *Coelocephalapion camarae* Kissinger (Brentidae), *Longitarsus* sp. AcSN 2440 (Alticinae), *Leptostales ignifera* Warren (Geometridae), and the flower-bud mite, *Aceria lantanae* (Cook) (Eriophyidae) (Urban *et al.* 2001; 2003). Research into new lantana agents for Africa is however ongoing.

For many invasive weed species biocontrol remains the most cost effective, sustainable option. For lantana biocontrol in South Africa, the cost benefit ratio was calculated as 1:22 for the year 2000 (from the year biocontrol research was initiated up to the year 2000) (van Wilgen *et al.* 2004). This was based on calculations, considering the rate of spread of the weed, the area that could potentially be invaded, the impact of the biocontrol agents on the weed populations and spread, the cost of biocontrol research and implementation, and quantified benefits of preventing further invasion, water use and land value loss (2% of land resale value loss at 100 % weed invasion). Benefits in terms of biodiversity were not captured in monetary value, unless directly utilized, e.g. harvesting of wildflowers. The benefit of biocontrol increased to 34 in relation to its cost

(1), as a future estimate, i.e., when calculated as a benefit, from the year the biocontrol project was initiated until the estimated date when the weed population has fully invaded its potential range (van Wilgen *et al.* 2004). These benefit ratios are achieved, even though the weed is not fully under control. The biocontrol has reduced the rate of invasion and the weed's competitiveness thus far, highlighting the need to improve on the success.

Table 1.1 Natural enemies released on *Lantana camara* in South Africa and their current status (taken from Anonymous 1999, updated to January 2006)

Natural enemy species	Origin	Main releases	Mode of attack	Status	Damage inflicted
Coleoptera: Cerambycidae					
Plagiohammus spinipennis (Thompson)	Mexico via Hawaii via Australia	1973	Stem-borer	Not established	-
Coleoptera: Chrysomelidae					
Alagoasa parana (Samuelson)	Brazil via Australia	1985	Leaf chewer	Not established	-
Octotoma championi (Baly)	Costa Rica via Australia Central America via Australia	1978 1995	Leaf miner	Establishment unconfirmed	Unknown
Octotoma scabripennis (Guérin-Méneville)	Mexico via Hawaii via Australia	1971 and 1974 and 1981	Leaf miner	Established in the moist, warm eastern range of lantana. Abundant in localized inland areas	Extensive defoliation, but localized
Uroplata girardi (Pic)	Paraguay via Hawaii via Australia	1974 1983	Leaf miner	Established, abundant in KwaZulu-Natal coastal regions. Present in low numbers in warm, moist inland range of lantana	Extensive defoliation in coastal regions
Uroplata lantanae (Buzzi and Winder)	Brazil via Australia	1984	Leaf miner	Not established	-
Uroplata fulvopustulata (Baly)	Costa Rica via Australia	1978	Leaf miner	Not established	-
Diptera: Agromyzidae					
Calycomyza lantanae (Frick)	Trinidad via Australia Florida USA	1982 1989	Leaf miner	Widely established in low numbers, heavily parasitized	Unknown
*Ophiomyia lantanae** (Froggatt)	Mexico via Hawaii	1961	Fruit miner	Widely established and abundant but heavily parasitized	Low impact on seed viability
Ophiomyia camarae (Froggatt)	Florida USA	2000	Leaf miner	Widely established	Still being studied
Diptera: Tephritidae					
Eutreta xanthochaeta (Aldrich)	Mexico via Hawaii	1983	Stem galler	Not established	-
Hemiptera: Miridae					
Falconia intermedia (Distant)	Jamaica	1999	Leaf sucker	Established at sites in the warm moist distribution range of lantana	Localy high, but limited by distribution and population fluctuations

Table 1.1 continued

Natural enemy species	Origin	Main releases	Mode of attack	Status	Damage inflicted
Hemiptera: Tingidae					
Teleonemia elata (Drake)	Brazil via Australia	1972	Flower and leaf sucker	Not established	-
Teleonemia scrupulosa (Stål)	Mexico via Hawaii, via Australia, via Mauritius Florida, USA	1961, 1971, 1984 1989	Flower and leaf sucker	Widely established in large numbers across the entire range of lantana	Severe damage sporadic, complete defoliation and abortion of flowers in subtropical regions
Leptobyrsa decora (Drake)	Colombia and Peru	1972	Leaf sucker	Not established	-
Lepidoptera: Gracillariidae					
Aristea onychote	?	?	Leaf miner	Widely established, present in low numbers, heavily parasitized	Unknown
Lepidoptera: Noctuidae					
Autoplusia illustrata (Guenée)	Colombia via Hawaii via Australia	1984	Leaf chewer	Not established	-
*Hypena laceratalis** (Walker)	Kenya and Zimbabwe via Hawaii	1961	Leaf chewer	Widely established. Larvae are only active during late summer and autumn and are often parasitized	Considerable damage to seedlings and new growth
Neogalea sunia (Guenée)	California, USA California, USA	1962 1969	Leaf chewer	Not established	-
Lepidoptera: Pterophoridae					
Lantanophaga pusillidactyla (Walker)	Mexico via Hawaii	1984	Flower, fruit and seed chewer	Widely established, but present in low numbers, possibly high levels of parasitism	Unknown
Lepidoptera: Pyralidae					
Salbia haemorrhoidalis (Guenée)	Florida and Cuba via Hawaii	1962	Flower and fruit feeder	Widely established in low numbers	Unknown
Lepidoptera: Tortricidae					
*Epinotia lantana** (Busck)	Hawaii	1984	Flower-peduncle and shoot-tip borer	Widely established	Unknown

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

1.3 Problems associated with biocontrol of lantana

Very poor control has been achieved, despite the long biocontrol history of lantana (Löyttyniemi 1982; Muniappan & Viraktamath 1986; Swarbrick *et al.* 1995; Baars & Neser 1999). The main causes for the lack of establishment and insufficient control are considered to be: (1) the biocontrol candidates that are not adapted to the weed's extreme variability, (2) the extensive climatic range it invades, and (3) high levels of parasitism on the natural enemies (Winder *et al.* 1984; Neser & Cilliers 1990; Cilliers & Neser 1991; Baars & Neser 1999; Day & Neser 2000; Baars 2002a; Williams 2003a). *Lantana camara* is a hybrid species that has been artificially bred from various species of lantana, from the South and Central American regions (Stirton 1977; Day & Neser 2000). This implies that insects associated with these lantanas are not necessarily adapted to feed and breed on the weedy lantanas. Day and Neser (2000) speculated that the effect of the original host species and lantana varieties, could have led to the failure to establish some insect species on lantana. They speculated that phenotype differences between countries caused agents to establish in some countries, but not in others. Of six species that have been shown to have variety preferences, namely, *Ectaga garcia* Becker, *Charidotis pygmaea* Klug, *Alogoasa parana* Sameulson, *T. scrupulosa*, *F. intermedia* and *A. compressa*, only two established, *T. scrupulosa* and *A. compressa* (Day & Neser 2000). Subsequently *F. intermedia*, has established. The statement about species with varietal preferences is however not complete, as, at the time, some of the older established agent's variety preferences were not studied experimentally. In later chapters in this thesis two of the agents previously untested, namely *C. lantanae* and *U. girardi* have shown to have variety preferences in the laboratory (Chapters 2 and 4). These figures reduce the relative weight of the statement, and will now read "Of eight species that have been shown to have variety preferences, 5 have established. The ratio therefore has changed from 2/6 to 5/8.

The numerous horticultural varieties of lantana that were bred differ in flower colour, hairiness of stems and leaves, thorniness, growth shape, and chemical composition (Stirton 1977). These varieties interbreed, forming new variations (Day & Neser 2000). Certain "colour forms" are dominant in a region, and agents with "colour preferences"

may control one variety of the weed, but it may simply be replaced by another variety (Cilliers & Neser 1991). The agents' reported preferences for certain colour varieties of lantana can however be misleading, as all the characteristics of the plant is certainly not linked to flower colour. Scott *et al*. (1997) demonstrated that geographical proximity of varieties with different flower colours contributed more to relatedness than flower colour, but colour can be a practical and simple tool as a point of reference to a variety, i.e., an insect may feed on one pink variety, but avoid another.

One of the most important factors for the limited establishment and control exerted by natural enemies of lantana is climate. Lantana is established over a wide climatic range in southern Africa, from 34°S, in the Western Cape region, with winter rainfall, along the warm moist, subtropical eastern coast of South Africa, with summer rainfall, up, beyond the equator (Henderson 2001). It ranges from sea level, to over 1500 m. Lantana becomes deciduous during cold or dry conditions, and causes populations of biocontrol agents dependent on leaves to be eliminated periodically, during these periods, or some species that are unable to cope with this leafless condition of their host plant not to establish (Day & Neser 2000; Baars & Heystek 2003a). The three most widely established agents in South Africa are *C. lantanae*, *O. lantanae*, both agromyzids, and the tingid, *T. scrupulosa*, (Baars & Heystek 2003a) but even *T. scrupulosa* outbreaks are restricted to drier areas, or to drier seasons (Harley 1974; Muniappan *et al.* 1996). Agents such as the leaf mining hispids, *O. scabripennis*, and *U. girardi* are limited to warm moist regions (Baars & Heystek 2003a).

Parasitism of agents has further hampered lantana biocontrol (Baars & Neser 1999). *Calycomyza lantanae* is heavily parasitized in the field in South Africa, so much so, that despite being found on virtually all lantana varieties, and over the whole of lantana's distribution range, its effect remains negligible (Baars 1999; Baars & Heystek 2003a). An unidentified eulophid species was recorded parasitising *U. girardi* and a few generalist predators have been recorded from field populations of *T. scrupulosa* (Cilliers 1987a). No recruitment of parasitoids has been recorded for *F. intermedia*, but predators like ants seem to become very damaging to populations (Heystek & Olckers 2003). Hill and

Hulley (1995) considered 62 agents released onto 22 weed species, and found 40% of agents to be parasitized. Poorly concealed endophagous biocontrol agents, such as *C. lantanae* and *U. girardi*, released into South Africa were most likely to be parasitized (62%), and followed by well concealed endophages (47%) and ectophages being least likely (20%). The same study revealed dipterans and lepidopterans to be most likely parasitized, but parasitism should not prevent the release of a biocontrol agent (Hill & Hully 1995).

1.4 Host specificity testing of lantana

Host specificity testing in quarantine is usually conducted by a series of no-choice, and choice trials, whereby some, or all the life stages of a candidate is presented with plant species, closely related to the target weed (Wapshere 1989; Edwards 1999; Heard 1999), and some plants of economic importance. These series of tests rank agents from specialists to generalists, or further refined to rank potential hosts relative to one another (van Klinken 2001). If agents accept non-target species, continuation trials are sometimes conducted. These measure if a candidate agent can sustain itself on these non-target species over several generations (Day 1999). Laboratory based host specificity testing is conservative, and may indicate an extended host range, from that realised in the field (van Klinken 2001). This may be due to the limitations the laboratory trials place on the insects normal host search and selecting behavior (Dunn 1976; van Klinken 2001). Under most conditions, the ovipositing females choose a suitable host, but some insects may have very mobile immature stages, that can also exert a choice on their food source.

Up to 1995, most of the host specificity testing for the lantana biocontrol programme in South Africa relied heavily on testing done in Australia. Indigenous Verbenaceae, especially from the genus *Lippia*, were not included in this early work (Neser & Cilliers 1990; Cilliers & Neser 1991). Nineteen agents have been introduced into the country this way (Anonymous 1999). The experiments that biocontrol practitioners conduct have also become more rigorous, and test species lists have expanded to include especially related indigenous plants that were omitted in the past. In recent studies, several other agents tested in quarantine showed an ability to feed and reproduce on these plants in the

laboratory. *Falconia intermedia* was able to reproduce about a fifth as well on *Lippia* species, as on lantana in the laboratory, but were still released into South Africa (Baars 2000b). Agents recently tested and rejected for release in South Africa, based on their laboratory host ranges are *Aconophora compressa* Walker (Hemiptera: Membracidae) (Heystek & Baars 2001; 2005)*, Alogoasa extrema* Jacoby (Coleoptera: Alticinae) (Williams 2003a) and*, Barella parvisaccata* Young (Hemiptera: Auchenorrhyncha: Cicadelidae) (Phenye & Simelane 2003).

The lantana biocontrol project still requires additional agents to increase the pressure exerted on the weed, considering the above mentioned shortcomings of the project. All potential biocontrol agents need to be proven host-specific, so their release won't lead to non-target impact (van Klinken 2001). Internationally the laws governing the release of agents are becoming stricter and South Africa's regulatory bodies have with right become stricter over the years in allowing the release of biocontrol agents. The releases of biocontrol agents have therefore been slow. Since the year 2000, 4 biocontrol agents for lantana have been found suitably safe for release, but false positives produced by laboratory conditions has slowed the process.

Several of the agents released prior to 1995 have been established in the field for several years. This presented an opportunity to reassess their laboratory host-specificity and extended the plant species list to include closely related species regularly utilized by agents that have been studied since 1995, and compare the results with that realized in the field.

1.5 The 5 agents of this study

Five insect species were selected for laboratory trials of this study. The tingid, *T. scrupulosa*, and the hispid beetle, *U. girardi*, two of the most damaging insects established in South Africa, the leaf blotch mining agromyzid fly, *C. lantanae,* the mirid, *F. intermedia* and the most recently released, herringbone leaf-mining agromyzid*, O. camarae*.

Four of these insects, *T. scrupulosa, U. girardi, F. intermedia* and *O. camarae* reach high population numbers at sites in the field, and two species, the tingid, and blotch-mining agromyzid, are widespread and will therefore aid the study. There are two damaging hispine species established in South Africa, and only one, *U. girardi*, was selected. Out of the three Agromyzid flies established on lantana in South Africa, *C. lantanae* was selected, as it is the most widespread and *O. camarae* as it is considered very damaging and recent host specificity tests conducted included native *Lippia* species. *Falconia intermedia* was also recently introduced into South Africa, and its laboratory host specificity trials therefore also included *Lippia* species. A short review of each agent follows.

1.5.1 Teleonemia scrupulosa Stål (Hemiptera: Tingidae)

Teleonemia scrupulosa was one of the first natural enemies deliberately introduced against lantana in Hawaii in 1902 (Perkins & Swezey 1924), into Australia in 1936 and South Africa in 1961 (Oosthuizen 1964). Subsequent releases in other countries, mostly lead to establishment (Greathead 1968; Haseler 1963; Löyttyniemi 1982; Muniappan & Viraktamath 1986; Waterhouse & Norris 1987), of which at least 25 countries are confirmed (Julien & Griffiths 1998).

Both Australia and South Africa sourced adults and nymphs from Hawaii, where it was introduced. The tingids were reported to show preferences for certain lantana varieties (Haseler 1963; Harley & Kassulke 1971; Radunz 1971; Harley *et al.* 1979; Cilliers 1987b; Cilliers & Neser 1991), and new genetic material was imported into both countries from Brazil, Honduras and Trinidad (Harley & Kassulke 1971; Neser & Cilliers 1990). About 6 000 insects were imported and released along the KwaZulu-Natal (KZN) coast in South Africa. The tingids established rapidly, and over 22 000 insects were later recollected and distributed to nearby and inland sites (Oosthuizen 1964). In the 1970's, *T. scrupulosa* was found to be well established (Neser & Annecke 1973) and had spread more than 400 km south, to East London in the Eastern Cape (EC) (Cilliers 1977). The insect was also redistributed to the northern distribution range of lantana (Neser &

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Annecke 1973; Cilliers 1977), where it established. By the early 1980s, *T. scrupulosa* was established throughout South Africa (Cilliers 1983).

Seasonally, populations are low during winter, but increase rapidly in warmer months to build up to very high numbers end summer (Willson 1968; Harley *et al.* 1979; Cilliers 1987a). Field populations of *T. scrupulosa* seem to prefer sunny exposed conditions, and populations decrease in very humid conditions (Harley 1974; Muniappan *et al.* 1996). A few generalist predators have been recorded from field populations of *T. scrupulosa* (Cilliers 1987a).

The damage caused by *T. scrupulosa* feeding is considered as severe, and cause periodic defoliation, and in cases branches to die back (Haseler 1963; Oosthuizen 1964; Haseler 1966; Greathead 1968; Harley & Kassulke 1971; Neser & Annecke 1973; Winder & Harley 1983; Muniappan & Viraktamath 1986; Cilliers 1987a; Baars & Neser 1999). *Teleonemia scrupulosa* damage indirectly reduces plant growth and flowering intensity (Oosthuizen 1964; Harley *et al.* 1979; Cilliers 1987b; Muniappan *et al.* 1996). The impact is however insufficient, as plants are able to compensate when growing conditions are favourable (Harley *et al.* 1979; Muniappan & Viraktamath 1986), and the reduction in seed production is insufficient to reduce plant regeneration (Harley *et al.* 1979). *Teleonemia scrupulosa* is currently considered the most effective biocontrol agent of lantana in the field in South Africa, despite its limitations (Baars & Neser 1999; Baars & Heystek 2003a).

There are a few documented cases where *T. scrupulosa* fed and bred on non-target species in the field in several countries. It was recorded feeding and breeding on *Sesamum indicum* Linn., and feeding on *Sesamum angustifolium* Oliv. in the field in Uganda (Davies & Greathead 1967). In India in host range tests, it was able to reproduce on *Tectona grandis* Linn. (Davies & Greathead 1967). In Hawaii, it was recorded attacking *Myoporum sandwicensis* (naio), and species of *Xanthium*, both native to Hawaii (Davies & Greathead 1967; Hight *et al*. 2003).

1.5.2 Uroplata girardi Pic (Coleoptera: Chrysomelidae)

Octotoma scabripennis was the first leaf-mining beetle introduced for biocontrol of lantana in South Africa in 1971, some three years later *U. girardi* was introduced, originally from Brazil, but was obtained via Hawaii (Cilliers & Neser 1991). Releases of beetles were made in the areas most affected by lantana in South Africa, with more than 49 000 insects released by 1981 (Cilliers 1983). It established only in the warm, moist regions, especially along the KZN coast in South Africa (Cilliers 1983; Cilliers 1987b). *Uroplata girardi* beetles were re-imported in 1983 from cooler regions in Argentina, to be better climatically suited to the inland conditions. These were released around Tzaneen in the Limpopo Province, and reportedly established (Cilliers & Neser 1991).

Uroplata girardi is presently well established in the warm, moist eastern range of lantana, where it is widespread in KZN but relatively sporadic in the Mpumalanga and Limpopo provinces (Baars & Neser 1999). It is uncertain if the genetic stock from Argentina influenced the present inland distribution range of *U. girardi*. This species is widely utilized as a biocontrol agent (Krauss 1962; Willson 1968; Scheibelreiter 1980; Löyttyiemi 1982; Muniappan & Viraktamath 1986; Waterhouse & Norris 1987; Denton *et al.* 1991; Swarbrick 1995; Muniappan *et al.* 1996), and is presently established in 22 countries worldwide (Julien & Griffiths 1998).

Along the east-coast of South Africa, *U. girardi* is the most abundant agent, where feeding causes excessive leaf loss, particularly by mid summer (Cilliers 1987a). Leaf damage causes a reduction in plant growth (Willson 1968; Cillers 1987b; Swarbrick *et al.* 1995) and a 38% reduction in berry production has been measured in Guam (Muniappan *et al.* 1996). In Australia significant control has also been reported (Swarbrick *et al.* 1995) and on five islands in Micronesia (Denton *et al.* 1991).

Adults are subject to facultative diapause and are relatively inactive during the winter months (Harley 1968). Populations build up slowly over the summer months to peak March and April in South Africa (Cilliers 1987a; b). The slow build-up of insects allows for damaged plants to recover (Baars & Neser 1999). Baars and Heystek (2003a) reported

the beetles to be abundant and damaging along the KZN coast, with no notable differences in adult abundance or mine densities on different lantana varieties.

Only one parasitoid, *Dicladocerus* sp. (Eulophidae), has been recorded from larval mines of *U. girardi* in Brazil (Krauss 1964), but additional species are likely, given that 11 species were reared from a related species, *U. lantanae* Buzzi & Winder, also in Brazil (Winder *et al.* 1984). In South Africa an unidentified eulophid species was recorded parasitising *U. girardi* (Cilliers 1987a).

1.5.3 Calycomyza lantanae (Frick) (Diptera: Agromyzidae)
The blotch leaf-mining fly oviposits on leaves of actively-growing plants, larvae hatch and tunnel between the leaf epidermises (Baars & Neser 1999). It was introduced into South Africa in 1982 (Baars & Neser 1999). The genetic stock originated from Trinidad and was imported via Australia (Harley & Kussulke 1974). Releases were made at Margate, along the KZN coast, where it established rapidly, and also in inland subtropical and temperate areas, but established only at Nelspruit in the Mpumalanga Province (Neser & Cilliers 1990; Cilliers & Neser 1991). By 1999, the fly occurred widely in South Africa (Baars & Neser 1999). The fly is now widely established, including in areas where no releases were made, the Eastern and Western Cape provinces, and assumed to have spread throughout the range of lantana in South Africa (Baars & Heystek 2003a). Establishment and spread have been equally as successful from introductions made in other countries (Ooi 1987; Swarbrick *et al.* 1995; Muniappan *et al.* 1996; Esguerra *et al.* 1997).

Swarbrick *et al.* (1995) reported that it causes minor to partial control in Australia. Neser and Cilliers (1990) reported that mines are more abundant during late summer, when fly populations have built up. The populations are reduced by high levels of parasitism in South Africa (Hill & Hulley 1995; Baars & Neser 1999), and Cilliers and Neser (1991) reported a preference for pink-flowering lantana varieties. From a survey by Baars and Heystek (2003a) fly numbers were equally distributed among different varieties, and

assumed the majority of varieties as suitable hosts. Population levels remained low country wide, and its impact therefore considered negligible (Baars & Heystek 2003a).

1.5.4 Falconia intermedia (Distant) (Hemiptera: Miridae)
The lantana mirid, a leaf sucking bug, was imported into South Africa in 1994 from Jamaica (Baars 2000a; b). This culture was subjected to rigorous host range testing and some feeding and development were recorded on indigenous species, belonging to the genus *Lippia*. It was still considered suitably specific for release into Africa, and permission for its release was granted in 1999 (Baars 2000a; b).

Releases were made from autumn 1999, throughout lantana's invasive range in South Africa. To date, more than 20 million mirids have been released throughout the Limpopo Province, Mpumalanga, Gauteng, KwaZulu-Natal, North-West, Eeastern Cape, and Western Cape provinces. It was mass reared and released by the Plant Protection Research Institute (PPRI), and mass rearing stations of the *Working for Water Programme* (Heystek & Olckers 2003). Initial releases of 1000 insects per site resulted in successful establishment at few sites, but many failed, subsequent larger releases at the same sites, however, resulted in successful establishment. Previous failures at these sites were ascribed to predation, or to population densities too low to notice (Heystek & Olckers 2003). Releases of 5 000-10 000 insects per site were advocated, but far greater numbers, generally in excess of 100 000, were released at sites.

It established at sites in the warmer, sub tropical regions of the weeds range, where populations built up rapidly, and severe chlorosis and leaf drop followed (Baars 2000a; b; Heystek & Olckers 2003). The mirid populations crash after defoliation, and build up after re-growth. Severe damage to seedlings was also observed. Parasitism was undetected, but predation by ants and spiders occurred (Heystek & Olckers 2003). Spillover onto adjacent *Lippia* species was observed when high population densities were achieved on lantana. At the maximum population density reached, a mean number of 20 eggs per leaf were measured and a leaf damage rating of 9, on a 0-12 scale, were

obtained. However, the minimum damage to leaves at the same sites was less than 0.5 on this scale at times. The impact at established sites indicated 40% to 100% reduction in fruit produced. However, this was limited to the climatically suitable areas and to site conditions (Heystek & Olckers 2003). It was released in Australia in 2000, but failed to establish at most sites by 2002, except in Southern Queensland, where it established on all varieties, except "the common pink" (Day *et al*. 2003).

1.5.5 Ophiomya camarae Spencer (Diptera: Agromyzidae)

This herringbone leaf-miner was imported into quarantine at PPRI, Pretoria for host specificity screening in 1997, from Florida (USA). It is widespread in Central and South Americas (Simelane 2002). The females oviposit under the leaf, where the eggs hatch, and the larvae tunnel along veins and into the midrib. The tunnelling eventually resembles a herringbone, disrupts translocation and leads to leaf abscission. *Ophiomya camarae* was found highly specific to *L. camara* and was released in November 2002 (Simelane 2002). By April 2002 an estimated 14 500 flies were released at 20 sites in 5 provinces (Simelane & Phenye 2004). Initially, most established, but the flies failed to persist at sites where plants became deciduous during cold or dry winter conditions. The fly is currently well established and highly damaging at sites below 900m altitude (Simelane & Phenye 2004). Simelane and Phenye (2004) also reported that the fly had established on all colour varieties of *L. camara*, but noted that higher populations were noted on pink rather than orange plants, in a mixed stand. The fly is regarded as almost as damaging as *T. scrupulosa*, showing a reduction in plant growth and flowering (Simelane & Phenye 2004). Parasitism is very low, even though it was expected to accumulate a very high rate of parasitism (Urban & Phenye 2005). It has not yet been released into other countries as a biocontrol agent for lantana, but Australia is currently investigating this possibility.

1.6 Aims of this study

The biocontrol project on *L. camara* has not achieved sufficient control of the weed and needs to improve to be an adequate alternative method of control. Fourteen natural enemies are established on the weed in South Africa, but their impact is limited amongst

others by climatic conditions, the variability of the weed and parasitism. Climate is widely accepted as the greatest limiting factor, especially to the largely leaf feeding insects in areas where plants become deciduous.

There is debate about the role of the lantana varieties in the establishment, population levels achieved and limitations on the distribution of agents. In Chapter 2, varieties of *L. camara* are presented to field-established agents in the laboratory, and performance and preference differences measured. In Chapter 4, field assessments are made to investigate varietal suitability which is noted from the field population levels of each of these agents and then compared to the results from laboratory based trials presented in Chapter 2.

To improve the level of control achieved, additional biocontrol agents are needed. Host specificity studies in the laboratory therefore need to be done before these are released into the country. Laboratory host specificity trials are considered conservative, and agents may show a wider host range under laboratory conditions than in the field. Chapter 3 investigates the laboratory host ranges of established lantana agents, most of which were previously not tested against several indigenous Verbenaceae. In Chapter 5, field assessments are conducted to determine the field non-target effects of each of these agents and the differences in the laboratory and field host ranges are discussed.

Chapter 6 provides a general discussion and conclusion about these factors, in which the future prospects for the biological control of *L. camara* in South Africa are discussed.

CHAPTER 2

Laboratory varietal performance and preference of *Falconia intermedia, Ophiomya camarae, Teleonemia scrupulosa, Calycomyza lantanae* and *Uroplata girardi*

2.1 Introduction

As previously mentioned in Chapter 1, several horticultural varieties of lantana, with differences in flower colour, hairiness of stems and leaves, thorniness, and chemical composition (Stirton 1977) have naturalized in South Africa. The ability of biocontrol candidates to persist on most or all of the *L. camara* varieties is considered to be important in successful establishment in a country of introduction, and for maintaining impact (Sands & Harley 1981; Winder *et al*. 1984; Neser & Cilliers 1990; Cilliers & Neser 1991; Palmer *et al*. 2000). These different varieties present a unique combination of characteristics that the biocontrol agents have to overcome, within the single weed species. Preference on a single variety may result in its eradication but one variety may then simply be replacing another.

Falconia intermedia has shown preferences for certain lantana varieties when African and Australian varieties were tested simultaneously. Up to 15 fold differences were recorded in offspring produced during laboratory no-choice performance trials (Urban & Simelane 1999; Urban *et al*. 2003). However, Baars (*et al*. 2003b) found that *F. intermedia* did not show variety performance differences, when four of the important South African varieties were tested. However, preferences were shown between varieties when the mirid was presented with a choice situation. In addition the herringbone miner, *Ophiomya camarae* did not show any varietal preferences when ten South African lantana varieties were tested (Simelane, unpublished data 2005). Furthermore, the inflorescence galling mite *Aceria lantanae* (Cook) (Acari: Eriophyidae) also showed variable performance on the Australian and South African *L. camara* varieties (Urban *et al*. 2001). In earlier biocontrol efforts varietal preferences were noted, where *T. scrupulosa* were reportedly persisting better on red flowering *L. camara* in Australia, and to a lesser extent on pink, white and orange varieties (Haseler 1966; Radunz 1971). Some pink varieties proved unsuitable to sustain a field population (Radunz 1971). In South

Africa on the other hand, *T. scrupulosa* was reported to feed on all the varieties, but lower populations were noted on orange lantanas (Cilliers 1987a). Cilliers and Neser (1991) reported the blotch leaf miner *C. lantanae* to have a preference for pink flowering lantana.

Recent studies showed that *Alogoasa extrema* Jacoby (Chrysomelidae: Alticinae) produced highest numbers of offspring on lantana variety 029 White pink and least on 010 Pink (variety descriptions: Table 2.1), which was only 13% as suitable (Williams 2003a). Although other varieties were suitable, the fact that the performance varied notably on the two pink varieties demonstrates the danger of referring to a flower colour as a variety.

This chapter describes the laboratory varietal preference trials conducted, using 3 agents on some of the important lantana varieties in South Africa. These results are combined with results from previous studies conducted on 2 agents using the same methods to assess the variability in the preference and performance of agents on lantana varieties.

2.2 Methods and Materials
The results included for *Falconia intermedia* and *Ophiomya camarae* are taken from published work (Baars 2000a; b) and unpublished data (Simelane, personal communication 2005) respectively. In addition to the data obtained for *F. intermedia* and *O. camarae,* trials were conducted on three additional species, namely *T. scrupulosa, C. lantanae* and *U. girardi*. All these biocontrol agents are leaf sucking or chewing species, and *T. scrupulosa* also feeds on the developing flower buds and mature florets. A similar method was used for all species and all laboratory experiments were conducted during the summer months at the PPRI facilities, Pretoria.

Plants were grown in large pots (11.5 *l*) under 50% shadenet, watered by an overhead irrigation system and fertilized, using (N:P:K) in 2:3:2/1:0:1/LAN (N), as required. Four of the most common *L. camara* varieties, listed in Table 2.1 and *Lippia wilmsii* were used for all the trials. The lantana varieties used included 017 Orange (017 O), 150 Orange

(150 O), 021 Light pink (021 P), 029 White pink (029 Wp), 012 Light pink (012 P). *Lippia wilmsii* was included as an experimental control, i.e.: as an indicator plant, whether the females were selecting oviposition sites, as this species should be less utilized by females for ovipositing. *Lantana camara* variety 012 P, was used in some trials, but later considered equivalent to 021 P, and therefore not cultivated in the PPRI nursery. Variety 012 P was thus unavailable to photograph, or describe for table 2.1. All test plants were selected with a large leaf surface area and present an equal leaf resource to the insect species, thereby reducing the possibility of bias during trials.

Table 2.1: Description of the *Lantana camara* reference varieties used in trials at PPRI.

Lantana camara 009 Light pink (009 LP)
(Sycamore turnoff, Past Waterval-onder, Mp. 25°35 13.7 S; 30°27 08.5 E)

Flower colour:
Closed: Light pink
Open: Yellow, dark yellow throat
Mature: Light pink, orange throat

Leaf:
Hairiness: Few

Stem
Hairiness: Med
Thorniness (old): Few
Thorniness (new): Med
Colour (new): Green
Growth shape: Shrub

Lantana camara 163 Light pink (163 LP)
(Coastal dune, N of Scotsburgh, KZN. 30°08 08.4 S; 30°49 39.7 E)

Flower colour:
Closed: Light pink
Open: Yellow, dark yellow throat
Mature: Light pink, orange throat

Leaf:
Hairiness: Few

Stem:
Hairiness: Med
Thorniness (old): Few
Thorniness (new): Med
Colour (new): Green
Growth shape: Shrub

Lantana camara 150 Orange (150 O)
(Along road Nr. La Merci, KZN. 29°38 45.9 S; 31°07 39.5 E)

Flower colour:
Closed: Orange-red
Open: Orange, yellow throat
Mature: Orange, orange throat

Leaf:
Hairiness: Few

Stem:
Hairiness: Med
Thorniness (old): Med
Thorniness (new): Very
Colour (new): Green and red
Growth shape: Shrub

Lantana camara 015 White-yellow (015 Wy)
(Kiepersol rd. close to Hazyview, Mp. 25°02 21.6 S; 31°02 19.8 E)

Flower colour:
Closed: Cream
Open: White, yellow throat
Mature: White, white throat

Leaf:
Hairiness: Few

Stem:
Hairiness: Few
Thorniness (old): Med
Thorniness (new): Med
Colour (new): Green
Growth shape: Shrub

Lantana camara 017 Orange (017 O)
(Main rd. 24 Km E. of Sabie, Mp. 25°03 17.1 S; 30°57 03.6 E)

Flower colour:
Closed: Dark pink
Open: Yellow, dark yellow throat
Mature: Orange-red, orange throat

Leaf:
Hairiness: Med

Stem:
Hairiness: Very
Thorniness (old): Few
Thorniness (new): Med
Colour (new): Green
Growth shape: Prostrate

Lantana camara 029 White pink (029 Wp)
(Rd. 6Km S of Hazyview, to Nelspruit, Mp. 25°08 10.6 S; 31°00 09.0 E)

Flower colour:
Closed: Light pink
Open: White, dark yellow throat
Mature: Light pink, yellow-pink throat

Leaf:
Hairiness: Few

Stem:
Hairiness: Med
Thorniness (old): Med
Thorniness (new): Med
Colour (new): Green
Growth shape: Shrub

Lantana camara 021 Total pink (021 P)
(Near main rd., next to Sabie river, 8km E. of Sabie, Mp. 24°59 30.2 S; 31°14 34.8 E)

Flower colour:
Closed: Light pink
Open: Pink edged white, yellow throat
Mature: Light pink, yellow throat

Leaf:
Hairiness: Few

Stem:
Hairiness: Few
Thorniness (old): Med
Thorniness (new): Very
Colour (new): Green
Growth shape: Shrub

Lantana camara 010 Dark Pink (010 Dp)
(Next to N4, 10 km E of Waterval boven, Mp. 25°37 08.3 S; 30°31 12.1 E)

Flower colour:
Closed: Light pink
Open: Pink, orange throat
Mature: Pink, orange-pink throat

Leaf:
Hairiness: Few

Stem:
Hairiness: Very
Thorniness (old): Very
Thorniness (new): Very
Colour (new): Green, little red
Growth shape: Shrub

Lantana camara 018 Dark pink (018 Dp)
(Next to rd. 5Km out Sabie to Long-Tom, Mp. 25°07 04.9 S; 30°45 39.2 E)

Flower colour:
Closed: Light pink
Open: White, dark yellow throat
Mature: Dark pink, pink throat

Leaf:
Hairiness: Med

Stem:
Hairiness: Med
Thorniness (old): Few
Thorniness (new): Few
Colour (new): Green
Growth shape: Shrub

Cultures of *T. scrupulosa* and *C. lantanae* were collected from the field in the surrounds of Pretoria, Gauteng, and kept on lantana varieties other than those exposed during the trials. *Uroplata girardi* adults were collected from the Amanzimtoti area in KwaZulu-Natal, and also cultured on lantana varieties other than those exposed during the trials. All the insects used in the experiments were mature and had comenced oviposition.

Both no-choice and multiple-choice trials were used in this study, and were employed to determine the performance and preference of the candidate under investigation. No-choice trials were conducted in quarantine glasshouse facilities, in which routine quarantine studies on the host range of potential candidate biocontrol agents are conducted. Isolation cages, measuring 0.55x 0.55x 0.9m were used to confine individuals onto potted plant species, but cages were still large enough to provide reasonable

movement. A fluctuating temperature regime was used in glasshouses, with temperatures ranging between 19°C and 30°C in a day and night cycle. Multiple-choice trials were conducted as 5x5 latin squares confined in 4x4x2m gauze covered walk in cages in an air flow-through fiberglass tunnel. Similar fluctuating temperatures were used, with minimum and maximum temperatures recorded in the tunnel ranging between 15°C and 32°C.

No-choice trials

Four of the five lantana varieties described earlier and a *Lippia* species were exposed to candidates during no-choice trials and a total of three replicates were conducted in glasshouse compartments. No-choice cages were assigned random positions to avoid the nearest neighbour potentially influencing the result of the performance trials. This was done to avoid volatile chemicals from a suitable variety stimulating insect candidates under trial to feed and oviposit on the variety they were confined to.

In no-choice trials ten male and ten female *T. scrupulosa* adults were confined to plants in each cage for a week. Adults were then recollected, recording their numbers and the plants were placed back into their respective cages. The progeny development were monitored every second day and the emerging adults were counted and removed. The numbers of progeny obtained was used as an indication of reproductive potential, and survival from first to fifth instar on the different plant species used, as a measure of reproductive success.

Trials on *C. lantanae* were conducted using 20 adults which were confined to plants for a week. Adults were then removed and the developing progeny was monitored. Developing mines were counted after 2 days, and the maturing larvae were later collected, by picking leaves in which the larvae have not yet dropped out to pupate. In addition, as a precaution, containers with damp filter paper were placed under the plant canopy to collect the larvae that had already emerged from leaves to pupate. Both methods were used on all cages, as excising the leaves may have influenced pupal survival, as larvae usually drop into the moist leaf litter found under plants in the field. Pupae collected in

the two ways were kept separate in the laboratory in plastic containers with gauze lids. Adults were collected every few days from the containers up to 24 days after the trial, at a point that all the pupae had either emerged or were noted to be desiccated.

In the no-choice trials on *U. girardi*, twenty adults were placed onto each of the plants for a week, and were then recovered. The number of eggs on each plant was recorded, and twenty days later mines were counted and emerging progeny collected. On two further occasions, each a fortnight later, the offspring were again collected to ensure that all the offspring were accounted for.

Multiple-choice trials
Multiple-choice trials were used to demonstrate adult preference for varieties, employed to simulate near-natural choice conditions. Each plant was measured for height, number of leaves and number of shoots off the main stem. In trials conducted on the tingid, *T. scrupulosa*, flowers were also counted. Each latin square was replicated twice. A total of 160 individuals were released for each of the candidate insects and were introduced from 16 vials that were equally spaced between plants on the cage floors.

Teleonemia scrupulosa adults were released into the cage for three days. After exposure plants were placed apart in the tunnel to prevent emerging nymphs to disperse between the varieties. After an initial one week period nymphs were counted every two days until the final instar. The final instar was removed from plants to reduce the risk of adults emerging and dispersing to nearby plants.

Calycomyza lantanae adults were released in each cage for a period of 5 days. After exposure the plants were removed from the cages, and placed in the fiberglass tunnel for the eggs to hatch and mines to develop. After ten days, the numbers of developing mines were counted.

Uroplata girardi adults were released and recollected after a week, recording the number of adults and eggs oviposited per plant exposed during the trial. The plants were removed

from the tunnel, and placed under a gauze-covered frame, and on tables to avoid predation by ants. One month later the number of mines were counted and after six weeks the mines were finally evaluated, and recorded as either emerged, containing larvae, pupae, or as dead (some larvae die at an early stage).

Data were analysed using the statistical program GenStat (2003). The experiments were designed as either random, or as 5x 5 latin squares that were repeated in two cages (once for *T. scrupulosa* multiple-choice trial). Differences between species and varieties were assessed using an analysis of variance (ANOVA). The data was acceptably normal with homogeneous treatment variances. The species and variety means were separated using Fishers' protected t-test least significant difference (LSD) at 1 and 5 % levels of significance (Snedecor & Cochran 1980), if the F- probability from the ANOVA was significant at 1 or 5% for the respective analyses.

2.3 Results

Falconia intermedia

Multiple-choice trials

The mirid showed significant varietal differences in feeding and oviposition (Table 2.2). The two *L. camara* varieties that were utilised most by *F. intermedia* during multiple-choice trials for both feeding and ovipositing were 150 Orange, and 163 Light pink, statistically significantly less were 018 Dark pink and 029 White pink (Table 2.2). It fed least and laid least eggs on the indigenous related *Li. wilmsii*.

Table 2.2 Feeding and oviposition preferences of *Falconia intermedia* adults in a multiple-choice trial involving 4 South African *Lantana camara* varieties and a related indigenous plant *Lippia wilmsii*. (adapted from Baars 2000a; b)

L. camara variety / test species	% Leaf damage	No. of eggs laid
018 Dark pink	20.3 a	457 b
029 White-pink	15.5 b	512 b
150 Orange	20.6 a	811 a
163 Light pink	21.5 a	939 a
Li. wilmsii	*9.9 c*	*206 c*
S.E.M.	*1.7*	*62*
LSD	*4.8*	*175*

S.E.M. = Standard error of mean; LSD = Least significant difference; means within a column followed by the same letter are not significantly different (P>0.05, Fisher's Protected LSD).

Ophiomya camarae
No-choice trials

Simelane and Phenye (Simelane, personal communication 2005) conducted varietal performance trials in no-choice cages. Ten of South Africa's prominent *L. camara* varieties, including dark- and light- pinks as well as oranges were exposed to adult flies. The F1 adult progeny were counted and compared, but no significant differences were found among any of the varieties tested.

Teleonemia scrupulosa
No-choice trials

The *T. scrupulosa* adult survival was similar on all the lantana varieties and *Li. wilmsii* (Table 2.3). The number of progeny developing subsequent to the adult exposure was significantly similar on all the lantana varieties and on *Li. wilmsii*.

Table 2.3 The mean number of *Teleonemia scrupulosa* adults recovered and the number of nymphs developing subsequent to exposure on the four *Lantana camara* varieties and on *Lippia wilmsii* during no-choice trials (n=3).

L. camara variety / test species	Adults recovered		Nymphs produced	
017 O	17.3 (± 1.33)	a	145.0 (± 54.51)	a
021 P	16.0 (± 1.53)	a	122.3 (± 47.67)	a
029 Wp	17.3 (± 0.67)	a	109.7 (± 41.48)	a
150 O	17.3 (± 1.76)	a	104.0 (± 41.24)	a
Li. wilmsii	16.0 (±1.53)	a	130.7 (± 51.27)	a
S.E.M.	1.414		3.32	
C.V.	14.6		32.0	
F	0.27		0.43	
F.p.	0.893		0.786	
LSD	--		--	

Means (± S.E.) in a column followed by the same letter do not differ significantly at the 1% level.

Multiple-choice trials

During multiple-choice trials, the numbers of *T. scrupulosa* adults recovered on each variety or test species was similar (Table 2.4) suggesting that the adults had no preference between plants tested. However, oviposition was most likely highest on on *L. camara* variety 029 White-pink, 017 Orange and 012 Pink, as the number of nymphs emerging was notably higher. The progeny numbers however did not differ significantly from those emerging from lantana variety 150 Orange, but were lower. Although the progeny emerging from *Li. wilmsii,* was not statistically significantly lower than that recorded on the 150 Orange and 029 White-pink lantana varieties, the numbers are still notably lower.

Table 2.4 The mean number of *Teleonemia scrupulosa* adults recovered, and subsequent emerging progeny on the different *Lantana camara* varieties and *Lippia wilmsii* exposed during a 5x5 latin square multiple-choice trial (n=5).

L. camara variety / test species	Adults recovered		Nymphs produced		F1 Adults produced	
012 P	5.8 (± 1.59)	a	486.0 (± 166.40)	ab	142.8 (± 48.70)	ab
017 O	7.4 (± 1.17)	a	498.4 (± 122.04)	ab	154.4 (± 39.07)	a
029 Wp	6.2 (± 1.74)	a	536.2 (± 117.22)	a	111.4 (± 32.74)	abc
150 O	3.2 (± 0.95)	a	207.6 (± 44.09)	bc	54.4 (± 10.15)	bc
Li. wilmsii	4.8 (± 1.59)	a	105.6 (± 26.67)	c	24.4 (± 7.75)	c
S.E.M.	1.076		101.7		30.9	
C.V.	43.9		62.0		70.8	
F	2.15		3.72		3.33	
F.p.	0.137		0.034		0.047	
LSD	---		313.2		95.1	

Means (± S.E.) in a column followed by the same letter do not differ significantly at the 5% level.

Calycomyza lantanae

No-choice trials

During all the no-choice trials the number of adults surviving was similar on the lantana varieties and the test species *Li. wilmsii* (Table 2.5). Significantly similar numbers of mines occurred on all the lantana varieties, while significantly fewer on *Li. wilmsii*. The number of progeny developing was on the whole very low and not statistically significantly different, but notably fewer adults emerged on *Li. wilmsii*. However the method of collection of the emerging pupae may have influenced the numbers obtained, and performance is probably best gauged from initial number of mines.

Table 2.5 The mean number of *Calycomyza lantanae* females recovered, and developing mines and F1 adults produced on different *Lantana camara* varieties and on *Lippia wilmsii* during no choice trials (n=3).

L. camara variety / test species	Females recovered		Mines produced		F1 Adults produced	
012 P	3.0 (± 2.52)	a	81.0 (± 14.57)	a	13.0 (± 8.74)	a
017 O	1.3 (± 0.67)	a	79.3 (± 18.32)	a	9.7 (± 4.67)	a
029 Wp	4.0 (± 1.53)	a	93.3 (± 8.19)	a	14.0 (± 2.65)	a
150 O	3.3 (± 1.86)	a	103.3 (±15.6)	a	14.3 (± 11.35)	a
Li. wilmsii	0.0 (± 0.00)	a	21.3 (± 6.89)	b	0.3 (± 0.33)	a
S.E.M.	1.585		13.46		6.84	
C.V.	117.6		30.8		115.4	
F	1.06		5.61		0.73	
F.p.	0.424		0.012		0.591	
LSD	---		42.43		---	

Means (± S.E.) in a column followed by the same letter do not differ significantly at the 5% level.

Multiple-choice trials

In choice trials the number of adults recovered on each plant suggests that there was a similar preference for the lantana varieties and the test species (Table 2.6). A significantly higher number of mines were recorded on the lantana varieties compared to the test species *Li. wilmsii*. Most larval mines were produced on lantana varieties 017 Orange, 029 White-pink and 012 Pink. Significantly fewer mines were recorded on 150 Orange than on 017 Orange, but not different from 029 White-pink, or 012 Pink. *Lippia wilmsii* produced least number of larval mines.

Table 2.6 The mean number of *Calycomyza lantanae* adults recovered, and mines produced on different *Lantana camara* varieties and *Lippia wilmsii* during two 5x5 latin square multiple-choice trials (n=10).

L. camara variety / test species	Adults recovered		Mines produced	
012 P	1.5 (± 0.45)	a	34.1 (± 5.14)	ab
017 O	1.0 (± 0.52)	a	54.6 (± 9.16)	a
029 Wp	1.7 (± 0.62)	a	48.5 (± 6.25)	ab
150 O	1.3 (± 0.42)	a	27.8 (± 4.66)	b
Li. wilmsii	0.5 (± 0.17)	a	0.4 (± 0.31)	c
S.E.M.	0.434		5.37	
C.V.	114.3		51.3	
F	1.17		2.05	
F.p.	0.346		< 0.001	
LSD	---		20.98	

Means (± S.E.) in a column followed by the same letter do not differ significantly at the 1% level.

Uroplata girardi

No-choice trials

During the no-choice trials *U. girardi* females survived and oviposited significantly similar numbers of eggs on all the lantana varieties and *Li. wilmsii* (Table 2.7). However, a fewer number of eggs hatched to produce larval mines on *Lippia wilmsii*. Fewer mines also developed on varieties 012 Light pink and 017 Orange when compared to 150 Orange and 029 White-pink. The number of adult progeny emergence was similar on the lantana varieties 029 White-pink, 012 Pink, and 150 Orange. Significantly fewer adult progeny were reared from lantana variety 017 Orange and the test species *Li. wilmsii*.

Table 2.7 The mean numbers of *Uroplata girardi* females recovered, eggs, mines and F1 adults produced on different *Lantana camara* varieties and *Lippia wilmsii* during no-choice trials (n=3).

L. camara variety / test species	Females recovered		Eggs laid		Mines produced		F1 Adults produced	
012 P	8.7 (± 0.88)	a	327.3 (±39.92)	a	125.7 (± 25.21)	bc	110.0 (± 13.61)	a
017 O	9.7 (± 0.33)	a	453.7 (±128.56)	a	173.0 (± 78.25)	abc	24.0 (± 2.65)	c
029 Wp	11.7 (± 0.33)	a	315.7 (± 38.60)	a	277.7 (± 37.00)	ab	117.7 (± 7.97)	a
150 O	13.7 (± 3.71)	a	554.3 (± 227.81)	a	288.0 (± 95.86)	a	72.0 (± 18.15)	b
Li. wilmsii	10.0 (± 4.62)	a	241.7 (± 108.90)	a	52.3 (± 19.63)	c	32.0 (± 8.50)	c
S.E.M.	2.69		129.1		59.5		11.47	
C.V.	43.4		59.1		56.2		27.9	
F	0.53		0.93		2.86		14.13	
F.p.	0.714		0.486		0.08		>0.001	
LSD	---		---		152.5		36.13	

Means (± S.E.) in a column followed by the same letter do not differ significantly at the 5% and 1% level.

Multiple-choice trials

During the multiple-choice trials *Uroplata girardi* adults were collected in higher numbers on 017 Orange, and 150 Orange lantana varieties compared to 029 White pink, 012 Light pink, and *Li. wilmsii* (Table 2.8). A similar number of eggs were oviposited on all the lantana varieties, and a significantly fewer number were recorded on *Li. wilmsii*. Egg hatch was relatively high with similar percentage mines developing on all the plants.

Table 2.8 The mean numbers of *Uroplata girardi* adults recovered, and resulting eggs oviposited and mines produced on different *Lantana camara* varieties and *Lippia wilmsii* exposed during two 5x5 latin square multiple-choice trials (n=10).

L. camara variety / test species	Adults recovered		Eggs laid		Mines produced	
012 P	7.0 (± 1.09)	b	176.0 (± 27.79)	a	77.3 (± 13.48)	bc
017 O	15.9 (± 3.03)	a	248.3 (± 35.01)	a	140.2 (± 18.65)	a
029 Wp	8.9 (± 2.12)	b	194.5 (± 32.82)	a	122.3 (± 18.45)	ab
150 O	9.9 (± 2.11)	ab	192.8 (± 31.10)	a	92.8 (± 15.21)	b
Li. wilmsii	5.4 (± 1.14)	b	66.2 (± 12.08)	b	32.6 (± 5.39)	c
S.E.M.	1.605		18.83		11.8	
C.V.	53.9		33.9		40.1	
F	6.26		12.62		12.53	
F.p.	< 0.001		< 0.001		< 0.001	
LSD	6.274		73.79		46.13	

Means (± S.E.) in a column followed by the same letter do not differ significantly at the 1% level.

2.4 Discussion and Conclusion

Baars (2000a; b) concluded that *F. intermedia* could perform equally well on the different varieties from results of no-choice and nymphal performance studies. He also argued that the mirids were likely to build up quicker on preferred varieties in a mixed stand of *L. camara,* from results obtained from the multiple-choice trials (Table 2.2). He further argued that the mirids were unlikely to be stimulated to disperse in search of the preferred varieties, as it was more likely that the motivational threshold that stimulates the mirid to feed and oviposit would be stronger than that to disperse.

Similarly, Simelane and Phenye (Simelane, personal communication 2005) found that *O. camarae* during no-choice varietal studies the females oviposited equally on the varieties tested, and that, equal numbers of offspring were produced on the different varieties.

Unfortunately however, no multiple-choice trials were conducted to determine whether the adults discriminate between the lantana varieties during feeding and oviposition.

During this study *T. scrupulosa,* during no-choice trials showed no differences in performance between the lantana varieties tested. However, exposed to a choice condition adults preferred two pink varieties and an orange variety, with notably fewer nymphs emerging on another orange variety. The control plant included in the choice arena was also selected as a potential host and the number of progeny recorded was similar to that on the least preferred lantana variety. This biocontrol agent was released into South Africa in 1961, (Anon 1999; Table 1.1), before host testing was routinely conducted on indigenous Verbenaceae. The current results suggest that *Lippia* species may be suitable hosts and would probably preclude its release into South Africa under the current guidelines. Ironically, *T. scrupulosa* is considered to be the most successful biocontrol agent established on lantana in South Africa (Baars & Neser 1999; Baars & Heystek 2003a; Simelane & Phenye 2004) and is also considered to be very valuable species worldwide for the biocontrol of lantana (Haseler 1963; Oosthuizen 1964; Greathead 1968; Haseler 1966; Harley & Kassulke 1971; Neser & Annecke 1973; Winder & Harley 1983; Muniappan & Viraktamath 1986). This could partly be due to little discrimination against certain lantana varieties. It was however reported to show preferences for certain lantana varieties (Haseler 1963; Harley & Kassulke 1971; Radunz 1971; Harley *et al.* 1979; Cilliers 1987b; Cilliers & Neser 1991). These trials have demonstrated that discrimination against lantana varieties are not solely linked to flower colour, as has been implied in the literature.

Calycomyza lantanae showed no differences in mine production on the different varieties tested in no-choice trials, while a significant reduction on the non-target plant was notable. The trial results reflected few F1 adults from the mines produced. This was however an artifact of the experimental design, as larvae that are ready to pupate, drop out of the leaves and pupate in the soil. The cage floors are less suitable and attempts were therefore made to collect the pupae. Few were recovered, and the results reflect a portion of the offspring. The same collection methods (2.2 *Methods and Materials*) were

used for all the plants, but the figures were still too low to show reliably the correct relationships obtained.

During multiple-choice trials, *C. lantanae* adults preferred three varieties. Here too, one orange was preferred to another, as in *T. scrupulosa* experiments. In both cases, variety 017 Orange was preferred to 150 Orange. Both of these varieties's leaves are sparsely haired (Table 2.1). This indicates that there are other factors, not necessarily obviously physical, that the insects use to discriminate between hosts. It is also interesting to note that most of the original lantanas in the countries of origin have orange flowers. This further suggests that secondary compounds in the plants may have a much greater role than flower colour or leaf hairiness.

During no-choice trials more *U. girardi* mines developed on varieties 150 Orange, 029 White-pink and 017 Orange. This time a pink variety, 012 Pink, produced significantly less mines. Less F1 adults were produced on variety 017 Orange, for the number of mines it produced. This indicates poor larval development on this variety that was one of the better varieties in general for the other insect species previously discussed. This could be an indication of insect specific deterrents that some varieties have evolved. This stresses the importance of using a suite of biocontrol agents against a weed as complex as *L. camara*.

Consistent with the other insect species, *U. girardi* produced most mines on variety 017 Orange in multiple-choice trials. The other well performing varieties were 029 White-pink, followed by 150 Orange. The worst performing was a pink (012 Light pink). The F1 generation of adults of *U. girardi* was unfortunately not recorded for this trial, and larval development to adulthood on variety 017 Orange was poor during no-choice trials. The mines produced on the varieties were relatively consistent with the no-choice trial, except for 150 Orange that performed better during multiple-choice trials. The trials indicated a high degree of specificity for *U. girardi*, ovipositing very little on *Li. wilmsii*.

The plants used in the trials all were grown under ideal conditions of shade, protected from cold, irrigated, fertilized and kept free of pests and competition. If grown in the field under varying conditions, the environmental factors may alter the preference rank insects exert on the *L. camara* varieties (Waddell & Mousseau 1996). Heshula (2005) found growth conditions to alter palatability of lantana varieties to *F. intermedia*.

The variety that was consistently preferred for oviposition during multiple choice trials was 017 Orange, where 150 Orange were much less preferred. One of the characters of variety 017 Orange is that it is more prostrate growing (Table 2.1). It is also slower growing in the nursery (observations, not measured). This variety is also rated to have few spines on its stem. Most of these characters resemble more "original" plants from the countries of origin that may have been parents to our current weedy forms. These may support arguments of Day and Neser (2000) that the biocontrol agents' lack of performance in introduced countries may indeed be strongly linked to variety differences. It also confirms lantana biocontrol agents' preferences for varieties, but not to flower colour alone.

CHAPTER 3

Laboratory host ranges of *Falconia intermedia, Ophiomya camarae, Teleonemia scrupulosa, Calycomyza lantanae* and *Uroplata girardi*

3.1 Introduction

Teleonemia scrupulosa, is considered to be one of the most effective biocontrol agents of lantana (Baars & Neser 1999; Baars & Heystek 2003a). It was introduced into South Africa in 1961, after host specificity trials conducted in Australia. The pre-release trials conducted in South Africa did not include many indigenous South African plants related to lantana (Cilliers & Neser 1991), a pre-requisite for any biocontrol candidate currently under investigation. Similarly, *U. girardi* and *C. lantanae* were released under the same circumstances*,* in 1974 and 1982 respectively after they were introduced via Australia. *Falconia intermedia* and *O. camarae* were released into South Africa in 1999, and 2000 respectively (Baars 2002a; Simelane 2002; Heystek & Olckers 2003). Both of these recent introductions involved rigorous host range tests to include several indigenous Verbenaceae. Although the laboratory trials indicate that these species have a narrow host range, these two species were both able to reproduce on some closely related indigenous species (Baars 2002a; Simelane 2002).

Several candidate biocontrol agents of *L. camara,* including *F. intermedia* have been recorded feeding on *Lippia* species in their country of origin (Harley & Kassulke 1971; Palmer *et al*. 1996). *Teleonemia scrupulosa* has been recorded feeding on non-target species worldwide (Davies & Greathead 1967; Hight *et al*. 2003; Chapter 1).

Few African Verbenaceae were used in laboratory host range tests of the species released prior to 1995 (Cilliers 1983, Cilliers & Neser 1991) and several of these agents have been established in the field for decades. This provided the opportunity to study their host ranges in trials, as conducted now in the laboratory in South Africa, and can provide information about the difference in laboratory and realised host ranges of lantana agents.

Laboratory based host specificity testing is conservative, due to the limitations the laboratory trials place on the insects normal host search and selecting behaviour (van Klinken 2001). Several candidate agents tested in quarantine also showed an ability to feed and reproduce on *Lippia* species in the laboratory (Baars & Neser 1999). Examples of rejected agents include *A. compressa, A. extrema*, and *B. parvisacata* (Heystek & Baars 2001; Williams 2003a; Phenye & Simelane 2003). Furthermore, *Falconia intermedia*, also reproduced on *Lippia* species in the laboratory, but were still released on the basis that the rate of reproductive success on *Lippia* species was significantly less than on lantana (Baars 2000a; b). Currently, applications for permission to release three agents on lantana have been submitted. Two of these, *Leptostales ignifera* Warren (Lepidoptera: Geometridae) (H.E. Williams, personal communication 2005), and *Coelocephalapion camarae* Kissinger (Coleoptera: Brentidae) (Baars & Heystek 2001; 2003b; Baars *et al*. 2003a) utilize *Lippia* species as alternative host plants in the laboratory to varying degrees. However, these were deemed safe for release due to the significant difference in development of these species on *L. camara*, compared to *Lippia* species (less than 20% as well as on lantana).

Assessing the extent to which the already released agents utilize the closely related plant species, particularly the indigenous *Lippia* species in the laboratory and in the field may establish the importance of this apparent host-range extension occurring for the majority of the candidate agents. This will aid in the decision making process, when new candidates are considered for release into South Africa, or other countries, and reduce the unnecessary rejection of potentially host specific agents. In this chapter the laboratory host ranges of five of these agents are described, and then compared to field assessments reported in Chapter 4.

3.2 Methods and Materials

The results of *F. intermedia* and *O. camarae* laboratory host specificity trials are extracted from published work of Baars (2000a; b; Baars *et al*. 2003b), and Simelane (2002). Trials on these species used similar methods as has been used in this study to

obtain the extent of the laboratory host range. In each case the results are summarized in the results section for ease of comparative purposes.

Specificity trials were conducted experimentally for the other insect species. Experiments were conducted during summer months at the PPRI facilities, Pretoria, using the same facilities and conditions as described for the variety performance and preference studies in Chapter 2. Test plant species that are closely related to *L. camara*, within Verbenaceae, were selected, including ones that are regularly accepted by other candidate biocontrol agents in quarantine host specificity trials. The species tested include *Lantana montevidensis, Lantana trifolia, Lippia javanica, Lippia rehmanii*, *Lippia wilmsii*, *Lippia scaberrima*, *Lippia* sp. A, *Lippia* sp. B (un described species) and *Priva meyeri*. One *L. camara* variety (Ref no. 017 Orange, (Chapter 2)) was used for all the trials (unknown varieties were used in *F. intermedia* and *O. camarae* trials). All test plants were selected to have a large leaf surface area and were of the same approximate area, to present an equal resource to these leaf-utilizing insect species.

Three replicates of no-choice trials (two for *U. girardi*) were conducted in one or two glasshouse compartments per insect species tested. Each replicate included the above five plant species, isolated into cages that were assigned to random positions in the glasshouse to reduce any effect volatiles of a neighboring plant may have had. The same numbers of insects, exposure periods and measurements were used as in the variety no-choice studies (Chapter 2).

Multiple-choice trials were also used to assess the extent of the host range of these insects in a choice condition, employed as a closer representation of a natural condition usually encountered in the field. Each plant was measured and trials conducted as described for the multiple-choice latin square trials in Chapter 2. Two latin square experiments were conducted for each of *T. scrupulosa, C. lantanae* and *U. girardi*.

Data was analysed using the statistical program GenStat (2003). The experiments were designed as either random, or as 5x 5 latin squares. Differences between species were

tested for in an analysis of variance (ANOVA). The data was acceptably normal with homogeneous treatment variances. Species means were separated using Fishers' protected t-test least significant difference (LSD) at 1, 5 and 10% levels of significance (Snedecor & Cochran, 1980), if the F- probability from the ANOVA was significant at 1, 5, or 10% for respective analyses. The means for mines produced by *C. lantanae* multiple-choice trials were log transformed to reduce variances.

3.3 Results

Falconia intermedia

No-choice trials

Under no-choice conditions the mirid oviposited the highest number of eggs on *Lantana camara*, but numerous eggs were also laid on *Li. scaberrima*, *Lippia* species A and B, *Li. javanica*, *Li. rehmanii* and *Li. wilmsii*, all listed in decreasing suitability, with least eggs laid on *Lantana trifolia* and *Priva meyeri* (Table 3.1). Oviposition on several *Lippia* species was still considered unacceptably high, and relatively similar to that on *L. camara*. Several more species were tested, but no oviposition was recorded (Baars 2002 a; b), and these are not listed.

Table 3.1 Oviposition of 20 *Falconia intermedia* adults, exposed to *Lantana camara* and related plants in no-choice trials for 48 days (adapted from Baars 2002 a; Baars *et al.* 2003b).

Plant species	n	Mean (± S.E.) no of eggs produced
L. camara	6	1698.8 ± 30.0 a
L. trifolia	3	9.8 ± 33.6 e
Li. javanica	3	1028.0 ± 41.8 cd
Li. rehmannii	3	978.8 ± 33.6 d
Li. scaberrima	3	1321.8 ± 33.6 b
Li. wilmsii	3	944.1 ± 33.6 d
Lippia sp.A	3	1122.8 ± 33.6 c
Lippia sp.B	3	1107.1 ± 33.6 c
P. meyeri	3	73.8 ± 33.6 e

Means (± S.E.) within a column followed by the same letter are not significantly different (P> 0.05, ANOVA, unbalanced design).

Multiple-choice trials

During multiple-choice trials, the mirids oviposited significantly more eggs on *L. camara* (Table 3.2). The species with the next highest number of eggs was *Lippia* sp. B, which yielded about 24% as many eggs as *L. camara*. In the choice condition the other *Lippia* species were not selected as potential hosts, with least eggs deposited on *Li. rehmanii* (approximately 2% of that on *L. camara*) and 0.3% on *Lantana trifolia*. *Lantana montevidensis* and *Duranta erecta* were not suitable for oviposition during choice trials.

Table 3.2 Oviposition preference of *Falconia intermedia* adults exposed to *Lantana camara* and related test plants in multiple-choice trials (adapted from Baars 2002a; Baars *et al*. 2003b).

Plant species *	n	Mean no of eggs deposited	
Lantana camara	9	870.4	a
L. montevidensis[*a]	9	0.0	
L. trifolia	9	2.5	e
Li. javanica	6	21.3	d
Li. rehmannii	8	15.6	d
Li. scaberrima	9	7.3	e
Li. wilmsii	3	48.4	cd
Lippia sp.A	3	92.9	bc
Lippia sp.B	3	208.4	b
P. meyeri	8	4.8	e
D. erecta[*a]	9	0.0	

* Test plants not included in statistical analysis because of zero values.

Means within a column followed by the same letter are not significantly different ($P > 0.05$, ANOVA, unbalanced design with log transformed data).

Ophiomya camarae

No-choice trials

Significantly more leaves developed larval mines on *L. camara* than on any of the test species. Eleven to15 times more adults were yielded from *L. camara* than on the best performing related indigenous *Lippia* species (Table 3.3).

Table 3.3 Plant species accepted for oviposition by *Ophiomya camarae*, and larval development to adulthood in no-choice trials (n=4) (adapted from Simelane 2002).

Plant species	Mean no. of larvae (± S.E.)	Mean no. of adults (± S.E.)
Lantana camara	131.5 ± 21.5	84.5 ± 18.60
L. trifolia	1.0 ± 1.0	0.6 ± 0.60
Lippia javanica	32.8 ± 9.5	4.0 ± 1.60
Li. rehmanii	0.8 ± 0.8	0.0 ± 0.00
Lippia sp. A	23.5 ± 15.4	7.6 ± 5.95
Lippia sp. B	15.8 ± 4.7	8.3 ± 2.90
Li. wilmsii	20.8 ± 6.1	5.5 ± 1.70

Multiple-choice trials

Ophiomya camarae oviposition led to the highest number of developing larvae on *L. camara*. After *L. camara*, *Lippia javanica* yielded the most mines and subsequent progeny but was only 16%, and 6% of the larvae and adult progeny on *L. camara* (Table 3.4).

Table 3.4 Host selection of *Ophiomya camarae* adults as determined by larval mining and subsequent adult emergence during paired-choice tests involving eight females (n=4) (Simelane 2002).

Plant spp. test pairs	Mean (± S.E.) no of leaves mined			Mean (± S.E.) F1 adult emergence		
L. camara	63.00	± 7.07	a	46.00	± 5.80	a
L. camara	59.25	± 8.82	a	55.75	± 6.24	a
L. camara	129.00	± 19.59	a	82.80	± 9.75	a
Li. javanica	20.40	± 13.01	b	5.00	± 3.26	b
L. camara	113.40	± 10.31	a	79.20	± 7.19	a
Li. wilmsii	16.60	± 11.30	b	2.00	± 1.76	b
L. camara	156.00	± 5.05	a	93.20	± 4.53	a
Lippia sp. A	4.20	± 1.91	b	1.20	± 0.73	b
L. camara	148.20	± 4.55	a	88.40	± 4.50	a
Lippia sp. B	7.20	± 2.73	b	1.00	± 0.45	b

Means compared by t-tests; those followed by the same letter are not significantly different (p> 0.05).

Teleonemia scrupulosa
No-choice trials

Adult survival was not significantly different between *L. camara, Lippia* species, or *Priva meyeri* (Table 3.5). It was however lower for *L. montevidensis*. The most number of nymphs were produced on *Li. rehmanii*, significantly fewer nymphs were recorded on *Li. javanica*, *L. camara* and *P. meyeri*. The least number of nymphs emerged on *L. montevidensis*, but not significantly lower than on *L. camara*, or *P. meyeri*.

Table 3.5 Mean number of *Teleonemia scrupulosa* adults recovered and nymphs produced on *Lantana camara* and different related test species during no-choice trials (n=3).

Species	Adults recovered		Nymphs produced	
L. camara	17.0 (± 1.73)	a	137.7 (± 14.4)	bc
L. montevidensis	2.0 (± 1.53)	b	23.7 (± 15.56)	c
Li. rehmanii	16.0 (± 2.08)	a	414.0 (± 63.52)	a
Li. javanica	15.7 (± 2.85)	a	167.3 (± 40.65)	b
P. meyeri	12.3 (± 2.67)	a	68.3 (± 31.38)	bc
S.E.M.	2.231		65.4	
C.V.	30.7		40.3	
F	7.67		<0.001	
F.p.	0.004		16.16	
LSD	7.030		118.9	

Means (± S.E.) in a column followed by the same letter do not differ significantly at the 5% and 1% level.

Multiple-choice trials

At the conclusion of multiple-choice trial 1, the highest numbers of *T. scrupulosa* adults were recovered from *L. camara,* and *Li. wilmsii*. A fewer number of adults were collected from *Li. rehmanii* and *P. meyeri*, and none were found on *L. montevidensis* (Table 3.6). In the second replicate, similar patterns were observed, but *Li. javanica* as apposed to *Li. wilmsii* was tested, and replaced it in order of preference. In replicate 1, no significant differences were shown in the number of nymphs produced, but the largest numbers of nymphs were recorded from *L. camara*. A large number of nymphs were recorded on *P. meyeri*, and only 21% of that occurring on lantana was recorded on the *Lippia* species. In the second replicate, the numbers of nymphs recovered were very low with the *Lippia* species and *L. camara* yielding very few nymphs.

Table 3.6 Mean number of *Teleonemia scrupulosa* adults recovered and nymphs produced on *Lantana camara* and different related test species during two different 5x5 latin square multiple-choice trials in walk in cages (cage 1: n=5, cage 2: n=5).

Species	Adults recovered, cage 1		Nymph progeny, cage 1		Adults recovered, cage 2		Nymph progeny, cage 2	
L. camara	14.0 (± 2.76)	a	56.6 (± 28.44)	a	9.0 (± 1.84)	a	17.6 (± 9.93)	a
L. montevidensis	0.0 (± 0.00)	c	0.0 (± 0.00)	a	0.0 (± 0.00)	b	0.0 (± 0.00)	a
Li. rehmanii	5.6 (± 0.40)	bc	16.2 (± 7.21)	a	0.8 (± 0.49)	b	16.2 (± 7.32)	a
Li. wilmsii*	8.2 (± 2.71)	ab	11.8 (± 5.09)	a	*--		*--	
Li. javanica*	*--		*--		6.4 (± 2.16)	ab	8.4 (± 5.49)	a
P. meyeri	6.0 (± 1.48)	bc	37.6 (± 16.93)	a	1.0 (± 0.55)	b	3.2 (± 1.62)	a
S.E.M.	1.481		12.81		1.534		5.97	
C.V.	49.0		117.2		100.3		147	
F	11.63		3.10		6.76		1.69	
F.p.	< 0.001		0.057		0.004		0.217	
LSD	6.399		---		6.666		---	

Means (± S.E.) in a column followed by the same letter do not differ significantly at the 1% and 5% level. * = Species not included in respective experiment.

Calycomyza lantanae

No-choice trials

Very few *C. lantanae* adults were recovered and survival was poor on all test species (Table 3.7). The test species with the most number of larval mines was *Li. javanica*, which produced only 11% as many larval mines as *L. camara*. *Priva meyeri* had less than 1% of the larvae on *L. camara*, and *Li. rehmanii*, produced less.

Table 3.7 Mean number of *Calycomyza lantanae* adults recovered and mines produced on *Lantana camara* and different related test species during no choice trials (n=3).

Species	Adults recovered		Mines produced*	
L. camara	2.0 (± 1.00)	a	235.7 (± 104.99)	a
L. montevidensis	2.0 (± 2.00)	a	0.0 (± 0.00)	c
Li. rehmanii	0.0 (± 0.00)	a	1.0 (± 0.58)	c
Li. javanica	0.0 (± 0.00)	a	25.7 (± 19.8)	b
P. meyeri	1.0 (± 1.00)	a	2.3 (± 1.45)	bc
S.E.M.	1.095		0.531	
C.V.	189.7		49.1	
F	0.83		15.78	
F.p.	0.534		<0.001	
LSD	--		1.674	

*Log transformed data used for analysis

Means (± S.E.) in a column followed by the same letter do not differ significantly at the 1% level.

Multiple-choice trials

In multiple-choice trials, adults preferred to sit on *L. camara*, over any of the test species (Table 3.8.). *Lantana camara* produced the most larval mines by a great margin, 31.7 times, that of *Li. rehmanii*, the best performing non-target species. No mines were found on *L. montevidensis* and on *P. meyeri*.

Table 3.8 Mean number of *Calycomyza lantanae* adults recovered and mines produced on *Lantana camara* and different related test species during two 5x5 latin square multiple-choice trials in walk in cages (n=10).

Species	Adults recovered		Mines produced	
L. camara	10.2 (± 1.47)	a	31.7 (± 5.47)	a
L. montevidensis	0.8 (± 0.51)	b	0.0 (± 0.00)	b
Li. rehmanii	1.1 (± 0.41)	b	1.0 (± 1.00)	b
Li. javanica	0.7 (± 0.40)	b	0.5 (± 0.50)	b
P. meyeri	0.3 (± 0.21)	b	0.0 (± 0.00)	b
S.E.M.	0.721		2.54	
C.V.	87.1		120.9	
F	34.66		30.46	
F.p.	< 0.001		< 0.001	
LSD	2.819		9.92	

Means (± S.E.) in a column followed by the same letter do not differ significantly at the 1% level.

Uroplata girardi

No-choice trials

There were no statistically significant differences found between species in terms of adult survival, and subsequent progeny produced on all of the plant species tested during no-choice trials (Table 3.9). However, this trial was only replicated twice (n=2) and from the values it seems that the other species are not as suitable as host plants in terms of sustaining a viable population. This, considering the 100% error of standard error of the mean in mines produced on *P. meyeri*, the next best performing species, and very little variation (7%, S.E.) in the larval mines on *L. camara*, that was more than two times the mines on *P. meyeri*.

Table 3.9 Mean number of *Uroplata girardi* adult survival and mines produced on *Lantana camara* and different related test species during no-choice trials (n=3).

Species	Adults recovered	Mines produced
L. camara	18.0 (± 1.00) a	14.0 (± 1.00) a
L. montevidensis	15.0 (± 4.00) a	3.5 (± 1.50) a
Li. rehmanii	13.0 (± 1.00) a	1.5 (± 0.50) a
Li. javanica	12.5 (± 3.50) a	0.0 (± 0.00) a
P. meyeri	15.0 (± 5.00) a	5.5 (± 5.50) a
S.E.M.	3.32	2.60
C.V.	32.0	75.0
F	0.43	4.47
F.p.	0.786	0.066
LSD	--	--

Means (± S.E.) in a column followed by the same letter do not differ significantly at the 1% level.

Multiple-choice trials

During multiple-choice trials the most number of adults were recorded on *L. camara*. In addition, the most number of eggs and subsequent larval mines were recorded on *L. camara* than any of the species tested (Table 3.10). *Lippia rehmanii* had the second most number of larval mines which totaled 17% of that recorded on *L. camara*.

Table 3.10 Mean number of *Uroplata girardi* adults recovered, eggs, and mines produced on *Lantana camara* and different related test species during two 5x5 latin square multiple-choice trials in walk in cages (n=10).

Species	Adults recovered		Eggs laid		Mines produced	
L. camara	17.1 (± 4.62)	A	223.0 (± 42.27)	a	102.0 (± 20.42)	a
L. montevidensis	1.4 (± 0.62)	B	10.5 (± 6.06)	b	4.0 (± 2.45)	b
Li. rehmanii	2.5 (± 0.92)	B	43.7 (± 10.28)	b	17.5 (± 4.25)	b
Li. javanica	3.2 (± 0.77)	B	29.4 (± 6.07)	b	8.0 (± 3.12)	b
P. meyeri	0.5 (± 0.22)	B	0.5 (± 0.27)	b	0.0 (± 0.00)	b
S.E.M.	2.052		17.82		9.09	
C.V.	131.4		91.8		109.3	
F	11.22		26.56		22.18	
F.p.	< 0.001		< 0.001		< 0.001	
LSD	8.020		69.66		35.52	

Means (± S.E.) in a column followed by the same letter do not differ significantly at the 1% level.

3.4 Discussion and Conclusion

Baars (2002a; Baars *et al*. 2003b) concluded on the host specificity studies of *F. intermedia*, that, no-choice trials indicated only *L. camara* and some species in the closely related genus *Lippia* were suitable host plants. From no-choice and multiple-choice trial results, he concluded that the oviposition by females was considerably higher on *L. camara* than on *Lippia* species, suggesting that the indigenous African species are inferior alternative hosts. *Falconia intermedia* posed no risk at all to other species in the Verbenaceae and Lamiaceae, or to 17 food crop species (Baars 2002a; b). Three indigenous *Lippia* species, *Li. wilmsii*, *Lippia* sp. A and *Lippia* sp. B, were shown to have a 6-21% chance of being used as alternative hosts. These species were thus predicted to be at risk of some damage by *F. intermedia*, especially in the absence of *L. camara*.

Simelane (2002) concluded that host range tests suggested *O. camarae* to be suitably host specific, and should pose no threat to non-target indigenous or commercially valuable

verbenaceous species in South Africa. He argued that the limited oviposition on native species of *Lippia* to which *O. camarae* has never previously been exposed to in the field, may be explained by the tendency of host specific insects to expand their host ranges under laboratory conditions.

During no-choice trials *T. scrupulosa* produced more progeny on some *Lippia* species, than on *L. camara*. The adults however were consistently recorded on *L. camara, Li. wilmsii* and *Li. javanica* rather than from other species tested in multiple-choice trials. High variation in the data however does not reflect clear statistical differences. By doing more replicates, it could be expected to be shown that *L. camara* is a superior host, as the data, despite high variation, show the best performing *Lippia* species having 21% of the nymphs that *L. camara* supported. These results show a slightly wider host range, but still similar to those of the previous two agents tested. It is also clear that the non-target host utilization decrease from no-, to multiple-choice trials as indicated by all three agents discussed thus far.

Calycomyza lantanae proved highly specific to *L. camara*, allowing only 11% as many larvae to develop on the best performing *Lippia* species than recorded on *L. camara* in no-choice conditions. Yet again, as with the other trials conducted for *F. intermedia, O. camarae* and *T. scrupulosa*, this non-target utilization margin was improved on from 11 % to 3% once the females were allowed to select hosts, rather than being confined on a single species but still in the confines of a cage.

The no-choice results of *Uroplata girardi* are unusual in that a test species otherwise usually relatively unsuitable as a host, namely *P. meyeri* supported the highest number of progeny (39% of that recorded on *L. camara*). *Lippia* species which are usually the second most suitable as host plants only supported 0-11% progeny of that recorded on *L. camara*. During the multiple-choice trials the adults were highly selective in their host choice with the most number of adults and subsequent progeny recorded on *L. camara*. The *Lippia* species had 8-17% progeny compared to *L. camara*, and none were recorded on *P. meyeri*.

Four of the five of the insect species tested proved suitably host specific to *L. camara* and *T. scrupulosa* could potentially be shown to be specific, by replicating experiments more, to reduce error in the data. If it however does not prove more specific than these limited results reflect, it would not have been released into South Africa under current regulations. All five, though, showed a good ability to develop on the indigenous *Lippia* species, especially in no-choice trials. The multiple choice trials are however more a true reflection of host-specificity under field conditions. The "improvement in specificity" from no-choice to choice trials for each insect, on the next best (or better) species to *L.camara*, expressed as a "% increase in specificity" is: ((Immature offspring of best performing species : *L. camara* (no –choice trial)) / (Immature offspring of best performing species : *L. camara* (multiple-choice trial)) x 100 =). The results were as follows; *F. intermedia*: 326%; *O. camarae*: 157%; *T. scrupulosa*: 1051%; *C. lantanae*: 340% and *U. girardi*: 228% "increase in specificity" fom no-choice to choice experiments for each insect respectively. This highlights the conservative nature of especially no-choice trials.

Even though multiple-choice trials allow the females to select species to oviposit on, conditions in cages and quarantine compartments disrupt the normal host searching behaviour of females. Laboratory based host specificity testing is therefore conservative, and may result in an extended host range, from that which would be realised in the field (van Klinken 2001). The close proximity of non-target species in cages, even the 4x4m walk in cages used in these trials, may have stimulated oviposition in nearest neighbour plants. Females may still have some difficulty in distinguishing between plant species in an atmosphere filled with positive stimuli from the target plants, even though this is reduced by the air flow-through system in the tunnel where trials were conducted. Because of the above factors, and the trend of specificity to increase with more freedom for the females, one can predict that there will be a further decrease in non-target species utilization, for all five insects, in the field.

CHAPTER 4

Field distribution and abundance of biological control agents established on different varieties of *Lantana camara* in South Africa

4.1 Introduction

Twenty two biocontrol agents have been introduced into South Africa for the biocontrol of *L. camara* (Julien & Griffiths 1998; Baars 2000c; Simelane 2002). Of these, 10 have established, and three of them were already in the country at the time, and two of these were considered indigenous (Anonymous 1999; Table 1.1, Chapter 1). The lack of establishment and low levels of control has been ascribed to several factors. These are considered to be climate, the wide variation in the host plant in the form of varieties and high levels of parasitism (Winder *et al.* 1984; Neser & Cilliers 1990; Cilliers & Neser 1991; Baars & Neser 1999; Broughton 2000; Day & Neser 2000).

The weedy lantana was bred through horticultural and natural hybridization, selection and somatic mutation, probably from several related tropical American species (Swarbrick *et al*. 1995, Chapter 1). This has given rise to varieties that continually interbreed and morphologically differ in flower colour, hairiness of leaves, stems and shoots, stem thorniness and also in chemical composition (Smith & Smith 1982). Spies and Stirton (1982 a; b) recognized more than 50 naturalised cultivars/ varieties in South Africa. The plants were described as polyploid, with a basic chromosome number of 11 or lower, or postspeciation evolution of the genome was speculated to have occurred. They found South African varieties to be mostly diploids and triploids, but found tetraploids, pentaploids as well as hexaploids, and confirmed further hybridization in the field.

Certain varieties dominate in areas, and if these are not controlled, they may simply out compete heavily attacked varieties (Cilliers & Neser 1991). Six biocontrol agents of lantana have been reported to have variety preferences, namely, *Ectaga garcia* Becker, *Chariditis pygmaea* Klug, *Alogoasa parana* Sameulson, *T. scrupulosa*, *F. intermedia* and *A. compressa* (Day & Neser 2000; Chapter 2). The lack of pressure from agents on some varieties has been one of the motivations for using a suite of agents for the biocontrol of lantana, as the different characters of the varieties may together or separately influence the performance of introduced agents that were collected from less diverse *Lantana* species in the region of origin (Chapter 2).

In this chapter, I rate what colour forms, and which of three levels of leaf hairiness of *L. camara* plants are spread over the largest area and are most densely populated (relative importance to one another) of the varieties occurring in South Africa. I then assess if biocontrol agents in the field prefer certain of these characteristics of *L. camara* varieties, by measuring the population levels of the agents in the field on the various lantana varieties that possess these characteristics.

4.2 Methods and Materials

Fourteen natural enemies associated with varieties of *L. camara* were sampled at sites throughout the weed's range in South Africa (Figure 4.1). These were mostly sites where *L. camara* infestations were visible from the road. The survey was conducted during several trips between November 2004 and March 2005. Most of this survey concentrated on the northern coast of the Eastern Cape, as this area had not been previously surveyed, but included all provinces where *L. camara* occurs (Figure 4.1). Earlier surveys (1998-2000) were conducted by Baars and Heystek (2003a), who used a single rating per insect species per site, focusing on insect distribution and parasitism. This new series of surveys included detailed descriptions of the lantana varieties present at each site, area infested and overall density ratings, site altitude and a search for lantana agents on non- target species present (ten non-target plants per species were searched at each site, and the non-target results are discussed in chapter 5).

Colour varieties were grouped by two main characters, namely corolla lobe and throat colours of mature flowers. The extent of leaf hairiness as a character were grouped into plants that had few leaf hairs (felt smooth to the touch, i.e. had few or very short leaf hairs), plants with medium hairy leaves (felt hairy to the touch, i.e. had medium number of leaf hairs per surface area, and were short) and plants that had very hairy leaves (felt very hairy, i.e. had many leave hairs and were relatively long), using the cultivated reference varieties at PPRI (Table 2.1, Chapter 2), as a point of reference. Factors such as bud and immature flower colour, stem hairiness and thorniness were described but disregarded in terms of the variety groupings, as it resulted in too many different varieties, that would have made grouping and comparing data impractical, and also required additional sampling. The varieties described were rated for "relative abundance". This was calculated from an estimated area (A) infested multiplied with an infestation level, or density (D) of the stand: (AxD). At each site a rating between

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0.1-100ha was assigned to the area (A) covered by the weed. A numerical value (1-2) was assigned to one of three levels of density (D) of the infestation, namely scarce (1), when plants grew singly scattered over an infested area, clumps (1.5), when large groups of plants formed clumps over an infested area, or thicket (2), when plants formed a dense impenetrable mass over most of the infested area. Sites with plants with similar flower colour groupings were added together to achieve a "Total relative abundance" ((A1xD1) + (A2xD2) + (A3xD3) = Total relative abundance). This was also calculated for the leaf hairiness characters of the plants. The top four colour groupings and all three levels of leaf hairiness obtained in this manner were mapped.

Ten plants of each of every variety present at the site were thoroughly searched and assessed for insect abundance and the level of damage inflicted on it per biocontrol agent present. The insect species surveyed were the twelve referred to in Table 1.1(Chapter 1), which are known to be established in South Africa. Two additional insects were surveyed, namely the mealybug, *Orthezia insignis* Browne, which sucks sap from stems of various plant species, especially at shady sites, and the noctuid moth, *Characoma submediana* Strand*, that feeds on lantana flowers and fruit. These two species are reportedly polyphagous but are commonly found on *L. camara*. The insects were rated for "relative insect abundance" using a five-category scoring system. The ratings were: 0, where no life stages were present or typical damage found on plant; 1, where very few individuals were encountered or few plant parts with characteristic damage at low intensities were found; 2, where individuals were found easily, but at low numbers, or characteristic damage easily noticed, but small proportions of the relevant plant parts were damaged; 3, where insects were readily present, with an even distribution over the plant and a large proportion of the relevant plant parts damaged and a section of the shrub showed signs of stress; 4, where large numbers of individuals were found on most of the shoots on each plant, with an even distribution and most of the relevant plant parts had characteristic damage and plants were noticeably stressed. This rating was therefore dependant on the size of each plant and is thus referred to as "relative insect abundance" as it is a measure of the number of insects which is dependant on the size of the plant. To illustrate this, a small plant with a rating 4 may have a very high population of the insect species and suffers maximum damage, but have fewer individuals than a large plant with a rating 4, that also suffers maximum damage.

Twenty damaged flowers and twenty damaged seed heads were collected per species per site. This was done at most sites except the very few sites where there were no flowers or seeds present. In this case the lantana varieties could not be described and the varietal preferences assessed. The plant material collected was brought back to the laboratory and immature stages of insects reared to the adult stage for identification. Field-collected and emerged insect material were accessioned and sent to the National Collection of Insects (NCI, PPRI) in Pretoria for confirmation of identity during the first round of surveys, and later specimens compared to these for identification.

Relative insect abundance on all the different varieties occurring within the insects' geographic distribution was compared. Some of the agents were localised in their established range and were only exposed to the lantana varieties in its geographic range. Analyses of the varietal association of these agents were conducted excluding the varieties occurring in other areas in the country.

4.3 Results

4.3.1 Distribution of Lantana camara varieties in South Africa

In total 774 *L. camara* plants were surveyed in 7 provinces of South Africa, (Figure 4.1). These consisted of 30 plants from 3 sites in Gauteng (GP), 42 plants from 5 sites in the North West Province (NW), 57 plants from 7 sites in Limpopo Province (LP), 155 plants from 16 sites in Mpumalanga (MP), 138 plants from 14 sites in KwaZulu-Natal (KZN), 292 plants from 30 sites in the Eastern Cape Province (EC) and 60 plants from 6 sites in the Western Cape Province (WC).
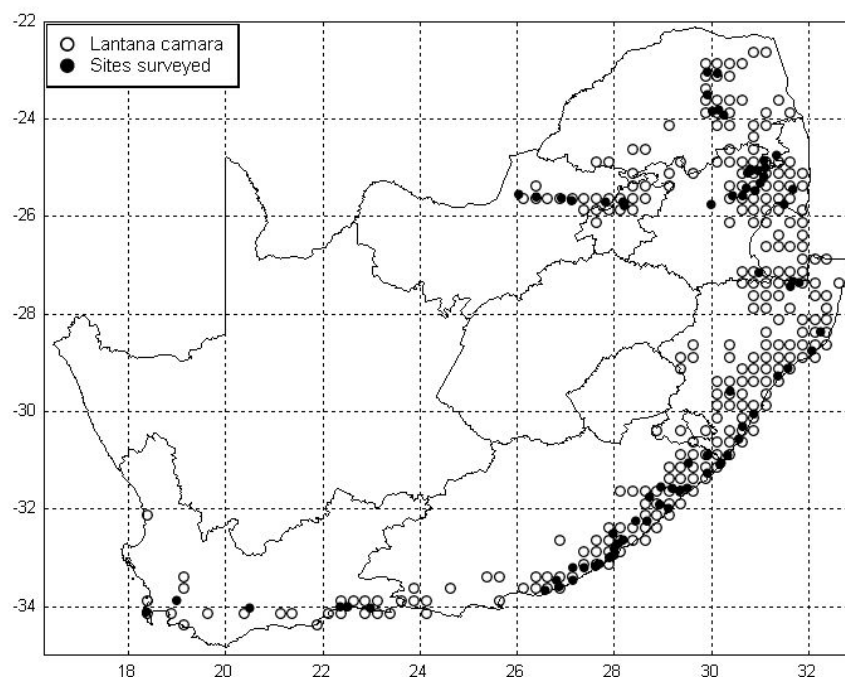
Figure 4.1 Distribution of *Lantana camara* in South Africa and sites surveyed for lantana varieties and natural enemies associated with the weed.

The mature flower corolla lobe colour that was rated most abundant was a pink *L. camara* (Figure 4.2). The most abundant corolla throat colour encountered during the survey was orange. The combinations of corolla lobe to throat colour encountered most was in the order Pink –orange (Po), Pink –pink (Pp), Light pink –pink (Lpp), Dark pink –orange (Dpo), Dark pink –red (Dpr), Light pink-orange (Lpo), Pink -yellow (Py), Dark pink – pink (Dpp), Red – red (Rr), Orange –orange (Oo), Dark pink –yellow (Dpy), Orange-red (Or) and White-white (Ww). These colour varieties each varied in leaf hairiness. The top two colour varieties, Po, and Pp, each had few (f), medium (m) and very hairy (v) leaves, whereas other colour varieties had only one or two degrees of leaf hairiness. One more colour combination was observed but not plotted on Figure 4.2, namely Pink-white (Pw), as hairiness of leaves was not described for this variety during the survey. It had a total abundance rating of 45 (Py>Pw>Rr).
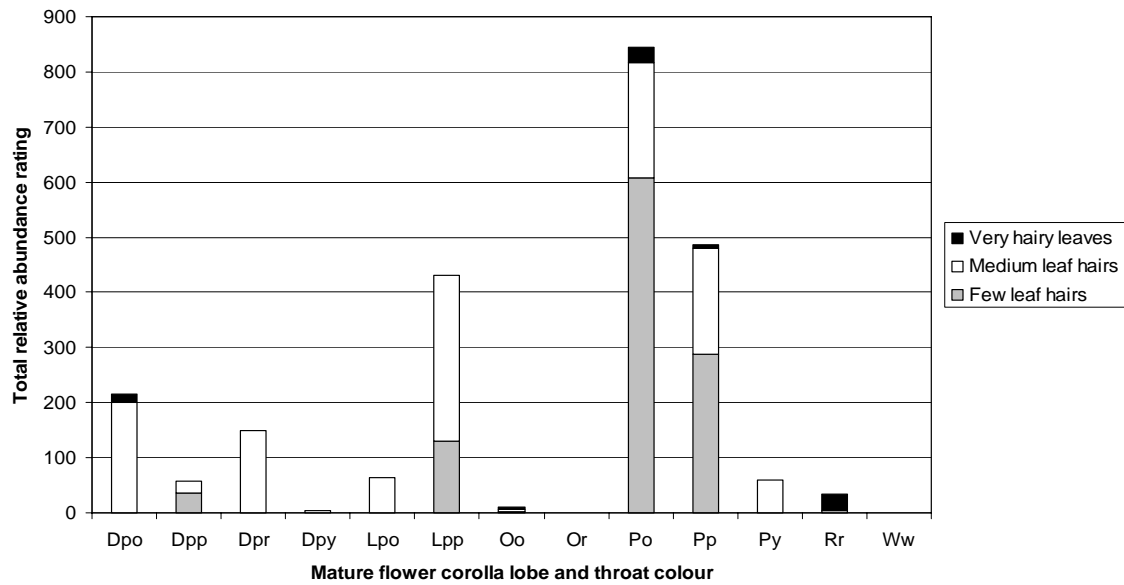
Figure 4.2 Total relative abundance ratings of different mature flower corolla lobe and throat colour combinations, and levels of hairiness of leaves among varieties of *Lantana camara* in South Africa, from the sites surveyed.

The most abundant colour form of *L. camara*, Po, had the widest geographic distribution (Figure 4.3). Pink-pink (Pp) occured in the highveld, lowveld, and coastal areas of the EC. Light pink –pink (Lpp) occurred on the coast of the EC and in MP. Dark pink –orange (Dpo) was only found on the coast in the three coastal provinces where *L. camara* occurs. The varieties were mixed, but one usually dominated a stand, or area.
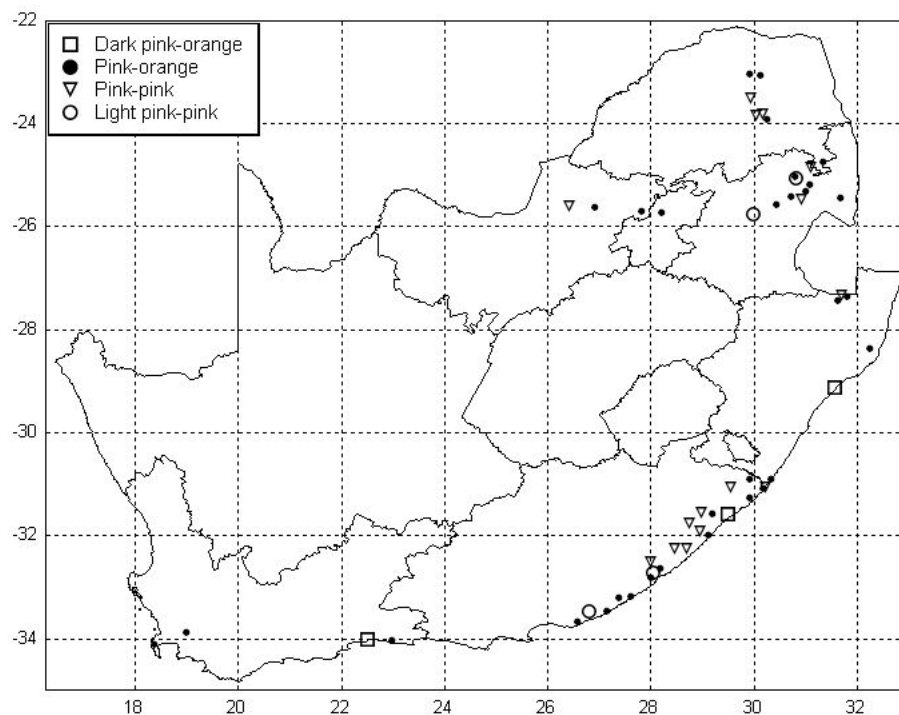
Figure 4.3 Distribution of the top four most abundant mature flower corolla lobe and throat colour combinations of *Lantana camara* in the country, from the areas surveyed.

In total, the lantana varieties with few leaf hairs were most abundant nationally; followed closely by plants with medium leaf hairs and less than 5% of all plants surveyed had very hairy leaves (Figure 4.4).
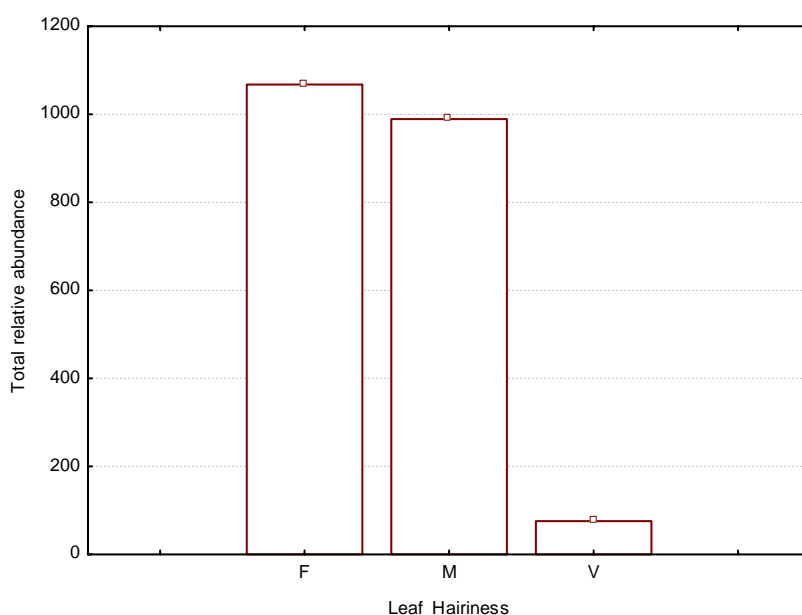
Figure 4.4 Total relative abundance of *Lantana camara* varieties in the country that had few (F), medium (M) and very hairy leaves (V) occurring at sites surveyed.

The two most abundant forms of *L. camara*, in terms of leaf hairiness, namely the few and medium haired leaved plants occurred throughout the distribution range of *L. camara* in South Africa (Figure 4.5). The plants that had few leaf hairs were more abundant in the EC, MP and LP, and were absent from GP and the NW provinces. The very hairy plants were encountered infrequently in all of the provinces with the exception of the NW, where it was absent. The hairy form was the most common variety in the WC.
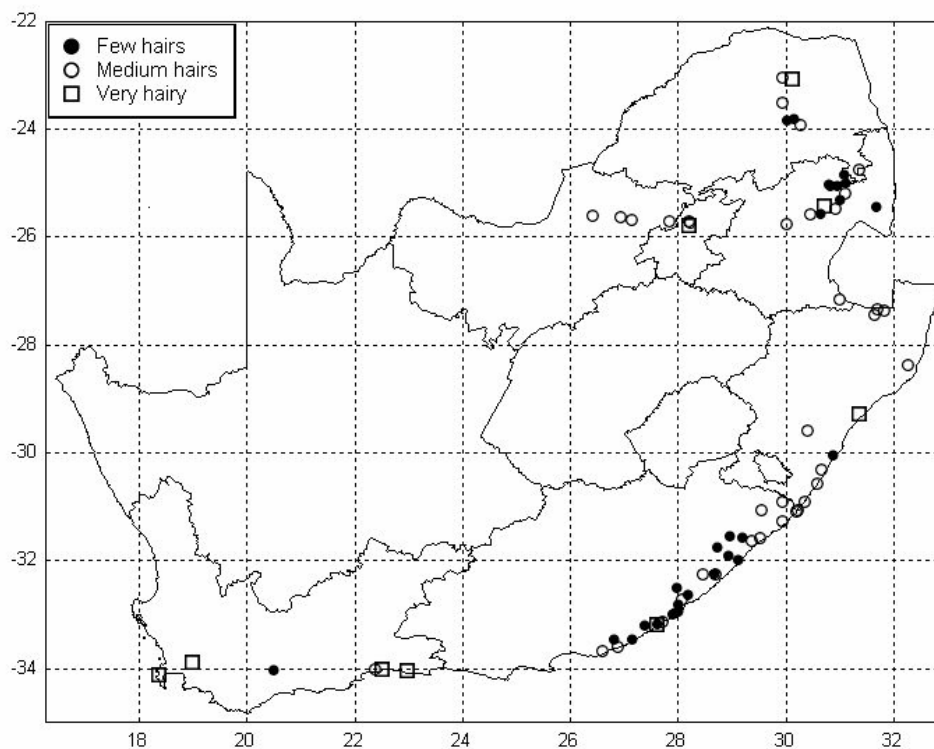
Figure 4.5 Distribution of *Lantana camara* varieties that had few, medium and very hairy leaves in South Africa, naturalized at the sites surveyed.

4.3.2 Distribution of biocontrol agents on varieties of Lantana camara in South Africa
The reported indigenous moth, *H. laceratalis* was the most abundant insect on *L. camara*, followed by *C. lantanae, O. lantanae, T. scrupulosa, O. camarae, A. onychote, O. insignis, F. intermedia, S. haemorrhoidalis, O. scabripennis, L. pusillidactyla*, and the least abundant species were *U. girardi* and *C. submediana* (Figure 4.6).
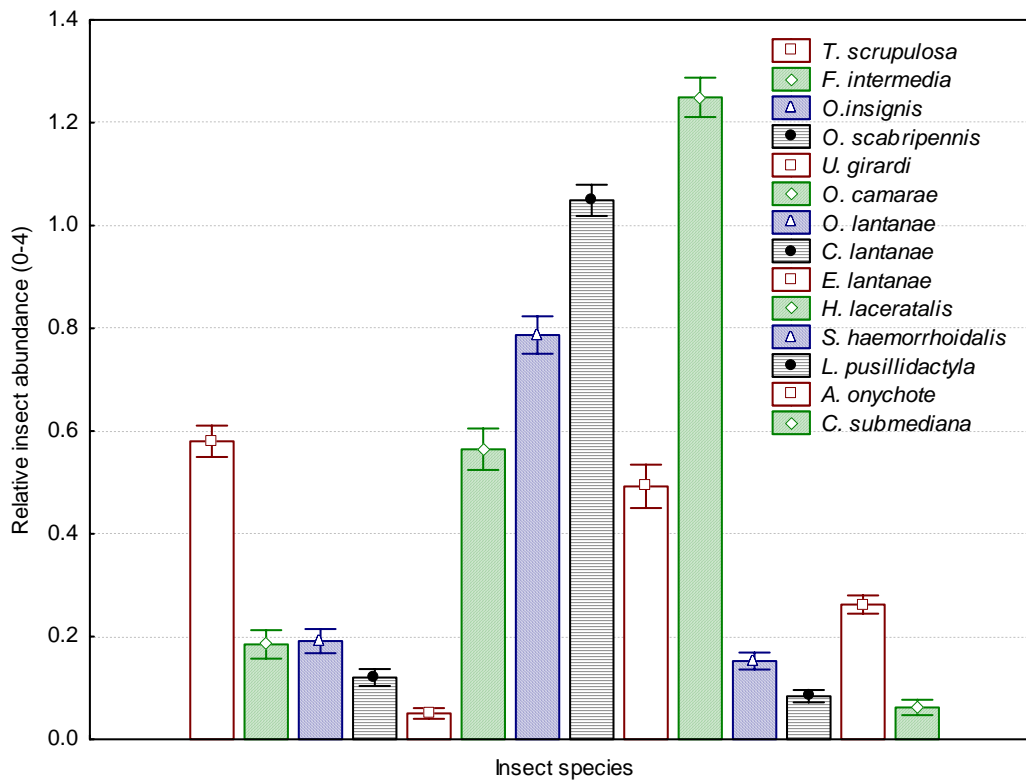
Figure 4.6 Mean relative abundance rating (0-4) per plant of all insect species associated with *Lantana camara* in South Africa, from the sites surveyed.

Teleonemia scrupulosa

Teleonemia scrupulosa was found at 80.5 % of the sites surveyed (Figure 4.7). It had a wide distribution and was found in all provinces of South Africa, where *L. camara* is problematic.
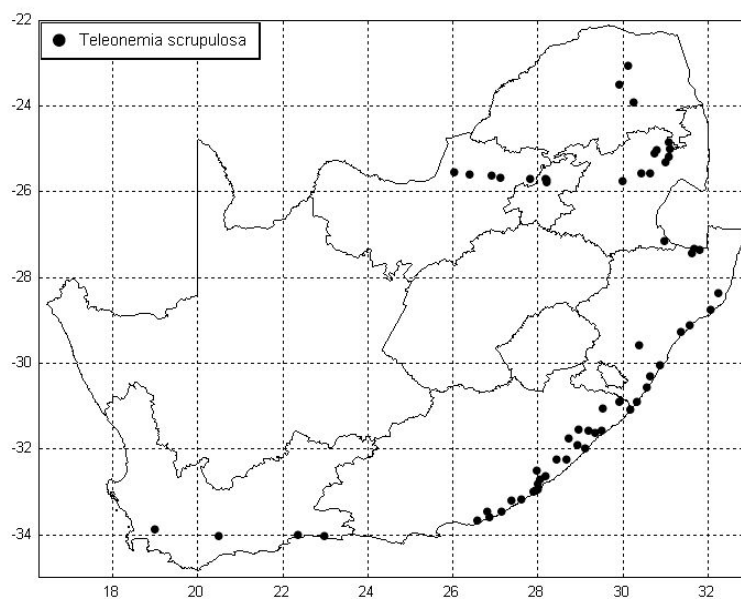
Figure 4.7 Distribution of *Teleonemia scrupulosa* at the *Lantana camara* sites surveyed.

Teleonemia scrupulosa abundance varied from 0 to over 30 as total relative abundance per site at most of the altitudes sampled (Figure 4.8). Most sites had a total rating of below 5 per site (at sites with less than ten plants, the total rating was converted to a value out of 40).
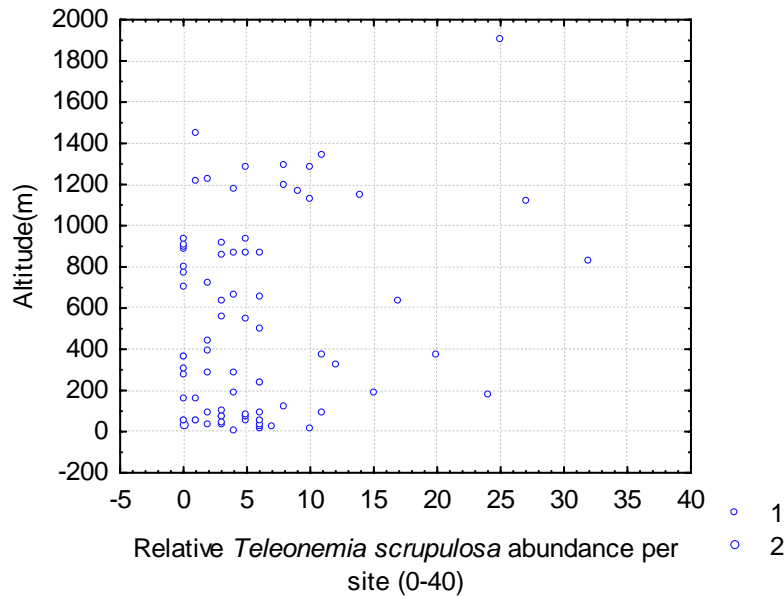
Figure 4.8 Relative *Teleonemia scrupulosa* abundance per site (0-40), at different altitudes during the survey (1 = 1 site at altitude with given abundance rating, 2 = 2 sites at altitude with same abundance rating).

Teleonemia scrupulosa was nationally most abundant on the Lpp flower variety of the 14 groupings that were encountered at the sites surveyed (Figure 4.9). The least number of tingids were on Ww. The top three varieties were all pink in colour. The fourth to sixth preferred varieties had pink corolla lobes, and yellow, orange and red throat colours respectively. The most abundant variety Po (Table 4.2), was rated as having ninth highest tinged populations.
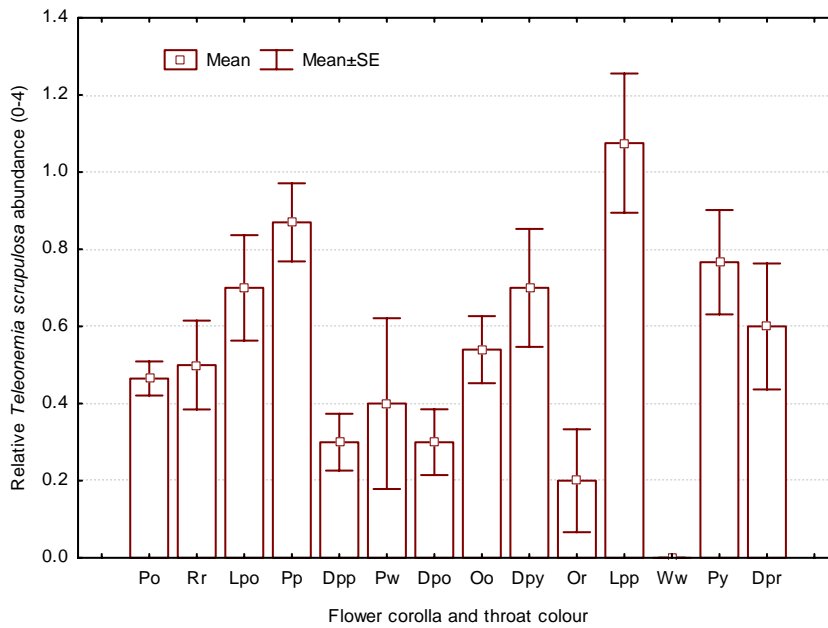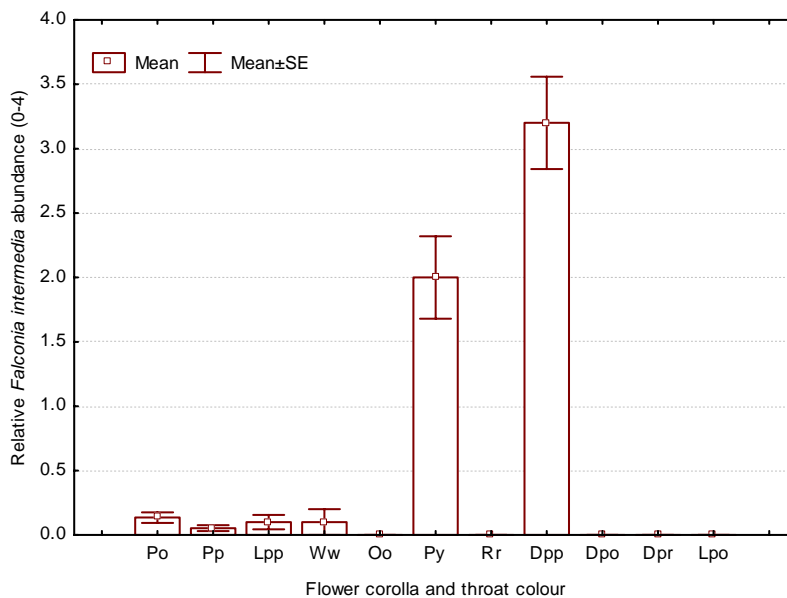
Figure 4.9 Mean relative *Teleonemia scrupulosa* abundance per plant on different mature flower corolla lobe and throat colour combinations of *Lantana camara* that occurred in the suitable geographic and altitudinal range for the species during the survey.

The tingid, *T. scrupulosa*, preferred varieties that had medium to few leaf hairs (Figure 4.10). The plants that had most leaf hairs were largely avoided.
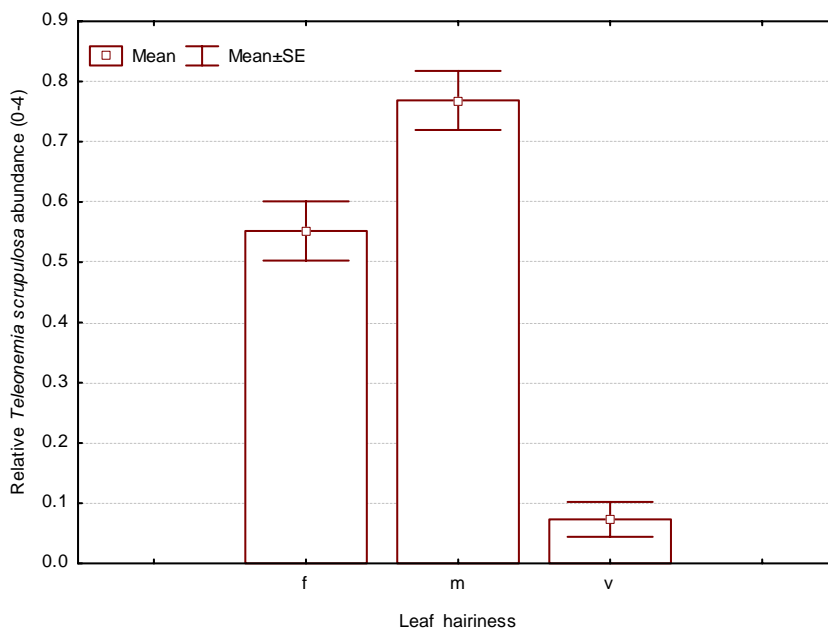
Figure 4.10 Mean relative *Teleonemia scrupulosa* abundance per plant on *Lantana camara* that had few (f), medium (m) and very hairy leaves (v) that occurred in the suitable geographic and altitudinal range for the species during the survey.

Falconia intermedia

Falconia intermedia were present at only 12.2 % of the sites surveyed (Figure 4.11). It was limited to few sites in the LP, MP and EC.
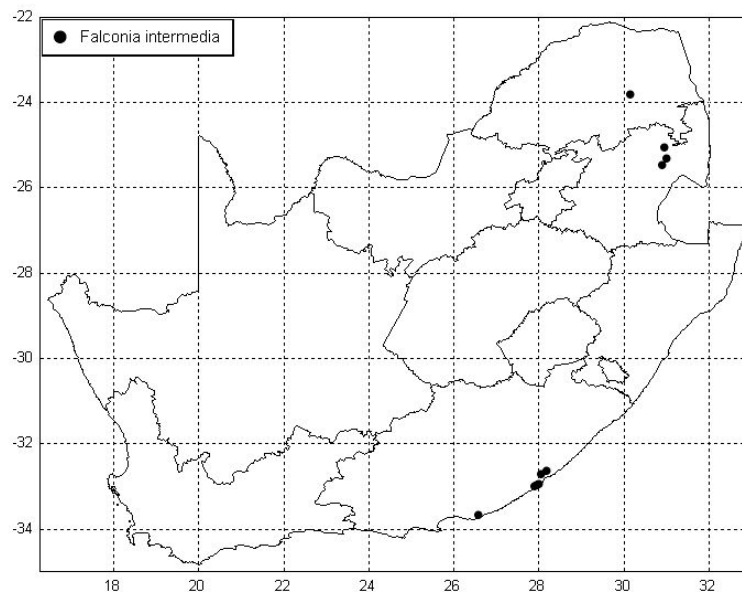
Figure 4.11 Distribution of *Falconia intermedia* at the *Lantana camara* sites surveyed.

The mirid, *F. intermedia*, was limited in distribution by high altitudes (Figure 4.12). It occurred only below 900m above sea level. The best population was found below 100m.
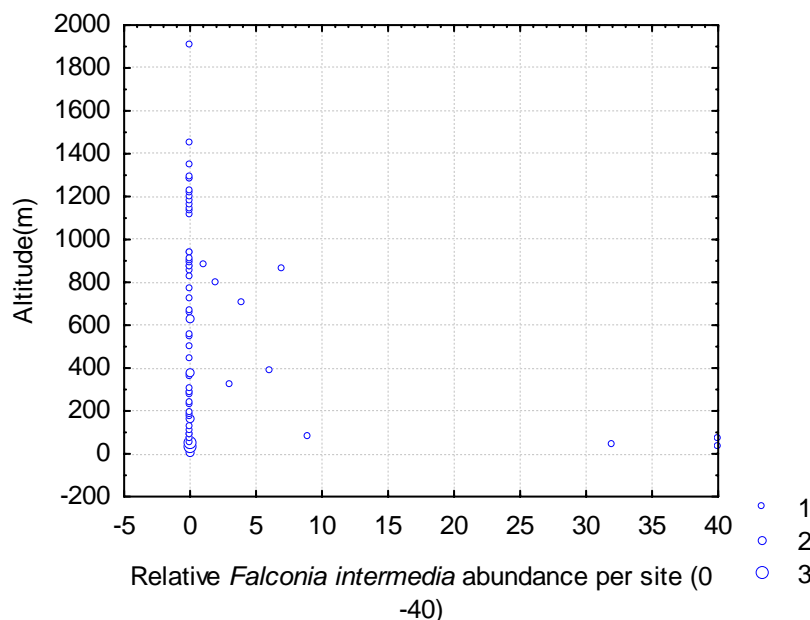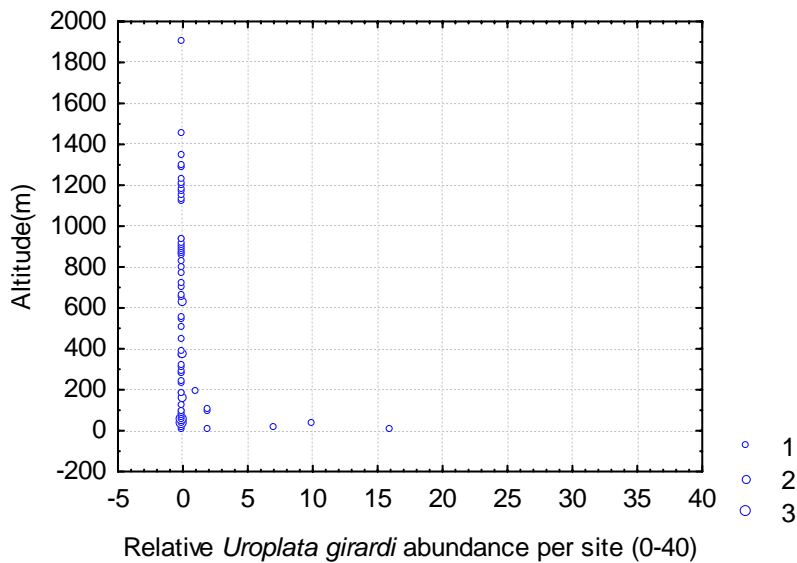

Figure 4.12 Relative *Falconia intermedia* abundance per site (0-40) at different altitudes during the survey (1 = 1 site at altitude with given abundance rating, 2 = 2 sites at altitude with same abundance rating, 3 = 3 sites at same altitude with same abundance rating).

The mirid, *F. intermedia,* occurred on only 6 colour varieties of the 11 that were found in the suitable geographic and altitudinal range for the species (Figure 4.13). It maintained high populations on only two, Dpp and Py, two colour forms that were rated to be very low in abundance throughout the range of lantana (Table 4.2).
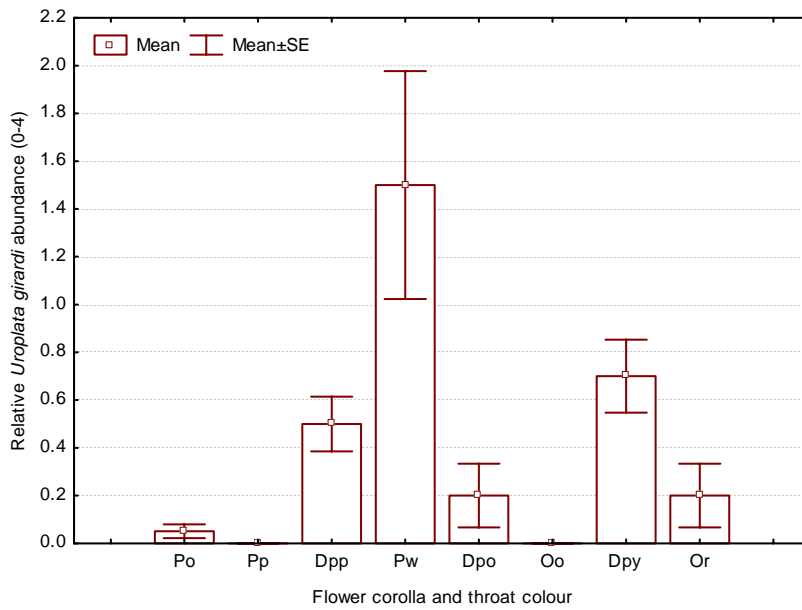

Figure 4.13 Mean relative *Falconia intermedia* abundance per plant on different mature flower corolla lobe and throat colour combinations of *Lantana camara* that occurred in the suitable geographic and altitudinal range for the species during the survey.

Falconia intermedia were highly limited by the leaf hairiness of plants (Figure 4.14). It only occurred in high numbers on varieties that had few hairs, and populations were low on plants with medium hairs, and did not occur on plants within its geographical and altitudinal range that had very hairy leaves.
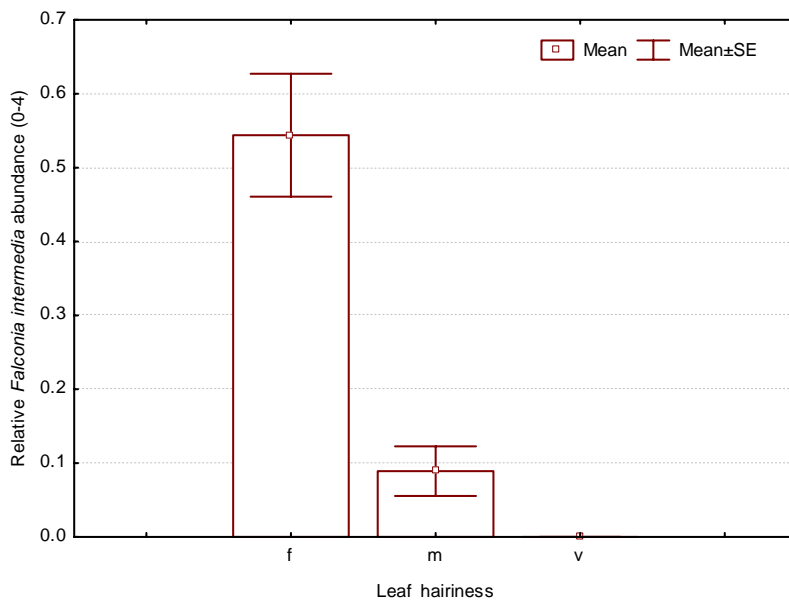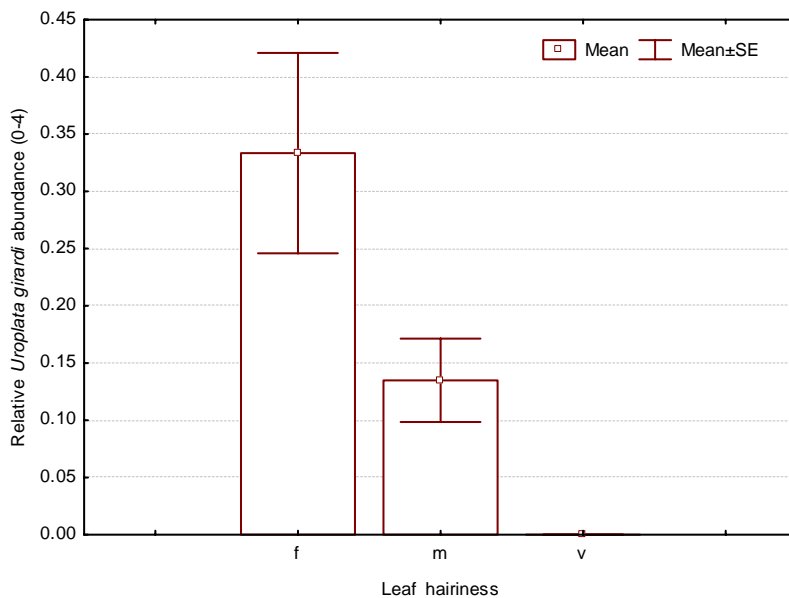
Figure 4.14 Mean relative *Falconia intermedia* abundance per plant on *Lantana camara* that had few (f), medium (m) and very hairy leaves (v) that occurred in the suitable geographic and altitudinal range for the species during the survey.

Uroplata girardi

Uroplata girardi, the second leaf mining hispid, was limited to the KZN province (Figure 4.15). It occurred at only 8.5% of all sites surveyed and in 54% of sites in KZN.
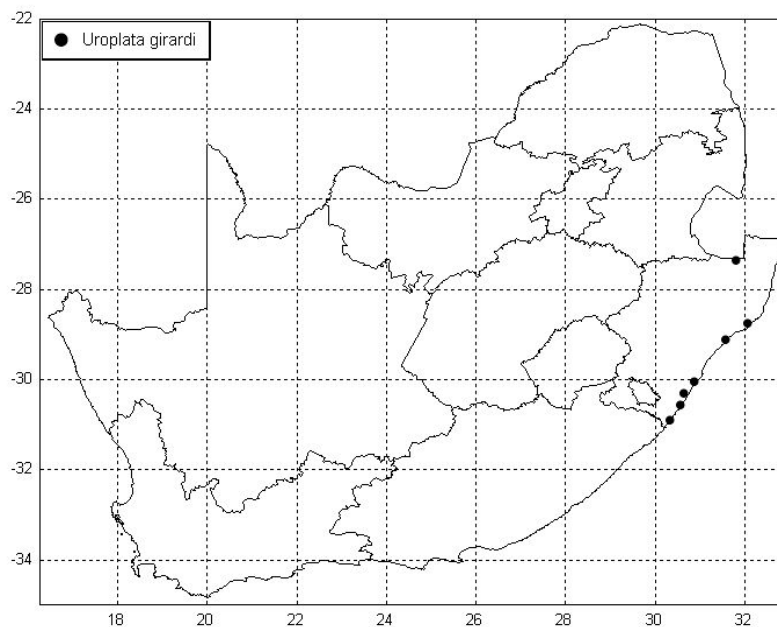
Figure 4.15 Distribution of *Uroplata girardi* at the *Lantana camara* sites surveyed.

Uroplata girardi only occurred below 200m above sea level (Figure 4.16). It reached a maximum population level of 16 out of a possible rating of 40 for a site.
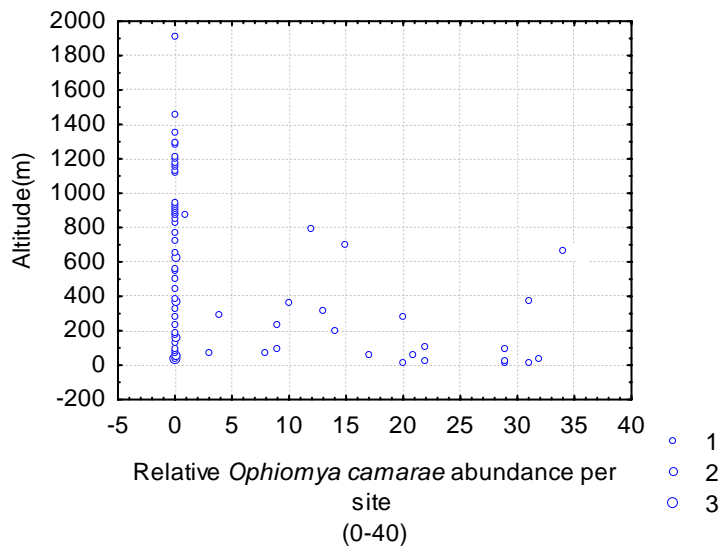

Figure 4.16 Relative *Uroplata girardi* abundance per site (0-40) at different altitudes during the survey (1 = 1 site at altitude with given abundance rating, 2 = 2 sites at altitude with same abundance rating, 3 = 3 sites at same altitude with same abundance rating).

Although *U. girardi* was only recorded at sites below 200m, the lantana varieties occuring below 400m in this survey were included in the analyses. This was done as this species was previously recorded from altitudes of approximately 400m. (Baars & Heystek 2003a). Of the varieties occurring below 400m, *U. girardi* was most abundant on Pw (Figure 4.17). Dark pink –yellow (Dpy) and Dpp had the next highest population levels followed by Dpo and Or. The variety Po had a mean population of just more than zero.
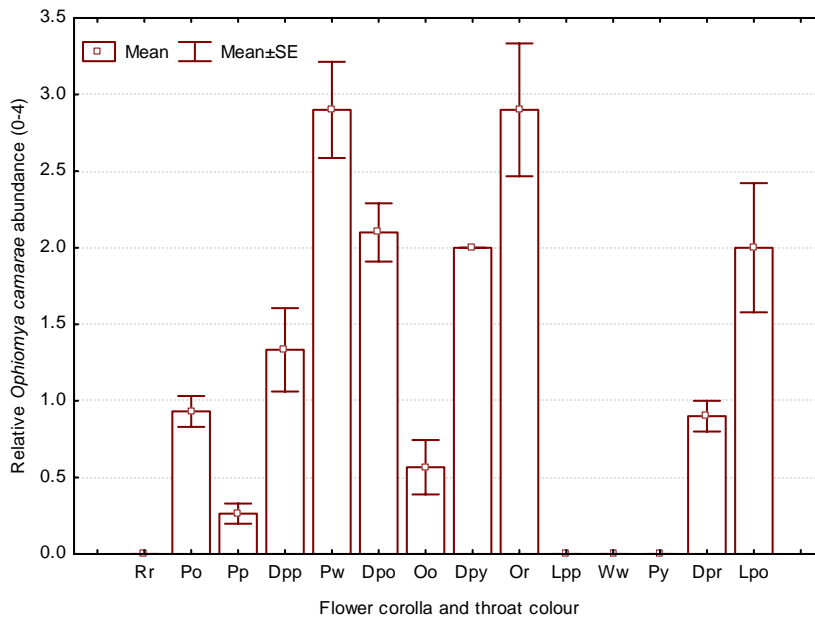
Figure 4.17 Mean relative *Uroplata girardi* abundance per plant on different mature flower corolla lobe and throat colour combinations of *Lantana camara* that occurred in the suitable geographic and altitudinal range for the species during the survey.

Calculated from potentially suitable sites, *U. girardi* built up the highest mean populations on plants that had least leaf hairs (Figure 4.18). Almost half the population mean was obtained on medium haired varieties, and very hairy plants were not utilized.
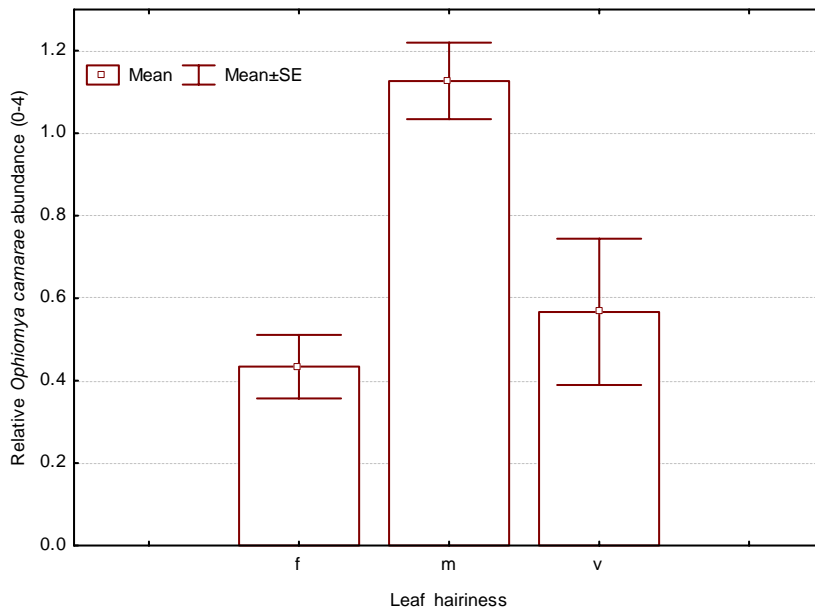

Figure 4.18 Mean relative *Uroplata girardi* abundance per plant on *Lantana camara* that had few (f), medium (m) and very hairy leaves (v) that occurred in the suitable geographic and altitudinal range for the species during the survey.

Ophiomya camarae

Ophiomya camarae populations were limited to the LP, MP, KZN, and EC provinces (Figure 4.19). Despite being in the field for only about 4 - 5 years at the time of the survey, it was established at 30.5% of all the sites surveyed.
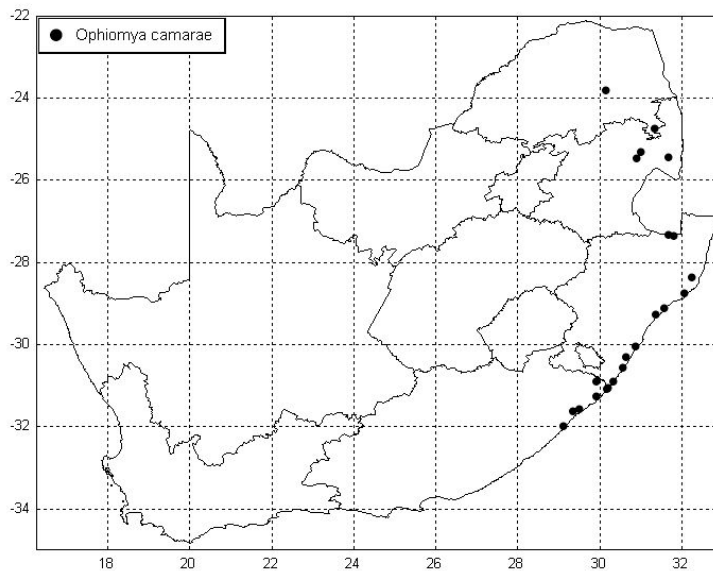
Figure 4.19 Distribution of *Ophiomya camarae* at the *Lantana camara* sites surveyed.

The herringbone leaf mining fly is limited by altitude and associated conditions (Figure 4.20). All of the populations occurred below 900m despite being released at higher altitudes. At a few sites it reached very high population levels of over 75% of the maximum abundance, severely impacting on the growth condition of the lantana infestations.

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Figure 4.20 Relative *Ophiomya camarae* abundance per site (0-40) at different altitudes during the survey (1 = 1 site at altitude with given abundance rating, 2 = 2 sites at altitude with same abundance rating, 3 = 3 sites at same altitude with same abundance rating).

The highest population densities of *Ophiomya camarae* were recorded on the colour varieties Pw and Or, that were all below 1300m, and E of 28º longitude, excluding those occurring in GP (Figure 4.21). The three next best varieties had yellow to orange throat colours, and were Dpo, Dpy and Lpo. The next three varieties had mean populations of less than half of the first two, and were Dpp, Dpr, and the most abundant colour variety Po. Orange -orange, and Pp, had less than a fifth of the populations recorded on the varieties that were most utilized.
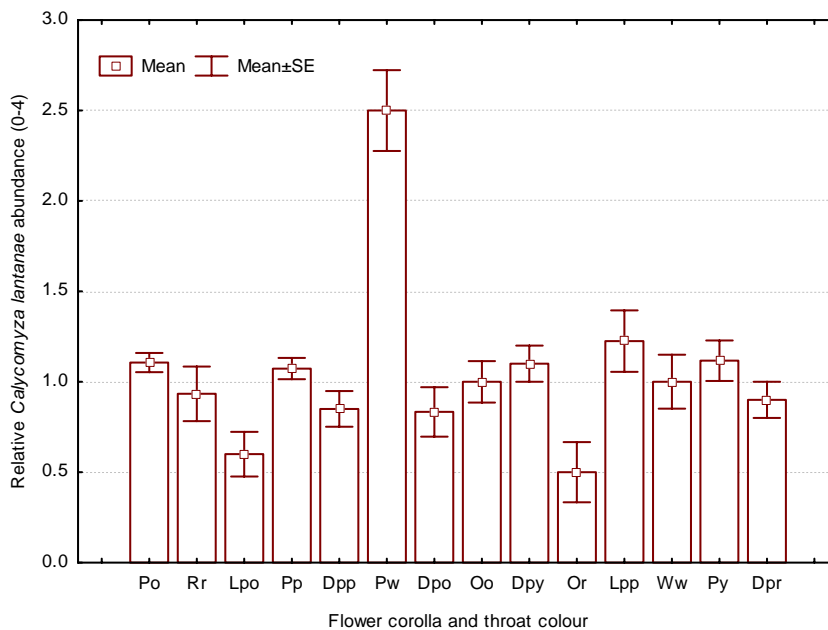
Figure 4.21 Mean relative *Ophiomya camarae* abundance per plant on different mature flower corolla lobe and throat colour combinations of *Lantana camara* that occurred in the suitable geographic and altitudinal range for the species during the survey.

The mean *O. camarae* abundance rating was highest on *L. camara* varieties that had medium haired leaves (Figure 4.22). The herringbone miner preferred plants with a medium hairiness rating over the densely haired and glabrous varieties.
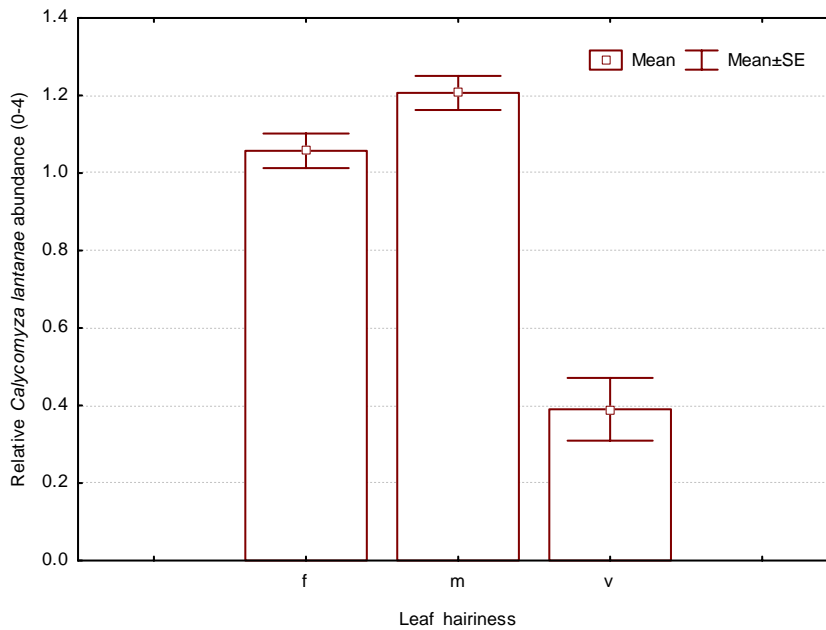

Figure 4.22 Mean relative *Ophiomya camarae* abundance per plant on *Lantana camara* that had few (f), medium (m) and very hairy leaves (v) that occurred in the suitable geographic and altitudinal range for the species during the survey.

Calycomyza lantanae

Calycomyza lantanae, the blotch leaf mining fly, occurred at 90.2 % of the sites surveyed (Figure 4.23). It was found in all the provinces where *L. camara* is problematic, but only at one site in the WC.
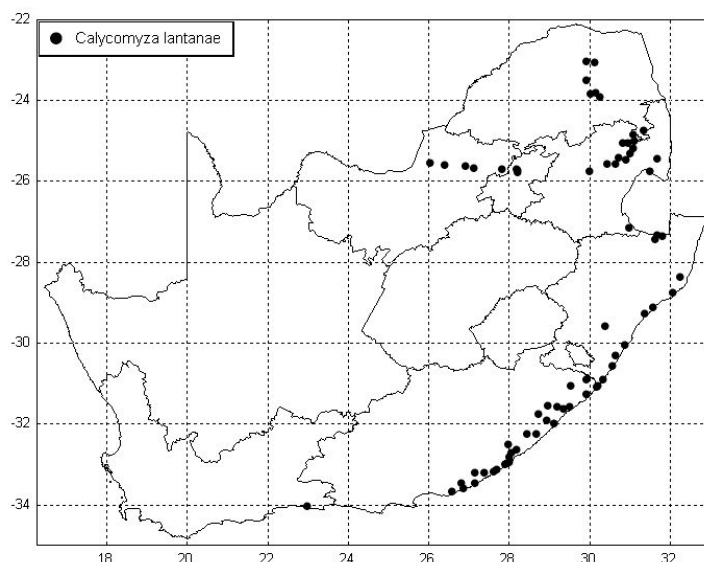
Figure 4.23 Distribution of *Calycomyza lantanae* at the *Lantana camara* sites surveyed.

Higher populations of the blotch miner occurred at coastal sites (Figure 4.24), but were found at most of the sites, even at the higher altitudes.
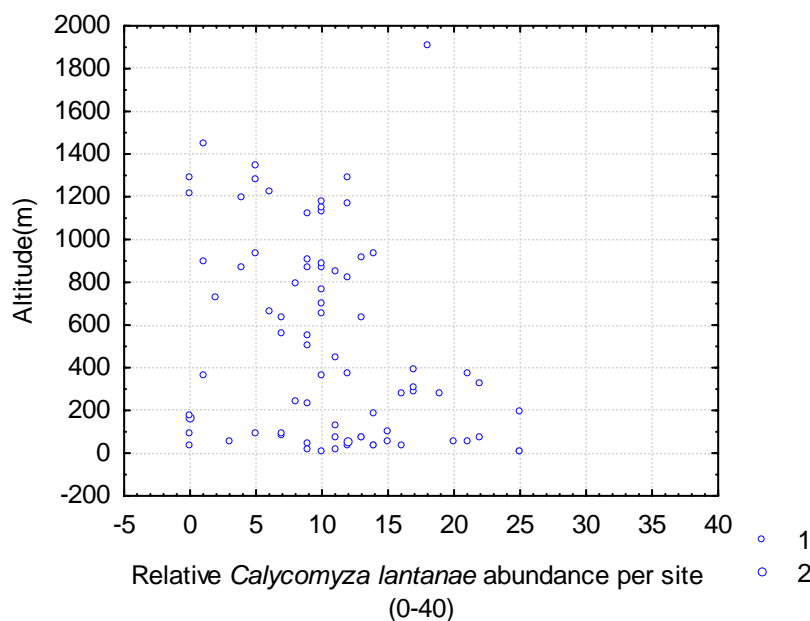
Figure 4.24 Relative *Calycomyza lantanae* abundance per site (0-40) at sites with different altitudes during the survey (1 = 1 site at altitude with given abundance rating, 2 = 2 sites at altitude with same abundance rating).

This leaf miner was recorded on most of the varieties surveyed showing little selectivity in the variety utilized at sites. However, the Pw coloured flower varieties had almost double the population levels compared to other colour forms (Figure 4.25). Light pink –orange (Lpo), and especially Or, varieties had noticeably lower population levels than all the other colour forms.

Figure 4.25 Mean relative *Calycomyza lantanae* abundance per plant on different mature flower corolla lobe and throat colour combinations of *Lantana camara* that occurred in the suitable geographic and altitudinal range for the species during the survey.

Nationally, *C. lantanae* preferred medium and few-haired varieties of *L. camara* (Figure 4.26). Very hairy varieties sustained about a third of the populations of the other two forms.

Figure 4.26 Mean relative *Calycomyza lantanae* abundance on *Lantana camara* plants that had few (f), medium (m) and very hairy leaves (v) that occurred in the suitable geographic and altitudinal range for the species during the survey.

Orthezia insignis

Orthezia insignis distribution was limited to the coast of the EC and KZN, but it occurred at a site inland in KZN and sites in LP and MP (Figure 4.27). It was present at 18.3 % of sites surveyed.
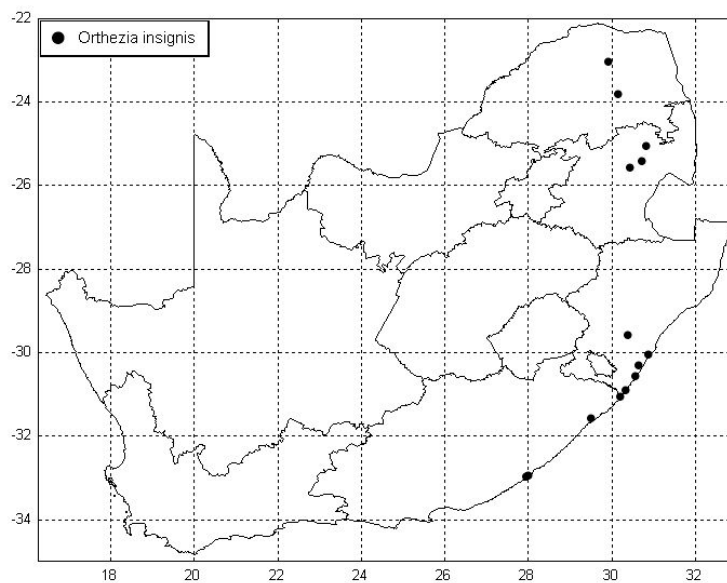

Figure 4.27 Distribution of *Orthezia insignis* at the *Lantana camara* sites surveyed.

Populations of the stem sucking mealybug, *O. insignis*, were not affected by altitude, and occurred from sea level to over 1200m (Figure 4.28).
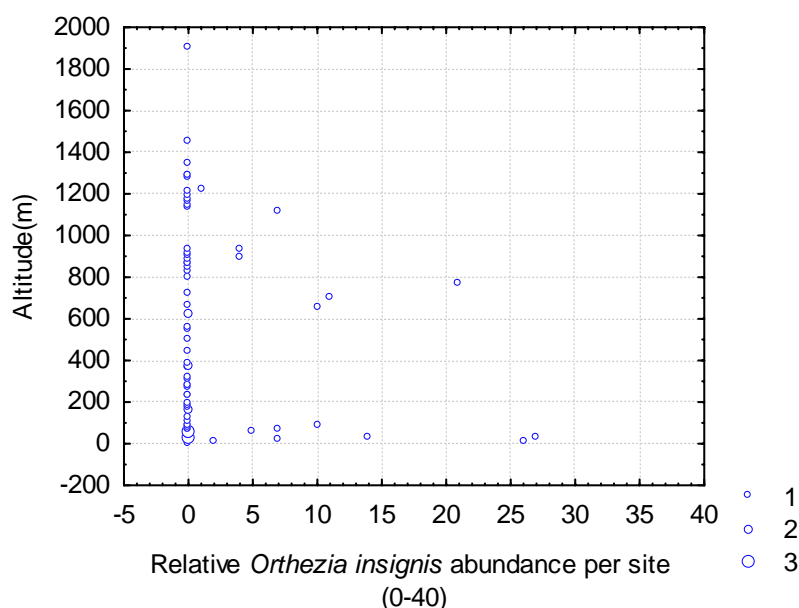
Figure 4.28 Relative *Orthezia insignis* abundance per site (0-40) at different altitudes during the survey (1 = 1 site at altitude with given abundance rating, 2 = 2 sites at altitude with same abundance rating, 3 = 3 sites at same altitude with same abundance rating).

All 14 varieties of flower colours occurred within the LP, MP, KZN and EC, the geographical range of *O. insignis* (Figure 4.29; 4.27). Of the 14 varieties occurring in its geographical range, the mealybug occurred in the highest numbers on Or followed by Dpy and Dpp. The varieties Py, Po and Oo also had relatively high population levels. The other varieties were relatively under utilized as host plants, only 25% of the maximum population levels recorded on the most susceptible varieties.
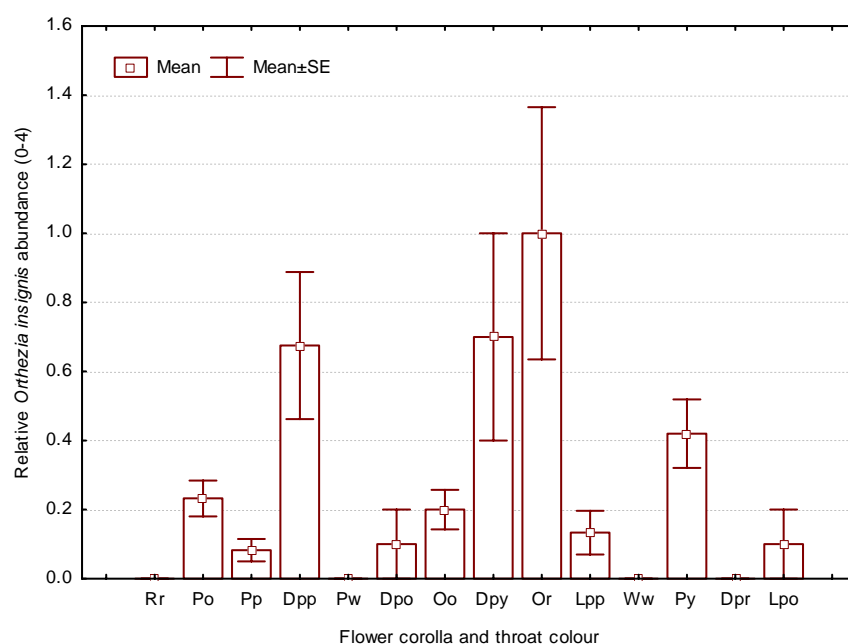
Figure 4.29 Mean relative *Orthezia insignis* abundance per plant on different mature flower corolla lobe and throat colour combinations of *Lantana camara* that occurred in the suitable geographic and altitudinal range for the species during the survey.

Orthezia insignis preferred the varieties with very hairy leaves (Figure 4.30). The varieties with either few or medium hairiness were equally utilized to about a third the level as the hairy varieties.
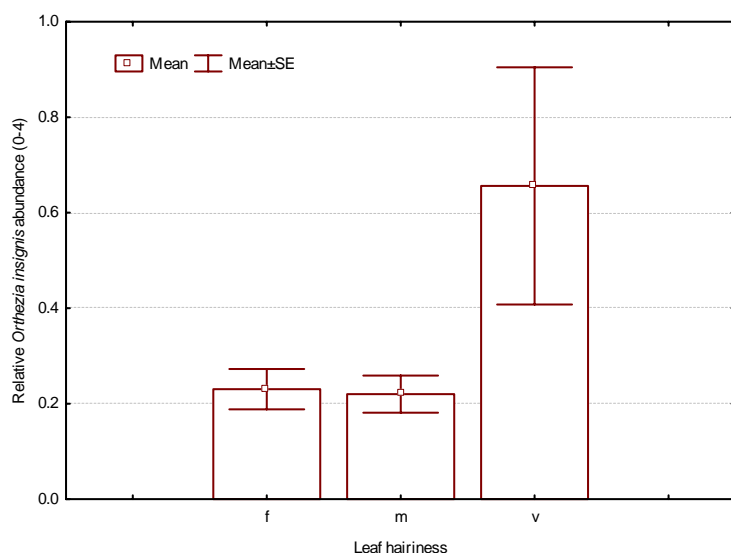

Figure 4.30 Mean relative *Orthezia insignis* abundance per plant on *Lantana camara* that had few (f), medium (m) and very hairy leaves (v) that occurred in the suitable geographic and altitudinal range for the species during the survey.

Octotoma scabripennis

Octotoma scabripennis, the leaf mining hispid beetle, was present at 18.3 % of the sites surveyed (Figure 4.31). It is considered to be the "inland" of the two hispine agents introduced onto lantana, as indicated by Figure 4.31. It does however also occur at the coast in the KZN and EC.
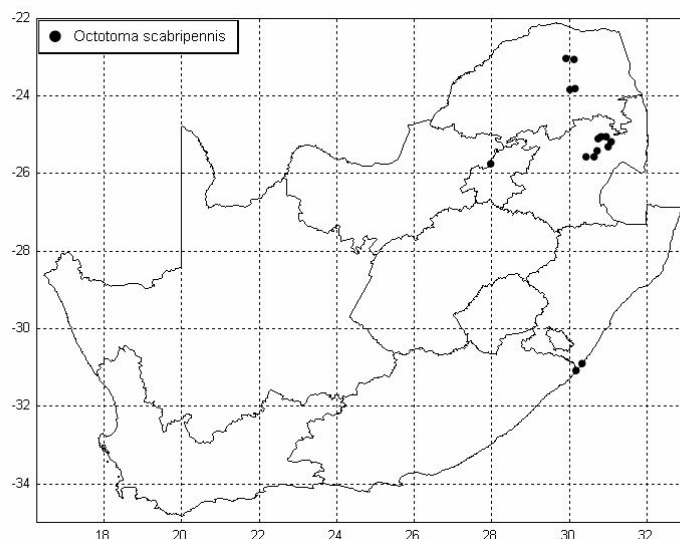

Figure 4.31 Distribution of *Octotoma scabripennis* at the *Lantana camara* sites surveyed.

This hispine occurred from 0 to 1900 m (Figure 4.32). It mostly occurred between 600 and 1000m, but the sites that had the highest populations were just above sea level and at about 1100m above sea level.
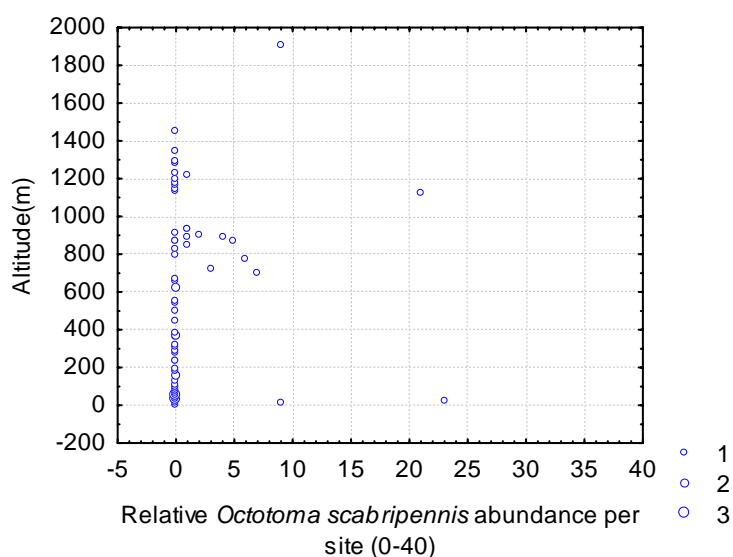

Figure 4.32 Relative *Octotoma scabripennis* abundance per site (0-40) at different altitudes during the survey (1 = 1 site at altitude with given abundance rating, 2 = 2 sites at altitude with same abundance rating, 3 = 3 sites at same altitude with same abundance rating).

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The data from outside the insects range (GP, WC and western side of the EC) were excluded and *O. scabripennis* preferred lightpink –pink (lpp) coloured varieties of *L. camara* (Figure 4.33). Two other varieties Po and Ww had the highest populations of the agent.
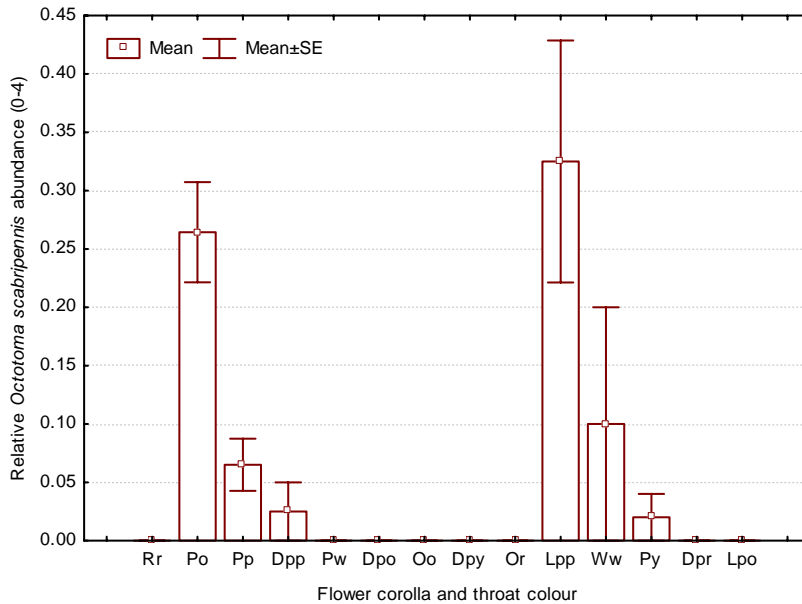

Figure 4.33 Mean relative *Octotoma scabripennis* abundance per plant on different mature flower corolla lobe and throat colour combinations of *Lantana camara* that occurred in the suitable geographic and altitudinal range for the species during the survey.

Despite being a leaf feeder, the leaf mining beetle, *O. scabripennis* preferred lantana varieties that had very hairy leaves (Figure 4.34). The medium leaves were next preferred and the lowest populations were recorded on smoother leaved varieties.
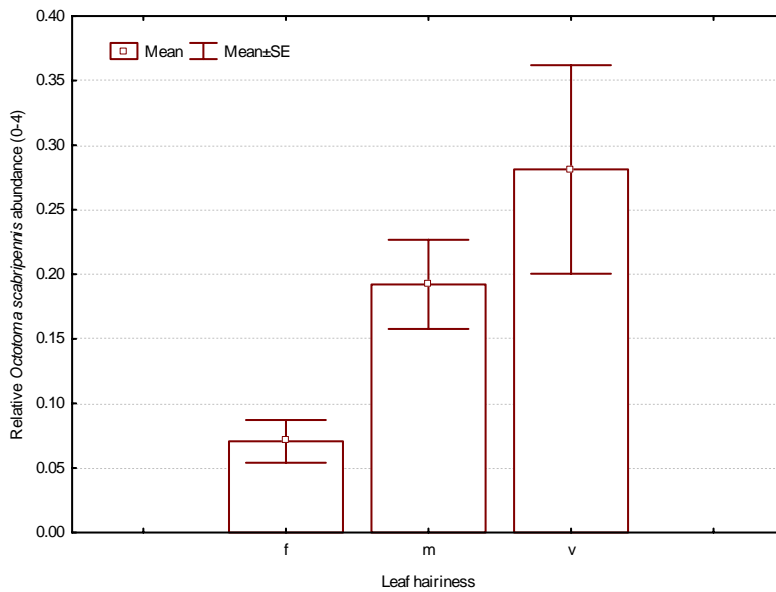
Figure 4.34 Mean relative *Octotoma scabripennis* abundance per plant on *Lantana camara* that had few (f), medium (m) and very hairy leaves (v) that occurred in the suitable geographic and altitudinal range for the species during the survey.

Ophiomya lantanae

Ophiomya lantanae, the fruit-mining fly, had a very wide distribution in South Africa (Figure 4.35). It occurred at 82.9 % of the sites surveyed and in all the provinces where *L. camara* is weedy.
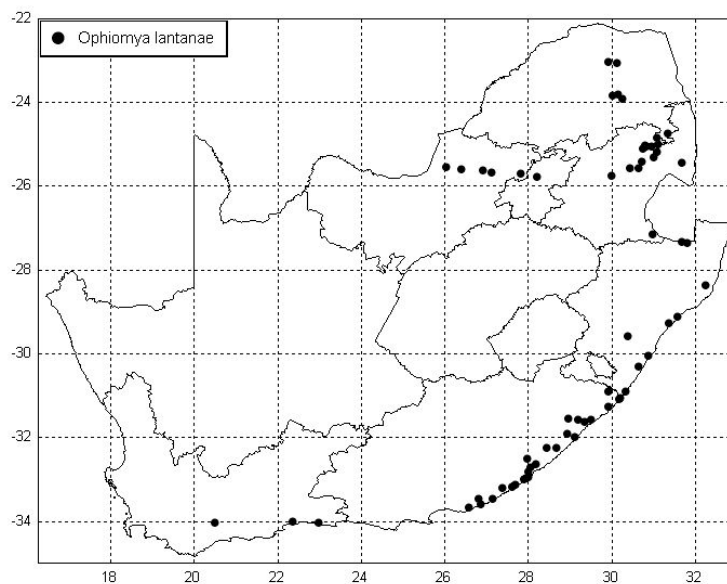

Figure 4.35 Distribution of *Ophiomya lantanae* at the *Lantana camara* sites surveyed.

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The fruit mining fly reached high population ratings (up to 30 of a maximum of 40 per site) (Figure 4.36). It was not affected by altitude, and built up equal populations both in the highveld and at the coast regions.
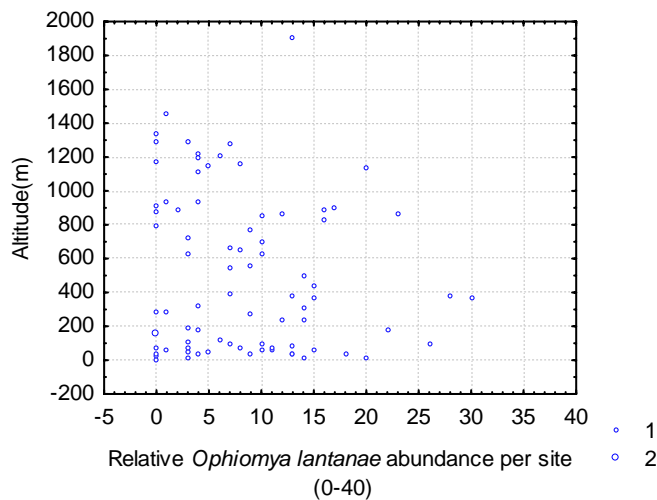

Figure 4.36 Relative *Ophiomya lantanae* abundance per site (0-40) at sites with different altitudes during the survey (1 = 1 site at altitude with given abundance rating, 2 = 2 sites at altitude with same abundance rating).

Nationally, *O. lantanae* populations were relatively high on most flower colour combinations (Figure 4.37). Two colour forms, Dpr, and Oo, sustained high populations, while Dpo, and Pw were considerably less suitable than other varieties.
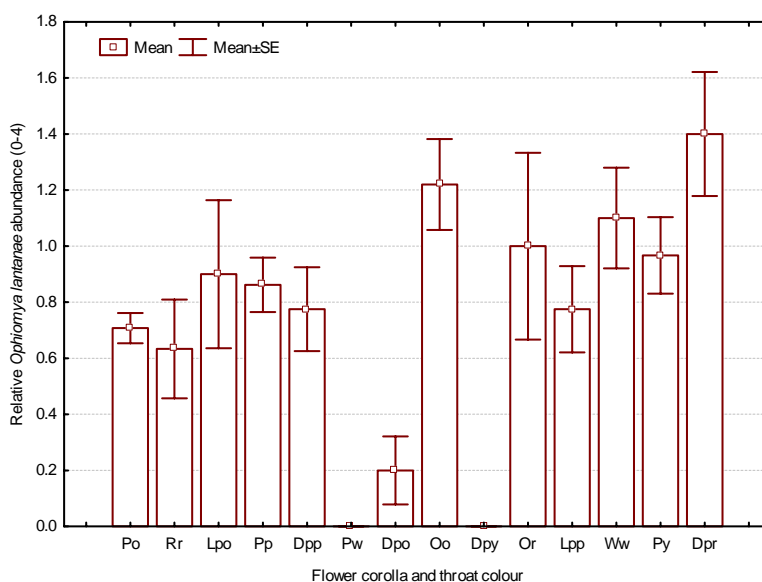

Figure 4.37 Mean relative *Ophiomya lantanae* abundance per plant on different mature flower corolla lobe and throat colour combinations of *Lantana camara* that occurred in the suitable geographic and altitudinal range for the species during the survey.

Nationally, *O. lantanae* built up the highest population levels on varieties with the least number of leaf hairs. This was followed by medium leaf hairiness, and varieties with very hairy leaves had about a quarter of the population levels occurring on the smoother leaf varieties (Figure 4.38).
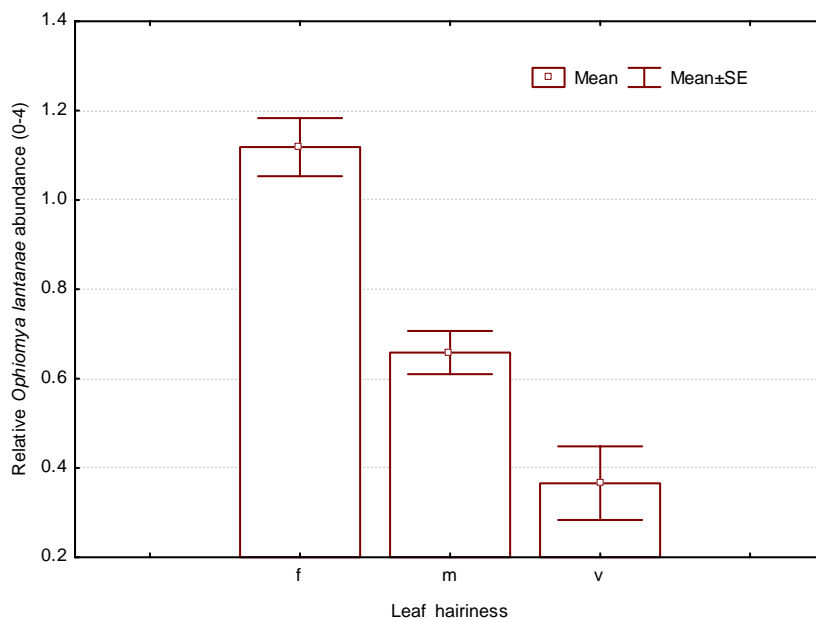

Figure 4.38 Mean relative *Ophiomya lantanae* abundance per plant on *Lantana camara* that had few (f), medium (m) and very hairy leaves (v) that occurred in the suitable geographic and altitudinal range for the species during the survey.

Epinotea lantanae

The flower and fruit mining moth, *E. lantanae*, were recorded from lantana throughout the weeds range (Figure 4.39). It was found at 58.5 % of all the sites surveyed, but notably absent from the NW province. However, Baars (2003) found this species around Hartebeespoort in the NW province during surveys in 1998-2000.
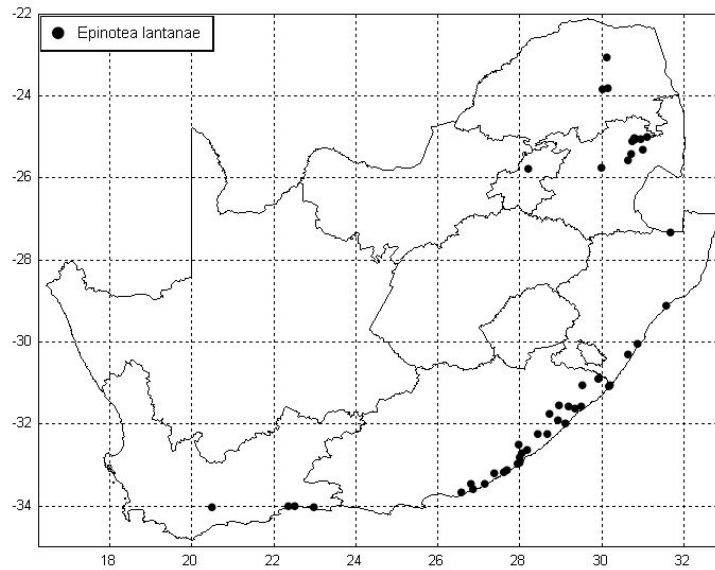
Figure 4.39 Distribution of *Epinotea lantanae* at the *Lantana camara* sites surveyed.

Epinotea lantanae occurred at all the altitudes surveyed (Figure 4.40). There was, however, a tendency to be present at more sites at lower altitudes.
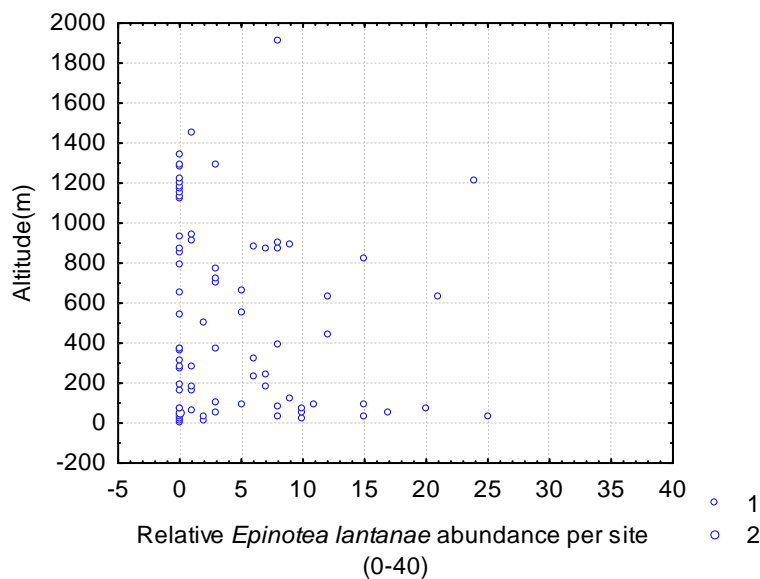
Figure 4.40 Relative *Epinotea lantanae* abundance per site (0-40) at different altitudes during the survey (1 = 1 site at altitude with given abundance rating, 2 = 2 sites at altitude with same abundance rating).

Epinotea lantanae populations were highest on Or coloured flowers of *L. camara* varieties (Figure 4.41). The standard error of this mean population was relatively high with the result that populations on the top 9 varieties were somewhat similar. Three lantana varieties had consistently slightly lower populations and two colour forms were not utilised.
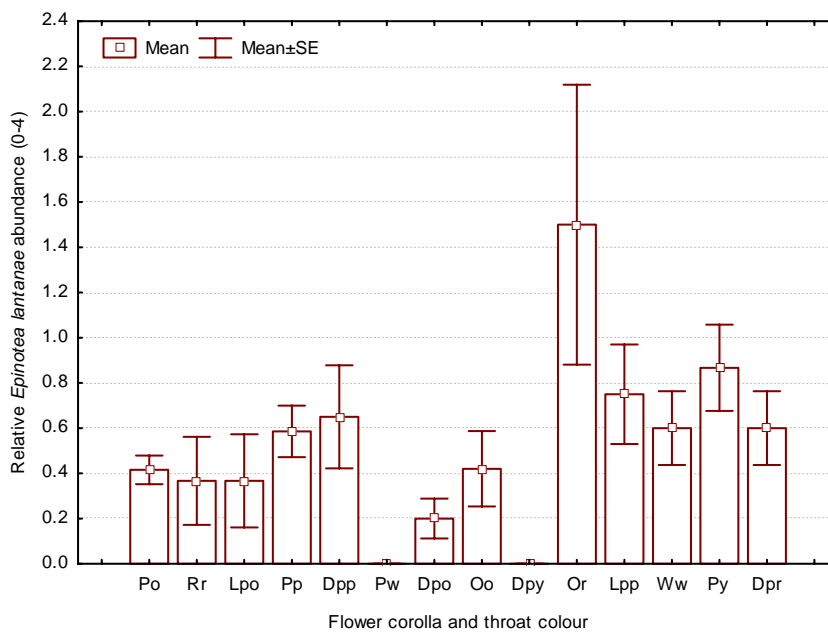
Figure 4.41 Mean relative *Epinotea lantanae* abundance per plant on different mature flower corolla lobe and throat colour combinations of *Lantana camara* that occurred in the suitable geographic and altitudinal range for the species during the survey.

Epinotea lantanae reached a mean population of about 0.7 out of a possible 4 on lantana varieties that were grouped as having few leaf hairs (figure 4.42). Half this population levels were achieved on varieties with medium hairy leaves, and very low populations (just more than 0.1) were recorded on the varieties with very hairy leaves.
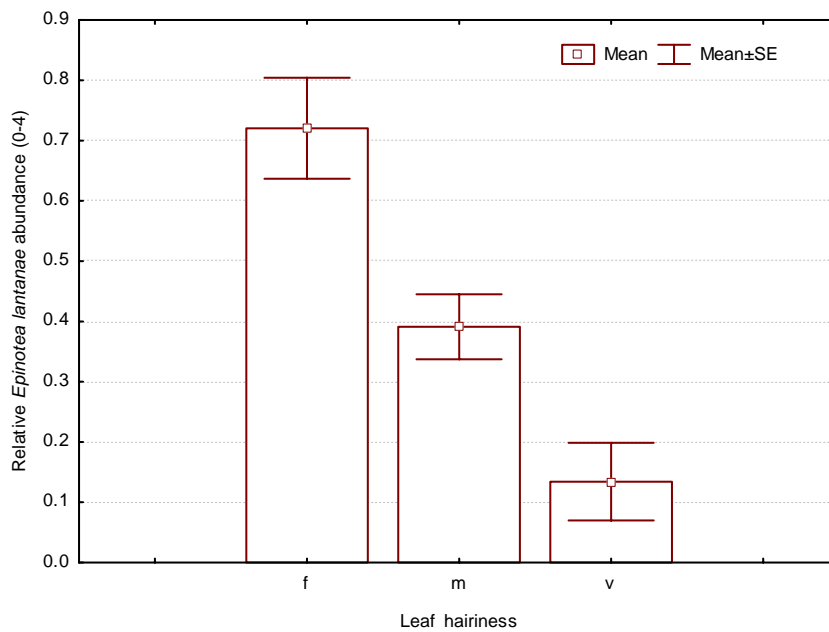
Figure 4.42 Mean relative *Epinotea lantanae* abundance per plant on *Lantana camara* that had few (f), medium (m) and very hairy leaves (v) that occurred in the suitable geographic and altitudinal range for the species during the survey.

Hypena laceratalis

Hypena laceratalis was found at 95.1 % of the sites surveyed (Figure 4.43). It occurred in all the provinces where lantana is naturalised.
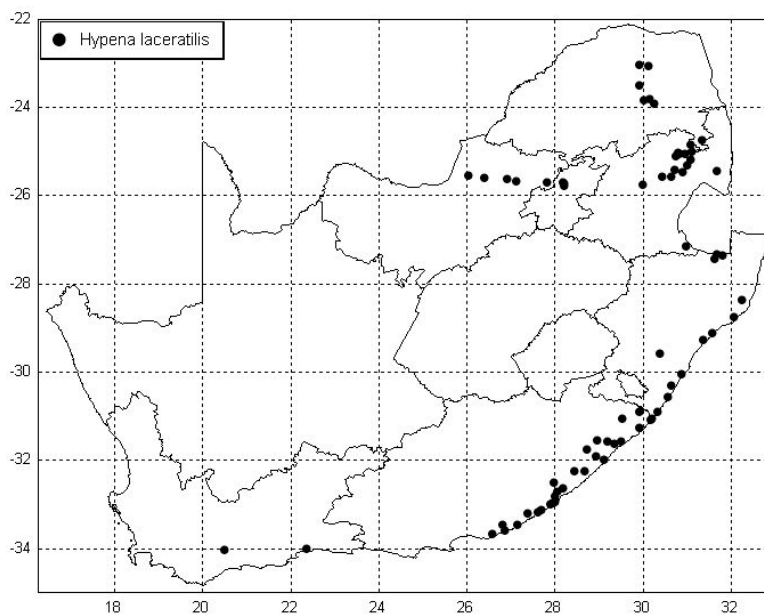
Figure 4.43 Distribution of *Hypena laceratalis* at the *Lantana camara* sites surveyed.

The leaf chewing lepidopteran, *H. laceratalis* was encountered at all the altitudes surveyed, but slightly higher population levels were noted to occur at lower lying sites (Figure 4.44).
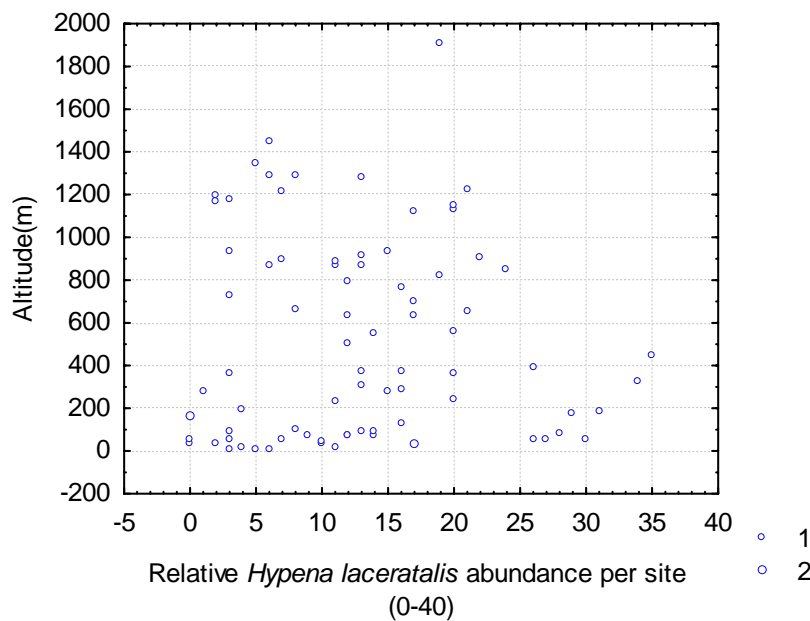
Figure 4.44 Relative *Hypena laceratalis* abundance per site (0-40) at different altitudes during the survey (1 = 1 site at altitude with given abundance rating, 2 = 2 sites at altitude with same abundance rating).

Nine colour forms of lantana had a mean national population rating over 1, but did not exceed a value of 2 (Figure 4.45). One colour variety, namely Dpp, had a rating of about 0.7 and the remaining 4 varieties all had low ratings of 0.4 or lower. This insect had populations on all of the colour varieties found during the survey.
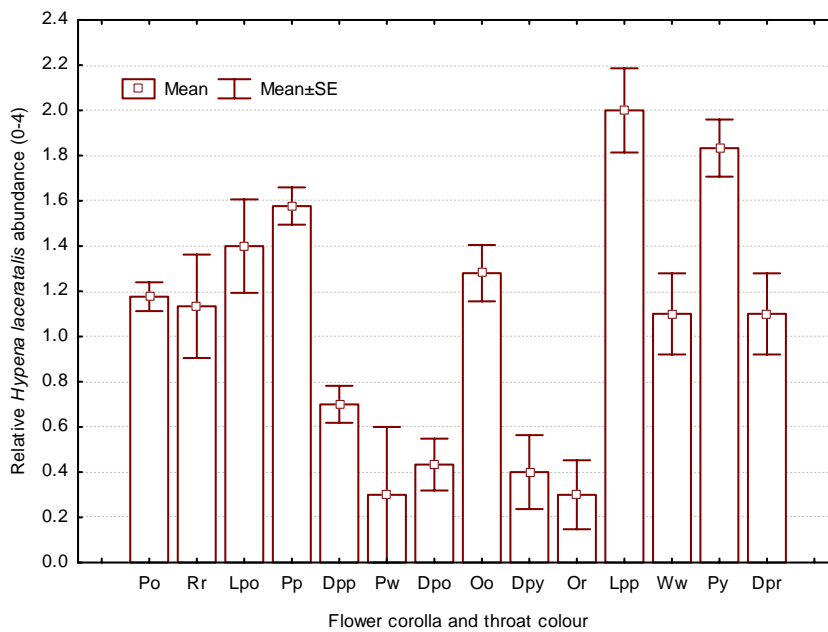
Figure 4.45 Mean relative *Hypena laceratalis* abundance on different mature flower corolla lobe and throat colour combinations per plant of *Lantana camara* that occurred in the suitable geographic and altitudinal range for the species during the survey.

Nationally, *H. laceratalis* populations almost reached a rating of 1.6 out of a potential 4, on lantana varieties with few leaf hairs (Figure 4.46). Slightly lower populations were recorded from lantana varieties grouped as medium hairy plants, and notably lower population levels were recorded on varieties with very hairy leaves.

Figure 4.46 Mean relative *Hypena laceratalis* abundance per plant on *Lantana camara* that had few (f), medium (m) and very hairy leaves (v) that occurred in the suitable geographic and altitudinal range for the species during the survey.

Salbia haemorrhoidalis

Salbia haemorrhoidalis, a leaf rolling and chewing moth, was recorded at 30.5 % of the sites surveyed (Figure 4.47). It was not found in three provinces during the survey, where lantana is naturalised.
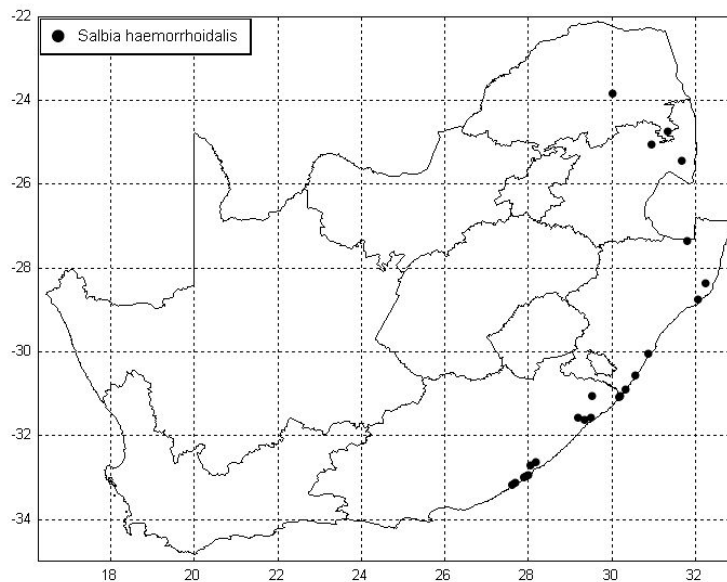
Figure 4.47 Distribution of *Salbia haemorrhoidalis* at the *Lantana camara* sites surveyed.

This moth mostly occurred at sites below 400m, where very high populations were sometimes recorded (Figure 4.48). At only a few sites this agent was found at higher altitudes, but seldom at sites higher than 900m.

Figure 4.48 Relative *Salbia haemorrhoidalis* abundance per site (0-40) at different altitudes during the survey (1 = 1 site at altitude with given abundance rating, 2 = 2 sites at altitude with same abundance rating).

Salbia haemorrhoidalis was found on 11 of the 14 flower colour groupings occurring in its range (below 1000m, E of 27° latitude, and excluding GP) (Figure 4.49). The highest population levels were recorded on Pw, followed by Lpo. The other varieties had much lower populations and the moth was not found to occur on the predominantly red and orange coloured varieties.

Figure 4.49 Mean relative *Salbia haemorrhoidalis* abundance per plant on different mature flower corolla lobe and throat colour combinations of *Lantana camara* that occurred in the suitable geographic and altitudinal range for the species during the survey.

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The leaf chewing moth, *S. haemorrhoidalis* were not found to occur on varieties with very hairy leaves (Figure 4.50). Varieties with medium to few hairs on the leaves were both suitable, with the highest populations recorded on the former.
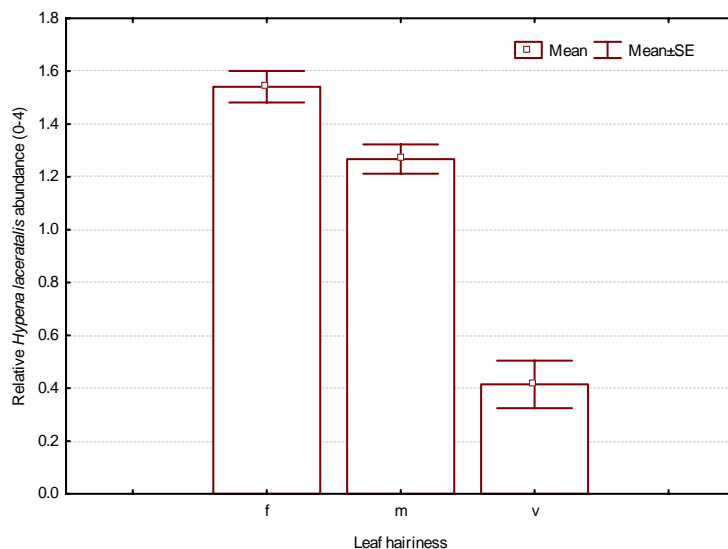

Figure 4.50 Mean relative *Salbia haemorrhoidalis* abundance per plant on *Lantana camara* that had few (f), medium (m) and very hairy leaves (v) that occurred in the suitable geographic and altitudinal range for the species during the survey.

Lantanophaga pusillidactyla

Lantanophaga pusillidactyla was present at 19.5% of sites surveyed (Figure 4.51). It was found in the LP, MP, GP, NW and EC provinces of South Africa. Baars (2003), however, found it at several sites in KZN, but not in GP or in the NW Province.
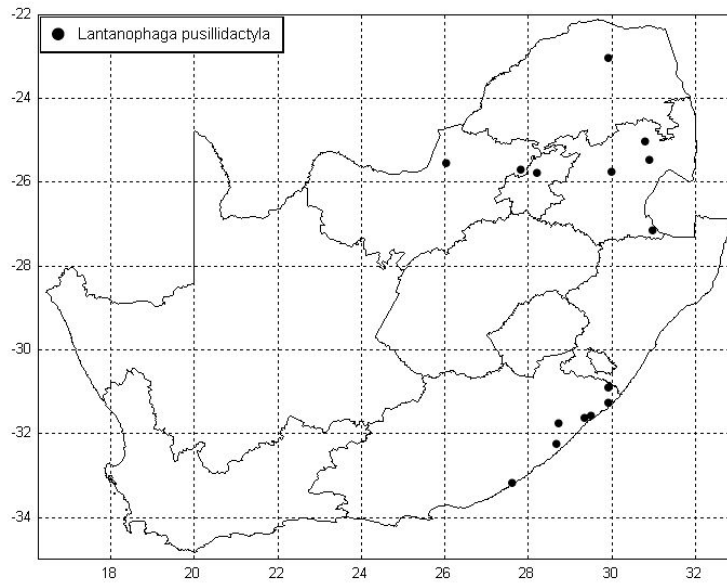
Figure 4.51 Distribution of *Lantanophaga pusillidactyla* at the *Lantana camara* sites surveyed.

The moth was found to occur at a wide range of altitudes (Figure 4.52). Population levels however, were mostly rare, but on some occasions reached slightly higher populations at sites that were below altitudes of 400m.
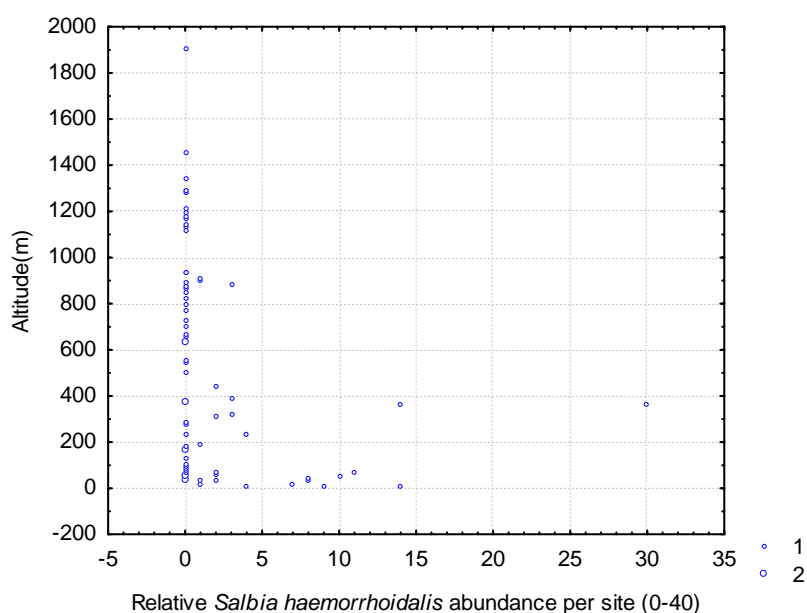

Figure 4.52 Relative *Lantanophaga pusillidactyla* abundance per site (0-40) at different altitudes during the survey (1 = 1 site at altitude with given abundance rating, 2 = 2 sites at altitude with same abundance rating, 3 = 3 sites at same altitude with same abundance rating).

All the fourteen colour variety groupings recorded during this study occurred at the sites where *L. pusillidactyla* was found, i.e. all the varieties occurring North of 26º S latitude, and East of 27º longitude (Figure 4.53). Populations were noted to be highest on the dark pink – red (Dpr) flower colour variety. Populations were less on Oo and Lpp, followed by Rr and Lpo. The varieties that supported the lowest populations included Dpo, Pp, and the most common colour form, Po (Figure 4.2).
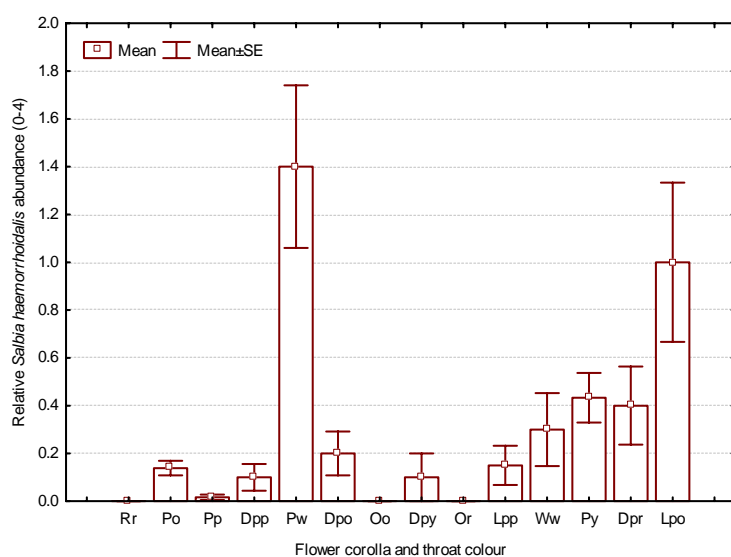

Figure 4.53 Mean relative *Lantanophaga pusillidactyla* abundance per plant on different mature flower corolla lobe and throat colour combinations of *Lantana camara* that occurred in the suitable geographic and altitudinal range for the species during the survey.

The moth populations were highest on the medium haired varieties, followed by varieties that had few leaf hairs (Figure 4.54). Varieties that had very hairy leaves had about a third of the population levels recorded on the other two lantana leaf hairiness groups.
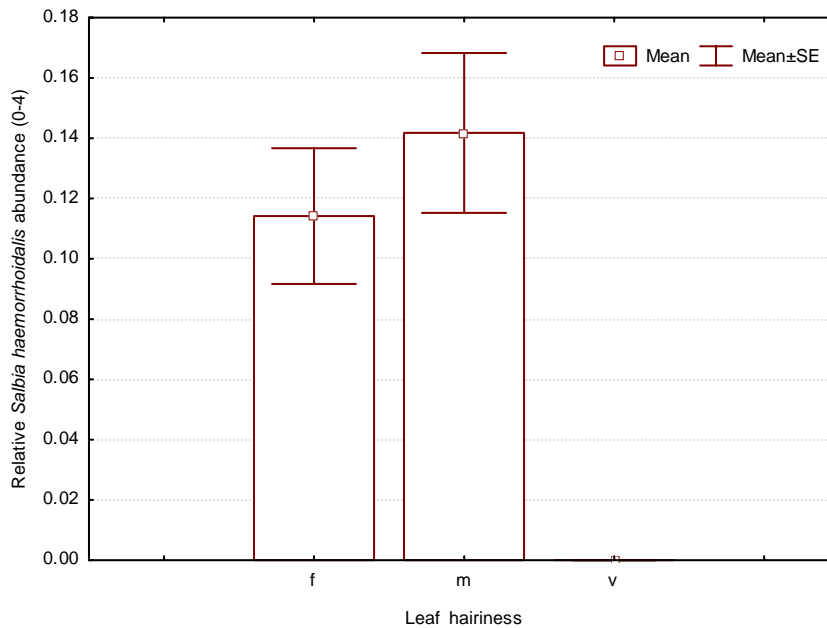
Figure 4.54 Mean relative *Lantanophaga pusillidactyla* abundance per plant on *Lantana camara* that had few (f), medium (m) and very hairy leaves (v) that occurred in the suitable geographic and altitudinal range for the species during the survey.

Aristea onychote

Aristea onychote, a leaf mining lepidopteran, reportedly indigenous, had a very wide distribution range (Figure 4.55). Excluding the WC, it occurred in all other provinces where *L. camara* is invasive. It was found at 72 % of the sites surveyed.
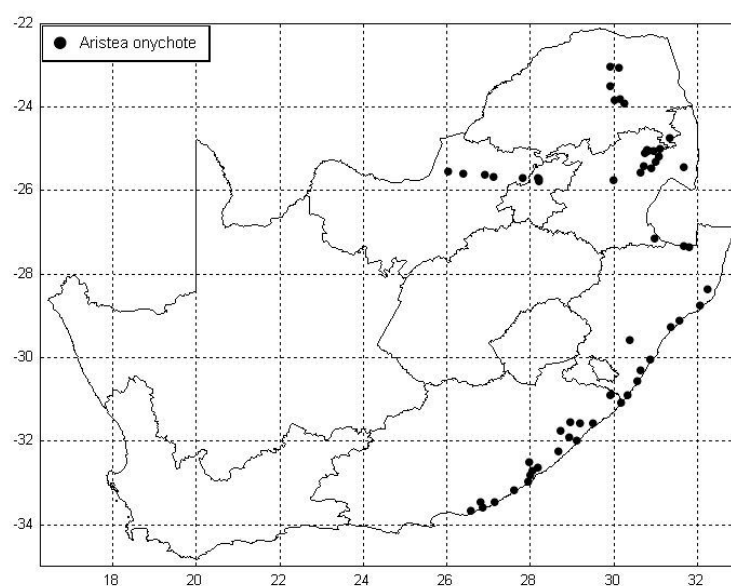
Figure 4.55 Distribution of *Aristaea onychote* at the *Lantana camara* sites surveyed.

The blister miner moth, *A. onychote*, had populations on lantana at all altitudes, where lantana is invasive in South Africa (Figure 4.56). Relatively similar population levels were recorded from coastal to highveld sites, across the altitude gradient.
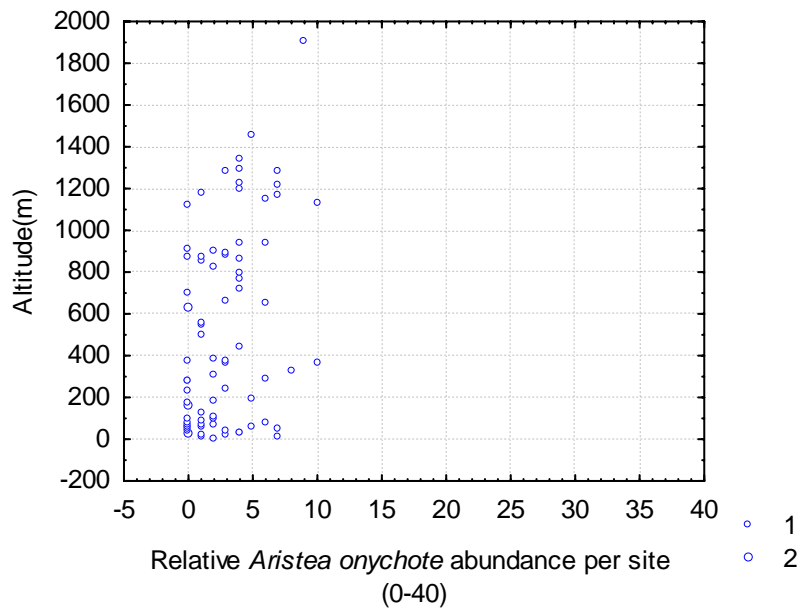
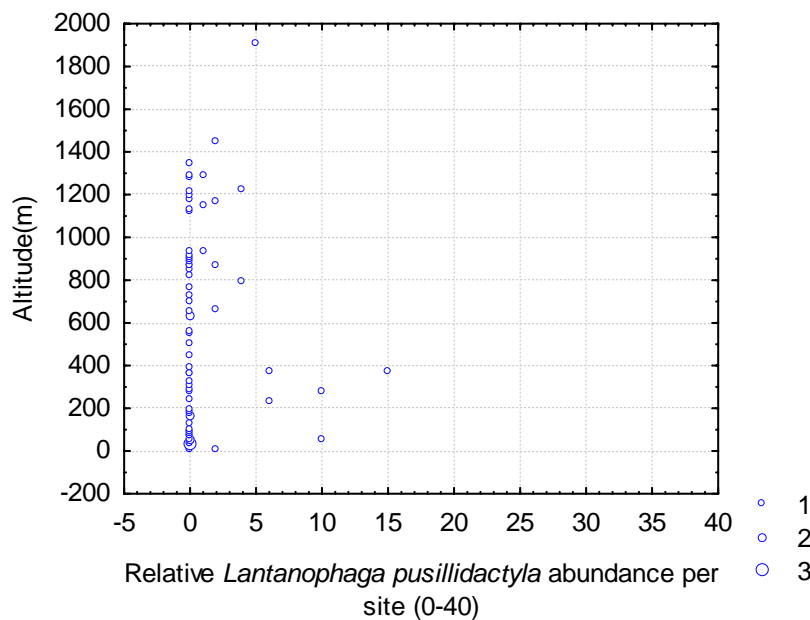**Figure 4.56** Relative *Aristaea onychote* abundance per site (0-40) at different altitudes during the survey (1 = 1 site at altitude with given abundance rating, 2 = 2 sites at altitude with same abundance rating).

All 14 colour varieties were recorded to occur in the moth's distribution range (i.e. N of 26º S latitude, and E of 26ºE longitude) (Figure 4.57). The highest number of mines were noted to occur on Lpp, and Rr flowering cultivars of lantana. Other varieties that supported the next best population levels were Lpo, Ddp, Ww, Po, Pw and Py. Slightly lower populations were found on Pp, Oo, Dpo, Dpy and Or. One of the varieties, namely Dpr had no blister mines present at any of the sites.
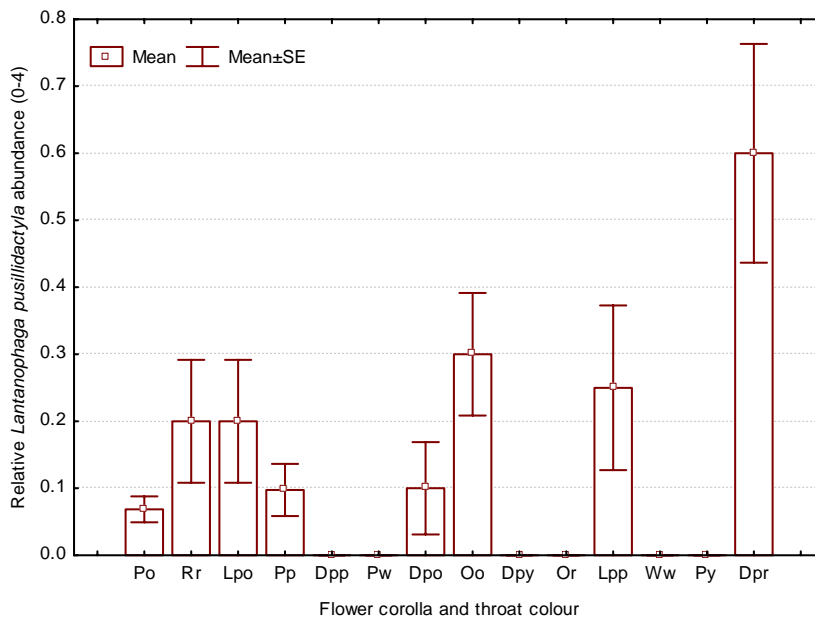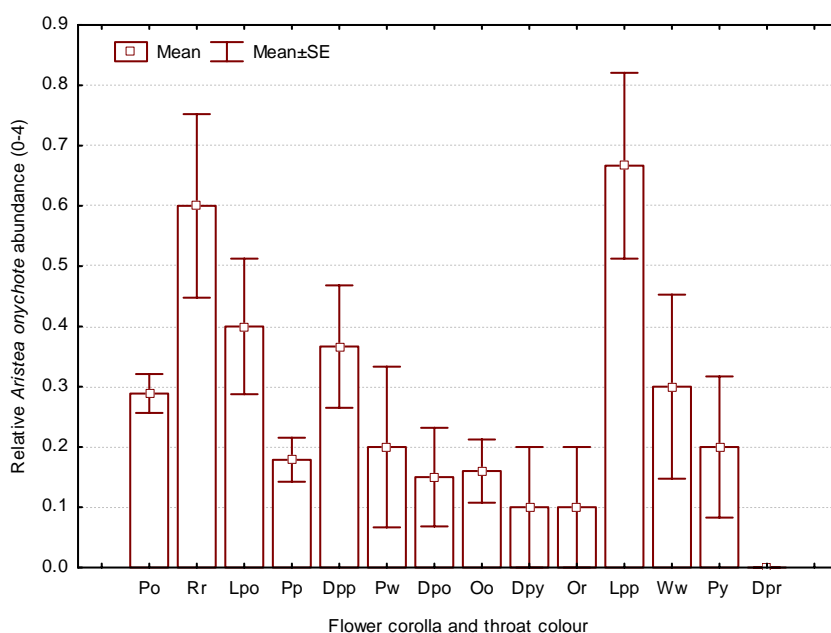
Figure 4.57 Mean relative *Aristaea onychote* abundance per plant on different mature flower corolla lobe and throat colour combinations of *Lantana camara* that occurred in the suitable geographic and altitudinal range for the species during the survey.

Aristaea onychote was most abundant on the most hairy varieties of *L. camara* (Figure 4.58). Populations on the medium hairy leaved varieties were nearly as high, and varieties with few leaf hairs had almost half as many mines on the leaves as that recorded on the hairy forms.
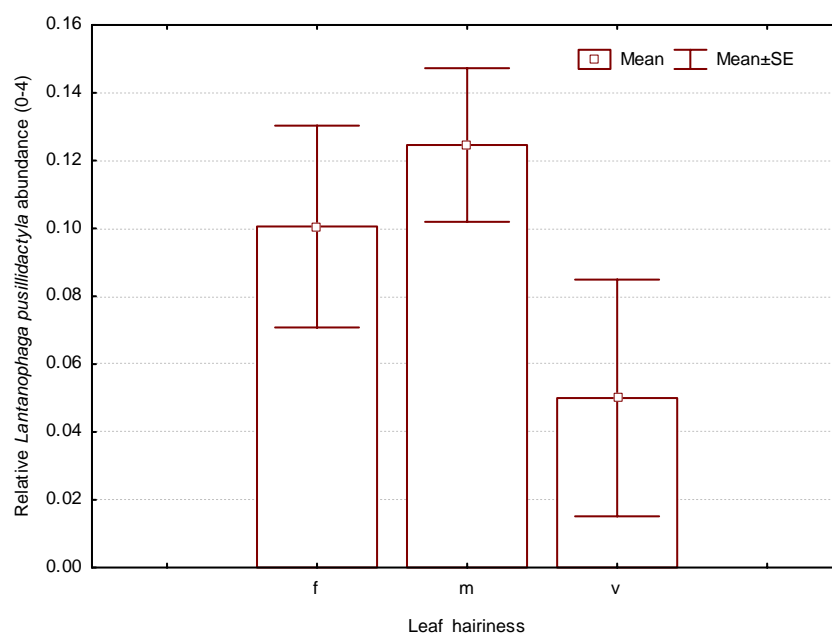

Figure 4.58 Mean relative *Aristaea onychote* abundance per plant on *Lantana camara* had few (f), medium (m) and very hairy leaves (v) that occurred in the suitable geographic and altitudinal range for the species during the survey.

Characoma submediana

Characoma submediana, the flower and fruit feeding noctuid moth, was present at 22.0 % of the sites surveyed (Figure 4.59). Despite the low occurrence, it was found at sites in all the provinces where lantana is naturalised.
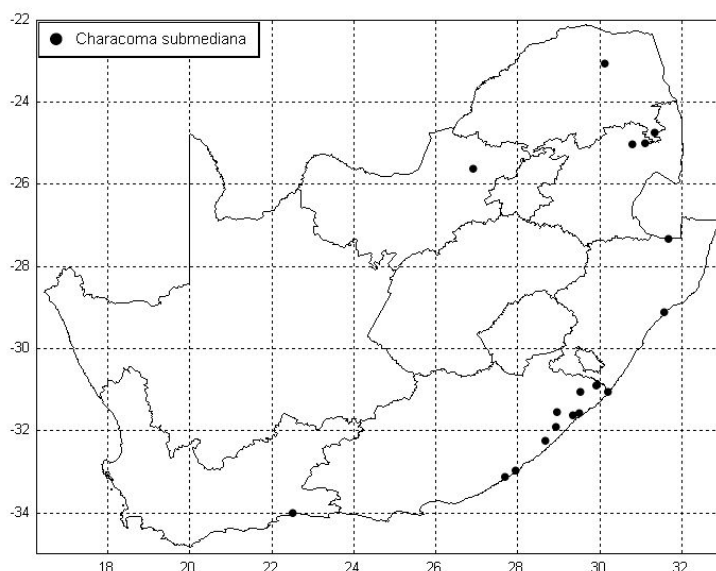
Figure 4.59 Distribution of *Characoma submediana* at the *Lantana camara* sites surveyed.

The moth maintained populations at sites from most altitudes (Figure 4.60). It was found from 0 to over 1300m, but always at low population levels.
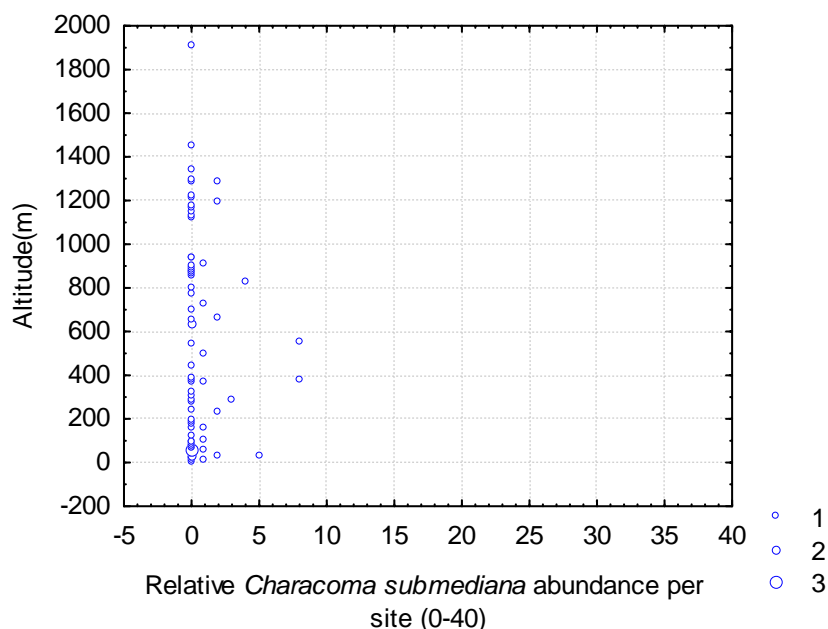
Figure 4.60 Relative *Characoma submediana* abundance per site (0-40) at different altitudes during the survey (1 = 1 site at altitude with given abundance rating, 2 = 2 sites at altitude with same abundance rating, 3 = 3 sites at same altitude with same abundance rating).

*Characoma submediana* populations were highest on Pp, and Dpr varieties of *L. camara,* occurring E of 22º E longitude, below 1400m altitude in South Africa (Figure 4.61). The next highest population levels were recorded on Py, and Dpo, which were half as numerous. Numbers recorded on Po, Dpp and Oo were about 25 % of those recorded on the varieties with the highest number of larvae. Seven of the varieties that co-occurred at sites were not utilized at all. These means are however very low and caution needs to be exercised, as populations may have been too low to be detected on all the varieties.
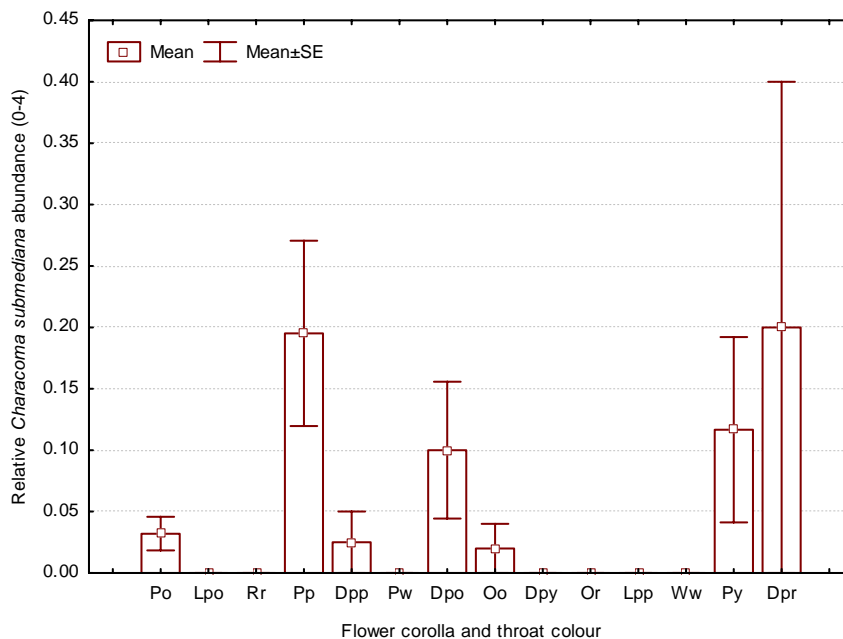
Figure 4.61 Mean relative *Characoma submediana* abundance per plant on different mature flower corolla lobe and throat colour combinations of *Lantana camara* that occurred in the suitable geographic and altitudinal range for the species during the survey.

Characoma submediana have little preferences between levels of hairiness of leaves of varieties (Figure 4.62). The populations were extremely low on all of the levels of hairiness of leaves, as the lowest, i.e. very hairy leaves, had a mean of 0.04, and the medium haired varieties had only about 0.075 out of a possible population of 4. These means are therefore too low, with a very small difference between varieties, to draw any meaningful trends in varietal suitability.

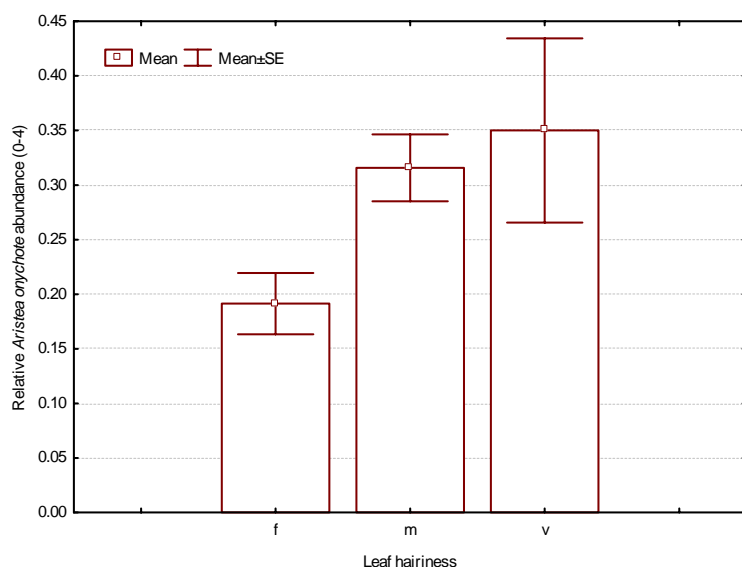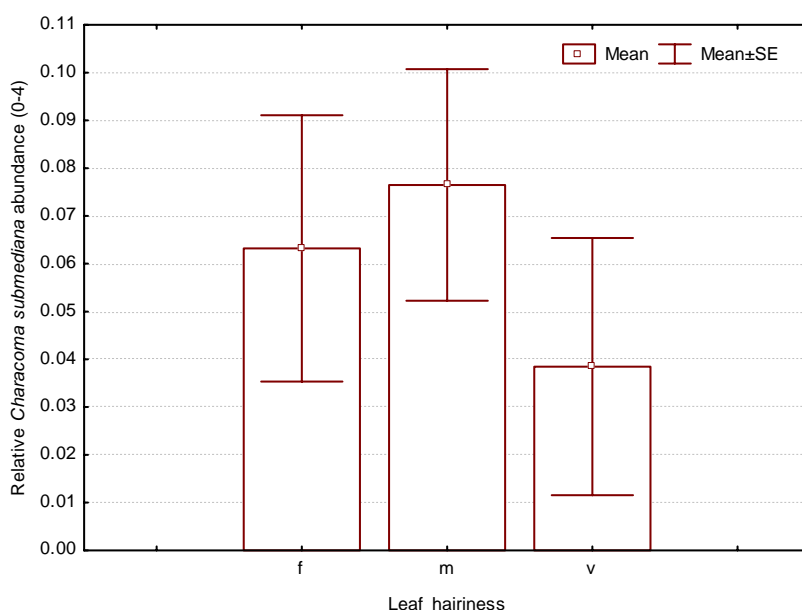**Figure 4.62** Mean relative *Characoma submediana* abundance per plant on *Lantana camara* that had few (f), medium (m) and very hairy leaves (v) that occurred in the suitable geographic and altitudinal range for the species during the survey.

4.4 Discussion and Conclusion

The field survey was conducted over the full geographical range of *L. camara.* A large proportion of the survey concentrated on areas that had not been surveyed previously. Almost 37 % of sites sampled were from the Eastern Cape, most of which had not been previously surveyed. New areas in the North West Province were also included in the survey. The relative abundance of *L. camara* in all provinces needs to be taken into account if abundance levels of agents should be directly compared using the current sampling methods. Care needs to be taken in the interpretation of the results, as the survey does not include the entire extent of the naturalized range of lantana. The intensity of the sampling did vary between areas, which may lead to some bias in assessing the abundance levels attained for some agents. However, lantana is considered to be most invasive and pervasive in areas such as the east coast and lowveld, which if taken into account may justify the overemphasis of these areas in this survey.

The Pink-orange flowering *L. camara* variety was rated as most abundant in South Africa during this survey, and is likely thus to be the most common. This, or similar variety has also been thought to be one of the most abundant varieties in Queensland, Australia (Smith and Smith 1982), referred to as the "common pink". Their description was however to a variety

with set characters, using flower colour only as a method of describing it, where this most common colour variant of lantana exhibited different characters towards leaf hairiness (Figure 4.2), and probably other characters too, as different methods were used in these variety descriptions.

The results of insect species abundance will undoubtedly vary annually and during the course of the growing season, and the results in this study reflect the situation for the period of the survey. As an example, Baars and Heystek (2003a) found *C. lantanae* to be very rare during surveys in 1998 to 2000, even though wide spread. These insects had a very good season during 2004 to 2005, and were rated as the second most abundant species (Figure 4.6). It was suggested that their numbers are severely suppressed by parasitism (Baars & Neser 1999; Day & Neser 2000; Baars & Heystek 2003a). It is therefore assumed that it may have been able to, at least temporarily, escape parasitism, or may have built up in numbers and may be part of a natural boom-bust cycle over a number of years. Baars and Heystek (2003a) and Baars (2003) did not rate insect abundance as a mean, or compared insect species, and three additional species were surveyed during this survey, and it is therefore difficult to note subtle changes in insect species abundance since that survey.

Teleonemia scrupulosa was one of the most widespread and damaging of the introduced agents in South Africa on *L. camara*. These results are similar to those of Baars and Heystek (2003a). *Teleonemia scrupulosa* did indeed show varietal preferences for colour forms of *L. camara*, as reported in the literature (Haseler 1963; Harley & Kassulke 1971; Radunz 1971; Harley *et al.* 1979; Cilliers 1987b; Cilliers & Neser 1991; Day & Neser 2000). Interestingly, the tingid had low populations on the most abundant colour form of *L. camara* (Po) in South Africa. Considering that it is a very widespread and abundant variety (rated about twice as abundant as Lpp), the impact on the weed by the tingid would have been rated considerably higher if this variety was utilised as much as the favoured variety (Lpp). It is fortunate that the *L. camara* varieties that have very hairy leaves were rated as least abundant by far, as the tingid considered the most damaging agent (Cilliers & Neser 1991, Baars & Neser 1999; Baars & Heystek 2003a), avoided these hairy-leafed lantana varieties.

Falconia intermedia has only established in a few areas (Heystek & Olckers 2003). This was confirmed by this survey, showing it was limited to a few sites in the Limpopo, Mpumalanga and Eastern Cape provinces. This insect was collected and imported from a single locality in

Jamaica (Baars 2002a; Heystek & Olckers 2003). This could explain the very specific and climatic narrow requirements of this species. Baars (2002a) has found *F. intermedia* to have varietal preferences between South African cultivars under laboratory conditions (Chapter 2), and has been shown to be the case during this survey (Figure 4.13). If the insect's climatic or other requirements are not met at more sites with different culivars, the varietal importance may be considerably less than reflected in Figure 4.13. Heshula (2005) found that plant condition plays a major role in the mirids performance, when Eastern Cape varieties of *L. camara* were compared both in the laboratory and the field. Because of the localized distribution, avoidance of plants that are very hairy should be interpreted with caution. The results probably reflect an accurate estimation, as the mirid has failed to establish on plants that were very hairy, after repeated extremely large releases (> 10 000 individuals), at two sites that otherwise should have been acceptable for the mirid, as it was in areas (Tzaneen, Limpopo Province) where mirid populations have persisted at extremely high levels for several years, shortly after releases in 2000 and 2001 (Heystek, unpublished data).

Orthezia insignis may have a more common distribution than reflected during the survey. At times, it has become a pest on potted plants in the PPRI nursery (personal observations). This species may have had more species specific environmental pressures, e.g. sensitivity to rainfall, temperature or parasitism, during the period of the survey and may have been undetected at low densities at some sites. The wide geographic area, and altitude range it occupies, may also suggest this species might be more common. The mealybug most likely does have strong varietal preferences as the data suggests (Figure 4.29). It was found on a large range of varieties, over a large area, with variable population densities, which suggest a definite association with certain varieties. The fact that very hairy plants had the highest population levels can be attributed to at least two possibilities. Hairy plants are less utilized by most biocontrol agents, this could potentially allow better quality of food for this species. Heshula (2005) reported a strong link between food quality and *F. intermedia* populations. *Orthezia insignis* are sucking sap from stems and is therefore less affected by the physical barrier of hairy leaves, which allowed it to build up to higher populations. The possibility of a correlation between hairy leaves and hairy stems are not excluded, which could then be an indication of a different character associated with hairiness of leaves as well.

The distribution of *O. scabripennis* recorded in this survey correlated strongly with that found by Cilliers and Neser (1991) and Baars and Heystek (2003a). It was however found at a site

in the North West Province, where it was thought not to occur. This species has shown very strong associations with at least three colour forms of lantana in South Africa (Figure 4.33). *Octotoma scabripennis*, like *O. insignis* has shown to have higher populations on hairy leaved varieties. This species therefore contributes to control of less utilized varieties of lantana. The phenomena of this species utilizing hairy leaved plants, despite being a leaf feeder can possibly be explained through its mode of feeding. Adults feed mostly at the top of leaves, while the leaves are most hairy on the undersurface (personal observations). Eggs are also laid on top of leaves, and larvae mine internally. Hereafter the preference for these varieties can be considered as diverse as any species' preference for any lantana variety. Biocontrol agents usually preferred least hairy plants, followed by medium hairy plants and least preferred very hairy plants, or the preference was the inverse hereof (Figures 4.14; 4.18; 4.34; 4.38; 4.42; 4.46 & 4.58). This reinforces the theory that a definite preference for either hairy or smooth leaves exists. This may be linked to another character of the plant though, but demonstrate an active choice executed by the biocontrol agents.

The second hispine leaf miner, *Uroplata girardi* that has been shown to have varietal preferences in the laboratory, also exhibited strong preferences in the field (Chapter 2; Figure 4.17 & 4.18).This species exhibits the opposite preferences towards colour varieties and leaf hairiness as *O. scabripennis*. This may support the theory of Day and Neser (2000), whom argued that lantana biocontrol agents are not necessarily pre-adapted to feed and breed on cultivars with characters very different from its original host. *Uroplata girardi* is native to countries in South America, and *O. scabripennis*, is native in the southern parts of the Northern continent (Cilliers & Neser 1991). These opposite preferences in variety qualities and distinct geographical distribution suggest South African varieties to have characters that may have originated from both areas. It also shows that agents have strong climatically specific requirements. For most species this is obvious, but these two hispines are extremely similar species, with seemingly exactly the same niche, that both have similar requirements to complete its life cycle. Authors have implied that biocontrol agents that have a wide geographical distribution in the country of origin, will exhibit that in the country of introduction (Baars 2000b; Day & Neser 2000; Baars & Heystek 2001). The difference in the geographical distribution of both these similar species supports the theory to collect biocontrol agents over a wide geographical range and to have a wide pre-adapted genetic pool in the population of the biocontrol agent. It suggests that re-introductions of established agents from another area may have value. These two agents, *O. scabripennis*, and *U. girardi*,

however complement one another and together contribute to the control of different varieties of lantana, in different regions in the country. Baars and Heystek (2003a) considered these insects, especially *U. girardi* at the KZN coast, to contribute significantly to the biocontrol of *L. camara*. The populations were however very low and the impact on the weed therefore low. The populations of *U. girardi* were much higher during 1998-2000 (Baars & Heystek 2003a), than during this survey. This can possibly be attributed to the high populations of *O. camarae* that built up on the KZN coast since its release in 2000. This leaf mining fly has a shorter life cycle than *U. girardi*, and cause premature leaf drop on *L. camara* (Cilliers 1987a; Simelane 2002). This possibly disrupts the life cycle of *U. girardi*, and may have caused their populations to decrease. The mines of both species have been observed on the same leaves, but the fly mines, far out numbered the hispine mines at these sites. This highlights the importance of such distribution surveys in order to assess these interactions in the future.

Ophiomya camarae has reached a wide distribution in South Africa, since its introduction in 2000 (Figure 4.19). Findings of this survey correlate with that of Simelane and Phenye (2004) that the fly only established at sites below 900m. *Ophiomya camarae,* like *U. girardi* had a preference for Pw varieties. This further strengthens the theory that *O. camarae* may be out competing *U. girardi*, under current environmental conditions as experienced during the survey, where both these species occur sympatrically. *Ophiomya camarae* is widespread in central and South America, but was introduced into South Africa, from Florida, where it also may have been introduced along with plant material (Simelane 2002). *Uroplata giradi* is a South American species, and both species may therefore be adapted to characters of similar varieties.

Ophiomya lantanae, the fruit mining fly, and *C. lantanae*, the blotch mining fly, were reported to have some of the widest distribution ranges in South Africa (Baars & Heystek 2003a), and was confirmed with this survey. Both of these fly species and the widespread flower chewing moth *E. lantanae* showed very little preferences for flower colour varieties of *L. camara*. All three species preferred plants that had few to medium hairy leaves. The fact that a fruit mining fly and a flower feeding moth avoided varieties with very hairy leaves, could be an indication of a link between hairy leaves of the varieties, and another anti-feedant or incompatibility with the chemical composition, that is expressed in the flowers and berries.

Of the two leaf chewing moths *Hypena laceratalis* occurred at three times as many sites as *S. haemorrhoidalis* (Figures 4.43 & 4.47). The results suggest that environmental conditions typically associated with higher altitudes in South Africa are unsuitable for *S. haemorrhoidalis* to sustain a viable population (Figures 4. 44 & 4.48). Alternatively *S. haemorrhoidalis* may only occur at lower altitudes as the varieties it is able to utilize occurs in this geographic range. In deed, the field survey suggests that this species has a strong association with certain colour forms of *L. camara.* (Figures 4.45 & 4.49). Baars (2003) found similar distribution ranges for these species, which indicates a state of equilibrium between these agents. The only obvious differences between some of the sites where *S. haemorrhoidalis* was absent, was varietal differences, as sites were inter-dispersed, where it occurred, with sites where it did not occur. Inter- species competition, along with small site specific conditions, may be a factor, allowing *H. laceratalis* to be more competitive. High levels of parasitism of *S. haemorrhoidalis* may be an unlikely possibility, as Baars (2003) reported both species to be parasitized frequently. *Salbia haemorrhoidalis* is however less parasitized than *H. laceratalis,* as *H. laceratalis* is considered an indigenous species to southern Africa (Table 1.1), and *S. haemorrhoidalis* is an introduced species from Florida and Cuba. To further support the theory that parasitism is the less likely factor for the low numbers of *S. haemorrhoidalis*, Baars (2003), recorded 9 parasitoid species from larvae of *H. laceratalis*, and only 2 for the introduced *S. haemorrhoidalis*.

Lantanophaga pusillidactyla and *C. submediana*, both flower feeding lepidopterans, were far less abundant than a third species, *E. lantanae* (Figures 4.51, 4.59 & 4.39).They seem to have a similar distribution range, but *E. lantanae* occurs at more coastal sites, relative to the other two species (Figures 4.52, 4.60 & 4.40). Both of the rarer species showed more differences in populations on various varieties than *E. lantanae* (Figures 4.53, 4.61 & 4.41). The extremely low population levels achieved by *C. submediana* do however make the results in terms of varietal preferences unreliable. *Epinotea lantanae* had the highest populations on colour varieties Or and Py, both of which supported no populations of *L. pusillidactyla*. This however will not explain the higher populations of *E. lantanae*, in South Africa, as both of these varieties are among the least abundant *L. camara* in South Africa (Figure 4.2). Baars (2003) reported similarly, that populations of all these species were widespread, and rated *E. lantanae* to be more abundant than the other two species. Parasitism is an unlikely explanation for the variation in population levels of these species, as Baars (2003) found no parasitoids from *L. pusillidactyla*, and 1 parasitoid species was reared on rare occasions from

larvae of *C. submediana.* He reasoned that heavy parasitism of *E. lantanae* reduced field populations of this moth. One of the most likely remaining factors limiting populations of these species are site specific microclimates.

Aristea onychote, an indigenous leaf mining lepidopteran, is widely established in South Africa (Figure 4.55). Baars (2003) found a similar range during surveys conducted in the period of 1998-2000. Consistent with the previous surveys, populations of this species remained low country wide (Figure 4.56). The low occurrence of this species during this survey may thus be a real representation of its normal levels and the varietal associations recorded may be a reliable reflection of its host choice (Figure 4.57). This indigenous lepidopteran, like the leaf mining hispine beetle, *O. scabripennis* has a preference for plants with hairy leaves (Figure 4.58).

To determine the role of varietal preferences of agents on their population levels in the field, other factors such as climate at sites and parasitism and predation need to be considered. These factors have been eliminated during laboratory studies for 5 of the species discussed here (Chapter 2). Results from both these chapters have demonstrated that varietal preferences very clearly do affect agent population levels. It can therefore be concluded that several characters of *Lantana camara*, that may be correlated to flower colour and leaf hairiness, either attract, or repel most of the biocontrol agents to affect their variatal association in the field in South Africa. Varietal preferences will remain a factor to be considered for future introductions of biocontrol agents of lantana and this survey will provide valuable insights into targeting agents that can survive and utilize the range of colour and hairiness of varieties. More effective release programmes can now be drawn up to maximize breeding and release programmes on *L. camara.*

CHAPTER 5

Survey for possible field non-target effects of fourteen species of the biological control agents established on *Lantana camara* in South Africa

5.1 Introduction

The biological control of weeds has a very good track record of success and safety (Cruttwell McFadyen 1998). There have been very few cases of non-target effects in weed biocontrol and virtually all non-target effects were anticipated (Pemberton 2000) or considered a fair tradeoff for the potential benefits derived from the release of the agent (Cruttwell McFadyen 1998; Baars & Neser 1999). However, despite the pre-release research, the release of every biological control agent worldwide brings with it some risk. These risks have been highlighted in recent times with the non-target effects on native thistles by *Rhinocyllus conicus* Frölich and rare cacti species by *Cactoblastis cactorum* (Berg) in the USA (Louda *et al*. 2000; Stiling and Moon 2001). Pemberton (2000) showed clearly that most non-target effects could be predicted and that indigenous species closely related to the target weed were most at risk.

There are several indigenous plant species within the family Verbenaceae, in South Africa. These include several *Lantana* and *Lippia* species (Retief 2003). In laboratory-based trials, these closely related species, especially in the genus *Lippia* were often utilized by candidate biological control agents under evaluation (Baars & Neser 1999; Heystek & Baars 2001; Simelane 2002; Baars *et al*. 2003a; b; Williams 2003; Heystek & Baars 2005). Baars and Neser (1999) argued that limited feeding on *Lippia* species had to be accepted as an ecologically and environmentally justifiable "trade-off" against the potential benefits to biocontrol of *L. camara*. If not, few candidate agents would ultimately be acceptable for release on *L. camara* in South Africa. Since the renewal of the lantana biocontrol programme in South Africa in 1995, only two insect species, *F. intermedia* and *O. camarae* and a fungus, *Micovelosiella lantanae* var. *lantanae* have been released (Baars *et al*. 2003b; Simelane 2002; Den Breeÿen 2000; Den Breeÿen & Morris 2003). The insect species were both able to develop on *Lippia* species under quarantine conditions but it was decided that these were inferior hosts and unlikely to

sustain significant field populations of the agents (Baars *et al*. 2003b; Simelane 2002). During the same ten year period, nine candidate lantana biocontrol agents have been rejected and quarantine populations destroyed, due to their utilization of especially *Lippia* species (Baars 2002a; Williams 2004; Williams & Hill 2004; Heystek & Baars 2001; 2005; Mabuda, submitted; Phenye & Simelane 2003; in prep.). Another four insect species that also utilize *Lippia* species to a lesser extent, were found "suitably host-specific", and applications for their release from quarantine have been made to the regulatory authorities (Williams; Simelane; Urban. personal communication 2005). This extension of the host range of agents to include *Li. rehmannii, Li. javanica* and *Li. scaberrima* (and probably all other *Lippia* species) may be as a result of at least 1 secondary chemical in common (van Wyk *et al.* 2002) and this chemical may be what the insects are cuing into.

Weed biological control practitioners often state that laboratory host specificity tests are conservative and frequently result in false positive results in which potentially safe agents are rejected due to the utilization of species in the quarantine laboratory that would otherwise not be suitable under field conditions (Baars & Neser 1999; Baars 2000; Van Klinken 2001). In the field there is no interference with the insect's natural host selection process, but there are additional stresses on an insect population. These include varying climatic conditions, plant condition, predators and parasitoids and other biotic resistance which further reduce the viability of long-term populations on non-target species.

There are a few documented cases of non-target effects in the field. *Teleonemia scrupulosa* fed and bred on non-target species in the field in several countries. It was recorded feeding and breeding on *Sesamum indicum* Linn., and feeding on *Sesamum angustifolium* Oliv. in the field in Uganda (Davies & Greathead 1967). In Hawaii, it was recorded attacking *Myoporum sandwicensis* Naio, and species of *Xanthium*, both native to Hawaii (Davies & Greathead 1967; Hight *et al*. 2003). In the Antilles it was recorded feeding on *Lippia alba* (Mill.) (Davies & Greathead 1967). In India in host range tests, it also was able to breed on *Tectona grandis* Linn, and its release was not recommended. However, after escaping into the field it was subsequently not found to feed on *T.*

grandis, despite establishing well on *L. camara* (Davies & Greathead 1967). Mostly non-target effects are argued to occur within close range of the target host, and described as spillover. Baars (2000a) explained the spillover of *T. scrupulosa* onto *Sesamum*, spp. by referring to survival and development of nymphs of the tingid on these species during no-choice trials, but using behavior of the adult female insects to indicate that this was a temporary spill over effect.

One argument that is used in motivations to have candidate biocontrol agents of lantana released is that the weed is probably more of a threat to the survival of *Lippia* species than some incidental levels of non-target feeding (Baars 2000a; b). However, possible non-target effects of *L.camara* biological control agents on native Verbenaceae have not been quantified. In this chapter, I attempt to quantify the non-target feeding of the established agents in the field. *Lippia* species occur sympatrically with *L. camara* in southern Africa (Figure 5.1) and it is therefore essential to determine to what level the established agents are utilizing plants in this genus. Furthermore this survey provided an ideal opportunity to test some of the predictions made during the pre-release host specificity testing of five important agents, *F. intermedia, O. camarae, T. scrupulosa, C. lantanae* and *U. girardi* (Chapter 3).
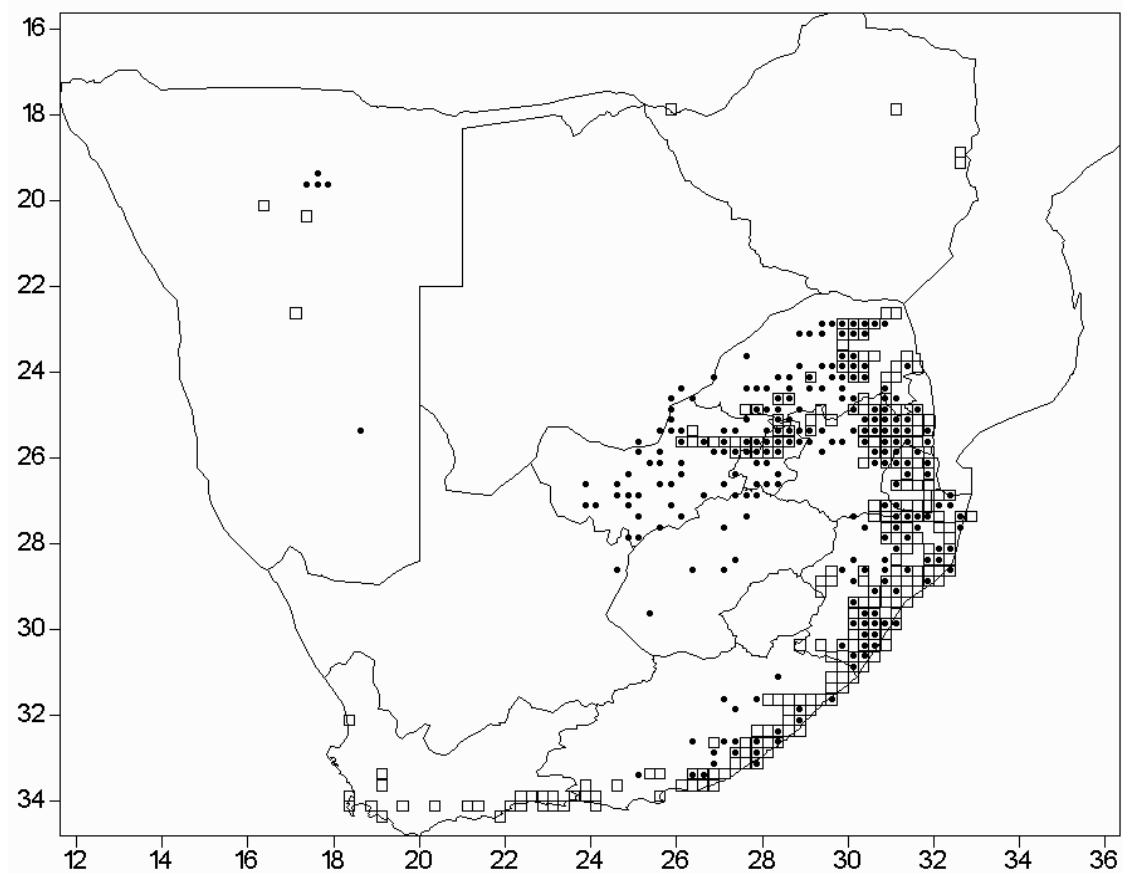
Figure 5.1 Distribution of *Lantana camara* (open blocks) and indigenous *Lippia* species (dots) in southern Africa. (Henderson 2001 and PRECIS database of National Botanical Institute).

5.2 Methods and Materials

The methods and materials used during the survey on insects on *L. camara* varieties were described in Chapter 4 and are the same for this study. Several indigenous species were incorporated during the field survey including *Lantana rugosa*, at least 5 *Lippia* species and *Priva meyeri*, (Figure 5.2), *Stachytarpheta* species and *Duranta erecta.* The damage and population levels were rated on as many plants as were found, totalling up to ten per species per site. The "relative insect abundance" ratings were done as described in Chapter 4, using a five-category scoring system. The ratings were: 0, where no life stages were present or typical damage found on plant; 1, where very few individuals were

encountered or few plant parts with characteristic damage at low intensities were found; 2, where individuals were easily noticed, but at low numbers, or characteristic damage easily noticed, but small proportions of the relevant plant parts were damaged; 3, where insects were readily present, with an even distribution over the plant and a large proportion of the relevant plant parts were damaged and a section of the shrub showed signs of stress; 4, where large numbers of individuals were found on most of the shoots on each plant, with an even distribution and most of the relevant plant parts had characteristic damage and plants were noticeably stressed. These ratings were therefore dependant on the size of each plant, and is noted as the "relative insect abundance" as it is a measure of the number of insects dependant on the size of the plant, i.e. a small plant with a rating of 4 has a very high population of the insect species and suffers maximum damage, but has fewer insects than a large plant with a rating of 4, that also suffers maximum damage.

The distance to the non-target species from the nearest *L. camara* shrub was recorded to determine relative insect populations on non-target species at varying distances from *L. camara*. This was conducted to determine if the population can be considered as a "spill over" effect or a viable established population. The non-target plants surveyed were therefore assigned to a "distance class" from the nearest *L. camara* plant (0-10m, 10-100m, 100-1000m, or >1000m). Most plants found and surveyed were in close proximity to an *L. camara* plant (0-10 and 10-100m). These measurements were estimated to save time, from a predominantly roadside survey. It would therefore have been possible that an *L. camara* plant were closer than 1000m to a non-target plant species, that was classified as >1000m, but errors in other classes are very unlikely. Twenty seed heads and / or inflorescences per species per site were collected, as was available, and immature stages of insects were reared out in the laboratory, for identification.

5.3 Results

In total, 774 *L. camara* plants from 82 sites, 99 *Lantana rugosa* plants from 12 sites, 563 *Lippia* plants from 46 sites (some sites had more than one *Lippia* species, of which up to ten were surveyed per species), 95 *Priva meyeri* plants from 10 sites, 12 plants from the

genus *Stachytarpheta* from 3 sites, and 8 *Duranta erecta* plants from 2 sites were found. The *Lippia* species were grouped for analysis, as the taxonomy of the genus *Lippia* is still unresolved. At most sites more than one non-target species was found. The numbers of *Lippia* and *L. rugosa* plants found in relation to the nearest *L. camara* is presented in Table 5.1.

Table 5.1 The number of *Lippia* and *L. rugosa* plants and number of sites they were found at, in relation to the distance from the nearest *L. camara* during the survey.

Plant species	Number of (n)	Distances plants occurred from *L. camara*			
		0-10m	10-100m	100-1000m	>1000m
Lippia species	Plants	451	27	29	56
	Sites	35	3	3	5
L. rugosa	Plants	69	10	10	10
	Sites	9	1	1	1

Due to the low number of sites in which especially *L. rugosa* occurred, the distance class data should not be considered a true reflection of the country as a whole, but is presented, as it still represents the trend of insect populations in relation to the distance it grows from *L. Camara.*

Sites where non-target species occurred were spread out over the northern and eastern range of the weed in South Africa (Figure 5.2).
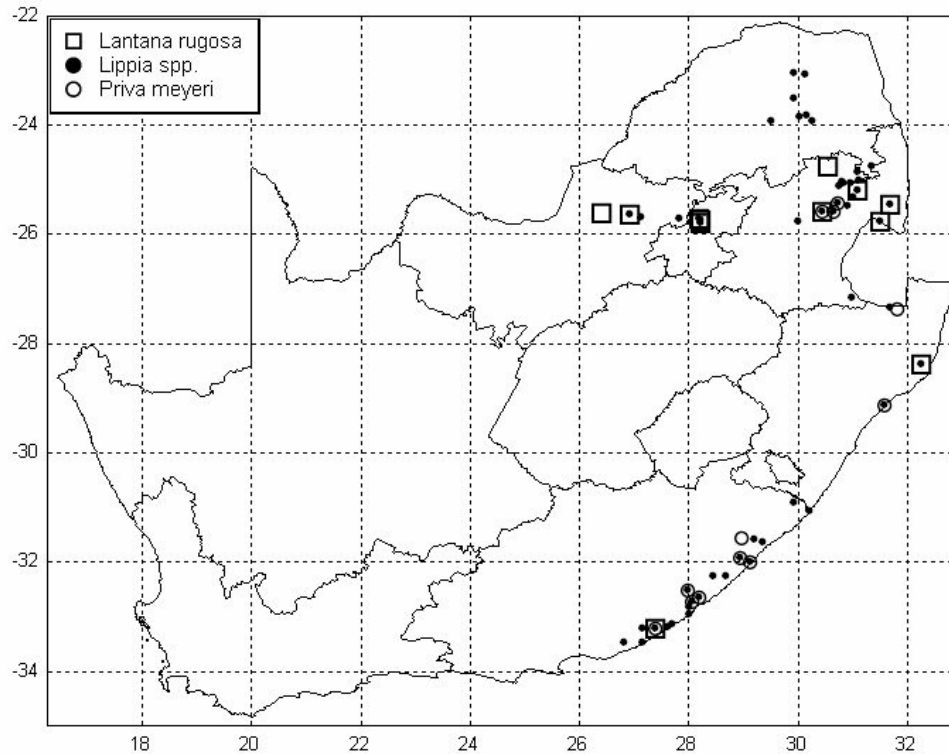
Figure 5.2 Localities in South Africa where *Lippia* species, *Lantana rugosa* and *Priva meyeri* were found and surveyed for insect species associated with *Lantana camara*.

Lantana camara supported populations of all 14 insect species known to be established in South Africa. Ten of these were recorded on *Lippia* species during the survey, six were found to occur on *L. rugosa* and only four were found on *Priva* (Figure 5.3). *Stachytarpheta* species supported only a small population of the indigenous moth *H. laceratalis* and no agents associated with *L. camara* were found on *D. erecta.* Insect species that were most common on most non-target species were the lepidopteran species thought to be indigenous, namely *H. laceratalis* and *A. onychote*. The introduced *T. scrupulosa* was the only other insect that had populations of any significance on *Lippia* and *L rugosa* plants in the field. Each insect species is dealt with individually, and populations on non-target species are compared to *L. camara* (Tables 5.1- 5.14, and figures 5.4- 5.19).
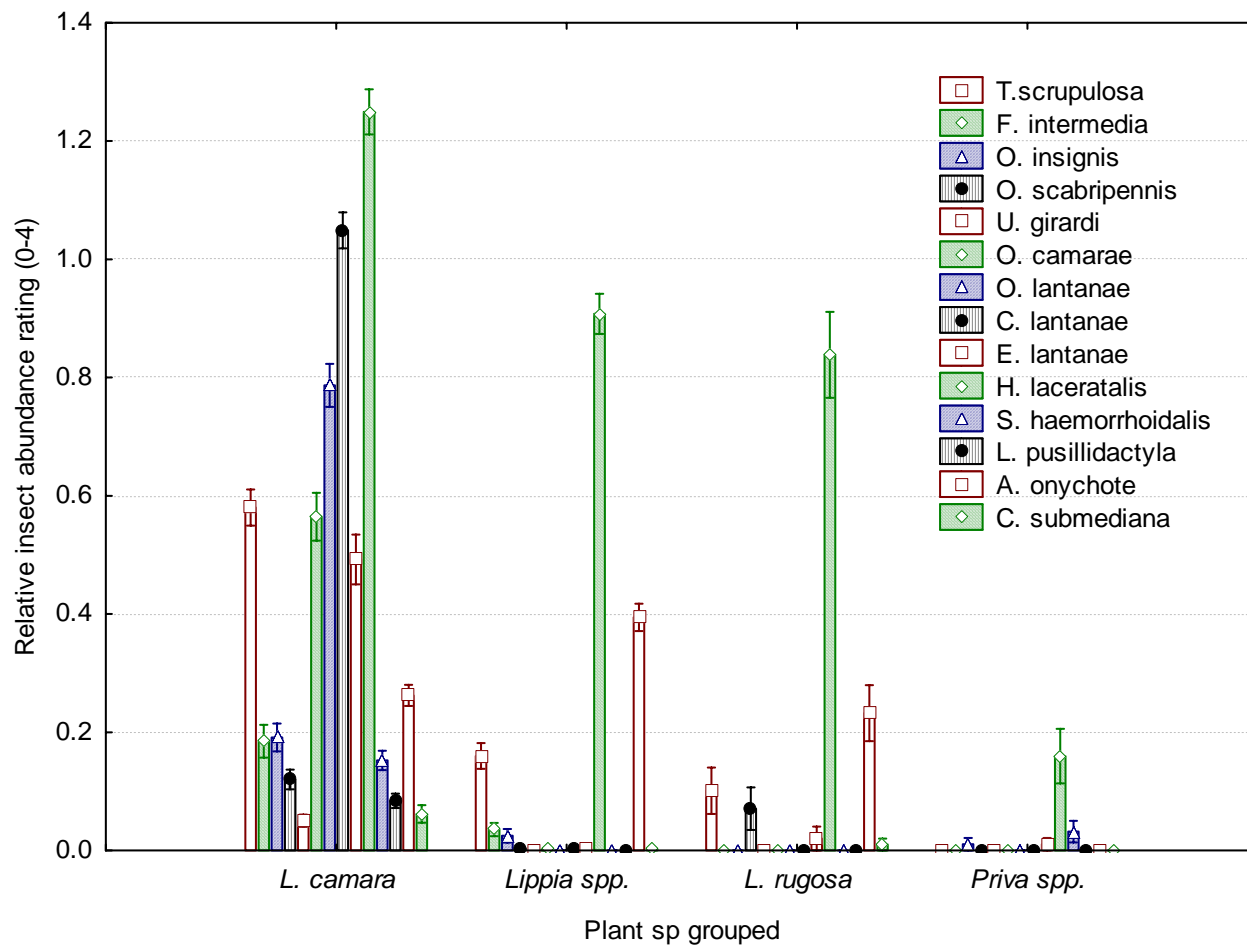
Figure 5.3 Mean relative insect species abundance rating (0-4) on *Lantana camara*, *Lippia* species, *Lantana rugosa* and *Priva meyeri* from the sites surveyed in South Africa.

Teleonemia scrupulosa

Lantana camara and *Lippia* species were the only plants that reached the maximum population rating (range) for *T. scrupulosa* (Table 5.2). The highest mean population were recorded from *L. camara*, followed by *Lippia* species with 28% and *L. rugosa* with 17 % of the population levels recorded on *L. camara*.

Table 5.2 Descriptive statistics of relative *Teleonemia scrupulosa* abundance on 6 plant species from the sites surveyed in South Africa and their percentage abundance relative to *Lantana camara*.

Plant species	n	Range (0-4)	Mode of abundance rating & frequency		Mean insect abundance (0-4)(±SE)		% Insect abundance : *L. camara*
L. camara	774	0-4	0	456	0.580	±0.031	100
L. rugosa	99	0-2	0	92	0.101	±0.039	17
Lippia spp.	563	0-4	0	499	0.160	±0.022	28
P. meyeri	94	0	0	94	0.000	±0.000	0
Stachytarpheta spp.	12	0	0	12	0.000	±0.000	0
D. erecta	8	0	0	8	0.000	±0.000	0

Teleonemia scrupulosa populations on *L. rugosa* were highest, closest to *L. camara*, and slightly lower up to 100m away (Figure 5.4). The 100-1000m site, had a mean population of just under 1 on *L. camara* (not presented in this Figure), but none was found on *L. rugosa* (Figure 5.4). *Lantana camara* did not grow anywhere near the site with the > 1000m group of plants, but were considered to occur in a climatically suitable range for *T. scrupulosa*.
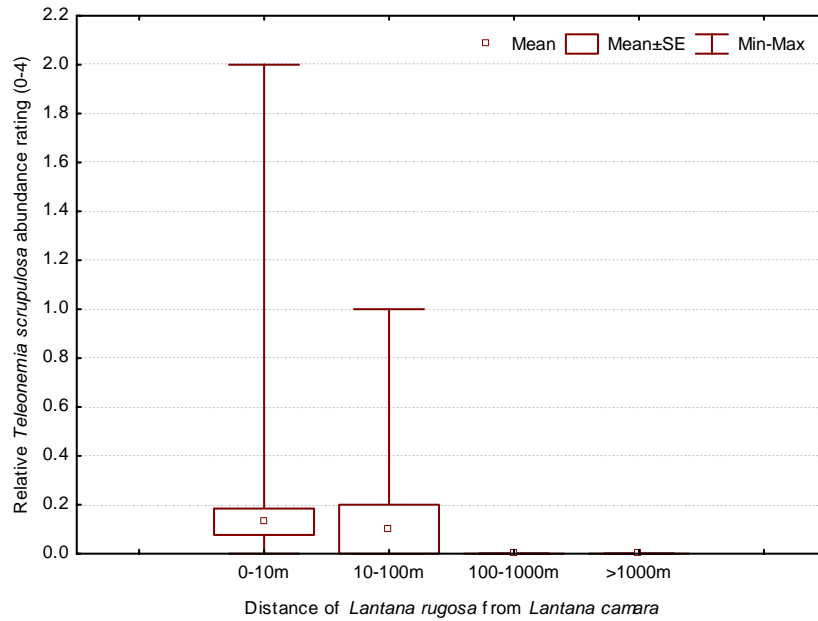
Figure 5.4 Mean (± standard error (SE), & range (Min-Max)) relative *Teleonemia scrupulosa* abundance rating (0-4) on *Lantana rugosa* at either 0-10m, 10-100m,100-1000m, or >1000m from the nearest *Lantana camara*, from the sites surveyed in South Africa.

A slightly higher mean population of *T. scrupulosa* was found on *Lippia* species growing next to *L. camara*, than those growing further away (Figure 5.5). The *Lippia* plants growing next to *L. camara* reached the highest population level rating of 4 and a population rating of 2 on plants over a kilometer away from *L. camara.* A maximum of 1 was reached at distance groupings in between the extremes.

Figure 5.5 Mean (± standard error (SE), & range (Min-Max)) relative *Teleonemia scrupulosa* abundance rating (0-4) on *Lippia* species at either 0-10m, 10-100m ,100-1000m , or >1000m from the nearest *Lantana camara*, from the sites surveyed in South Africa.

Falconia intermedia

Lantana camara and *Lippia* species were the only plants that supported populations of *F. intermedia* (Table 5.3). Most plants of all species usually had no populations of the mirid. The highest mean population was recorded off *L. camara*, followed by *Lippia* species with 19% of that of the population achieved on *L. camara*.

Table 5.3 Descriptive statistics of relative *Falconia intermedia* abundance on 6 plant species from the sites surveyed in South Africa and their percentage abundance relative to *Lantana camara.*

Plant species	n	Range (0-4)	Mode of abundance rating & frequency		Mean insect abundance (0-4)(±SE)		% Insect abundance : *L. camara*
L. camara	774	0-4	0	723	0.185	±0.028	100
L. rugosa	99	0	0	99	0.000	±0.000	0
Lippia spp.	563	0-2	0	553	0.036	±0.011	19
P. meyeri	94	0	0	94	0.000	±0.000	0
Stachytarpheta spp.	12	0	0	12	0.000	±0.000	0
D. erecta	8	0	0	8	0.000	±0.000	0

Falconia intermedia populations were only found on *Lippia* plants growing next to *L. camara* (Figure 5.6). The populations of the mirid were however very rare, even on *L. camara* and therefore, are these results unreliable.

Figure 5.6 Mean (± standard error (SE), & range (Min-Max)) relative *Falconia intermedia* abundance rating (0-4) on *Lippia* species at either 0-10m, 10-100m,100-1000m, or >1000m from the nearest *Lantana camara*, from the sites surveyed in South Africa.

Uroplata girardi

Lantana camara was the only plant species on which *U. girardi* populations were recorded during the survey (Table 5.4). The insect was found on only 30 of the 774 *L. camara* plants searched countrywide, with only sites occurring within its previously known distribution range sustaining insect populations.

Table 5.4 Descriptive statistics of relative *Uroplata girardi* abundance on 6 plant species from the sites surveyed in South Africa and their percentage abundance relative to *Lantana camara.*

Plant species	n	Range (0-4)	Mode of abundance rating & frequency		Mean insect abundance (0-4)(±SE)		% Insect abundance : *L. camara*
L. camara	774	0-4	0	744	0.050	±0.010	100
L. rugosa	99	0	0	99	0.000	±0.000	0
Lippia spp.	563	0	0	563	0.000	±0.000	0
P. meyeri	94	0	0	94	0.000	±0.000	0
Stachytarpheta spp.	12	0	0	12	0.000	±0.000	0
D. erecta	8	0	0	8	0.000	±0.000	0

Ophiomya camarae

Lantana camara and *Lippia* species were the only plants that had populations of *O. camarae* (Table 5.5). The maximum population level of 4 was recorded on *L. camara* and 1 on *Lippia* species. Most plants had no insects. The mean *O. camarae* population recorded on *L. camara,* was 0.565, and *Lippia* species had less than 1% of that.

Table 5.5 Descriptive statistics of relative *Ophiomya camarae* abundance on 6 plant species from the sites surveyed in South Africa and their percentage abundance relative to *Lantana camara.*

Plant species	n	Range (0-4)	Mode of abundance rating & frequency		Mean insect abundance (0-4)(±SE)		% Insect abundance : *L. camara*
L. camara	774	0-4	0	580	0.565	±0.040	100
L. rugosa	99	0	0	99	0.000	±0.000	0
Lippia spp.	563	0-1	0	562	0.002	±0.002	0
P. meyeri	94	0	0	94	0.000	±0.000	0
Stachytarpheta spp.	12	0	0	12	0.000	±0.000	0
D. erecta	8	0	0	8	0.000	±0.000	0

Opiomya camarae was only recorded once from a *Lippia* species, and this was within 10 m of the nearest *L. camara* (Figure 5.7).
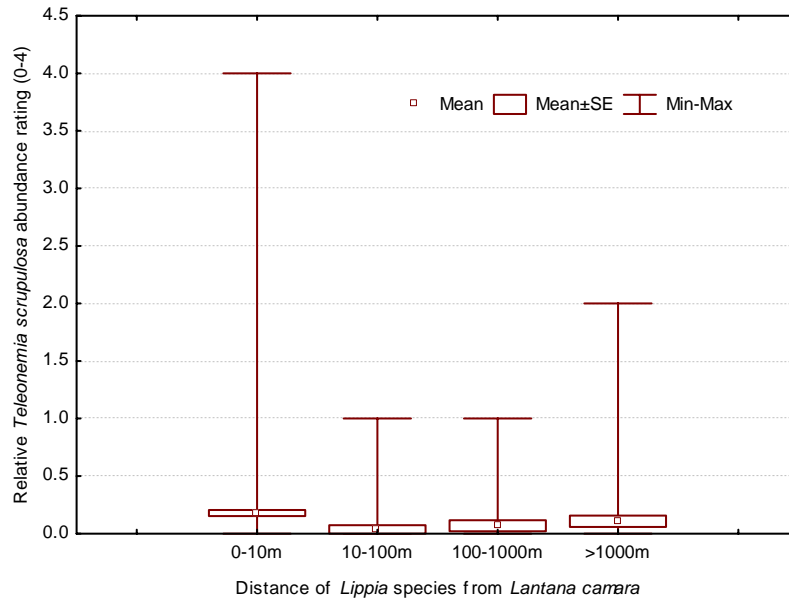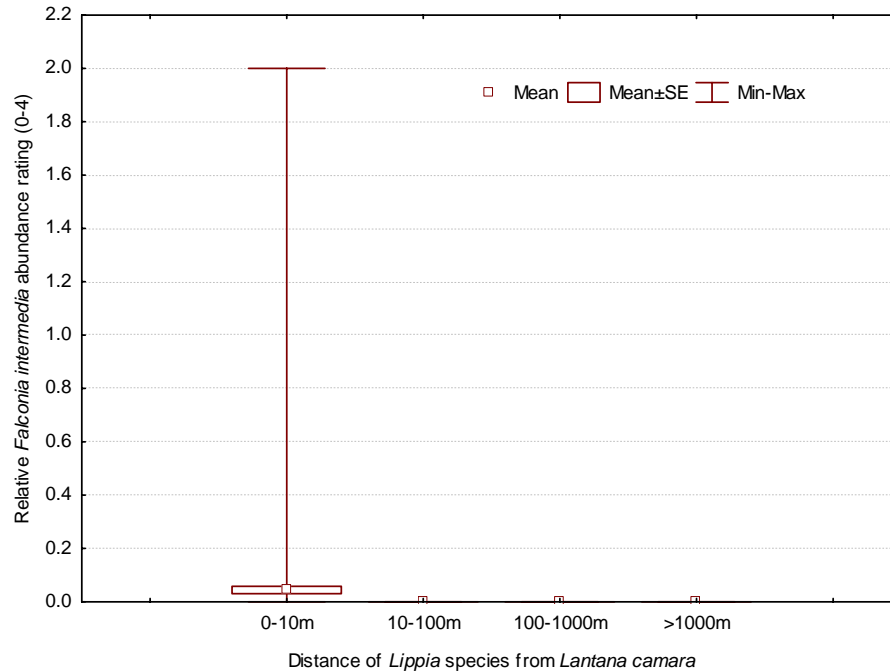

Figure 5.7 Mean (± standard error (SE), & range (Min-Max)) relative *Ophiomya camarae* abundance rating (0-4) on *Lippia* species at either 0-10m, 10-100m,100-1000m, or >1000m from the nearest *Lantana camara*, from the sites surveyed in South Africa.

Calycomyza lantanae

The blotch leaf-mining fly *C. lantanae,*were recorded on most *L. camara* plants, and on *Lippia* species on two occasions (Table 5.6). It occasionally reached the maximum rating on *L. camara*, but was only rated as rare (1) on the two *Lippia* plants. Both these non-target species were within 10 m from the nearest *L. camara* (Figure 5.8).

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Table 5.6 Descriptive statistics of relative *Calycomyza lantanae* abundance on 6 plant species from the sites surveyed in South Africa and their percentage abundance relative to *Lantana camara.*

Plant species	n	Range (0-4)	Mode of abundance rating & frequency		Mean insect abundance (0-4)(±SE)		% Insect abundance : *L. camara*
L. camara	774	0-4	1	379	1.049	±0.030	100
L. rugosa	99	0	0	99	0.000	±0.000	0
Lippia spp.	563	0-1	0	561	0.004	±0.003	0
P. meyeri	94	0	0	94	0.000	±0.000	0
Stachytarpheta spp.	12	0	0	12	0.000	±0.000	0
D. erecta	8	0	0	8	0.000	±0.000	0

Figure 5.8 Mean (± standard error (SE), & range (Min-Max)) relative *Calycomyza lantanae* abundance rating (0-4) on *Lippia* species at either 0-10m, 10-100m,100-1000m, or >1000m from the nearest *Lantana camara*, from the sites surveyed in South Africa.

Orthezia insignis

Orthezia insignis reached ratings of 4 on *Lantana camara*, and occurred on *Lippia* species and *P. meyeri*, reaching a maximum rating of 1 (Table 5.7). The stem sucking

scale insect was however absent from the majority of the sites. The highest mean populations was recorded on *L. camara*, followed by *Lippia* species with 13% of that and *P. meyeri* with 6 % of the population levels recorded on *L. camara*.

Table 5.7 Descriptive statistics of relative *Orthezia insignis* abundance on 6 plant species from the sites surveyed in South Africa and their percentage abundance relative to *Lantana camara*.

Plant species	n	Range (0-4)	Mode of abundance rating & frequency		Mean insect abundance (0-4)(±SE)		% Insect abundance : *L. camara*
L. camara	774	0-4	0	693	0.191	±0.024	100
L. rugosa	99	0	0	99	0.000	±0.000	0
Lippia spp.	563	0-4	0	558	0.025	±0.011	13
P. meyeri	94	0-1	0	93	0.011	±0.011	6
Stachytarpheta spp.	12	0	0	12	0.000	±0.000	0
D. erecta	8	0	0	8	0.000	±0.000	0

Orthezia insignis was only found on *Lippia* plants growing up to 10m away from *L. camara* and none were recorded on these non-target species in the other distance categories (Figure 5.9).
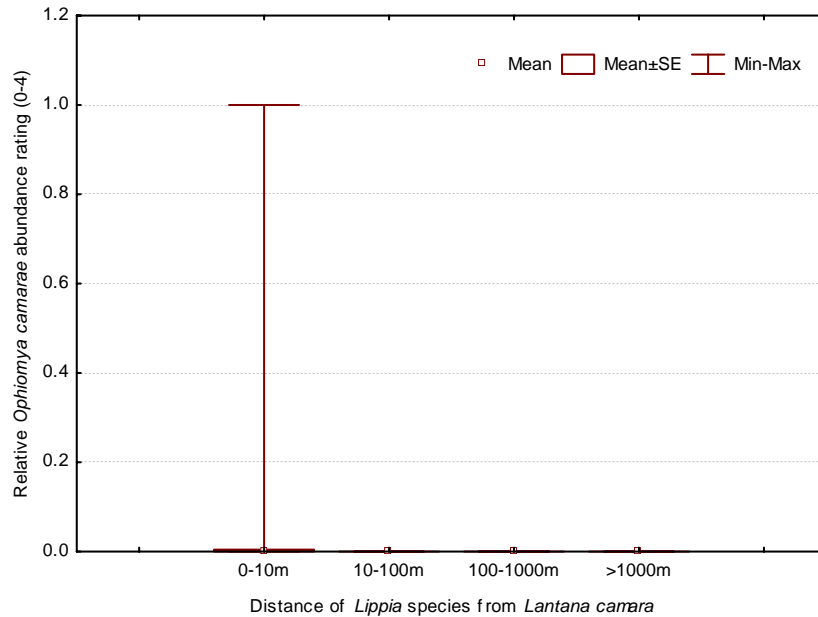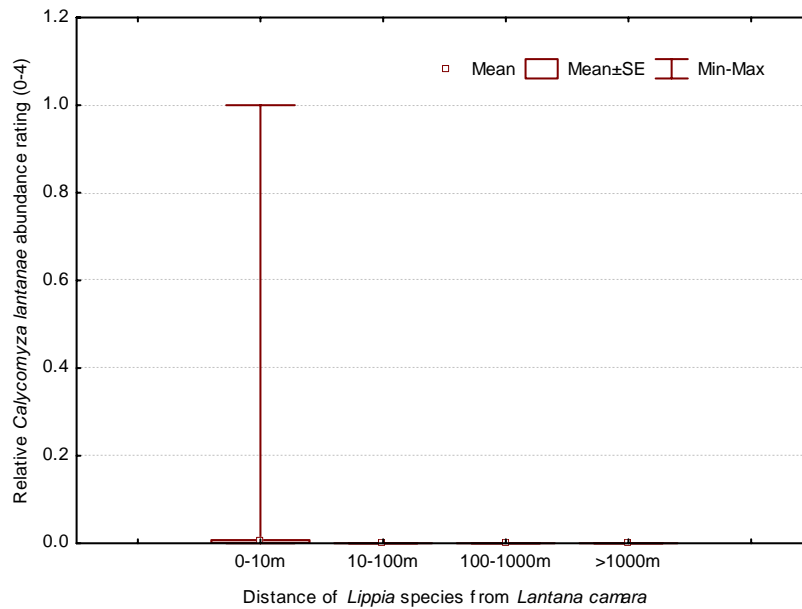
Figure 5.9 Mean (± standard error (SE), & range (Min-Max)) relative *Orthezia insignis* abundance rating (0-4) on *Lippia* species at either 0-10m, 10-100m,100-1000m, or >1000m from the nearest *Lantana camara*, from the sites surveyed in South Africa.

Octotoma scabripennis

Octotoma scabripennis reached the maximum population rating on *Lantana camara* (Table 5.8). Populations on *Lantana rugosa* reached a maximum rating of 2 and 1 on *Lippia* species The populations on all plant species were usually 0. The highest mean population was recorded on *L. camara*, followed by *L. rugosa* with 59% of that, which was obtained from populations on only 4 plants (resulting in a high standard error). Population levels of *Lippia* species were low, only 3 % of that recorded on *L. camara*.

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Table 5.8 Descriptive statistics of relative *Octotoma scabripennis* abundance on 6 plant species from the sites surveyed in South Africa and their percentage abundance relative to *Lantana camara*.

Plant species	n	Range (0-4)	Mode of abundance rating & frequency		Mean insect abundance (0-4)(±SE)		% Insect abundance : *L. camara*
L. camara	774	0-4	0	712	0.120	±0.017	100
L. rugosa	99	0-2	0	95	0.071	±0.036	59
Lippia spp.	563	0-1	0	561	0.004	±0.003	3
P. meyeri	94	0	0	94	0.000	±0.000	0
Stachytarpheta spp.	12	0	0	12	0.000	±0.000	0
D. erecta	8	0	0	8	0.000	±0.000	0

Octotoma scabripennis populations on *L. rugosa* occurred only within 10m of *L. camara* (Figure 5.10).
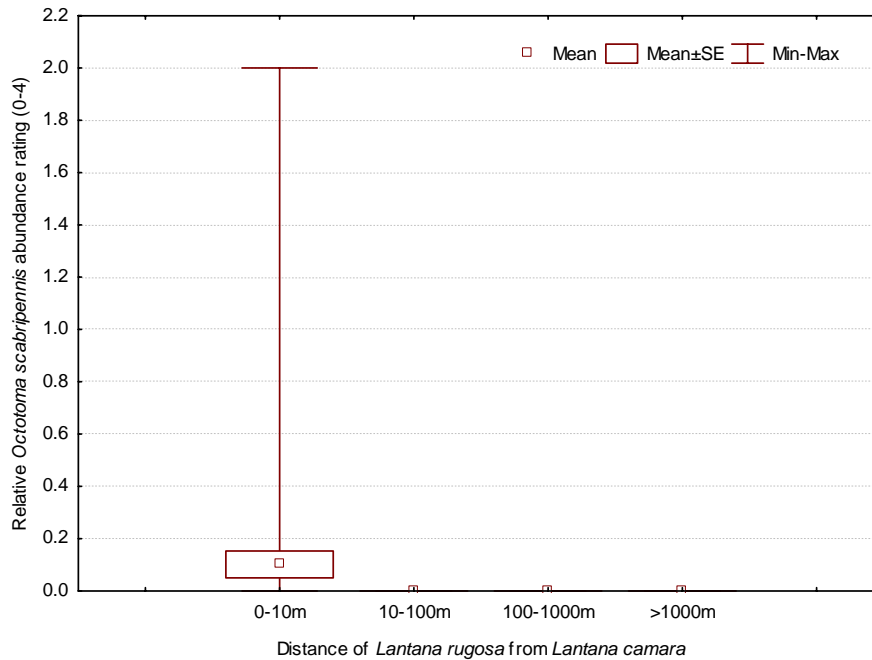
Figure 5.10 Mean (± standard error (SE), & range (Min-Max)) relative *Octotoma scabripennis* abundance rating (0-4) on *Lantana rugosa* at either 0-10m, 10-100m,100-1000m, or >1000m from the nearest *Lantana camara*, from the sites surveyed in South Africa.

The *O. scabripennis* populations on *Lippia* plants were all recorded within 10 m from *L. camara* (Figure 5.11).
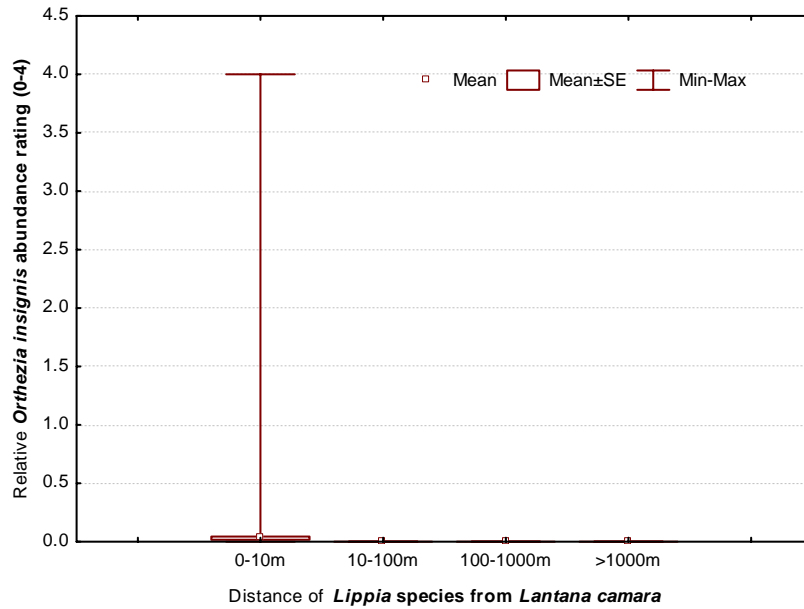

Figure 5.11 Mean (± standard error (SE), & range (Min-Max)) relative *Octotoma scabripennis* abundance rating (0-4) on *Lippia* species at either 0-10m, 10-100m,100-1000m, or >1000m from the nearest *Lantana camara*, from the sites surveyed in South Africa.

Ophiomya lantanae

Lantana camara was the only plant species that supported populations of *O. lantanae* (Table 5.9). It reached the maximum rating on *L. camara* at times, yet none were found on any non-target species.

Table 5.9 Descriptive statistics of relative *Ophiomya lantanae* abundance on 6 plant species from the sites surveyed in South Africa and their percentage abundance relative to *Lantana camara.*

Plant species	N	Range (0-4)	Mode of abundance rating & frequency		Mean insect abundance (0-4)(±SE)		% Insect abundance : *L. camara*
L. camara	774	0-4	0	405	0.787	±0.036	100
L. rugosa	99	0	0	99	0.000	±0.000	0
Lippia spp.	563	0	0	563	0.000	±0.000	0
P. meyeri	94	0	0	94	0.000	±0.000	0
Stachytarpheta spp.	12	0	0	12	0.000	±0.000	0
D. erecta	8	0	0	8	0.000	±0.000	0

Epinotea lantanae

Populations of *E. lantanae* reached maximum levels at times on *L. camara* (Table 5.10). It reached a population rating of 2 on one occasion on *L. rugosa*, and was rated as rare on one occasion each for *Lippia* species and for *P. meyeri*. The mean populations on *L. rugosa* were 4% and *P. meyeri* only 2% of that on *L. camara*. Populations on the non-target species *L. rugosa* and *Lippia* species occurred within 10m from *L. camara* plants (Figures 5.12 & 5.13).

Table 5.10 Descriptive statistics of relative *Epinotea lantanae* abundance on 6 plant species from the sites surveyed in South Africa and their percentage abundance relative to *Lantana camara.*

| Plant species	n	Range (0-4)	Mode of abundance rating & frequency		Mean insect abundance (0-4)(±SE)		% Insect abundance : *L. camara*
L. camara	774	0-4	0	625	0.492	±0.042	100
L. rugosa	99	0-2	0	98	0.020	±0.020	4
Lippia spp.	563	0-1	0	562	0.002	±0.002	0
P. meyeri	94	0-1	0	93	0.011	±0.011	2
Stachytarpheta spp.	12	0	0	12	0.000	±0.000	0
D. erecta	8	0	0	8	0.000	±0.000	0

Figure 5.12 Mean (± standard error (SE), & range (Min-Max)) relative *Epinotea lantanae* abundance rating (0-4) on *Lantana rugosa* at either 0-10m, 10-100m,100-1000m, or >1000m from the nearest *Lantana camara*, from the sites surveyed in South Africa.
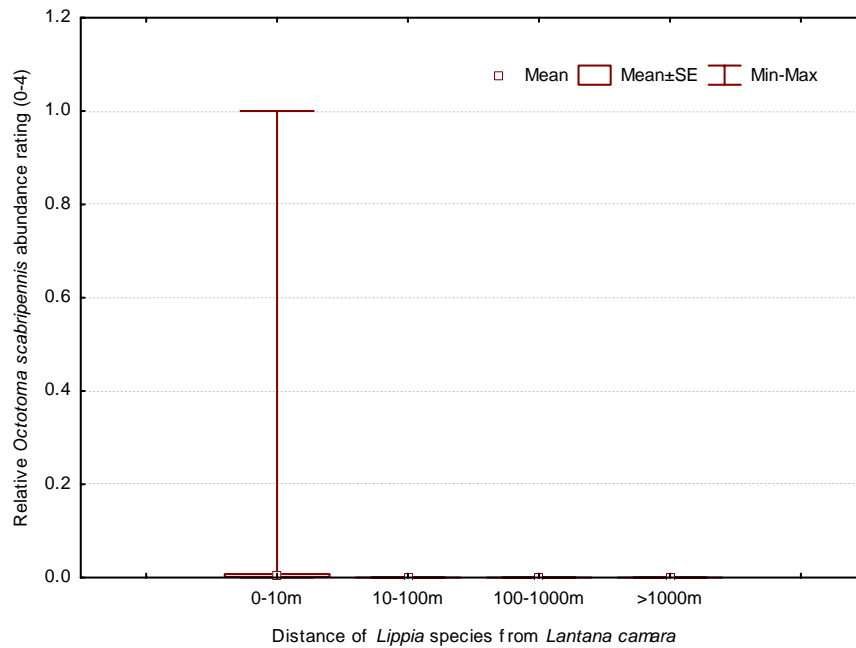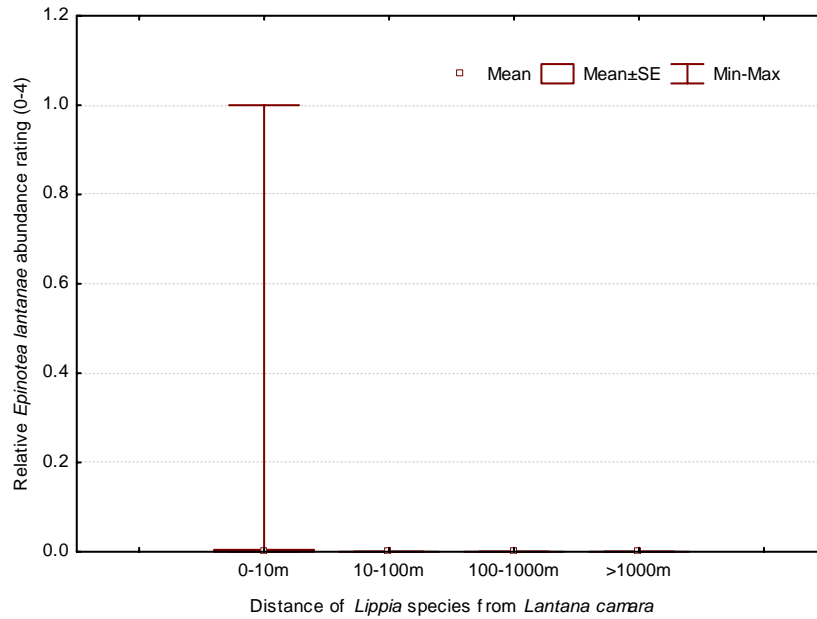
Figure 5.13 Mean (± standard error (SE), & range (Min-Max)) relative *Epinotea lantanae* abundance rating (0-4) on *Lippia* species at either 0-10m, 10-100m,100-1000m, or >1000m from the nearest *Lantana camara*, from the sites surveyed in South Africa.

Hypena laceratalis

The indigenous moth *H. laceratalis* reached population ratings of 4 on both *L. camara* and *Lippia* species (Table 5.11). *Lantana rugosa* and *P. meyeri* reached ratings of 3, and one *Stachytarpeta* plant had a rating of rare (1). The highest mean population level of about 1.2 (max of 4) was recorded on *L. camara*. This was followed by *Lippia* species with 73%, *L. rugosa* with 67 %, *P. meyeri* with 13% and *Stachytarpheta* species with 7% of the population levels found to occur on *L. camara*.

Table 5.11 Descriptive statistics of relative *Hypena laceratalis* abundance on 6 plant species from the sites surveyed in South Africa and their percentage abundance relative to *Lantana camara.*

Plant species	n	Range (0-4)	Mode of abundance rating & frequency		Mean insect abundance (0-4)(±SE)		% Insect abundance : *L. camara*
L. camara	774	0-4	1	324	1.249	±0.038	100
L. rugosa	99	0-3	1	51	0.838	±0.073	67
Lippia spp.	563	0-4	1	275	0.908	±0.034	73
P. meyeri	94	0-3	0	81	0.160	±0.046	13
Stachytarpheta spp.	12	0-1	0	11	0.083	±0.083	7
D. erecta	8	0	0	8	0.000	±0.000	0

Hypena laceratalis populations were found on *L. rugosa* at all the distance categories away from *L. camara* (Figure 5.14). Mean populations were slightly higher at 10-100m and closest to *L. camara*, than further away.
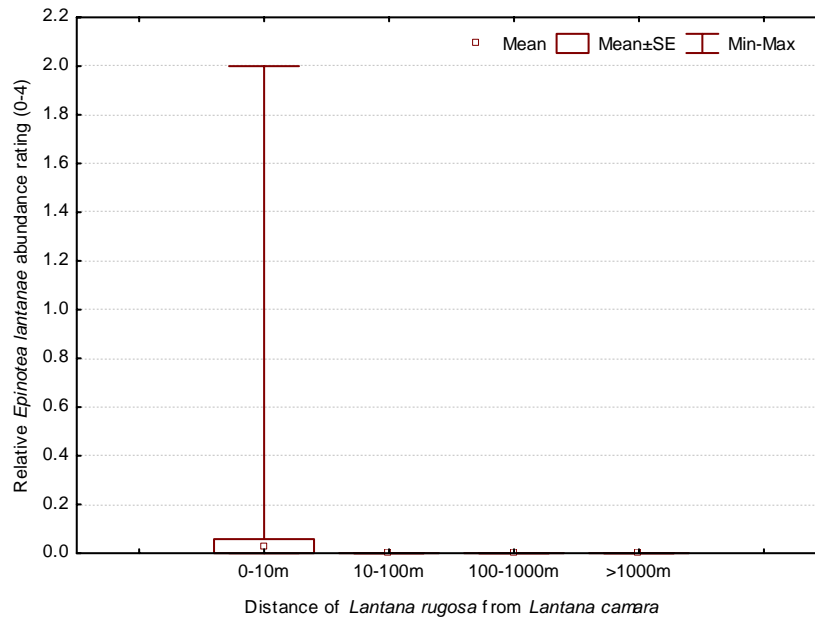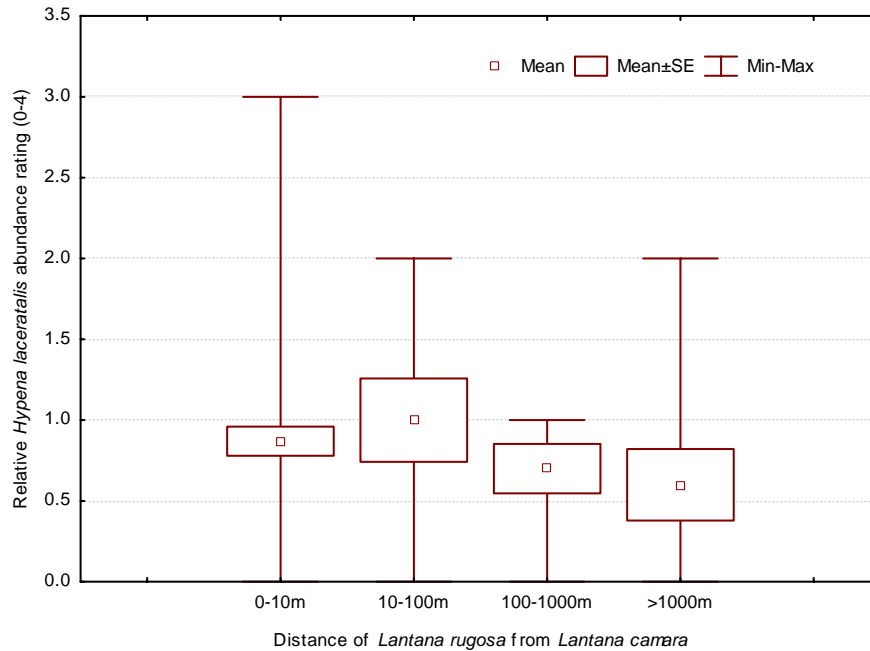
Figure 5.14 Mean (± standard error (SE), & range (Min-Max)) relative *Hypena laceratalis* abundance rating (0-4) on *Lantana rugosa* at either 0-10m, 10-100m,100-1000m, or >1000m from the nearest *Lantana camara*, from the sites surveyed in South Africa.

Hypena laceratalis populations were found on *Lippia* species in all the distance classes from *L. camara* shrubs (Figure 5.15). Mean populations were slightly higher on *Lippia* plants at 100-1000m and 10-100m away, than on the remaining categories.
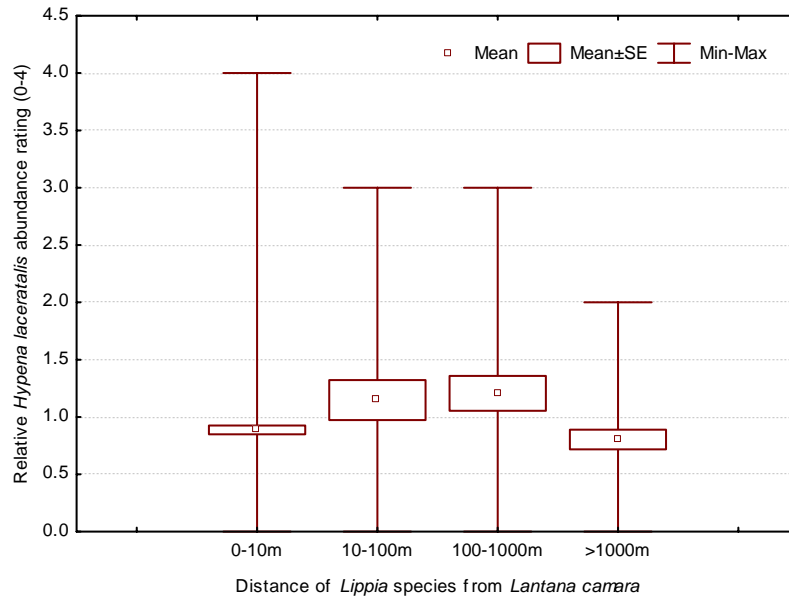
Figure 5.15 Mean (± standard error (SE), & range (Min-Max)) relative *Hypena laceratalis* abundance rating (0-4) on *Lippia* species at either 0-10m, 10-100m,100-1000m, or >1000m from the nearest *Lantana camara*, from the sites surveyed in South Africa.

Salbia haemorrhoidalis

Ninety-one *L. camara* and 3 *P. meyeri* plants supported populations of *S. haemorrhoidalis* (Table 5.12). The populations on all plant species were usually rated as 0. The highest mean population was recorded on *L. camara*, and *P. meyeri* had only 21% of that on *L.camara*.

Table 5.12 Descriptive statistics of relative *Salbia haemorrhoidalis* abundance on 6 plant species from the sites surveyed in South Africa and their percentage abundance relative to *Lantana camara.*

Plant species	n	Range (0-4)	Mode of abundance rating & frequency		Mean insect abundance (0-4)(±SE)		% Insect abundance : *L. camara*
L. camara	774	0-3	0	683	0.152	±0.016	100
L. rugosa	99	0	0	99	0.000	±0.000	0
Lippia spp.	563	0	0	563	0.000	±0.000	0
P. meyeri	94	0-1	0	91	0.032	±0.018	21
Stachytarpheta spp.	12	0	0	12	0.000	±0.000	0
D. erecta	8	0	0	8	0.000	±0.000	0

Lantanophaga pusillidactyla

L. pusillidactyla was only recorded on *L. camara*, occurring on 51 plants surveyed and reaching levels of occasional (value of 2) per plant (Table 5.13).

Table 5.13 Descriptive statistics of relative *Lantanophaga pusillidactyla* abundance on 6 plant species from the sites surveyed in South Africa and their percentage abundance relative to *Lantana camara.*

Plant species	n	Range (0-4)	Mode of abundance rating & frequency		Mean insect abundance (0-4)(±SE)		% Insect abundance : *L. camara*
L. camara	774	0-2	0	723	0.084	±0.012	100
L. rugosa	99	0	0	99	0.000	±0.000	0
Lippia spp.	563	0	0	563	0.000	±0.000	0
P. meyeri	94	0	0	94	0.000	±0.000	0
Stachytarpheta spp.	12	0	0	12	0.000	±0.000	0
D. erecta	8	0	0	8	0.000	±0.000	0

Aristea onychote

Aristea onychote, an indigenous leaf mining moth, were recorded on *L. camara*, *L. rugosa* and *Lippia* species (Table 5.14). The highest mean population was recorded on *Lippia* species, followed by *L. camara*, which had two thirds as many insects as *Lippia* plants, followed by *L. rugosa* that had almost nine tenths the population level of that on *L. camara*.

Table 5.14 Descriptive statistics of relative *Aristea onychote* abundance on 6 plant species from the sites surveyed in South Africa and their percentage abundance relative to *Lantana camara*.

Plant species	n	Range (0-4)	Mode of abundance rating & frequency		Mean insect abundance (0-4)(±SE)		% Insect abundance : *L. camara*
L. camara	774	0-3	0	588	0.262	±0.018	100
L. rugosa	99	0-2	0	78	0.232	±0.047	89
Lippia spp.	563	0-3	0	357	0.394	±0.023	150
P. meyeri	94	0	0	94	0.000	±0.000	0
Stachytarpheta spp.	12	0	0	12	0.000	±0.000	0
D. erecta	8	0	0	8	0.000	±0.000	0

Populations of *A. onychote* were found on *L. rugosa* from 0, up to and beyond 1km from the nearest *L. camara* shrub (Figure 5.16). The highest mean populations were found to occur 100-1000m from *L. camara*, then 10-100m, followed equally by populations very close to, or far removed from *L. camara*. There was however less variation in population abundance closest to *L. camara* (largest number of observations was made here). The highest rating of 4 was however recorded in the *L. rugosa* groups closest (0-100m) to *L. camara*.

Figure 5.16 Mean (± standard error (SE), & range (Min-Max)) relative *Aristea onychote* abundance rating (0-4) on *Lantana rugosa* at either 0-10m, 10-100m,100-1000m, or >1000m from the nearest *Lantana camara*, from the sites surveyed in South Africa.

Populations of *A. onychote* were also found on *Lippia* from 0, up to and beyond 1km from the nearest *L. camara* shrub (Figure 5.17). The highest mean populations were found to occur both closest and furthest from *L. camara*, the 100- 1000m category then followed by populations on *Lippia* plants 10-100m from *L. camara*. The highest population rating of 4 was however only recorded closest to *L. camara*. The second highest population rating reached was found to occur furthest away from *L. camara*.

Figure 5.17 Mean (± standard error (SE), & range (Min-Max)) relative *Aristea onychote* abundance rating (0-4) on *Lippia* species at either 0-10m, 10-100m,100-1000m, or >1000m from the nearest *Lantana camara*, from the sites surveyed in South Africa.

Characoma submediana

The indigenous moth *C. submediana* was recorded on *Lantana camara, L. rugosa* and *Lippia* species (Table 5.15). Populations only reached levels of 4 on *L. camara*, and only occurred at levels of 1 on the other species. The highest mean population was recorded on *L. camara*, followed by *L. rugosa* with 16% of that, and *Lippia* species with 3% of that occurring on *L. camara*.

Table 5.15 Descriptive statistics of relative *Characoma submediana* abundance on 6 plant species from the sites surveyed in South Africa and their percentage abundance relative to *Lantana camara.*

Plant species	n	Range (0-4)	Mode of abundance rating & frequency		Mean insect abundance (0-4)(±SE)		% Insect abundance : *L. camara*
L. camara	774	0-4	0	751	0.062	±0.015	100
L. rugosa	99	0-1	0	98	0.010	±0.010	16
Lippia spp.	563	0-1	0	562	0.002	±0.002	3
P. meyeri	94	0	0	94	0.000	±0.000	0
Stachytarpheta spp.	12	0	0	12	0.000	±0.000	0
D. erecta	8	0	0	8	0.000	±0.000	0

Characoma submediana only occurred on *L. rugosa* and *Lippia* species within 10m from *L. camara* (Figures 5.18 & 5.19).
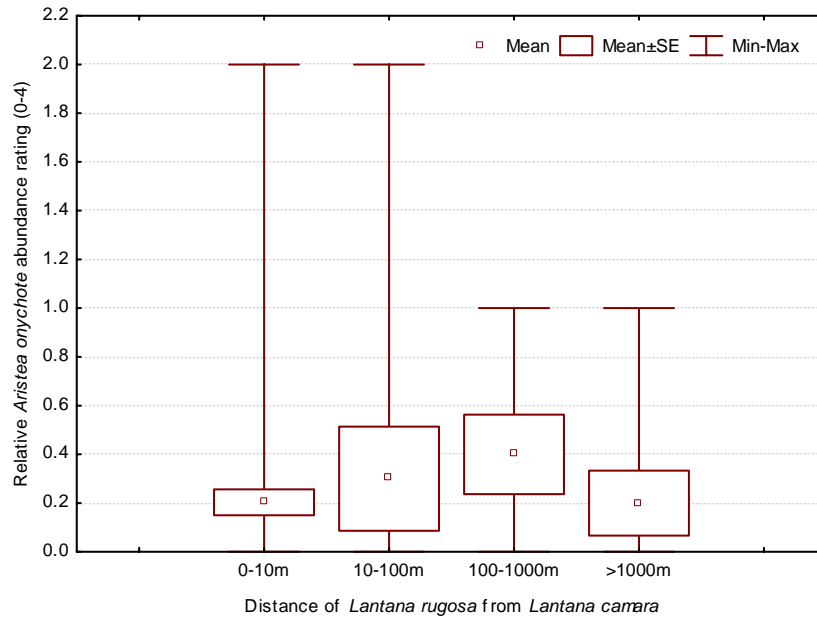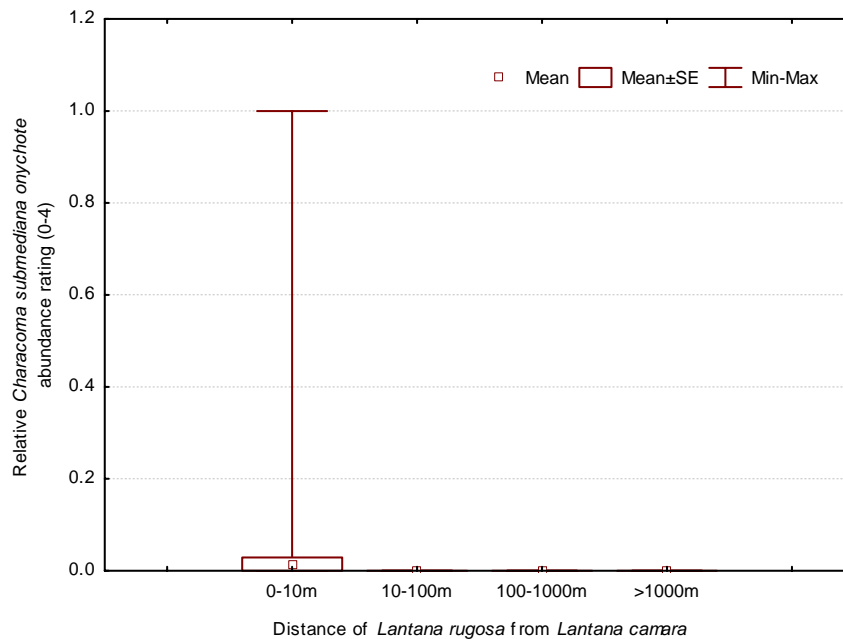

Figure 5.18 Mean (± standard error (SE), & range (Min-Max)) relative *Characoma submediana* abundance rating (0-4) on *Lantana rugosa* at either 0-10m, 10-100m,100-1000m, or >1000m from the nearest *Lantana camara*, from the sites surveyed in South Africa.
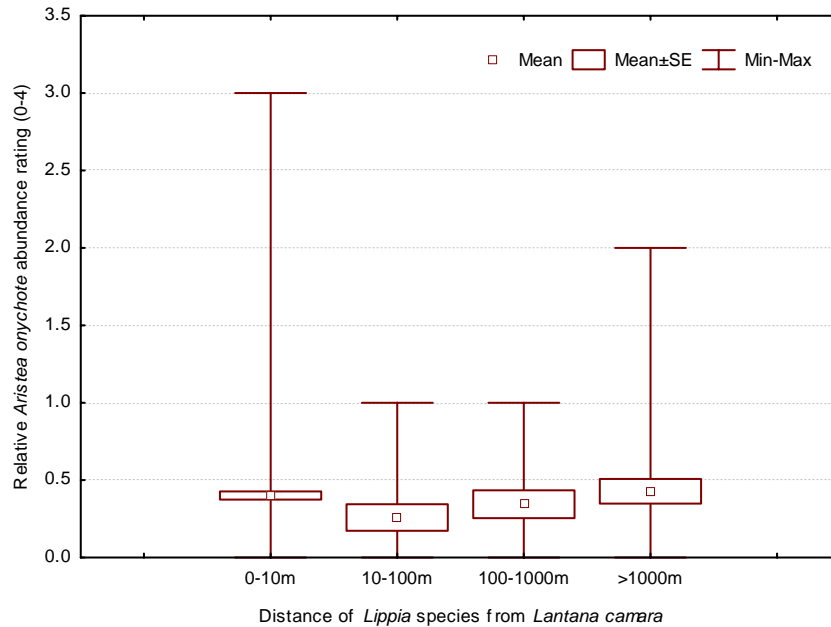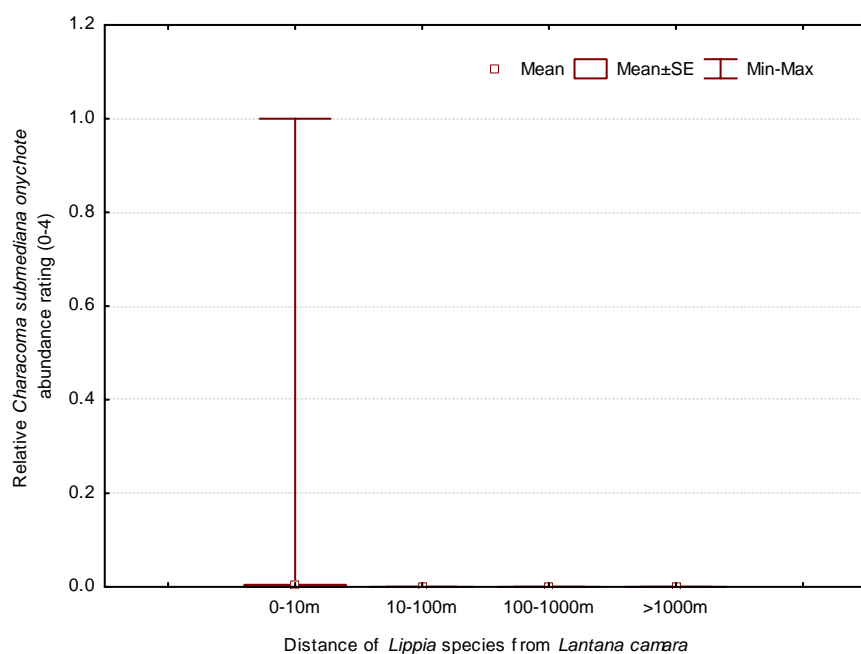
Figure 5.19 Mean (± standard error (SE), & range (Min-Max)) relative *Characoma submediana* abundance rating (0-4) on *Lippia* species at either 0-10m, 10-100m,100-1000m, or >1000m from the nearest *Lantana camara*, from the sites surveyed in South Africa.

5.4 Discussion and Conclusion

Of the 14 agents associated with *L. camara*, only 3, *U. girardi, O. lantanae* and *S. haemorrhoidalis* were not recorded on the non-target species surveyed. Excluding the indigenous insect species, all the others were found to occur on non-target species, though, mostly at a much reduced rate, compared to *L. camara.* The non-target species utilized most by all the agents were *Lippia* species, followed by *Lantana rugosa and Priva meyeri., Stachytarpheta* species was utilised by the indigenous leaf chewing lepidopteran, *Hypena laceratalis*, and *Duranta erecta* not utilized. More indigenous *Lantana* species occur in southern Africa than were included in this survey, these include *Lantana mearnsii* Moldenke var. *latibracteolata* Moldenke, *Lantana dinteri* Moldenke and *Lantana angolensis* Moldenke. Of these, only *L. mearnsii* occurs within the geographic distribution of naturalized lantana stands in South Africa, in the LP, NW, and MP provinces. The other two species occur further north and west of the South African

border (PRECIS database of the National Botanical Institute). Unfortunately, no *Lantana* species other than *L rugosa* were found in the field during the survey, but from several other biocontrol candidates from laboratory trials (see also Chapter 3), these species will probably reflect very similar patterns of utilization as *L. rugosa* has shown in this survey. It was reported that plants closely related to the target weed are more at risk of non-target effects, than those genetically further removed (Louda *et al*. 1997; Pemberton 2000; Louda 2000; Louda *et al*. 2005). This is the case to some extent for the lantana biocontrol agents, but the fact that plants from the genus *Lippia* are utilized more than those from the genus *Lantana* remains unexplained. The apparent utilization of *Lippia* species in the field, more so than *Lantana* species correlates strongly with the laboratory host specificity results of most lantana candidate agents tested in quarantine in recent years (Baars & Neser 1999; Simelane 2002; Baars *et al*. 2003a; b; Williams 2003a; b; Heystek & Baars 2005; Chapter 3).

The introduced biocontrol agents, with the exception of *T. scrupulosa* were not able to sustain populations on non-target plants in the absence of *L. camara*, as indicated by the data reflecting populations on non-target species, relative to the nearest *L. camara* (Figures 5.4 -5.19).This implies that any record of their association with native species should be considered as a spill-over effect. It is likely that the *L. camara* shrubs were under utilized in the field by any one species, but the combined effect of all the species on the weed may have led to *L. camara* plants becoming less palatable, increasing host searching behaviour of the agents leading to them utilizing the non-target species. As expected, the indigenous lepidopteran species, *H. laceratalis* and *A. onychote*, were recorded on *Lippia* and *L. rugosa* irrespective of its occurance relative to *L. camara* shrubs. The host range of these species seems to include these closely related species in the family Verbenaceae. The occurance of these insects on *L. camara* may constitute a new association but native species in the genus *Lantana* were likely to have been part of their physiological host range. The reported indigenous lepidopteran, *C. submediana* was only recorded on *L. rugosa* and *Lippia* species close to *L. camara* shrubs. *Characoma submediana* is however polyphagous, and was reported to have been recorded from fungus galls of *Ravenelia macrowaniana* Pazschke on *Acacia karoo* Hayne (Fabaceae),

L. camara in South Africa, Zambia, Kenya and Mauritius and has also been recorded feeding on pigeon peas, *Cajanus cajan* (L.) Millsp. (Baars 2002a).

One concern that was not addressed in this study is the damage the biocontrol agents, specifically *T. scrupulosa* cause to the non-target species. *Teleonemia scrupulosa* is a leaf feeding agent, but has been reported to reduce flowering, and may cause branches to die back on *L. camara* (Haseler 1963; Oosthuizen 1964; Haseler 1966; Greathead 1968; Harley & Kassulke 1971; Neser & Annecke 1973; Winder & Harley 1983; Muniappan & Viraktamath 1986; Cilliers 1987a; Baars & Neser 1999). The impact it has on *Lippia* species is still undetermined. *Lippia* plants in particular need be exposed to *T. scrupulosa*, to similar levels as found in the field, and impact on these plants be assessed in the future.

The impact of *Rhinocyllus conicus* Frölich on native thistles in the USA is of great concern, as it was able to reduce viable seed on Platte thistle by more than 80% (Louda 2000). Of much concern is that some *Cirsium* species are already threatened by extinction, without the added pressure of *R. conicus* (Louda 2000). The impact on *Lippia* species in South Africa is however not this severe, as the populations of biocontrol agents are very low, and *Lippia* species are abundant and weedy. *Lippia rehmannii*, *Li. javanica* and *Li. scaberrima* are all, although indigenous, considered to be problem species in South Africa (van Wyk *et al*. 2002). They compete for space, light, water and nutrients, take the place of preferred, grassy vegetation used as natural pasturage, taint milk, and sometimes (though rarely) poison livestock through liver damage and photo-sensitivity (Wells *et al*. 1986; van Wyk *et al*. 2002). These species, apart from being indigenous and deserving of conservation, are also used as a tea. Infusions of the leaves and twigs of *Lippia javanica*, *L. scaberrima* and *L. rehmannii*, are used, either weak, as a general-purpose health tea, or strong, as a topical treatment for ectoparasites. A commercial product of minor economic importance, Mosukujane tea bags, is made from *Lippia scaberrima* (van Wyk *et al*. 2000). Because of their toxicity, the prolonged use of high doses of these plants is potentially harmful (van Wyk *et al*. 2002).

Baars and Neser (1999) suggested limited feeding on non-target species in the field, especially on *Lippia* species had to be accepted as an ecologically justifiable 'trade-off ' against the benefits of releasing agents that have the potential to suppress such an environmentally damaging target weed. For the established agents in South Africa, this seems to be more the case for *T. scrupulosa*, and other agents, only within close range of *L. camara*.

CHAPTER 6

General Discussion

6.1 Introduction

This study showed that *L. camara* plants exhibit various characteristics (including flower colour, leaf and stem hairiness and thorniness), expressed in different combinations in different varieties of *L. camara* (Stirton 1977; Chapters 1; 2; 4). This variability was reported by Day and Neser (2000) to have affected populations of biological control agents, preventing establishment in some cases. In chapter 2, the varietal studies of 5 agents were conducted experimentally in the laboratory, and confirmed that varietal preference was a reality. In chapter 4, the distribution and population levels of the established agents on lantana varieties was measured in the field and showed that varietal preference influences population levels of agents on the weed.

There are also constraints placed on the biocontrol programme against the weed in terms of host specificity testing in quarantine. Biological control agents show a wider host range under confined quarantine conditions than in the field (Hill *et al*. 2000; Olckers 2000; van Klinken 2001; Chapter 1, 3 and 5). To test if the extended host ranges seen in the laboratory were a true reflection of the situation in the field, the host specificity of 5 lantana biocontrol agents were compared in the laboratory, and found that all of these, as many others (Chapter 3) accepted non-target species from the family Verbenaceae under these conditions. It was also evident that the agents showed a higher level of specificity, when taken from a no-choice situation to a more natural multiple choice situation. In chapter 5, it was concluded that field non-target effects from introduced biocontrol agents did occur. However not all species showed non-target effects. The populations of agents on non-target species were lower than on *L camara*, and in close proximity to the weed, with the exception of *T. scrupulosa*. In this chapter, these laboratory and field measured variety and host specificity are compared and discussed in terms of the effect it has on the biological control programme of the weed.

6.2 Varietal performance and preferences in the laboratory versus the field

All the biocontrol agents for *L. camara* that have shown varietal preferences in the laboratory (Chapter 2) have shown to have similar preferences in the field. *Falconia intermedia* adults were predicted to still have a high potential for biocontrol of *L. camara* in South Africa, despite its preferences for some varieties over others (Baars 2002) Other factors limiting establishment and impact of the mirid, such as acquired resistance of *L. camara* are currently being investigated (Heshula, U-N.L.P. 2005, personal communication). *Teleonemia scrupulosa*, the agent that is considered most damaging to *L. camara*, attained high populations on several varieties of *L. camara*, and few were not well utilized, those mostly discriminated against were hairy leaved varieties. *Uroplata girardi* showed varietal preferences in the laboratory, and also exhibited strong preferences in the field (Chapters 2; 4).This species exhibits the opposite preferences towards colour varieties and leaf hairiness as *O. scabripennis,* which pefers Light pink-pink and Pink-orange colour forms as well as hairy leaved varieties. This supports the theory of Day and Neser (2000), who argued that lantana biocontrol agents are not necessarily pre-adapted to feed and breed on cultivars with characters very different from its original host.

Coelocephalapion camarae Kissinger (Brentidae), a petiole and peduncle galling weevil on *L. camara*, was tested on a series of South African varieties, as well as the original plant that the weevil was collected from in Mexico (Baars 2002). The Mexican plant and three South African *L. camara* varieties were exposed to the weevil in no-choice trials, and two South African varieties and the Mexican plant in paired choice trials. All these varieties and the original host produced equal numbers of eggs in no-choice and paired choice conditions. In multiple-choice trials done by Baars (2002), only South African varieties were tested, but this weevil species showed no differences between varieties. He used this example and speculated on the potential of *F. intermedia*, and argued that the role of varietal preferences and performance differences were over-estimated. All of these studies have shown that it is important that newly introduced agents perform well on several varieties, at least to the same or slightly lower level than the original host, to ensure establishment and increase chances of high impact on the weed.

This study showed that the established agents in South Africa showed differences in preference for certain varieties, as specific populations built up much better on certain flower colour form groupings and plants with different levels of hairiness of leaves, both in the laboratory (Chapter 2) and field (Chapter 4). Laboratory studies were echoed in the field and most agents have varietal preferences and this has lead to reduced establishment and impact levels on the weed.

6.3 Effect of insect reaction to varieties for the future of biocontrol of Lantana camara

The biocontrol agents assessed in both the laboratory and in the field have shown species specific preferences for characters of the different varieties. These complement each other and the biocontrol agents collectively exert pressure on the weed complex as a whole. More biocontrol agents are still needed to control the weed sufficiently. The screening of these agents should not only include traditional host range tests, but the agent's performance and preferences on the weedy varieties needs to be tested, in comparison to its performance on its original host. This will allow predictions of potential establishment and potential population levels on varieties of the weed. It is hard to specify varieties that specifically need more pressure from agents, as all the characters are not independent. One measure that can be very useful for future assessments is to ensure that the most abundant forms are well utilized. These include mature flower corolla lobe and throat colour combinations of Pink-orange, Pink –pink, Light pink-pink and Dark pink-orange, and plants with few to medium leaf hairs. By targeting these varieties with highly successful biocontrol agents, the fastest decline in the weed populations will be achieved. The candidate's potential as a whole though, needs to be assessed, and impact studies on the weed and on non-target plants need to be quantified.

6.4 Host specificity in the laboratory versus in the field

Host-specificity tests have indicated that most of the natural enemies currently under evaluation for the biocontrol of *L. camara* accepted related native plants, especially *Lippia* species as alternative hosts. Some insect species (e.g. *A. compressa* and *C. pygmae*a), which were acceptable for release in Australia (Julien & Griffiths 1998), were

rejected for South Africa, because of their utilization of indigenous species in the laboratory (Heystek & Baars 2001; 2005). *Falconia intermedia*, *O. camarae*, *T. scrupulosa*, *C. lantanae* and *U. girardi*, have all shown some non-target impact during laboratory studies (Chapter 3). Of these, only *T. scrupulosa* and *F. intermedia* have however shown non-target impact in the field (Chapter 5) and these were very similar to that "predicted" by multiple-choice trials. This study provided further evidence of the conservative nature of laboratory tests, especially no-choice trials and suggests that extended laboratory host ranges are often not realized under field conditions. The conservative nature of these trials however contributes to the safety of biocontrol. Each agent therefore need be assessed individually and once its potential as a biocontrol agent is assessed, a decision can be made as to accept the risk of releasing such an agent with limited non-target effects measured under laboratory conditions.

6.5 Effect of candidate specificity for future biocontrol of Lantana camara

The challenge to biocontrol of lantana is to find agents that attack all the varieties of *L. camara*, yet, are specific enough not to cause any negative non-target feeding, once released. Baars and Neser (1999) suggested limited feeding on non-target species in the field, especially *Lippia* species had to be accepted as an ecologically justifiable "trade-off" against the benefits of releasing agents that have the potential to suppress such an environmentally damaging target weed. This has to be interpreted with caution, as the level of limited feeding in the lab, may not be realised in the field. The analysis of the non-target feeding in the field in South Africa is therefore important, to improve our predictive ability of further agent introductions, based on laboratory studies. It also validates predictions made, for agents recently released, and retrospectively for some of the established agents, tested in Chapter 3. The likelihood of non-target effects as well as the scale or impact of non-target effects should be predicted from laboratory studies for the field (Louda *et al.* 2005).

6.5.1 Predictability of non-target effects.

It was predicted that *F. intermedia* would be an effective agent, with very little damage to non-target species, and only in close proximity to *L. camara* (Baars 2002a). *Falconia*

intermedia is not as effective an agent as predicted, but it is as specific as predicted. It did utilise *Lippia* species, and as predicted, only in close proximity to the weed (Chapter 3 & 5). *Ophiomya camarae* was predicted to be very host specific, and very little non-target effects were anticipated. It was predicted not to be very efficient as a biocontrol agent, as high levels of acquired parasitism were expected (Simelane 2002). *Ophiomya camarae* proved to be very host specific (Chapter 3), and virtually no non-target effects were recorded (Chapter 5). It is however an efficient agent and high populations that are damaging to the weed are achieved in the field (Chapter 5). It has not acquired the level of parasitism as anticipated. Predictions for both these species were correct in terms of non-target effects, but incorrect in terms of the potential impact on the weed.

Retrospective host specificity predictions for the established agents, *T. scrupulosa*, *C. lantanae* and *U. girardi*, were all correct, or slightly conservative (Chapters 3 & 5). All of these insects showed a higher utilization of non-target species during no-choice trials, but reflected the field situation closely in multiple choice situations. The host specificity results from laboratory studies, measuring candidates physiological host range therefore does predict its ecological host range in the field correctly.

6.5.2 Impacts of non-target use
This study has unfortunately omitted to measure the impact on *Lippia* species, and interactions between species associated with *Lippia* species and introduced biocontrol agents. The non-target feeding on *Lippia* plants by *T. scrupulosa* was 28 % of that on *L. camara* in the field (Chapter 5), but what damage it caused to the plant in terms of growth and reproduction of the plant and its population numbers are still unknown. The effects of interactions between fauna species associated with *Lippia* species and the introduced agents also remain unclear. *Rhinocyllus conicus*, an introduced biocontrol agent on invasive thistles, population numbers had an inverse effect on *Paracantha culta* (Diptera: Tephritidae) populations, developing on inflorescences of native thistles in the USA (Louda et al. 2005). Several indigenous tephritid species, for example, were reared from flower and seed collections of *Lippia* species, a tingid species associated with this indigenous plant was also found on leaves of *Lippia* plants, on rare occasions. The

introduced tingid *T. scrupulosa* may indeed have had a negative effect on populations of these species, but remains unknown. The indigenous lepidopteran species naturally associated with the *Lippia* species, on the other hand, may now reach higher populations on *L. camara*, and may increase herbivory on the *Lippia* species in turn, but also remained undetermined.

Another factor influencing non-target feeding, that needs consideration, is whether the site specific situation in the field presents an insect biocontrol agent with its physiological host range or ecological. The first will be a no-choice situation, and the second a choice condition, as usually experienced in the field. The no-choice condition in the field may be experienced, as indicated by the plants far removed from *L. camara*, or a potential situation that the weed is reduced severely through effective control. Hill *et al.* (2000) found that *Eccritotarsus catarinensis* (Carvahlo) (Heteroptera: Miridae) was unable to establish on Pickerelweed, a non-target species, in the absence of the target weed, water hyacinth. Similarly, the data in this study indicated all the introduced agents, except *T. scrupulosa,* did not persist when removed from *L. camara* in the field.

The competition between weed and non-target species, aided by insects, is however, another unanswered question posed by Louda *et al.* (2005). In comparison between cases, lantana and thistles, the weeds were in direct competition with the non-target species for natural resources. The weeds in both cases were more competitive. *Rhinocyllus conicus* was acknowledged or implied to be an effective biocontrol agent (Louda 2000; Pemberton 2000; Louda *et al.* 2005). *Teleonemia scrupulosa* is considered the most effective biocontrol agent of *L. camara* (Haseler 1963; Oosthuizen 1964; Greathead 1968; Haseler 1968; Harley & Kassulke 1971; Neser & Annecke 1973; Winder & Harley 1983; Muniappan & Viraktamath 1986; Cilliers 1987a; Baars & Neser 1999; Baars & Heystek 2003a). The thistle was however not as invasive (Louda *et al.* 2005) as *L. camara*, which through allelopathy, out compete several indigenous species (Cilliers & Neser 1991; Swarbrick *et al.* 1995; Baars & Neser 1999). The real impact of non-target feeding on indigenous verbenaceae populations could only be quantified using manipulative exclusion experiments. The question can be posed: what would the weed

and non-target plant populations have been now, if the candidate biocontrol agents were not released. This same question was posed by Olckers (2000), in relation to a biocontrol candidate, *Gargaphia decoris* Drake (Tingidae), of *Solanum mauritianum* Scopoli (Solanaceae) This weed also competes directly with an indigenous *Solanum* species, on which non-target feeding and breeding occurred in laboratory studies. This however requires further study, and impact trials on *Lippia* species, as well as long-term competition studies between *L. camara* and *Lippia*, with potential agents on it.

6.6 Future prospects

Biocontrol of the lantana weed complex relies on the use of several biocontrol agents. More agents are being evaluated as potential agents for lantana and four insect species that were evaluated were considered safe for release but are still in quarantine. These are *C. camarae*, *Aceria lantanae* (Eriophyidae), *Leptostales ignifera* (Geometridae) and a *Longitarsus* species (Alticinae) (Baars & Heystek 2001; Baars, *et al*. 2004; Urban *et al*. 2001; Williams 2003; Simelane, in prep). *Coelocephalapion camarae* has been described as a promising agent, in terms of potential impact on the weed, and limited expected non-target effects and wide acceptance of South African lantana varieties (Baars & Heystek 2001; Baars, *et al*. 2004). *Aceria lantanae* is highly host specific, but shows high levels of varietal preferences, and will be limited to high humidity areas in South Africa, once released (Urban *et al*. 2001). *Leptostales ignifera* is highly damaging, and specific to *L. camara*, but is expected to suffer high levels of parasitism and predation (Williams 2003). The impact of this species can however not be ruled out, as the lepidopteran species, *H. laceratalis* and *S. haemorrhoidalis* do reach high, damaging population levels on *L. camara* in South Africa and previous predictions on *O. camarae*, attracting high levels of parasitism, were incorrect. *Longitarsus* sp., a root-feeding agent, and therefore utilizing a new niche on *L. camara* in lantana biocontrol worldwide, may make a valuable contribution towards the control of lantana. It may however be limited by soil types as well as soil moisture conditions. This agent, like the others have a very narrow host specificity and very little non-target feeding on *Lippia* species in close proximity to *L. camara* is anticipated (Simelane, unpublished data).

Further unexplored niches on *L. camara* also need to be addressed, and stem attacking agents as well as pathogens should be explored further as additional biocontrol agents for this weed.

6.7 Conclusion

Lantana camara is a complex weed and therefore requires several biocontrol agents to address each aspect of the weed in all the climatic conditions of South Africa. It is a strong competitor against several indigenous plants, and successful control is therefore essential. The success of the lantana biocontrol programme may be under-estimated, as it is such a long running programme that has had little post release studies in terms of the impact of the agents on the weed and non-target species. It is clear that success of the programme needs to be improved further, and the best solution seems to be the introduction of more biocontrol agents.

Variety differences in the weed remain a challenge that can be overcome with the use of multiple agents, as the different agents have demonstrated a preference to characteristics of varieties of the weed that are species specific. There are a few candidate biocontrol agents that hold promise to contribute to the biocontrol of the weed, once released from quarantine.

Potential non-target effects must be avoided, not only to preserve *Lippia* species, but to preserve the credibility of biocontrol as a whole. Limited feeding on *Lippia* plants in the field in close proximity to the weed may have to be accepted, but the impact it has on *Lippia* species needs to be addressed, before further introductions are made of agents with the potential to damage these indigenous species. The potential non-target effects can also be predicted relatively accurately, as the multiple choice trials in the laboratory closely resemble the situation in the field.

REFERENCES

Anonymous. 1999. Releases of introduced biological control agents against weeds in South Africa. In: T. Olckers and M.P. Hill (eds.). *Biological Control of Weeds in South Africa (1990-1998). African Entomology Memoir* 1: 175-182.

Baars, J-R. 2000a. Emphasizing behavioural host-range: the key to resolving ambiguous host-specificity results on *Lantana camara* L. In: N.R. Spencer (ed.). *Proceedings of the Xth International Symposium on Biological Control of Weeds*. July 4-14 1999, Montana State University, Bozeman, Montana, USA. pp. 887-896.

Baars, J-R. 2000b. Biology and host range of *Falconia intermedia* (Hemiptera: Miridae), a potentially damaging natural enemy of *Lantana camara* in South Africa. In: N.R. Spencer (ed.). *Proceedings of the Xth International Symposium on Biological Control of Weeds*. July 4-14 1999, Montana State University, Bozeman, Montana, USA. p. 673.

Baars, J-R. 2000c. A cure for lantana at last? *Plant Protection News* 57: 8-11.

Baars, J-R. 2002a. 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. Department of Zoology and Entomology. Rhodes University, South Africa.

Baars, J-R. 2002b. The life history and host specificity of *Teleonemia vulgata* Drake & Hambleton (Hemiptera: Tingidae) a biocontrol agent of *Lantana camara* L. (Verbenaceae). *African Entomology* 10(2): 315-324.

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. and F. Heystek. 2001. Potential contribution of the petiole galling weevil, *Coelocephalapion camarae* Kissinger, to the biocontrol of *Lantana camara* L. In: T. Olckers and D.J. Brothers (eds.). *Proceedings of the Thirteenth Entomological Congress.* July 2-5, Pietermaritzburg, South Africa. p. 79.

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

Baars, J-R. and F. Heystek. 2003b. Release of the Mexican weevil, *Coelocephalapion camarae*, from quarantine in Pretoria, for biological control of the noxious weed, *Lantana camara,* in South Africa. *Host Specificity Report to the National Department of Agriculture: Directorate of Plant Health & Quality*. 36 pp.

Baars, J-R., F. Heystek and M.P. Hill. 2003 (Baars *et al*. 2003a). Potential of petiole galling weevil to improve lantana biocontrol. In: J. M. Cullen, D.T. Briese, D.J. Kriticos, W.M. Lonsdale, L. Morin and J.K. Scott (eds.) *Proceedings of the eleventh International Symposium on Biological Control of Weeds*, April 27- May 2, 2003. CSIRO, Canberra, Australia. p. 604.

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

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

Briese, D.T., T.A. Heard, R.E. Mcfadyen, A.W. Sheppard and H. Spafford Jacob. 2003. The selection, testing and evaluation of weed biological control agents: Is there still room for improvement? In: Spafford Jacob, H. and Briese, T. (eds.) *Improving the selection, testing and evaluation of weed biological control agents. Proceedings of the CRC for Australian Weed Management Biological Control of Weeds Symposium and Workshop.* September 2002. Pert, Australia. Technical Series 7: 1-3.

Broughton, S. 1999. Impact of the seed-fly *Ophiomyia lantanae* (Froggatt)(Diptera: Agromyzidae), on the viability of lantana fruit in South-East Queensland, Australia. *Biological Control* 15: 168-172.

Broughton, S. 2000. Review and evaluation of lantana biocontrol programs. *Biological Control* 17: 272-286.

Buzzi, Z.J. and J.A. Winder. 1981. A new species of *Uroplata* (Coleoptera, Chrysomelidae, Hispinae) mining leaves of *Lantana tiliaefolia* in Paraña State, Brazil. *Revista Entomologia do Brasil* 25(1): 49-52.

Cilliers, C.J. 1977. On the biological control of *Lantana camara* in South Africa. In: A.A. Balkema (ed.) *Proceedings of the Second National Weeds Conference of South Africa*. 2-4 February 1977. Cape Town, South Africa. pp. 341-344.

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

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

Cruttwell McFadyen, R.E. 1998. Biological control of weeds. *Annual Review of Entomology* 43: 369-393.

Davies, J.C. and D.J. Greathead.1967. Occurrence of *Teleonemia scrupulosa* on *Sesamum indicum* Linn. In Uganda. *Nature* 213(5071): 102-103.

Day, M. D. 1999. Continuation trials: their use in assessing the host range of a potential biological control agent. *Host specificity testing in Australia: towards improved assays for biological control*: pp11-19.

Day, M. D. and S. Neser. 2000. Factors influencing the biological control of *Lantana camara* in Australia and South Africa. In: N.R. Spencer (ed.). *Proceedings of the tenth International Symposium on Biological Control of Weeds*. July 4-14 1999, Montana State University, Bozeman, Montana, USA. pp. 897-908.

Day, M.D., C.J. Wiley, J. Playford and M.P. Zalucki. 2003. *Lantana: Current Management Status and Future Prospects*. ACAIR, Canbera, ACT, Australia.

Den Breeÿen, A. 2000. *Mycovellosiella lantanae* var. *lantanae*, a potential biocontrol agent of *Lantana camara* in South Africa. In: *Proceedings of the third International Weed Science Congress* – IWSC, June 6-11 2000, Foz Do Iguassu Brazil. pp. 172.

Den Breeÿen, A. & M.J. Morris. 2003. Pathogenicity and host specificity of *Mycovellosiella lantanae* var. *lantanae*, a potential biocontrol agent for *Lantana camara* in South Africa. *Biocontrol Science and Technology* 13: 313-322.

Denton, G.R.W., R. Muniappan. and M. Marutani. 1991. Status and natural enemies of the weed, *Lantana camara*, in Micronesia. *Tropical Pest Management* 37: 338-344.

Dunn, P.H. 1976. Shortcommings in the Classic tests of Candidate Insects for the Biocontrol of Weeds. In: T.E. Freeman (ed.). *Proceedings of the fourth International Symposium on Biological control of Weeds.* Center for Environmental Programs, Institute of Food and Agricultural sciences, University of Florida. Gainesville. pp.51-56.

Edwards, P.B. 1999. The use of choice tests in host-specificity testing of herbivorous insects. *Host specificity testing in Australia: towards improved assays for biological control*: pp 35-43.

Esguerra, N.M., K.J. Diopulos, R.P. Samuel. and J. William. 1997. Establishment of the leaf mining fly, *Calycomyza lantanae* Frick, on the weed *Lantana camara* L. on Pohnpei. *Micronesica* 30 (2): 417-419.

GenStat ® for Windows® (2003) (7th Edition) - Introduction (Editor R.W. Payne), *VSN International*, ISBN 1-904375-08-1.

Gentle, C.B. and J.A. Duggin. 1997a. Allelopathy as a competitive strategy in persistant thickets of *Lantana camara* L. in three Australian forest communities. *Plant Ecology* 132: 85-95.

Gentle, C.B. and J.A. Duggin. 1997b. *Lantana camara* L. invasions in dry rainforest-open ecotones: the role of disturbances associated with fire and cattle grazing. *Australian Journal of Ecology* 22: 298-306.

Greathead, D.J. 1968. Biological control of *Lantana*. A review and discussion of recent developments in East Africa. *PANS* 14: 167-175.

Grobler, H., J.B. Vermeulen and K. van Zyl. 2000. *A Guide to the use of herbicides*. Seventeenth edition. National Department of Agriculture, Republic Of South Africa. Directorate: Agricultural Production Inputs. 154 pp.

Harley, K.L.S. 1968. The suitability of *Octotoma scabripennis* Guér. and *Uroplata girardi* Pic (Col., Chrysomelidae) for the control of *Lantana* (Verbenaceae) in Australia. *Bulletin of Entomological Research* 58: 835-843.

Harley, K.L.S. 1974. Biological control of *Lantana* in Australia. In: A.J. Wapshere (ed.). *Proceedings of the third International Symposium of the Biological Control of Weeds*. September 1973. Commonwealth Institute of Biological Control Franham, UK, Montpellier, France. pp. 23-29.

Harley, K.L.S. and R.C. Kassulke. 1971. Tingidae for biological control of *Lantana camara* (Verbenaceae). *Entomophaga* 16: 389-410.

Harley, K.L.S. and R.C. Kassulke. 1974. The suitability of *Phytobia lantanae* Frick for biological control of *Lantana camara* in Australia. *Journal of the Australian Entomological Society* 13: 229-233.

Harley, K.L.S., J.D. Kerr and R.C. Kassulke. 1979. Effects in S.E. Queensland during 1967-72 of insects introduced to control *Lantana camara*. *Entomophaga* 24: 65-72.

Haseler, W.H. 1963. Progress in insect control of lantana. *Queensland Agricultural Journal* 89: 65-68.

Haseler, W.H. 1966. The status of insects introduced for the biological control of weeds in Queensland. *Journal of the Entomological Society of Queensland* 5: 1-4.

Heard, T.A. 1999. Methods for host testing insects that use an oviposition deterring pheromone or other host discrimination system. *Host specificity testing in Australia: towards improved assays for biological control*: pp30-34.

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

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. Msc thesis. Department of Zoology and Entomology. Rhodes University, South Africa.

Heystek, F. and J-R. Baars. 2001. The treehopper, *Aconophora compressa*, unsuitable for biocontrol of the weed *Lantana camara* in South Africa. In: T. Olckers and D.J. Brothers (eds.). *Proceedings of the Thirteenth Entomological Congress.* July 2-5, Pietermaritzburg, South Africa. p. 29.

Heystek, F. and J-R. Baars. 2005. Biology and host range of *Aconophora compressa*, a candidate considered as a biocontrol agent of *Lantana camara* in Africa. *BioControl* 50: 359-373.

Heystek, F. and T. Olckers. 2003. Impact of the lantana mirid in South Africa. In: J. M. Cullen, D.T. Briese, D.J. Kriticos, W.M. Lonsdale, L. Morin and J.K. Scott (eds). *Proceedings of the eleventh International Symposium on Biological Control of Weeds*, April 27- May 2, 2003. CSIRO, Canberra, Australia. p. 606.

Hight, S.D., R.W. Pemberton, P. Conant and T. Johnson. 2003. Attack on and use of a native Hawaiian plant by the biological control agent *Teleonemia scrupulosa* introduced against *Lantana camara*. In: J. M. Cullen, D.T. Briese, D.J. Kriticos, W.M. Lonsdale, L. Morin and J.K. Scott (eds). *Proceedings eleventh International Symposium on Biological Control of Weeds*, CSIRO, Canberra, Australia. p. 350.

Hill, M.P. and P.E. Hulley. 1995. Host-range extension by native parasitoids to weed biocontrol agents introduced to South Africa. *Biological Control* 5: 297-302.

Hill, M.P., T.D. Center, J.N. Stanley, H.A. Cordo, J. Coetzee and M.J. Byrne. 2000. The performance of the water hyacinth mirid, *Eccritotarsus catarinensis*, on water hyacinth and pickerelweed: a comparison of laboratory and field results. In: N.R. Spencer (ed.). *Proceedings of the tenth International Symposium on Biological Control of Weeds*. July 4-14 1999, Montana State University, Bozeman, Montana, USA. pp. 357-366.

Julien, M.H. and M.W. Griffiths. 1998. *Biological Control of Weeds: A World Catalogue of Agents and their Target Weeds*. 4th Edition. CABI Publishing, UK.

Kellerman, T.S., T.W. Naude and N. Fourie. 1996. The distribution, diagnoses and estimated economic impact of plant poisonings and mycotoxicoses in South Africa. *Onderstepoort Journal of Veterinary Research* 63: 65-90.

Krauss, N.L.H. 1962. Biological control investigations on lantana. *Proceedings of the Hawaiian Entomological Society* 18: 134-136.

Krauss, N.L.H. 1964. Some leaf-mining chrysomelids of lantana (Coleoptera). *Coleopterist's Bulletin* 18: 92-94.

Louda, S.M. 2000. Rhinocyllus conicus – Insights to improve Predictability and Minimize Risk of Biological Control of Weeds. In: N.R. Spencer (ed.). *Proceedings of the tenth International Symposium on Biological Control of Weeds.* July 4-14, 2000. Montana State University, Bozeman, Montana, USA. pp. 187-193.

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

Louda, S.M., T.A. Rand, F.L. Russell and A.E. Arnett. 2005. Assessment of ecological risks in weed biocontrol: Input from retrospective ecological analyses. Biological Control 35 (3): 253-264.

Löyttyniemi, K. 1982. Evaluation of the use of insects for biological control of *Lantana camara* L. (Verbenaceae) in Zambia. *Tropical Pest Management* 28: 14-19.

Mabuda, K. (Submitted). *Eutreta xanthochaeta* (Diptera: Tephritidae) unsuitable for biocontrol of *Lantana camara* in Africa. *Biocontrol Science and Technology*.

Muniappan, R. and C.A. Viraktamath. 1986. Status of biological control of the weed, *Lantana camara* in India. *Tropical Pest Management* 32: 40-42.

Muniappan, R., G.R.W. Denton, J.W. Brown, T.S. Lali, U. Prasad. and P. Singh. 1996. Effectiveness of the natural enemies of *Lantana camara* on Guam: a site and seasonal evaluation. *Entomophaga* 41: 167-182.

Neser, S. and D.P. Annecke. 1973. Biological control of weeds in South Africa. *Entomology Memoir* 28: 1-27.

Neser, S. and C.J. Cilliers. 1990. Work towards biological control of *Lantana camara*: perspectives. In: E.S. Delfosse (ed.). *Proceedings of the seventh International Symposium on Biological Control of Weeds*. March 6-11 1988, Rome, Italy. Istituto Sperimentale per la Pathologia Vegetale, Ministero dell'Agricoltura e delle Foreste. pp. 363-369.

Olckers, T. 2000. Biology, host specificity and risk assessment of *Gargaphia decoris*, the first agent to be released in South Africa for the biological control of the invasive tree *Solanum mauritianum*. *BioControl* 45: 373-388.

Ooi, P.A.C. 1987. A fortuitous biological control of *Lantana* in Malaysia. *Tropical Pest Management* 33: 234-235.

Oosthuizen, M.J. 1964. The biological control of *Lantana camara* L. in Natal. *Journal of the Entomological Society of Southern Africa* 27: 3-16.

Palmer, W.A., B.W. Willson and K.R. Pullen. 1996. The host range of *Aconophora compressa* Walker (Homoptera: Membracidae): a potential biological control agent for *Lantana camara* L. (Verbenaceae). *Proceedings of the Entomological Society of Washington* 98: 617-624.

Palmer, W.A., B.W. Willson and K.R. Pullen. 2000. Introduction, rearing, and host range of *Aerenicopsis championi* Bates (Coleoptera: Cerambycidae) for the biological control of *Lantana camara* L. in Australia. *Biological Control* 17: 227-233.

Pemberton, R.W. 2000. Predictable risk to native plants in weed biological control. *Oecologia* 125: 489-494.

Perkins, R.C.L. and O.H. Swezey. 1924. The introduction into Hawaii of insects that attack lantana. *Hawaii Sugar Planters Association Experimental Station Bulletin* (*Entomological Series*) 16: 1-83.

Phenye, M.S. & D.O. Simelane. 2003. An arid-adapted leafhopper, *Barela parvisaccata* (Hemiptera:Auchenorrhyncha: Cicadellidae), rejected as a biological control agent for *Lantana camara* in South Africa. In: R. Stals (ed.). *Proceedings of the fourteenth Entomological Congress organised by the Entomological Society of Southern Africa*, Pretoria, July 6-9, 2003. p. 64.

Phenye, M.S. & D.O. Simelane. Arid-adapted leafhopper, *Barela parvisaccata* (Hemiptera: Cicadellidae), unsuitable for biocontrol of *Lantana camara* in Africa. (In prep.)

Radunz, L.A.J. 1971. Some aspects of the preferencial behaviour of *Teleonemia scrupulosa* Stål, towards its host plant *Lantana camara*. Honours thesis, University of Queensland, 53 pp.

Retief, E. 2003. Verbenaceae. In Germishuizen, G. And Meyer, N.L. (eds), Plants of southern Africa: an annotated checklist. *Strelitzia* 14: 941-944. National Botanical institute, Pretoria.

Sands, D.P.A. and K.L.S. Harley. 1981. Importance of geographic variation in agents selected for biological control of weeds. In: E.S. Delfosse (ed.). *Proceedings of the fifth International Symposium on the Biological Control of Weeds*, July 22-27, 1980, Brisbane, Australia. CSIRO, Melbourne, pp.81-89.

Scott, L.J., G.C. Graham, M. Hannan-Jones and D.K. Yeates. 1997. DNA profiling resolves the limited importance of flower colour in defining varieties of *Lantana camara*. *Electrophoresis* 18: 1560-1563.

Scheibelreiter, G.K. 1980. Biological control of *Lantana camara* L. (Verbenaceae) in Ghana. *Zeitschrift für angewante Entomologie* 90: 99-103.

Schreiner, J.A. 1989. Biological control introductions in the Caroline Islands. *Proceedings of the Hawaiian Entomological Society* 29: 57-69.

Simelane, D.O. 2001. Laboratory host range of *Longitarsus* sp., a flea beetle on *Lantana camara* L. In: T. Olckers and D.J. Brothers (eds.). *Proceedings of the Thirteenth Entomological Congress.* July 2-5 2001 Pietermaritzburg, South Africa. p. 59.

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. 2003. Preliminary assessment of release and establishment of lantana herringbone leafminer, *Ophiomyia camarae* (Diptera: Agromyzidae), in South Africa. In: J. M. Cullen, D.T. Briese, D.J. Kriticos, W.M. Lonsdale, L. Morin and J.K. Scott (eds). *Proceedings eleventh International Symposium on Biological Control of Weeds*, April 27- May 2, 2003. CSIRO, Canberra, Australia. pp. 447-450.

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.and M.S. Phenye. 2004. Suppression of growth and reproductive capacity of the weed *Lantana camara* (Verbanaceae) by *Ophiomyia camarae* (Diptera: Agromyzidae) and *Telionemia scrupulosa* (Heteroptera: Tingidae). *BioControl Science and Technology* 15 (2): 153-163.

Smith, L.S. and D.A. Smith. 1982. The naturalized *Lantana camara* complex in eastern Australia. *Queensland Botanical Bulletin No. 1.* Queensland Department of Primary Industry, Brisbane.

Snedecor, G.W. and W.G. Cochran. 1980. *Statistical methods (7ᵗʰ Ed.).* Iowa State University Press.

Spies, J.J. and C.H. Stirton. 1982a. Chromosome numbers of the South African plants. *South African Journal of Botany* 48: 21-22.

Spies, J.J. and C.H. Stirton. 1982b. Meiotic studies of some South African cultivars of *Lantana camara* (Verbenaceae). *Bothalia* 14: 101-111.

Stilling, P and D.C. Moon. 2001. Protecting Rare Florida Cacti from attack by the Exotic Cactus Moth, *Cactoblastis cactorum* (Lepidoptera:Pyralidae). *Florida Entomologist* 84 (4): 506-509.

Stirton, C.H. 1977. Some thoughts on the polyploid complex *Lantana camara* L. (Verbenaceae). *Proceedings of the Second National Weeds Conference of South Africa*. February 2-4, 1977. A.A. Balkema, Cape Town pp. 321-344.

Swarbrick, J.T., B.W. Willson and M.A. Hannan-Jones. 1995. The biology of Australian weeds: *Lantana camara* L. *Plant Protection Quarterly* 10: 82-95.

Urban, A.J., P.F Mpedi, S. Neser, and C. Creamer. 2001. C. Potential of the flower gall mite, *Aceria lantanae* (Cook) (Acari: Eriophyidae) for biocontrol of the noxious weed *Lantana camara* L. (Verbenaceae). In: T. Olckers and D.J. Brothers (eds.). *Proceedings of the thirteenth Entomological Congress,* July 2-5, 2001. Pietermaritzburg, South Africa. pp. 67-68.

Urban, A. J., S. Neser, J-R. Baars, D.O. Simelane, A. den Breëyen, K. Mabuda, H. Klein, H.E. Williams, F. Heystek, M.S. Phenye, G.A. Samuels and L.G. Madire. 2003. New Biocontrol Agents Developed for Lantana. In: R. Stals (ed.). *Proceedings of the fourteenth Entomological Congress*, July 6-9, 2003. Pretoria, South Africa. p. 106.

Urban, A.J. and M.S. Phenye. 2005. Impact of *Ophiomya camarae* (Diptera: Agromyzidae) on *Lantana camara* (Verbenaceae): prediction and realization. In: M.H. Villet (ed.). *Proceedings of the fifteenth Entomological Congress*. Grahamstown, South Africa. July 10-13, 2005. Grahamstown, South Africa. p. 84.

Urban, A.J. and D.O. Simelane. 1999. Performance of a candidate biocontrol agent, *Falconia intermedia* (Hemiptera: Miridae), on selected biotypes of *Lantana camara* (Verbenaceae). In: J.B.J. Van Rensburg and J. Van Den Berg (eds.). *Proceedings of the twelfth Entomological Congress*, July 12-15, 1999. Potchefstroom, South Africa. p. 120.

Van Klinken, R.D. 2001. Host specificity testing: Why do we do it and how we can do it better. In: R. Van Driesche, T.A. Heard, A.S. McClay and R. Reardon (eds.). *Proceedings of Session: Host specificity testing of exotic Arthropod Biological Control agents – the biological basis for improvement in Safety*. USDA Forest Service, August 2000, pp. 54-68.

Van Wilgen, B.W., M.P.de Wit, H.J. Anderson, D.C. Le Maitre, I.M. Kotze, S. Ndala, B. Brown and M.B.Rapholo. 2004. Costs and benefits of biological control of invasive alien plants: case studies from South Africa. *South African Journal of Science* 100: 113-122.

Van Wyk, B-E., F. Van Heerden and B. van Oudtshoorn. 2002. *Poisonous plants of South Africa*. Briza Publications, Pretoria. 288 pp.

Van Wyk, B-E., B. Van Oudtshoorn and N Gericke. 2000. *Medicinal plants of South Africa*. Briza Publications, Pretoria. 304 pp.

Waddell, K.J. and T.A. Mousseau. 1996. Oviposition preference hierarchy of *Brachys tessellatus* (Coleoptera: Buprestidae). *Population Ecology* 25: 63-67.

Wapshere, A.J. 1989. A testing sequence for reducing rejection of potential biological control agents for weeds. *Annals of Applied Biology* 114: 515-526.

Waterhouse, D.F. and K.R. Norris. 1987. *Biological Control Pacific Prospects*. Inkata Press, Melbourne, Australia.

Wells, M.J., A.A. Balsinhas, H. Joffe, V.M. Engelbrecht, G. Harding and C.H. Stirton. 1986. A catalogue of problem plants in South Africa. *Memoirs of the Botanical Survey of South Africa No.* 53. Botanical Research Institute, Department of Agriculture and Water Supply, Pretoria, South Africa.

Williams, H.E. 2003a. The Suitability of *Alagoasa extrema* Jacoby (Coleoptera: Chrysomelidae: Alticinae), as a biological control agent for *Lantana camara* L. in

South Africa. Msc thesis. Department of Zoology and Entomology. Rhodes University, South Africa.

Williams, H.E. 2003b. Release of the Mexican leaf-feeding inch-worm, Leptostales *ignifera*, from quarantine in Pretoria, for biological control of the invasive weed, *Lantana camara*, in South Africa. *Host specificity report to the National Department of Agriculture: Directorate of Plant Health and Quality.*

Williams, H.E. 2004. Unsuitability of *Charidotis pygmaea* for biocontrol of *Lantana camara* in Africa. *BioControl* 49: 211-223.

Williams, H. and M.P. Hill. 2003. The trimorphic lantana flea-beetle *Alagoasa extrema* not suitable for release in Africa, is suitable for biocontrol in Australia. In: J. M. Cullen, D.T. Briese, D.J. Kriticos, W.M. Lonsdale, L. Morin and J.K. Scott (eds). *Proceedings of the eleventh International Symposium on Biological Control of Weeds*, CSIRO, April 27- May 2, 2003. Canberra, Australia. p. 357.

Willson, B.W. 1968. Insects on trial in fight against lantana. *Queensland Agricultural Journal* 94: 748-751.

Winder, J.A. and K.L.S. Harley. 1983. Phytophagous insects on lantana in Brazil and their potential for biological control in Australia. *Tropical Pest Management* 29: 346-362.

Winder, J.A., K.L.S. Harley and R.C. Kassulke. 1984. *Uroplata lantanae* Buzzi & Winder (Coleoptera: Chrysomelidae: Hispinae), a potential biological control agent of *Lantana camara* in Australia. *Bulletin of Entomological Research* 74: 327-340.