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.

THESIS

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ABSTRACT

Lantana camara (lantana), a thicket-forming shrub, a number of different varieties of which were introduced into South Africa as ornamental plants but which has become a serious invasive weed. Conventional control measures for lantana are expensive and ineffective and it has therefore been targeted for biological control since 1961.

To date, eleven biological control agent species have become established on lantana in South Africa. However, most agents persist at low densities and only occasionally impact plant populations. Three species regularly cause significant damage, but only reach sufficiently high numbers by midsummer after populations crash during the winter. Overall, the impact of the biological control programme on the weed is negligible and this has been ascribed to the poor selection of agents for release, the accumulation of native parasitoids, differences in insect preference for different varieties of the weed and variable climatic conditions over the weed's range. This study suggests that the importance of varietal preferences has been over-estimated.

A predictive bioclimatic modelling technique showed that most of the agents established in South Africa have a wide climatic tolerance and that the redistribution and importation of new climatypes of these agents will not improve the level of control. Additional agents are required to improve the biocontrol in the temperate conditions, and also to increase damage in the sub-tropical areas where most of the agents are established and where the weed retains its leaves year round. New candidate agents that possess biological attributes that favour a high intrinsic rate of increase, a high impact per individual and that improve the synchrony between the weed and the agent in climatic conditions that promote the seasonal leaflessness of plants should receive prior consideration.

A survey in Jamaica indicated that additional biological control agents are available in the region of origin but that care should be taken to prioritise the most effective agents. The various selection systems currently available in weed biocontrol produce contradictory results in the priority assigned to candidate agents and a new selection system is proposed.

The biology and host range of two new candidate natural enemies, the leaf-galling weevil, *Coelocephalapion camarae* and the leaf-sucking mirid, *Falconia intermedia* were investigated for the biocontrol of lantana. The studies indicated that these have considerable biocontrol potential, in that the weevil has a wide climatic tolerance and has the potential to survive the host leaflessness typical of temperate conditions, while the mirid has a high intrinsic rate of increase, and the potential for several generations a year. Both agents caused a high level of damage to the leaves, with the weevil galling the vascular tissue in the leaf-petiole and the mirid causing chlorotic speckling of the leaves. During laboratory trials both agents accepted indigenous species in the genus *Lippia*. However, under multiple choice conditions these agents showed a significant and strong oviposition preference for lantana. A risk assessment and post release field trials indicated that *F. intermedia* is likely to attack some *Lippia* species in the presence of lantana, but the levels of damage are predicted to be relatively low. A possible low incidence of damage to indigenous species was considered a justifiable 'trade-off' for the potentially marked impact on *L. camara*.

Preference and performance studies on the two candidate agents suggested that most of the South African lantana varieties are suitable host plants. The mirid preferred certain varieties in multiple choice experiments, but this is unlikely to affect its impact under field conditions. Permission for release was accordingly sought for both species.

Finally, the challenges facing the biological control programme and the potential for improving the control of *L. camara* in South Africa are considered.

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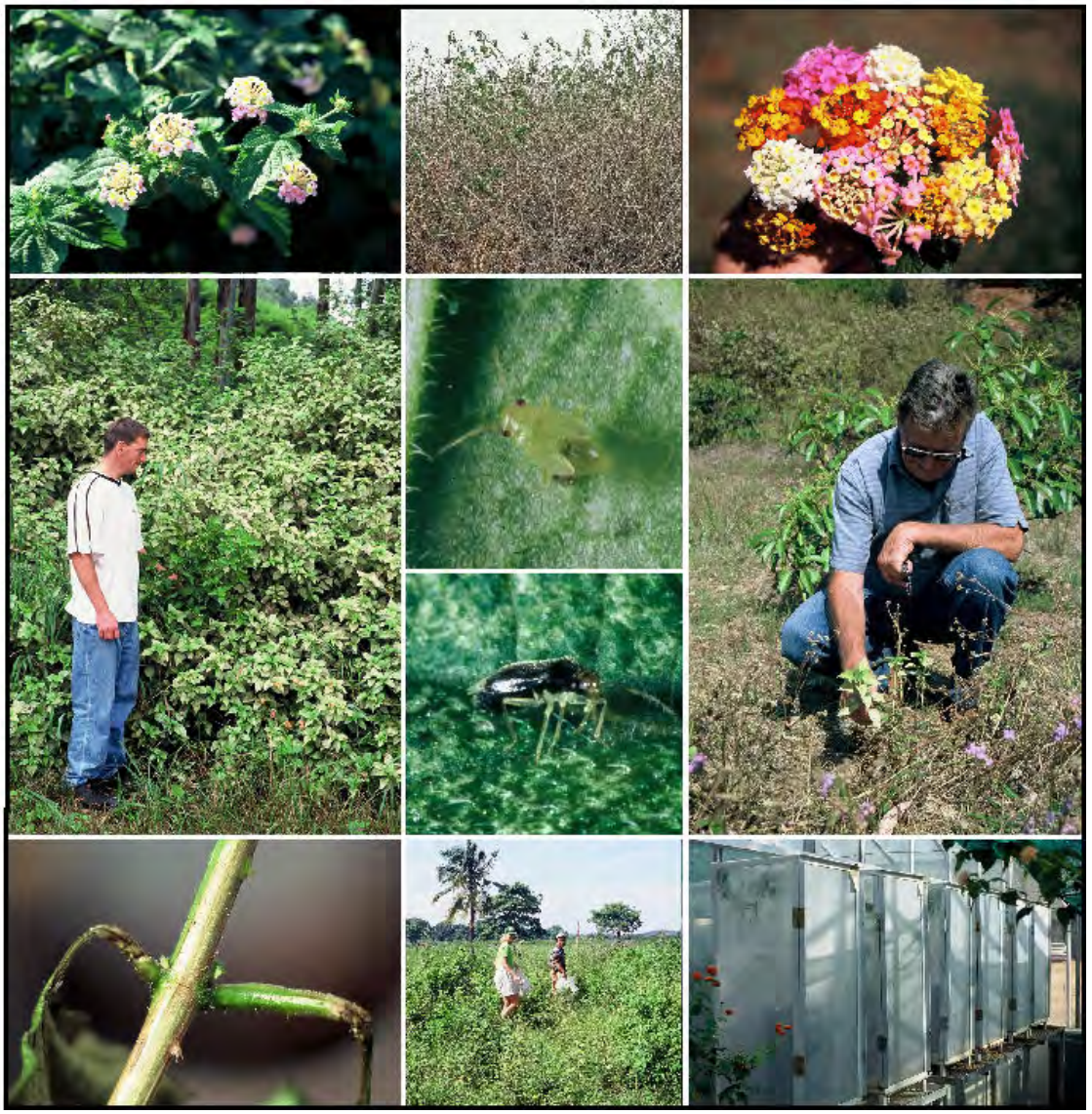

Top row (Left to Right): *Lantana camara* (lantana); state of *L. camara* in dry and cold winter conditions; Flower colour of South African lantana varieties.

Second row (Left to Right): *Falconia intermedia* impact on lantana (Tzaneen, South Africa); *F. intermedia* nymph and adult; feeding damage on lantana seedlings.

Bottom row (Left to Right): *Coelocephalapion camarae* petiole galls on lantana; *L. camara* in native range (Mexico); No-choice host-specificity trials (Pretoria, South Africa).

PUBLICATIONS ARISING FROM THIS STUDY

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GENERAL INTRODUCTION

1.1 *Biology and Invasiveness*

Lantana camara sensu lato (Verbenaceae; Fig. 1.1), a floriferous, prickly, thicket-forming shrub, which is commonly known as lantana, originates from tropical South and Central America (Stirton, 1977). As a popular ornamental plant, several varieties (cultivars) of lantana have been widely distributed throughout the tropics, subtropics and warm temperate regions of the world. Lantana has become naturalized in some 50 countries and is rated as one of the world's worst weeds (Holm *et al.*, 1977).

Lantana is an aggressive, vigorously growing weed that tolerates a wide variety of environmental conditions, but thrives better in humid than in dry regions. In South Africa it is presently naturalized in the warm, moist subtropical and temperate regions (Oosthuizen, 1964; Stirton, 1977) of the Northern, Gauteng, Mpumalanga and KwaZulu-Natal, as well as the southern coastal regions of the Eastern and Western Cape provinces (Fig. 1.2). Lantana invades river-banks, mountain slopes and valleys, pastures and commercial forests where it forms impenetrable stands that obstruct access and utilization. Through allelopathic suppression of indigenous plant species, lantana invasions also interrupt regeneration processes (Gentle and Duggin, 1997a) and reduce the biodiversity of natural ecosystems.

Lantana leaves, stems and fruit contain toxic compounds, notably the pentacyclic triterpenes (Kellerman *et al.*, 1996), lantadene A and B (Morton, 1994), which if consumed can cause photosensitization, liver and kidney damage, paralysis of the gall bladder, intestinal haemorrhage and death in 1-4 days in cattle, sheep and horses. Livestock previously exposed to lantana are less likely to suffer from the acute symptoms caused by ingestion, while those with no previous exposure are likely to be severely affected. The expected annual impact of cattle mortalities from lantana poisoning in South Africa was estimated to be in excess of R 1 728 000 (exchange rate of R3.965 to US$1 in 1996) (Kellerman *et al.*, 1996).

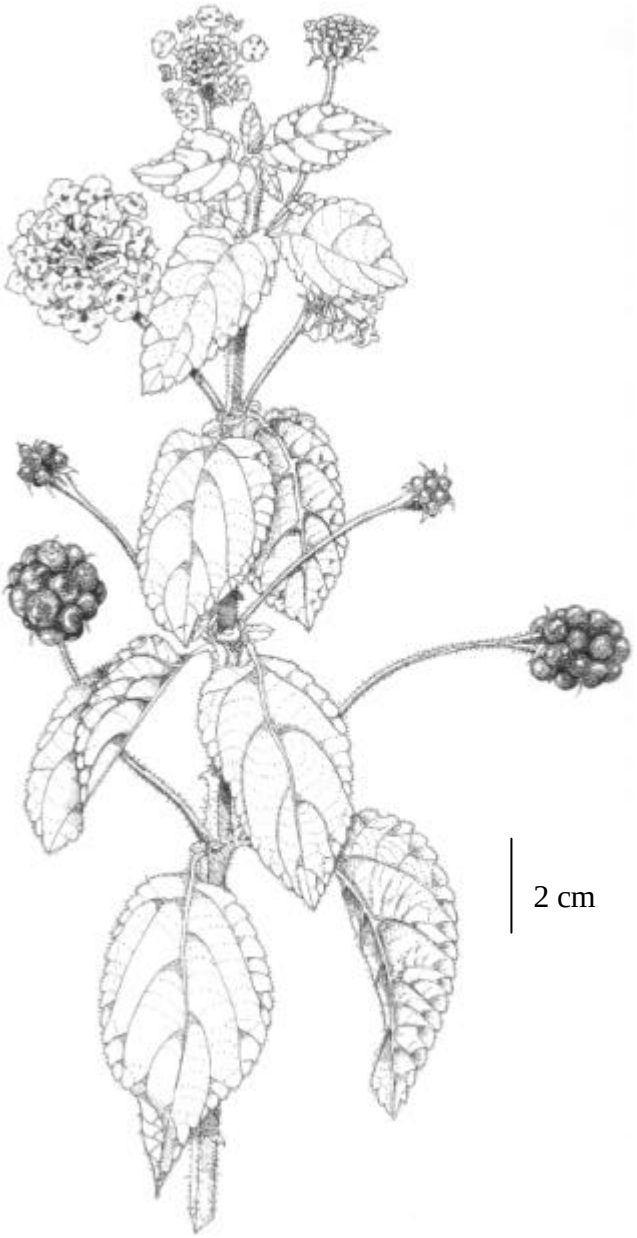


Fig. 1.1 Branch of *Lantana camara* L. (Verbenaceae) with flowers and fruit. Drawn by R. Weber (National Botanical Institute, Pretoria).

1.2 *Taxonomy*

Lantana camara is an extremely variable entity that presents a complex taxonomic problem (Munir, 1996). Its conspicuous variability in general morphology (Howard, 1970; Smith and Smith, 1982; Cilliers, 1987a; Munir, 1996), cytology and genetic composition (Spies and Stirton, 1982a, 1982b; Spies, 1984; Spies and Du Plessis 1987),

has led to the recognition of hundreds of cultivars or varieties, all of which are classified as *L. camara*. The weed is a polyploid complex, presumably derived from the deliberate hybridization of species in the genus *Lantana* (Stirton, 1977) and, subsequent to naturalization in South Africa, hybridization has continued to occur in the field (Spies and Stirton, 1982a, 1982b, 1982c). Spies (1984) argued that *L. camara* is in an active phase of evolution, with intermediates in a transitional stage of speciation. Although the species is considered to be unstable, certain varieties dominate in localized areas and remain relatively stable through space and time. Following ecological disturbances, lantana stands may recolonize as a mixture of varieties, but soon revert to the former dominant condition (Spies and Du Plessis, 1987).

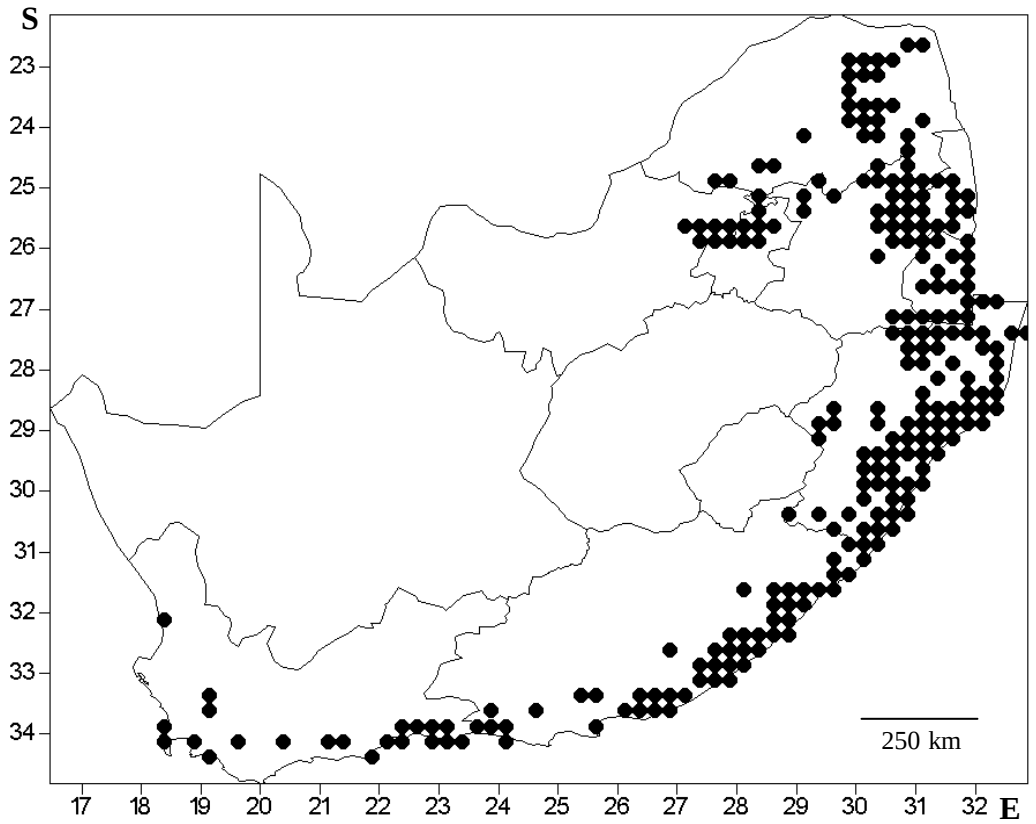


Fig. 1.2 Distribution of *Lantana camara* in South Africa, Swaziland and Lesotho. Drawn by L. Henderson (Southern African Plant Invaders Atlas Database, Plant Protection Research Institute, Pretoria).

In KwaZulu-Natal Province alone there are 17 *L. camara* varieties that have been differentiated using floral and vegetative characters (Stirton and Erasmus, 1990). Although some of the varieties naturalized in other provinces are comparable in general morphology to those in KwaZulu-Natal, several additional varieties are expected to occur. It is unlikely, due to the isolation of populations and continued hybridization in the field, that the varieties of similar morphology are genetically and chemically homogeneous throughout the geographic distribution in South Africa. Indeed, it is possible that although varieties are morphologically similar the factors governing their susceptibility to natural enemies may differ. Furthermore, due to the range in morphological and physiological characteristics the varieties within the weed complex have been referred to as distinct weed species (Neser and Cilliers, 1990; Cilliers and Neser, 1991).

1.3 *Control*

Chemical and mechanical control of lantana was reviewed by Cilliers and Neser (1991) who concluded that, although very effective, these methods are often labour-intensive and expensive. These control measures usually only offer temporary relief unless continual follow-up treatments are used. Controlled low- to moderate-intensity fires reduce invasion by lantana, and can be an effective, preventative management strategy (Gentle and Duggin, 1997b). However, the use of fires is not always a suitable option as infestations are often close to, or in, indigenous forests, grazing lands and plantations. The difficulties and expense incurred by conventional control measures have fostered the hope that biological control may provide a solution.

1.3.1 *Biological Control*

Biological control of lantana in South Africa was initiated during 1961/62, with the introduction of *Hypena laceratalis* Walker, *Ophiomyia lantanae* (Froggatt), *Neogalia sunia* (Guenée), *Salbia haemorrhoidalis* Guenée and *Teleonemia scrupulosa* Stål (Oosthuizen, 1964). However, two of these, *H. laceratalis* (= *H. strigata* (F.)) and *O.*

lantanae were already established in South Africa (Table 1.1) and were misidentified during a pre-introductory survey conducted by Oosthuizen (1964). The leaf feeding moth, *Hypena jussalis* Walker was subsequently found to be synonymous with *H. laceratalis*, while specimens of the fruit mining fly, *O. lantanae* were misidentified as *O. rhodesiensis* Spencer, which had been described from Zimbabwe (Cilliers and Neser, 1991). *Hypena laceratalis* is widespread and damages seedlings and new growth in summer (Table 1.1). *Ophiomyia lantanae* is widespread and abundant, but has little impact on seed viability (Cilliers and Neser, 1991)(Table 1.1).

Table 1.1

Status of natural enemies present on *Lantana camara* in South Africa prior to the initiation of the biological control programme in 1961/62 ('generalists' not included).

Order/Family	Natural enemy species	Mode of attack	Status	Limitations
Diptera				
Agromyzidae	*Ophiomyia lantanae* (Froggatt)	Fruit miner	Widely established, and abundant	Low impact on seed viability; possible high levels of parasitism
Lepidoptera				
Noctuidae	*Hypena laceratalis* Walker	Leaf feeder	Widely established; causes considerable damage to seedlings and new growth	Larvae are only active during late summer and autumn and are often parasitized
Pterophoridae	*Lantanophaga pusillidactyla* (Walker)	Flower and seed feeder	Established range unknown; present in low numbers	Low abundance and possible high levels of parasitism
Tortricidae	*Epinotia lantana* (Busck)	Flower peduncle and shoot-tip borer	Established range unknown	Unknown
Gracillariidae	*Aristaea onychote* (Meyrick)	Leaf-blister miner	Widely established, but present in low numbers	Possible high levels of parasitism

The third species, the noctuid moth *N. sunia* (= *N. esula* (Druce)), failed to establish despite introductions from Trinidad in 1962 and 1968 (Cilliers, 1977) and later from Australia in 1983 (Cilliers and Neser, 1991). The remaining two species, *S. haemorrhoidalis* and *T. scrupulosa* became established following the initial releases (Table 1.2).

The range and impact of the leaf-tying moth, *S. haemorrhoidalis* is unknown. The sap-sucking lace bug *T. scrupulosa*, has become widely established and abundant throughout the range of *L. camara* in South Africa. It is uncertain whether the later

importation and release of additional genetic material from various countries (Table 1.2) has influenced the efficacy of the tingid in South Africa (Cilliers and Neser, 1991). Feeding damage is often extensive, causing periodic defoliation of *L. camara* populations, with damaged plants producing less seed (Cilliers, 1983, 1987a). Tingid populations and their resultant damage peak in midsummer but rapidly decline towards winter. *Teleonemia scrupulosa* is considered to be the most effective natural enemy of lantana in South Africa at present.

Two leaf-mining hispine beetles, *Octotoma scabripennis* Guérin-Méneville and *Uroplata girardi* Pic, released in the early 1970s (Cilliers and Neser, 1991), have become established and coexist in the moist, subtropical areas of the country (Table 1.2). *Uroplata girardi,* is most successful in the coastal regions of KwaZulu-Natal Province, where it is widely established and reaches high population densities. By contrast, *O. scabripennis* is most abundant at inland sites, where populations peak sporadically in a few areas. Populations of both species reach damaging levels during midsummer and often cause the defoliation of lantana stands (Cilliers, 1987b), but the plants recover rapidly. Although a third leaf-mining beetle, *Octotoma championi* Baly, failed to establish after releases in 1978 (Cilliers and Neser, 1991), further releases were made at an inland, low altitude site near Nelspruit (Mpumalanga Province) in 1995. The population persisted in low numbers for the following two seasons, but its present status is unknown (Table 1.2).

The leaf-mining fly, *Calycomyza lantanae* (Frick), released in 1982, has established at a few sites in the moist, subtropical areas of the country (Table 1.2), but the result of additional releases made in the temperate regions is unknown (Cilliers and Neser, 1991). The blotch mines are most abundant on actively growing plants, seedlings and coppice growth. Additional material was released in 1989 (Table 1.2) but its effect on the established populations is also unknown. The impact of *C. lantanae* populations on lantana infestations is unknown, but may be reduced by larval parasitism. Besides the three species already mentioned, a further two moths *Lantanophaga pusillidactyla* (Walker) and *Epinotia lantana* (Busck) were released in South Africa via Hawaii in 1984 (Cilliers and Neser, 1991) but were later found to be already present (Table 1.1). Their present range and impact is unknown.

Table 1.2
Status of the natural enemies released and established on *Lantana camara* in South Africa.

Order/ Family	Natural enemy species	Origin	Main releases	Mode of attack	Status	Damage inflicted
Coleoptera						
Chrysomelidae	*Octotoma scabripennis* Guèrin-Mèneville	Mexico via Hawaii via Australia	1971	Leaf miner	Established in the warm, moist eastern range of lantana. Abundant in localized inland areas	Extensive defoliation, but localized
	Octotoma championi Baly	Costa Rica via Australia	1978 1995	Leaf miner	Persisted in low numbers for two seasons after the last release. Establishment unconfirmed	Unknown
	Uroplata girardi Pic	Paraguay via Hawaii via Australia	1974 1983	Leaf miner	Present in low numbers in the warm, moist inland range of lantana. Abundant in KwaZulu-Natal coastal regions	Extensive defoliation in localized coastal regions
Diptera						
Agromyzidae	*Calycomyza lantanae* (Frick)	Trinidad via Australia	1982	Blotch leaf miner	Established locally in warm moist eastern range of lantana. Heavily parasitized	Unknown
		Florida, USA	1989			
Heteroptera						
Tingidae	*Teleonemia scrupulosa* Stål	Mexico via Hawaii	1961	Flower and leaf sucker	Widely established in large numbers across the entire range of lantana; severe damage sporadic	Complete defoliation, and abortion of flowers
		via Australia	1971			
		via Mauritius	1984			
		Florida, USA	1989			
Lepidoptera						
Pyralidae	*Salbia haemorrhoidalis* Guenée	Cuba via Hawaii	1962	Leaf feeder	Range and impact unknown	Unknown

Although never deliberately introduced, the gracillariid leaf-mining moth *Aristaea onychote* is widely established in South Africa (Table 1.1). Populations are usually low, suffer heavy parasitism, and also cause negligible damage to lantana. It is possible that *A. onychote, L. pusillidactyla, E. lantana* and perhaps even *H. laceratalis* were inadvertently introduced into South Africa together with lantana plants. The geographic range, correct identities and status of the different Lepidoptera associated with lantana in South Africa are in need of revision.

Despite the establishment of several natural enemies on *L. camara* in South Africa (Oosthuizen, 1964; Cilliers and Neser, 1991), the biological control programme in South Africa has had limited success. This has largely been attributed to the genetic

diversity of lantana, which has made it an extremely variable target weed. The diversity of varieties presents the natural enemies with several morphological and physiological barriers to utilization (Cilliers, 1983; Neser and Cilliers, 1990; Cilliers and Neser, 1991). Indeed, the introduced complex of *L. camara* is a man-made polyploid entity (Stirton, 1977), which is far removed from the parent species occurring in its native range. Introduced natural enemies are therefore poorly adapted to cope with the diversity of 'new' entities naturalized in the countries of introduction.

Several natural enemies *(e.g. T. scrupulosa*, *C. lantana*e and *Leptobyrsa decora* Drake) were reported to display preferences for certain varieties of lantana (Harley, 1971; Radunz, 1971; Harley and Kassulke, 1974; Cilliers, 1977; Harley *et al.*, 1979; Cilliers, 1987b; Cilliers and Neser, 1991). In some cases, agents have reportedly failed to establish because of the phenomenon of varietal resistance or reduced compatibility (*e.g. Uroplata lantanae* Buzzi and Winder and *Eutreta xanthochaeta* Aldrich) (Winder *et al*., 1984; Cilliers and Neser, 1991). Agents from different isolated varieties of *L. camara,* or from other species such as *L. tiliaefolia* Cham. (Winder and Harley, 1983) in the region of origin, may thus all be considered as 'new' associations (Hokkanen and Pimental, 1984) when deployed against the plants in South Africa. Consequently, the interactions between the various natural enemies and the different lantana varieties are complex and difficult to predict.

Harley and Kassulke (1974) and Cilliers and Neser (1991), amongst others, have emphasized the need to import different 'strains' of the natural enemies already released, so as to increase the number of lantana varieties attacked. However, the distinction must be made between agents previously established and those that failed to establish. Since little is known about the extent to which established agents have realized their potential, importations of new genetic material may not necessarily alter an agent's status in South Africa (*e.g. T. scrupulos*a). Unless the impacts of established natural enemies are well quantified, it seems inappropriate to introduce new material. Neser and Cilliers (1990) highlighted the difficulties of monitoring new genetic material in large, well-established populations, which may result in rapid dilution of the new genes and in inconclusive evidence unless suitable techniques are used. By contrast, the re-importation of new 'strains' of species that failed to establish, collected from different species in the lantana

complex and from climates that match target release sites, is expected to improve the chances of establishment (Neser and Cilliers, 1990; Cilliers and Neser, 1991).

To increase the number of lantana varieties attacked in South Africa, Neser and Cilliers (1990) suggested that local varieties should be exposed to the natural enemies within the native range of *L. camara*. This could have several advantages but would depend on detailed comparative performance studies on the South African lantana varieties. These would indicate the natural enemies that can cope with the different varieties as well as the degree of suitability for their 'new' hosts.

High rates of parasitism in the field have been observed for *C. lantanae, A. onychote, O. lantanae* and *H. laceratalis* and appear to significantly reduce the efficacy of these natural enemies in South Africa. *Eutreta xanthochaeta* is also known to be heavily parasitized in other countries where it has established (Daun and Messing, 1996), suggesting that the recruitment of native generalist parasitoids may have contributed to its failure to establish in South Africa.

1.3.2 *Global biocontrol initiatives*

Lantana camara is a serious weed in most parts of Africa, the Indo-Malaysian region, eastern Australia and the South Pacific Islands (Muniappan and Viraktamath, 1986; Waterhouse and Norris, 1987; Denton *et al*., 1991; Baars and Neser, 1999; Day and Neser, 2000), and has been targeted by a global biocontrol programme. The attempts at biocontrol were pioneered in Hawaii when insects were introduced from Mexico in 1902 (Swezey, 1923; Perkins and Swezey, 1924). These biocontrol agents were the focus of introductions made to Australia between 1914 and 1935 (Wilson, 1960). The promising agents, such as *T. scrupulosa* and *O. lantanae* were then introduced to some Pacific countries, Indonesia and southern parts of Asia (Waterhouse and Norris, 1987; Esguerra *et al*., 1997). Through close collaboration with scientists in Hawaii and Australia, biocontrol agents were introduced to many African countries in the 1950s and 1960s (Greathead, 1968, 1971). Many agents deliberately released in South Africa were obtained from the then Department of Lands, Queensland (Queensland Department of Natural Resources) and CSIRO, Brisbane in Australia (Cilliers, 1983).

The biological control research on *L. camara* in most countries was intermittent during the 20th century, which in part was dictated by funding support but also by the delay in determining the potential of agents between their release and impact in the field. It was only in the 1960s when the true potential of *O. scabripennis* and *U. girardi* was realised in Hawaii and Australia (Davis and Krauss, 1967; Harley, 1974) that global interest in biological control of lantana was sparked. These two beetles were then distributed around the world during the early 1970s and 1980s (Greathead, 1968; Scheibelreiter, 1980; Löyttyniemi, 1982; Cock and Godfray, 1985; Muniappan and Viraktamath, 1986; Waterhouse and Norris, 1987; Schreiner, 1989; Julien and Griffiths, 1998). The interest in new candidates for both Australia and South Africa was initiated when new candidates were collected during surveys in Mexico and the Caribbean region in the 1990s (Palmer and Pullen, 1995; S. Neser, personal communication) probably due to the realisation that additional agents were required to increase damage levels on *L. camara* in the field and to new funding becoming available.

The host specificity tests conducted by biocontrol scientists in Hawaii and Australia were sufficient for permission to be granted for the release of agents by regulatory bodies in South Africa (Cilliers, 1983), and most other countries, with the notable exception of India (Khan, 1944; Muniappan and Viraktamath, 1986). However, with the increase in the global concern for the potential irreversible effects of biocontrol, emphasis has shifted in the last decade to incorporating a larger complement of related indigenous plants in host-specificity tests. Collaboration between countries remains good, with the frequent exchange of biocontrol agents, but countries are required to perform host range evaluations on their local flora. For example, the stem sucking bug, *Aconophora compressa* Walker (Homoptera: Membracidae) was found safe for release in Australia (Palmer *et al*., 1996), but a few plants indigenous to South Africa were found acceptable during laboratory studies and this agent has been rejected for release in Africa (Heystek and Baars, 2001). The new initiatives in the 1990s include the evaluation of agents previously released but which failed to establish, and candidates new to lantana biocontrol (Baars and Neser, 1999; Day and Neser, 2000), and also includes the evaluation of promising pathogens (Morris *et al*., 1999; Den Breeÿen, 2000; Thomas and Ellison, 2000).

1.4 *Aims of research*

The biocontrol of *L. camara* in its introduced range relies almost entirely on a hand full of agents despite the release of 35 species (Julien and Griffiths, 1998). Three agents, *T. scrupulosa, O. scabripennis* and *U. girardi* are consistently regarded the most effective agents that cause significant levels of damage to the growth and reproductive rate of lantana. Almost certainly, without the suite of agents already released, lantana would persist as a more formidable weed. Nevertheless, the impact of agents is largely insufficient to reduce lantana to manageable levels. Biocontrol scientists are therefore faced with many challenges to improve the biocontrol programme, notably: (i) evaluating the biocontrol agents established to determine the factors which reduce their biocontrol potential; (ii) using these factors and new trends in biocontrol to improve the survey and selection techniques in the native range and thereby to invest in the most promising candidates, (iii) importing and conducting host-specificity tests on new candidate agents, and include related indigenous plants in the test list; (iv) evaluate the importance of the key factors reducing the potential of established agents using the new candidates in quarantine. Therefore, the main objectives of this research were firstly, to determine the present status of the biocontrol agents established on *L. camara* in South Africa, and the factors that influence their efficacy. Secondly, to investigate the prospects of improving the biological control programme using additional candidate biocontrol agents.

Information on the present range, and impact of the biocontrol agents established in South Africa is largely unsubstantiated, and based on opportunistic observations conducted during fieldwork. These observations have proved very valuable, but have not been quantified using field surveying techniques. In addition, most of the agents introduced to South Africa were released more than twenty years ago, making previous observations outdated and very possibly resulting in a misrepresentation of the present situation. Chapters 2 and 3, therefore, investigate the identity, geographic range and impact of the natural enemies established on *L. camara* in South Africa, and the factors that influence their effectiveness.

Climate is identified as one of the contributing factors reducing the established range and impact of some of the biocontrol agents on lantana (Neser and Cilliers, 1990;

Swarbrick *et al*., 1995; Broughton, 2000; Day and Neser, 2000), and is implicated in preventing the establishment of others (Cilliers and Neser, 1991; Baars, 2000c). The impact of the established agents is also severely reduced because insect populations only attain high densities by midsummer, and maintain damage levels until late summer (Harley *et al*., 1979; Cilliers, 1987a). In the first few months of the growing season (September to December) therefore, plants are relatively free from attack and compensate for cumulative insect damage sustained in the previous season. Chapter 4 is an investigation into how climate influences the distribution of the natural enemies in South Africa using predictive distribution modelling techniques (Robertson *et al*., 2001). Understanding the role climate plays in the range of the established agents, may identify the strategies that should be considered when redistributing these agents, and the strategies that possibly promote the establishment of new biocontrol candidates.

Due to the extreme variability of the weed, and the diverse climate conditions over the naturalised range, early assessments of the biocontrol of *L. camara* acknowledged that the success of the programme relied on the introduction of a suite of natural enemies (Oosthuizen, 1964; Neser and Annecke, 1973; Cilliers, 1977, 1983, 1987a). Comprehensive lists of the phytophagous organisms associated with the parent species (Winder and Harley, 1983; Palmer and Pullen, 1995) have been the source of recent evaluations and introductions (Palmer *et al*., 1996; Palmer and Pullen, 1998; Day *et al*., 1999; Palmer *et al*., 2000; Baars, 2000b, 2000c, 2001, 2002; Simelane, 2001, 2002), but the species pool of many countries in the native region have not been investigated. In particular, several Caribbean islands have not been surveyed, and may be a valuable source of new candidates. Chapter 5 investigates the phytophagous organisms associated with *Lantana* species in Jamaica and evaluates the standard selection procedures used in classical biological control programmes to select new biocontrol candidates.

The use of natural enemies that have acceptably restricted host ranges remains central in the effective application of biological weed control (Cruttwell McFadyen, 1998). The host range of an agent is determined through extensive host specificity testing, the design of which might vary with each agent (Wapshere, 1974, 1989; Cullen, 1990; Shepherd, 1990; Marohasy, 1998; Baars, 2000a). Fundamental to the design of

tests is the biology of agents, which also influences the stage and abundance of host plants in trials (Forno *et al*., 1994; Baars, 2000a), and ultimately the accuracy of the host range prediction. As the safety of biocontrol comes under increasing scrutiny, biocontrol scientists call for the justification of increased importation rates and lack in post release surveys (Samways, 1997; Thomas and Willis, 1998; McEvoy and Coombs, 1999; Cory and Myers, 2000). It is accepted that with every release there is an element of risk, and a release should only be considered if in all probability the agent would have a positive impact. Chapters 6 to 11 are the focus of the thesis, and deal with the biology, and host range of two new agents, *Coelocephalapion camarae* Kissinger (Coleoptera: Brentidae) (Chapters 6, 8) and *Falconia intermedia* (Distant) (Heteroptera: Miridae) (Chapter 9). Chapters 7 and 10 investigate the host preferences of these agents, to evaluate their potential for biological control of the varieties of *L. camara* naturalized in South Africa. Chapter 11 evaluates the potential non-target effects of *F. intermedia* by conducting post-release open-field trials.

The final discussion (Chapter 12) examines the prospects for the biological control of *L. camara* in South Africa based on the results of this study, and highlights the critical factors that require further research.

Chapter 2

Geographic range, impact and parasitism of lepidopteran species associated with *Lantana camara* in South Africa

2.1 INTRODUCTION

Prior to the initiation of the biological control programme in the early 1960s, four Lepidoptera species were already established on *L. camara* in South Africa (Oosthuizen, 1964). Two of these, *Epinotia lantana* and *Lantanophaga pusillidactyla* are thought to have been inadvertently introduced with the weed, while *Hypena laceratalis* and *Aristaea onychote* are believed to be native to South Africa and to have extended their host range from native verbenaceous plants to include *L. camara* (Oosthuizen, 1964; Cilliers and Neser, 1991).

Amongst the species deliberately released only *Salbia haemorrhoidalis* became established. Although these species have been established for over forty years their geographic ranges and contribution to the control of lantana in South Africa has not been investigated. Cilliers and Neser (1991) concluded that lepidopteran species do not reach large enough population levels in South Africa to cause significant levels of control. However, Muniappan *et al*. (1996) indicated that *E. lantana* reduced the seed production of *L. camara* in Guam. Baars and Neser (1999) thus suggested that the geographic range and contribution of these species of Lepidoptera to the biocontrol programme on lantana in South Africa should be investigated.

This chapter evaluates the status of the lepidopteran species associated with *L. camara* in South Africa and is based on a field survey that was conducted to confirm their identity and describe their life history, geographic range and the occurrence of native parasitoids.

2.2 MATERIALS AND METHODS

All species of Lepidoptera associated with *L. camara* were sampled at selected sites over most of the weed's geographic range in South Africa (Fig. 2.1). Sites where sampled

opportunistically and were selected where the weed was abundant and comprised the dominant species in the plant community. The survey was conducted over three years (1998-2000), and sampling was restricted to the growing season (October to April). In total, 141 sites covering 66 quarter degree squares were sampled during the survey (Fig. 2.1), which included roadsides, riparian zones, arable land borders, plantations and natural vegetation. A minimum of 10 plants were sampled at each site, and plants were initially assessed for insect abundance and the level of damage inflicted, each of which were assessed using a five-category scoring system (Table 2.1). Two to three sections of each sampled plant were shaken above a beating tray to dislodge any insects. Where possible, at least 20 each of damaged flowers and green, mature and dry seed heads were collected per site. Where endophagous insect stages were present (e.g. leaf-mining larvae), the affected plant material was collected. All samples were brought back to the laboratory, where immature stages were reared to adulthood on lantana bouquets. The field-collected and emerged moths, as well as their associated parasitoids, were lodged at the National Collection of Insects (NCI, ARC- Plant Protection Research Institute) in Pretoria for identification.

2.3 RESULTS

2.3.1 *Aristaea onychote* (Gracillariidae)

Eggs of this leaf-mining moth were deposited on leaves. The emerging larvae initiated serpentine mines that later developed into 'blister' mines in which a cavity was produced in the mesophyll tissue. The larvae were pale cream in colour and attained a length of about 5mm (Fig. 2.2.1a). The late instar larvae pupated in ribbed and spindle-shaped cocoons, which were suspended within the 'blister', and the emerging moths breached the lower epidermis of the 'blister'. Adults were small, predominantly tan-grey in colour with light bands on the forewings (Fig. 2.2.1b). The 'blister' mines, at most, damaged about 50% of the leaves, but usually far less, leaving the remainder of the leaves to function normally. This suggested that high densities of mines were required before significant damage was caused.

Aristaea onychote occurred at 70% of the sites (Table 2.2), which were distributed throughout the geographic range of *L. camara* in South Africa (Fig. 2.3a). The moth populations were always found to be 'rare' (Table 2.2). As a result, the few mines recovered, usually less than 2 per shrub, probably had no significant impact on the growth of plants. Very few adults were reared from the field-collected mines, which may have been due to the high incidence of larval parasitism. Although a large proportion of the larvae were attacked by ectoparasitic hymenopteran parasitoids, none were successfully reared for identification.

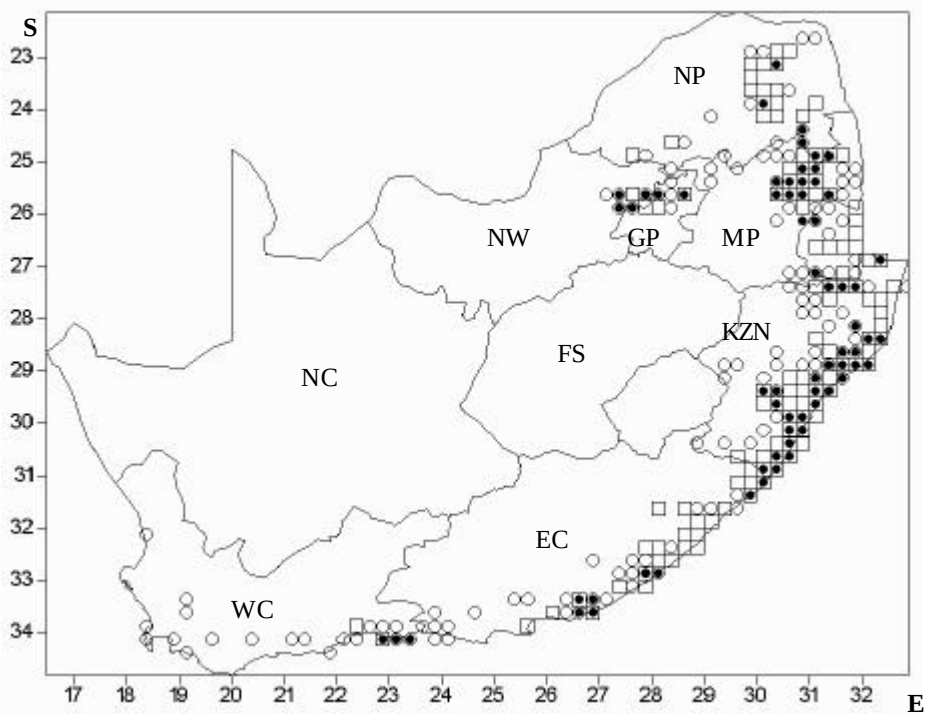


Fig. 2.1 Distribution and relative abundance of *Lantana camara* in Southern Africa (Southern African Plant Invaders Atlas (SAPIA) Database, ARC-PPRI), and the sites sampled during a survey of the Lepidoptera associated with this weed. Open squares = *L. camara* abundant, open circles = *L. camara* present, closed circles = field sites sampled. Key to the provinces: NP, Northern; GP, Gauteng; MP, Mpumalanga; NW, North West; KZN, KwaZulu-Natal; FS, Free State; NC, Northern Cape; WC, Western Cape; EC, Eastern Cape.

TABLE 2.1

Definition of categories used during a field survey of the Lepidoptera attacking *Lantana camara* in South Africa.

Site Parameter	Categories	V[a]	Definition of categories
Insect Abundance	None	0	No life stages present
	Rare	1	Very few individuals encountered, and patchy in distribution at the site sampled
	Occasional	2	Individuals present on most plants surveyed; most life stages encountered regularly
	Frequent	3	Individuals present on all plants; most life stages regularly encountered with an even distribution over site sampled
	Abundant	4	Plants with large numbers of individuals on most of the shoots on each plant; even distribution of individuals in large numbers
Relative Damage	None	0	No damage on plants; characteristic signs of damage not present
	Minimal	1	Few leaves/flowers or shoot tips with characteristic damage on plants; damage at low intensity
	Minor	2	Characteristic damage easily noticed; small proportion of the relevant plant parts damaged
	Partial	3	Large proportion of the relevant plant parts damaged; section of shrubs show signs of stress; damage intense but patchy
	Significant	4	Most of the relevant plant parts with characteristic damage; large portion of shrubs with intense damage; plants noticeably stressed

[a] Values assigned to the categories.

2.3.2 *Epinotia lantana* (Tortricidae)

Larvae of *E. lantana* attacked the flowers, hollowed out the receptacles, and also burrowed into the young shoot tips. Larvae were dark grey-brown in colour, and frequently moved between the different plant parts that were attacked, reaching a length of about 10mm (Fig. 2.2.2a) before pupating on the ground. The adult moth had mottled dark grey forewings and dirty cream coloured hind-wings (Fig. 2.2.2b).

Epinotia lantana occurred throughout the range of *L. camara* in South Africa (Fig. 2.3b), and although it was absent from 39% of the sites (Table 2.2), this may be attributed to seasonal and spatial variability. The moth populations were found to be 'occasional' at most sites, but ranged from 'rare' to 'abundant' (Table 2.2). The damage induced by this this species over its geographic range was rated as less than 'minimal' (Table 2.2). However, damaged attributed to this species varied from 'none' to 'partial', with outbreaks of large populations occurring in some localised areas. *Epinotia lantana* was therefore considered to contribute to the damage of the flowers of lantana, throughout its range, although this was insufficient to reduce the weedy status of lantana.

Three hymenopteran parasitoid species (Braconidae) were reared from *E. lantana* larvae feeding on *L. camara* (Table 2.3), and each occurred throughout the moth's geographic range in South Africa. Although the incidence of parasitism was not quantified, parasitoids were regularly reared from samples and thus seem likely to have reduced field populations of the moth.

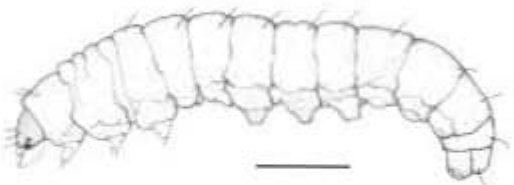
TABLE 2.2

The abundance and impact of the Lepidoptera established at sites sampled on *Lantana camara* in South Africa.

Natural enemy species	Present[a]	Absent[a]	Mean insect abundance[b]	Mean damage[b]
Aristaea onychote	98	43	1.0 (-)	0 (-)
Epinotia lantana	86	55	1.6 (1-4)	0.5 (0-3)
Hypena laceratalis	133	8	1.7 (1-4)	0.7 (0-3)
Lantanophaga pusillidactyla	27	114	1.4 (1-3)	0.1 (0-1)
Characoma submediana	48	93	1.4 (1-3)	0.7 (0-1)
Salbia haemorrhoidalis	55	86	1.5 (1-4)	1.3 (0-4)

[a] Number of sites during the survey where the natural enemy species were present or absent.

[b] Abundance and damage means were calculated only from sites where the biocontrol agent was present, using the categories specified in Table 2.1. The range is given in parentheses.



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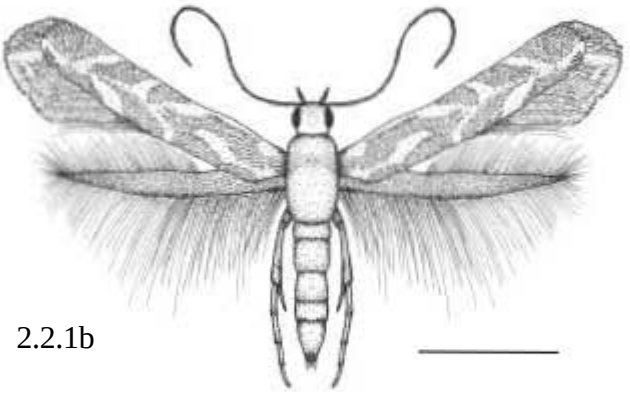

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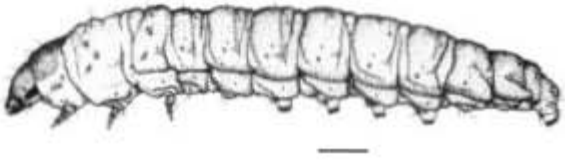

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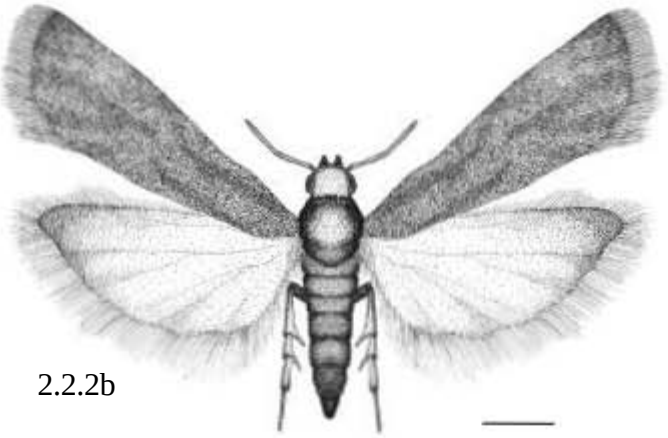

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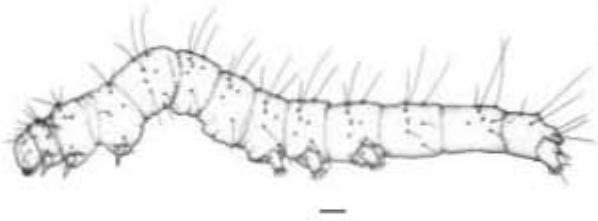

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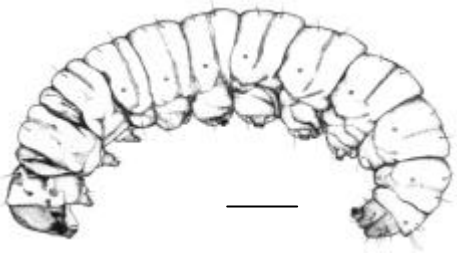
2.2.3b



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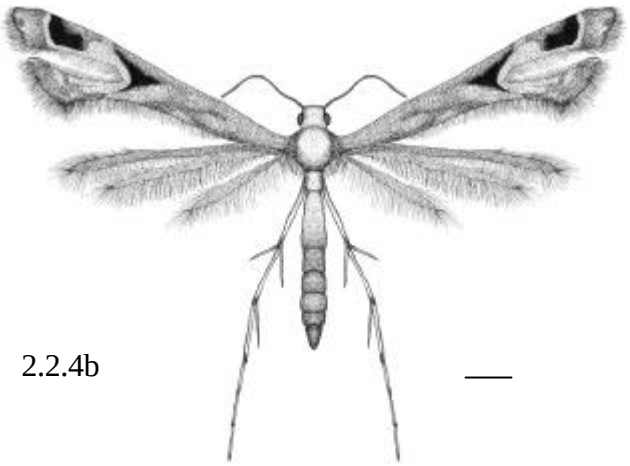

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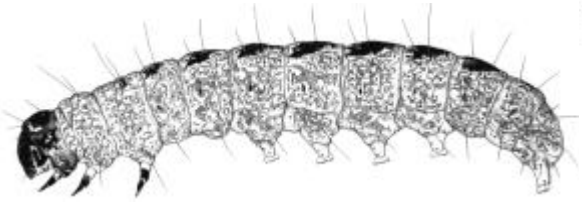

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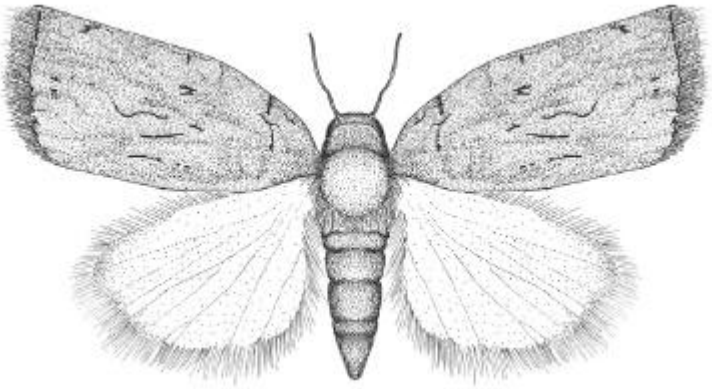

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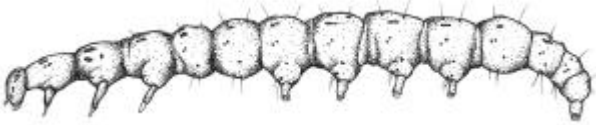

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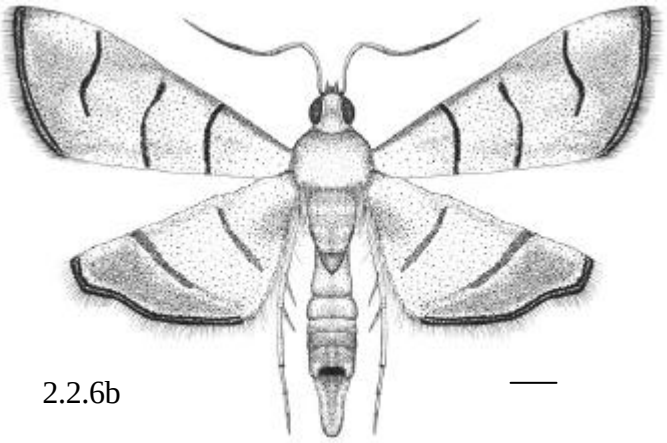


2.2.6b

Fig. 2.2 The larvae (a) and adults (b) of the species of Lepidoptera associated with *Lantana camara* in South Africa. 1. *Aristaea onychote* (Gracillariidae); 2. *Epinotia lantana* (Tortricidae); 3. *Hypena laceratalis* (Noctuidae); 4. *Lantanophaga pusillidactyla* (Pterophoridae); 5. *Characoma submediana* (Noctuidae); 6. *Salbia haemorrhoidalis* (Pyralidae). Scale bars = 1mm.

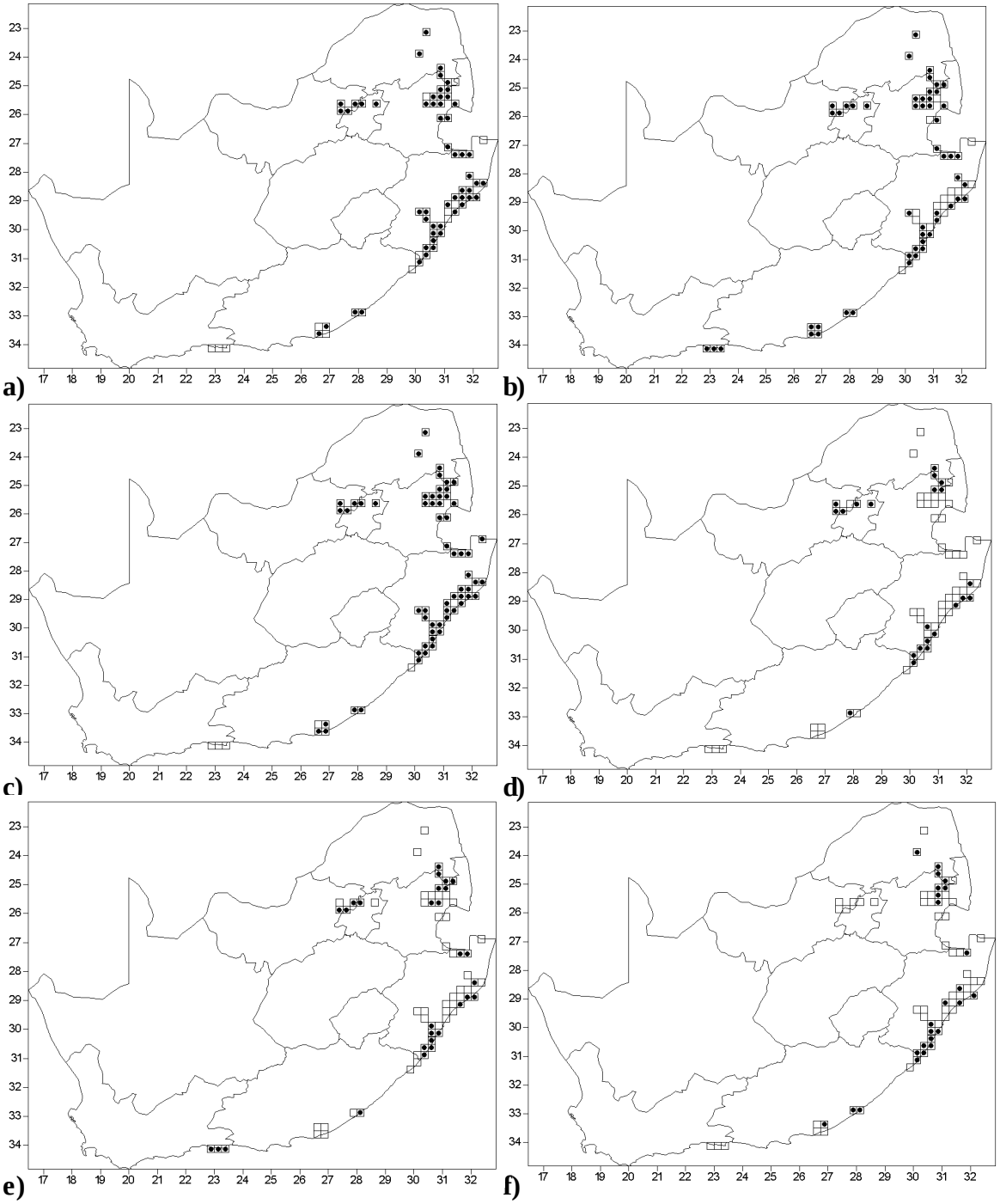


Fig. 2.3 The geographic range of six Lepidoptera species established on *Lantana camara* in South Africa, in relation to the sites sampled during a survey: (a) *Aristaea onychote*; (b) *Epinotia lantana*; (c) *Hypena laceratalis*; (d) *Lantanophaga pusillidacyla*; (e) *Characoma submediana*; (f) *Salbia haemorrhoidalis*. Open squares = sampled site with biocontrol agent absent; closed squares = sampled site with biocontrol agent present.

2.3.3 *Hypena laceratalis* (Noctuidae)

The larvae of *H. laceratalis* were solitary external leaf feeders, causing large 'windows' in the leaf, which left the upper epidermis intact. The larvae were large (Fig. 2.2.3a), bright green with a pale lateral stripe, and attained a length of about 35 mm before dropping to the ground to pupate in the leaf litter. The forewings of the adults were mottled brown and black, while the hind wings were uniform dark brown (Fig. 2.2.3b). The labial palpi were characteristically large and protruded in front of the head.

Hypena laceratalis occurred throughout the range of lantana in South Africa (Fig. 2.3c), and was absent from only 5.7% of the sites surveyed (Table 2.2). Populations of *H. laceratalis* were usually rated as 'occasional' and this species was typically the most abundant lepidopteran at the sampled sites, and its abundance ranged between 'rare' and 'abundant' (Table 2.2). However, the damaged attributed to this moth was generally rated to be less than 'minimal' over its range, but when populations were either 'frequent' or 'abundant', the damage was rated as 'minor' or 'partial' respectively. Field collected larvae were occasionally infected with a bacterial disease, and were often parasitised by a suite of parasitoids, including four species of Braconidae, one each of Eulophidae and Ichneumonidae and three of Tachinidae (Table 2.3).

2.3.4 *Lantanophaga pusillidactyla* (Pterophoridae)

Larvae of the plume moth *L. pusillidactyla* fed on the bases of the flowers, binding them together. Feeding damage caused a typical 'streak' of aborted flowers amongst the flower heads, leaving the other flowers to develop normally. The larvae were small (Fig. 2.2.4a), cream coloured, and reached a length of about 7mm before pupating amongst the clusters of damaged flowers. The adults were relatively small and predominantly light brown or tan in colour (Fig. 2.2.4b).

Although *L. pusillidactyla* was only recorded from 19.2% of the sites sampled (Table 2.2), these sites were scattered throughout most of the range of *L. camara* in South Africa (Fig. 2.3d). Populations of this species were usually 'rare', but on occasion were 'frequent' (Table 2.2). Larval damage was restricted to only a section of the entire flower

head, allowing the undamaged flowers on the infested cluster to mature and set fruit. As a result, its impact on the flowers of lantana was likely to be negligible. No parasitoids were reared from the collected material.

TABLE 2.3
List of the parasitoid species and their host Lepidoptera species collected from *Lantana camara* in South Africa.

Natural enemy species	Parasitoid Order: Family	Parasitoid species[a]	Stage[b]
Epinotia lantana	Hymenoptera: Braconidae	*Ascogaster* sp. (2231)	L
		Prob. *Bassus* sp. (2216)	L
		Bracon sp. (2217)	L
Hypena laceratalis	Diptera: Tachinidae	*Peribaea* sp. 1 (2273)	L
		Carcelia sp. (2272)	L
		Phorinia sp. (2282)	L
	Hymenoptera: Braconidae	*Apanteles* sp. 1 (2249)	L
		Apanteles sp. 2 (2268)	L
		Meteorus sp. (2266)	L
	Microgastrinae	Undet. sp. 1 (2254)	L
	Hymenoptera: Eulophidae	*Platypectrus* sp. (2259)	L
	Hymenoptera: Ichneumonidae	Undet. sp. (2261)	L
Characoma submediana	Diptera: Tachinidae	*Exorista* sp. (2283)	L
Salbia haemorrhoidalis	Diptera: Tachinidae	*Peribaea* sp. 2 (2278)	L
	Hymenoptera: Braconidae: Microgastrinae	Undet. sp. 2 (2258)	L

[a] Accession numbers (AcSN) of the undetermined species are given in parentheses. All material lodged with the National Collection of Insects, Pretoria, South Africa.
[b] Life stage of the biocontrol agent attacked; L= larva.

2.3.5 *Characoma submediana* (Noctuidae)

Larvae of the noctuid moth, *Characoma submediana* Wiltshire fed on the fruit and flowers, and flower receptacles. Small larvae were pale beige (tan) in colour, while developing larvae either retained this colouration or became dark grey (Fig. 2.2.5a). Larvae had a distinctive black head capsule and dark chevron markings on the dorsum, which was more pronounced in the dark grey colour form. Small larvae were often found in flower heads, where they bound the mature florets together and fed on the base of the corolla and developing seeds. Feeding damage caused flowers to abort, and extensive

feeding also caused damage to the receptacle. Larger larvae attacked the developing green berries and damaged the receptacle, which on occasion caused the other seeds to abort. Larvae pupated on the plant, by spinning a cocoon amongst the flowers and binding flower debris to the cocoons. Adult moths were about 10mm in length and had grey forewings and dirty white hind wings (Fig. 2.2.5b). At rest, the adults were bullet shaped and were predominantly grey in colour.

Characoma submediana occurred at 34% of the sites (Table 2.2) that were distributed throughout the geographic range of *L. camara* in South Africa (Fig. 2.3e). Moth populations were usually rated between 'rare' and 'occasional', with larvae and affected flowers found with relative regularity (Table 2.2). However, due to the low densities of field populations, the relative damage attributed to this moth was considered to be 'minimal'. Only one parasitoid species, *Exorista* sp. (Tachinidae) was occasionally reared from the larvae of *C. submediana* (Table 2.3), suggesting that the larvae did not suffer extensively from parasitism.

2.3.6 *Salbia haemorrhoidalis* (Pyralidae)

Larvae of the leaf-tying moth, *S. haemorrhoidalis* were external feeders, and tied the leaves with silk threads and caused them to fold or partially roll. Larval feeding caused epidermal windows, and recently damaged leaves were characterised by frass caught up within the threads. The larvae were relatively large (Fig. 2.2.6a), had a dirty cream colour, and attained a length of 20mm before dropping to the ground to pupate. Larvae were mobile and frequently moved between leaves, and at low population levels caused patchy damage to plants in the field. The adult was yellow-brown in colour, with darker bands on the forewings (Fig. 2.2.6b).

Salbia haemorrhoidalis displayed a more restricted distribution and occurred at 39% of the sites (Table 2.2), mainly in the warm moist areas in the Northern, Mpumalanga, KwaZulu-Natal and Eastern Cape provinces (Fig. 2.3f). Although the density of the moth populations usually varied between 'rare' and 'occasional', they reached densities rated as 'abundant' (Table 2.2). The moth populations were particularly abundant along the eastern coastline, from the towns of East London (Eastern Cape

Province) to Umhalanga (KwaZulu-Natal Province). The damage to plants attributed to this moth was usually rated between 'minimal' and 'minor', but along the coastal range their damage was usually 'partial' or 'significant'. In these areas, *S. haemorrhoidalis* reached high enough population densities to potentially contribute to the control of lantana.

The larvae were parasitized by an undetermined species of Braconidae and the tachinid *Peribaea* sp. (Table 2.3). Larvae collected during the survey were often parasitised, and cocoons of the braconid larvae were occasionally observed attached to lantana leaves in the field. Despite the relatively high frequency of parasitoid pressure, the moth populations persisted in large enough numbers to cause considerable impact to lantana in South Africa.

2.4 DISCUSSION

There are six species of Lepidoptera commonly associated with *L. camara* in South Africa. Three of these species are exotic, of which *S. haemorrhoidalis* was released as part of a biocontrol initiative (Oosthuizen, 1964), while *E. lantana* and *L. pusillidactyla* were probably inadvertently introduced with the weed (Baars and Neser, 1999). Although attempts were made to introduce the leaf-chewing moth *H. laceratalis*, this species was found to be native to South Africa (Oosthuizen, 1964) and other African countries (Krauss, 1962). The flower- and fruit-boring moth *C. submediana* is an indigenous species previously recorded from fungus galls of *Ravenelia macrowaniana* Pazschke on *Acacia karoo* Hayne (Fabaceae) (McGeoch and Krüger, 1994). This species is synonymous with *Pardasena virgulana* (Mabille) (M. Krüger, personal communication), which has been recorded from *L. camara* in South Africa (Swain and Prinsloo, 1986; Kroon, 1999), and is widespread, occurring in Zambia, Kenya and Mauritius (Löyttyniemi, 1982; Zhang, 1995). This species has also been recorded feeding on pigeon peas, *Cajanus cajan* (L.) Millsp. (Zhang, 1995), and thus appears to be polyphagous.

The serpentine leaf-miner, *A. onychote* is considered to be an indigenous species (Vári, 1961), which has also been recorded from the native *Lantana rugosa* Thunb. and *Lippia javanica* (Burm.f.) Spreng. (both Verbenaceae) in South Africa (Kroon, 1999). It

is assumed that this moth has subsequently extended its host range to include the varieties of *L. camara* in South Africa (Cilliers and Neser, 1991) and Zambia (Löyttyniemi, 1982). *Aristaea onychote* is comparable to the biocontrol agent *Cremastobombycia lantanella* Busck (Gracillariidae), which was released in Hawaii and which is also a serpentine leaf-miner that occupies an equivalent niche in the weed's natural range (Perkins and Swezey, 1924; Palmer and Pullen, 1995). It is suspected that this species may have been inadvertently introduced with the weed, as occurred with *E. lantana* and *L. pusillidactyla*, and was then described as *A. onychote* and presumed to be indigenous to South Africa. However, specimens from the present survey were initially identified as *A. onychote* (M. Krüger, personal communication) and subsequently as *Aristaea eurygramma* Vári (D.R. Davis, personal communication), when they were compared to *C. lantanella* type specimens in the Smithsonian National Museum of Natural History. Due to the small number of adults successfully reared for identification, additional specimens are needed to confirm the taxonomic status of this species.

Two other lepidopteran species, *Neogalea sunia* and *Autoplusia illustrata* Guenée (both Noctuidae) were released in South Africa (Oosthuizen, 1964; Cilliers and Neser, 1991), although neither became established. During this survey, no individuals of either *N. sunia* or *A. illustrata* were recorded, supporting the contention that these two species have not established in South Africa.

The leaf-tying moth, *S. haemorrhoidalis* is the only species that is not widespread and appears to be restricted to the moist eastern range of *L. camara* in South Africa (Fig. 2.3f). This species became established following a small release of 114 individuals in the Durban Bluff area in KwaZulu-Natal Province (Oosthuizen, 1964). Originally thought not to have established (Neser and Annecke, 1973), it was later recovered over a wide area in the KwaZulu-Natal Province coastal belt (Cilliers and Neser, 1991). No subsequent releases were made, and there have been no attempts at collecting and redistributing *S. haemorrhoidalis* to other areas. The present restricted range of the moth may thus either reflect an ecoclimatic limitation, or it may still be in the process of dispersing to new areas within the geographic range of *L. camara* in South Africa.

Worldwide, the impact of these lepidopteran biocontrol agents has been regarded as minor (Haseler, 1966; Willson, 1968; Harley, 1974; Cilliers and Neser, 1991;

Swarbrick *et al*., 1995; Baars and Neser, 1999), but some of these agents have been effective in some countries (Waterhouse and Norris, 1987; Muniappan *et al*., 1996). During the period of this survey, the populations of all the lepidopteran agents were usually low, with few individuals encountered per plant, and the resultant damage to the plants was thus minor. The moth population densities varied between sites, and on occasion some species reached levels where few to many individuals were present on all of the plants at the site, with resultant damage noticeable and sometimes severe. However, as the moth population densities were usually low, their relative damage was considered to be 'minimal' to 'minor', and thus insufficient to reduce the growth rate and reproductive output of lantana. Where populations become locally abundant, as with *S. haemorrhoidalis* along the east coast and *H. laceratalis* in localised areas, their levels of damage were considerable. Due to the minor nature of the damage caused by *L. pusillidactyla* and *A. onychote*, large moth populations are required to cause a significant impact, and the observed populations are therefore most likely to be relatively ineffective.

The accumulation of native parasitoids is thought to have reduced the efficacy of some biocontrol agents introduced to South Africa (Hill and Hulley, 1995; Baars and Neser, 1999). Broughton (2000) suggested that ectophagous Lepidoptera should not be considered for release against lantana in view of their potential to be parasitized. The high levels of parasitism amongst field collected larvae of all but one of the lepidopteran species surveyed, may well reduce the biocontrol potential of these moths in South Africa. Indeed, the low population densities of *N. sunia* and *S. haemorrhoidalis* in Australia were attributed to high levels of parasitism (Willson, 1968). These considerations suggest that the evaluation of additional lepidopteran species for release against *L. camara* in South Africa should not be considered. However, the indirect impact of such species through the introduction of plant pathogens should be consideration.

These surveys showed that neither the native nor introduced species of Lepidoptera associated with *L. camara* in South Africa contribute significantly to the control of this weed. Occasionally, moth population densities are high enough to cause appreciable damage and have a considerable impact, but this effect is usually only localized. Additional natural enemy species, particularly from different feeding guilds thus need to be considered for the biological control of *L. camara*.

Chapter 3

Geographical range and impact of five non-lepidopteran biocontrol agents established on *Lantana camara* in South Africa

3.1 INTRODUCTION

Despite a long history of extensive research done worldwide on *L. camara*, the levels of control achieved are a poor return for the investment made. Substantial control has been achieved, particularly on relatively small islands (Waterhouse and Norris, 1987; Denton *et al.*, 1991; Swarbrick *et al.*, 1995; Muniappan *et al.*, 1996), but the natural enemies in most countries have not reduced lantana infestations to acceptable levels (Löyttyniemi, 1982; Muniappan and Viraktamath, 1986; Swarbrick *et al.*, 1995; Baars and Neser, 1999). The degree of control achieved in most countries is negligible, where in spite of damage inflicted by agents the control of the weed remains entirely reliant on the implementation of other control measures (Hoffmann, 1995; Anonymous, 1999). The lack of control has been attributed to many factors, but predominantly to the extreme variability of the weed, the extensive climatic range over which it is naturalized and high levels of parasitism of released biological control agents (Winder *et al.*, 1984; Neser and Cilliers, 1990; Cilliers and Neser, 1991; Baars and Neser, 1999; Day and Neser, 2000; Chapter 2).

In this chapter, the present distribution, abundance, relative impact and levels of parasitism of five non-lepidopteran biocontrol agents established on lantana in South Africa are described. Consideration is then given to: (i) the factors which limit the effectiveness of these natural enemies, (ii) the relative importance of each factor, and (iii) their influence on the evaluation of new candidate biocontrol agents.

3.2 MATERIALS AND METHODS

The biocontrol agents of *L. camara* were sampled at the same 141 sites covering 66 quarter degree squares throughout the weed's geographic range in South Africa as in

Chapter 2 (Fig. 2.1). The survey was conducted opportunistically over three years (1998-2000), and the sampling techniques used were as described in Chapter 2.

The lantana varieties occurring at sampled sites were differentiated using distinctive morphological characters, including the variation in shoot tip, flower colour, leaf, stem and growth characteristics (see Smith and Smith, 1982; Stirton and Erasmus, 1990). A representative sample of the varieties occurring in South Africa was collected, each from a single plant in homogenous stands in the field and were planted in the grounds of the Plant Protection Research Institute in Pretoria (the dominant varieties are detailed in Chapter 6: Table 6.1). The varieties at sampled sites were compared to the reference collection to identify similarities, and thus provide a conservative estimate of the number of varieties sampled during the survey.

3.3 RESULTS

The status of each natural enemy species is given separately and includes its geographic range, abundance, relative damage, and limiting factors such as parasitism and *L. camara* variety preferences.

3.3.1 *Calycomyza lantanae* (Agromyzidae)

The blotch leaf-mining fly *C. lantanae* occurred at 94% of the sites sampled (Table 3.1). Field abundance usually varied between 'rare' and 'occasional', but intermittently reached a rating of 'frequent' (Table 3.1). Because the fly was recorded from areas where no releases were made, notably in the Eastern and Western Cape provinces, it is assumed to have spread throughout the range of lantana in South Africa (Fig. 3.1a). Field surveys suggest that the fly is not inducing significant levels of damage to lantana, even though populations ranged from 'rare' to 'frequent' (Table 3.1).

Small leaf mines induced by the early larval instars often contained parasitoids. Although only one eulophid, ?*Chrysonotomyia* sp. was reared from larval mines (Table 3.2), high levels of parasitism were noted in the field, and additional parasitoid species may occur. Fly mines were observed on 14 varieties during the survey (Table 3.3). On 7 varieties fly populations were consistently 'rare', but on each of 7 other varieties fly

populations ranged from 'rare' to 'abundant'. At 15% of the sites where two or more varieties occurred simultaneously, the fly populations were above 'rare' and occurred in equal proportion on the varieties present. This suggests that a range of lantana varieties are susceptible and equally acceptable to *C. lantanae*. Fly populations were usually rated as 'rare', and observations on varietal preferences could only be based on the small number of sites where it occurred in higher densities.

Table 3.1
The abundance and impact of the biocontrol agents established at sites sampled on *Lantana camara* in South Africa.

Family Biocontrol agent	Present[a]	Absent[a]	Mean abundance[b]	Mean damage[b]
Chrysomelidae				
Octotoma scabripennis	49	92	2.2 (1-4)	2.2 (1-4)
Uroplata girardi	44	97	2.2 (1-4)	2.2 (1-4)
Tingidae				
Teleonemia scrupulosa	123	18	1.4 (1-4)	1.4 (1-4)
Agromyzidae				
Calycomyza lantanae	133	8	1.2 (1-3)	0.02 (0-1)
Ophiomyia lantanae	132	9	2.4 (1-4)	unknown

[a] Number of sites during the survey where the natural enemy species was present or absent.
[b] Abundance and damage means were calculated only from sites where the biocontrol agent was present, using the categories specified in Chapter 2 (Table 2.1). The range is given in parentheses.

3.3.2 *Octotoma scabripennis* (Chrysomelidae)

The leaf-mining beetle, *O. scabripennis* only occurred at 35% of the sampled sites, and was restricted to the warm, moist eastern parts of the range of lantana in South Africa (Fig. 3.1b). Although it was previously thought to thrive only in inland areas, large beetle populations were observed along the southern coast of KwaZulu-Natal. Where present, *O. scabripennis* abundance was usually 'occasional' or 'frequent' (Table 3.1), but ranged from 'rare' to 'abundant'. The damage attributed to *O. scabripennis* was generally 'minor' to 'partial', but could become 'significant' (Table 3.1). Because the distribution of *O. scabripennis* is restricted, its damage to lantana populations can, at best, be considered 'significant' but localised.

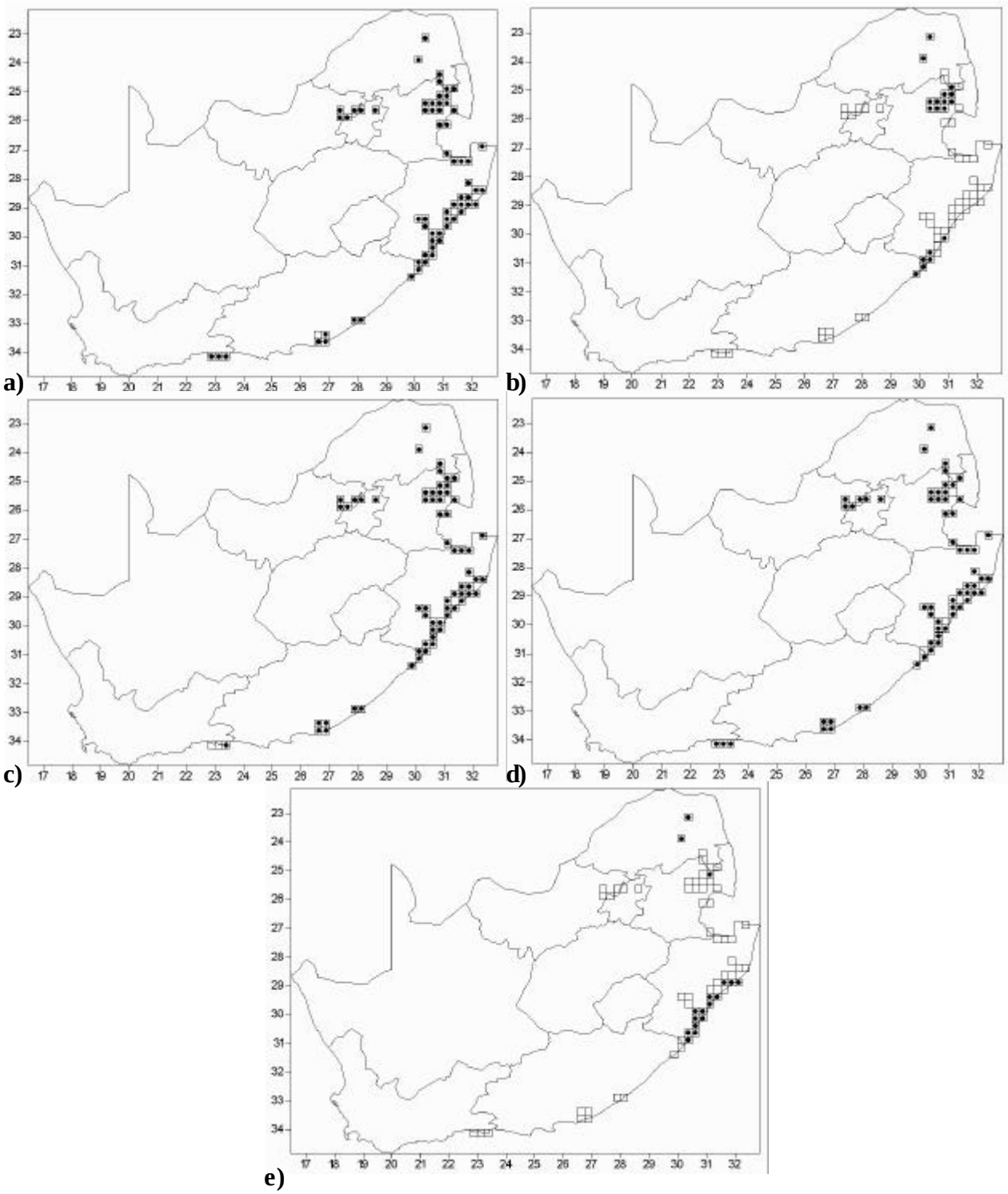


Fig. 3.1 The geographic range of the biocontrol agents established on *Lantana camara* in South Africa: (a) *Calycomyza lantanae*; (b) *Octotoma scabripennis*; (c) *Ophiomyia lantanae*; (d) *Teleonemia scrupulosa*; (e) *Uroplata girardi.* Open squares = sampled site with biocontrol agent absent; closed squares = sampled site with biocontrol agent present.

Predation and parasitism of the larvae of *O. scabripennis* was not observed during the survey, and probably has little influence on field populations. *Octotoma scabripennis* adults and larval mines were observed on 11 lantana varieties (Table 3.3). The abundance of adults and intensity of larval mines ranged between 'rare' and 'abundant' on each of 8 of these varieties. Beetle populations were observed at seven sites where two or more varieties co-existed. At five of these sites, insect populations were rated larger than 'rare', and occurred in equal abundance on the different varieties present. The results suggest that the lantana varieties in South Africa are equally acceptable to *O. scabripennis*.

Table 3.2
List of parasitoid species of biocontrol agents associated with *Lantana camara* in South Africa.

Biocontrol agent	Parasitoid Family	Parasitoid species[a]	Host stage attacked
Octotoma scabripennis	-	None found during survey	-
Uroplata girardi	Braconidae	Undet. sp. (2252)	Larvae
	Eulophidae	*Cirrospilus* ?*ambiguus* (2250)	Larvae
		?*Notanisomorphella* sp. (2251)	Larvae
	Pteromalidae	*Agiommatus* sp. (2253)	Larvae
Teleonemia scrupulosa	-	None found during survey	-
Calycomyza lantanae	Eulophidae	?*Chrysonotomyia* sp. (2257)	Larvae
Ophiomyia lantanae	Braconidae	*Bracon* sp. (2242)	Larvae and/or
		Opius sp. (2230)	pupae?
	Eucoilidae	Undet. sp. (2223)	Larvae
	Eupelmidae	*Eupelmus* sp. (2219)	Hyperparasitoid
		Eupelmus sp. (2221)	Hyperparasitoid
	Eulophidae	*Euderus* sp. (2220)	Hyperparasitoid

[a] Accession numbers (AcSN) of the undetermined species are given in parentheses. All material lodged with the National Collection of Insects, Pretoria, South Africa.

3.3.3 *Ophiomyia lantanae* (Agromyzidae)

The fruit-boring fly, *O. lantanae* occurred at 94% of the sampled sites (Table 3.1), and can thus be expected to occur throughout the range of lantana in South Africa (Fig. 3.1c). Fly populations were usually between 'occasional' and 'frequent' (Table 3.1).

Several hymenopteran parasitoids were reared from the larvae of *O. lantanae* collected throughout the range of the weed (Table 3.2). Some such as *Opius* sp. had a wide distribution, whereas *Bracon* sp. was restricted to the eastern regions of KwaZulu-Natal Province. Fly infestations in the field are relatively high, which suggests that the parasitoids may not be having a dramatic impact on *O. lantanae* population levels in South Africa. However the influence of hyperparisitism by *Eupelmus* spp. and *Euderus* sp. on parasitoid densities remains undetermined. Fly populations occurred on 14 lantana varieties (Table 3.3). On each of 12 of these varieties the fly populations ranged between 'rare' and 'abundant' (Table 3.3). At 20 of the sites where two or more varieties co-existed, the fly populations occurred in equal densities on the varieties present. These results suggest that *O. lantanae* shows no preferences between lantana varieties.

3.3.4 *Teleonemia scrupulosa* (Tingidae)

The sap-sucking lace bug was collected at 87% of the sites sampled, occurring throughout the geographic range of lantana (Fig. 3.1d). The field populations were low during the survey, and were rated as 'rare' to 'occasional' (Table 3.1), but infrequently reached 'abundant' levels. As a result, the damage attributed to this species was low and was rated between 'minimal' and 'minor' (Table 3.1). Where *T. scrupulosa* was abundant its relative damage to the weed was 'significant'. The reason for the low abundance of *T. scrupulosa* during the survey is unknown, but may have been due to unusually high rainfall experienced during the sampling period.

No incidences of predation or parasitism were observed during the survey. *Teleonemia scrupulosa* occurred on 14 lantana varieties (Table 3.3). On each of 9 of these varieties the insect populations were rated to range between 'rare' and 'abundant'. Although *T. scrupulosa* populations were usually 'rare' at the sites where two or more varieties co-existed, at 8 of these the insect densities were rated to be equal between the varieties present. This suggests that a range of varieties were susceptible to *T. scrupulosa*, and that preferences between varieties do not occur.

Table 3.3

The number of *Lantana camara* varieties on which the biocontrol agents are established, and their relative abundance on each over the range in South Africa and at sites with two or more varieties present.

Biocontrol agent	Number of varieties with insect rating:			Number sites with 2 or more varieties	Percentage sites where agent > 'rare'[b]	Preference between varieties at sites with 2 or more varieties present
	'Rare' only[a]	Range of 'Rare' to 'Abundant'[a]	'Frequent' or 'Abundant' only[a]			
O. scabripennis	3	8	0	7	71	No preference
U. girardi	1	7	0	12	67	No preference
T. scrupulosa	5	9	0	16	50	No preference
C. lantanae	7	7	0	20	15	No preference, but based on small sample
O. lantanae	1	12	1	20	70	No preference

[a] The insect abundance ratings are defined in Chapter 2: Table 2.1.
[b] Sites with 2 or more *L. camara* varieties present.

3.3.5 *Uroplata girardi* (Chrysomelidae)

This second species of leaf-mining beetle, *U. girardi* occurred at only 31% of the sampled sites and its distribution was restricted to the warm, moist eastern range of lantana (Fig. 3.1e). Where present, field populations were usually 'occasional' to 'frequent' in abundance, but ranged between 'rare' and 'abundant' (Table 3.1). The damage to plants attributed to this beetle was usually between 'minor' and 'partial', but 'abundant' populations often caused 'significant' levels of damage, causing the defoliation of entire lantana plants. However, due to the restricted distribution of the beetle in South Africa the 'significant' levels of damage are localised. Populations were usually abundant in the KwaZulu-Natal coastal region, but also in some restricted areas in Mpumalanga Province, such as areas close to the town of Hazyview. Beetle populations in the inland areas of its distribution range were usually 'rare'.

During the survey four parasitoid species were reared from larval mines (Table 3.2), but the incidence of parasitism was extremely low. Adults and larval mines of *U. girardi* were observed on 8 lantana varieties (Table 3.3). On each of 7 of the varieties the insect populations were rated between 'rare' and 'abundant', on one variety the insect populations were consistently 'rare'. The beetle occurred at 12 sites where two or more varieties co-existed, and at 8 of these the insect populations were rated to occur in equal densities on each variety present. These results suggest that *U. girardi* accepts a range of varieties, and that it generally shows no preference between lantana varieties in South Africa.

3.4 DISCUSSION

3.4.1 *Geographic range in South Africa*

Three of the five biocontrol agents considered in this paper, namely *T. scrupulosa*, *O. lantanae* and *C. lantanae* seem to be established throughout the geographical range of *L. camara* in South Africa (Fig. 3.1a, c, d), and are reported to have similarly wide geographic ranges in other countries (Greathead, 1968; Löyttyniemi, 1982; Muniappan and Viraktamath, 1986; Ooi, 1987; Waterhouse and Norris, 1987; Swarbrick *et al.*, 1995;

Muniappan *et al*., 1996; Esguerra *et al*., 1997). Although large releases of the two remaining species, *O. scabripennis* and *U. girardi*, were made in different geographic regions in the weed's range in South Africa (Cilliers, 1977, 1983), populations persist in only restricted areas in the warm, subtropical regions within its eastern range (Fig. 3.1b, e). The field survey confirms previous observations (Cilliers, 1987b; Cilliers and Neser, 1991) that, of the two hispine beetles, *O. scabripennis* is generally the dominant inland species, while *U. girardi* is dominant along the coastal range. However, there were several exceptions with large populations of *O. scabripennis* recorded in the KwaZulu-Natal Province coastal areas, and similarly, large populations of *U. girardi* recorded in the inland areas of Mpumalanga Province. The lack of established populations of *U. girardi* between the Northern and Mpumalanga provinces, with a gap in establishment in excess of 200 km, dismisses the assumption made that the 'cold adapted' Argentina stock released in Tzaneen (Northern Province) dispersed to inland areas in the Mpumalanga Province (Cilliers and Neser, 1991). It is likely that the initial releases made in Mpumalanga (Cilliers, 1983) persisted in low numbers after dispersing to surrounding areas, and were undetected until larger field populations were reached. Indeed, no signs of establishment of *O. scabripennis* were noted until 10 years after its release at a site near Nelspruit (Cilliers and Neser, 1991).

Field populations of these beetles are subject to facultative diapause (Harley, 1969) and are relatively inactive during the winter months, between May and September in South Africa (Cilliers, 1987a). Adults surviving the winter give rise to the spring generation, and thus the subsequent beetle numbers are directly proportional to the size of the over wintering population. In the temperate regions and exposed habitats in sub-tropical areas the dry and cold winter conditions induce plants to become dormant and predominantly leafless. Under these conditions beetle populations suffer high mortality rates and are unable to initiate viable spring populations. The restricted distribution of the beetles therefore appears to be attributable to their response to the condition of the weed, which becomes unsuitable under climatic conditions that promote seasonal leaflessness. On the other hand, the adults and nymphs of *T. scrupulosa* are able to persist under these conditions by feeding on the small buds on the stems, and *O. lantanae* and *C. lantanae*

survive by diapausing in the pupal stage, but also only persist in large numbers by the middle of the growing season.

No recoveries were made of the previously released biocontrol agents (this excludes the Lepidoptera) that apparently failed to establish in South Africa (Cilliers and Neser, 1991). The stem-boring beetle *Plagiohammus spinipennis* (Thompson) (Coleoptera: Cerambycidae) that was reported as 'established' in a garden (Cilliers and Neser, 1991), and the leaf-mining beetle *Octotoma championi* Baly (Coleoptera: Chrysomelidae), the status of which was unknown (Baars and Neser, 1999), both do not appear to have established.

3.4.2 *Biocontrol impact*

In South Africa, *T. scrupulosa, U. girardi* and *O. scabripennis* are considered the most damaging biocontrol agents on *L. camara* (Cilliers, 1983; Baars and Neser, 1999). Typically, over-wintering populations of these species are low, but increase during the growing season to peak in late summer (Cilliers, 1987a; Cilliers and Neser, 1991). As populations increase they cause extensive feeding damage that results in the periodic defoliation of plants and the die back of branches (Oosthuizen, 1964; Greathead, 1968; Haseler, 1966; Cilliers, 1977, 1987a; Baars and Neser, 1999). Substantial insect damage also reduces plant growth and flowering intensity (Oosthuizen, 1964; Harley *et al.*, 1979; Cilliers, 1987b; Muniappan *et al.*, 1996). However, during favourable growing conditions plants are able to compensate (Harley *et al.*, 1979; Muniappan and Viraktamath, 1986) and the reduction in seed production is probably not enough to reduce regeneration by seedlings (Harley *et al.*, 1979). During this survey the lace bug *T. scrupulosa* and the two hispine beetles *O. scabripennis* and *U. girardi* were considered to induce the highest levels of damage to *L. camara*. However, the restricted distributions of the two hispine beetles limited their damage induced to localized areas, whereas *T. scrupulosa* has the potential to induce more widespread damage. Early in the growing season the lack of feeding pressure from these agents provided a window of opportunity for plants to compensate for cumulative agent damage accruing at the end of the previous growing season.

The fruit-boring fly, *O. lantanae* was the most abundant biocontrol agent present at sites during the survey, but fly populations are known to fluctuate with the percentage fruits attacked ranging from 4 to 94% (Oosthuizen, 1964; Cilliers, 1987a; Graaff, 1987). However, studies on the impact of fly larvae on embryo viability (Cilliers, 1987a; Broughton, 1999), seed dormancy and germination (Graaff, 1987) remain inconclusive. To further understand the damage attributable to the fly additional aspects of the ecology of the weed need studying, such as the influence of cross pollination between different lantana varieties on seed viability, seasonal changes in seed development and viability, and the role of the digestive tract of birds in seed germination. Although the fly feeding damage leave the embryos intact, the impact on the seed endosperm may reduce the competitive ability of seedlings. Until we understand these processes the biocontrol impact of the fly remains uncertain. Due to the low field populations, the blotch leaf miner, *C. lantanae,* is considered to be ineffective in South Africa.

3.4.3 *Preference for* L. camara *varieties*

Varietal resistance, or the poor performance of biocontrol agents on certain lantana forms, has often been invoked to explain the lack of success in the biocontrol programme against *L. camara*. The disparity between the naturalized forms of *L. camara* and the different *Lantana* species in their native range (Baars and Neser, 1999; Day and Neser, 2000), raises the possibility that introduced agents are not preadapted to cope with the local varieties of lantana. The reports of *T. scrupulosa* and *C. lantanae* showing preferences for certain lantana varieties (Radunz, 1971; Harley *et al.*, 1979; Cilliers, 1987b; Cilliers and Neser, 1991) have supported this argument. Following these studies, varietal resistance has been widely accepted as an important factor that limits the effective biocontrol of lantana. Elaborate strategies, such as the exposure of the introduced forms of lantana to the suite of natural enemies in their native range (Neser and Cilliers, 1990) and the importation of better-adapted insect biotypes (Harley, 1974; Neser and Cilliers, 1990) have been proposed to alleviate this problem. However, the results of this survey indicate that the five biocontrol agents occur on a wide range of lantana varieties in South Africa. In addition, at a small but significant sample of sites,

where two or more varieties co-existed, the results suggest that these agents show no preference between the varieties. Populations of *T. scrupulosa* were uncharacteristically low during the survey, but the reported preference for different lantana varieties (Haseler, 1964; Radunz, 1971; Harley *et al*., 1979; Cilliers, 1987b; Cilliers and Neser, 1991) was not supported by the results of this survey. The apparent lack of varietal preference shown by these biocontrol agents, which has also been shown to be the case in Australia (Broughton, 2000; Day and Neser, 2000), suggests that varietal resistance may have been over-estimated and that its relative importance in explaining the poor performance of agents should be reconsidered. Varietal preference and performance studies in the laboratory and in field plots using these agents provide the opportunity to clarify the importance of this phenomenon.

3.4.4 *Parasitism and predation*

The high incidence of predation and parasitism of agents on *L. camara* appears to significantly reduce their effectiveness, particularly amongst Lepidoptera species (Baars and Neser, 1999; Broughton, 2000; Chapter 2). Various hymenopteran parasitoids have been recorded from *O. lantanae* in South Africa (Oosthuizen, 1964), including species of Braconidae, Cynipidae, Eupelmidae and Eulophidae (Cilliers, 1987a; Hill and Hulley, 1995). Unlike the impact of parasitism on fly populations in Brazil (Winder, 1982), population densities in South Africa appear relatively unaffected. The common parasitoid, *Opius* sp., may reduce fly populations, but in turn the effect of hyperparasitism is unknown. The blotch leaf miner, *C. lantanae*, is under heavy parasitoid pressure, which is likely to be the cause of the low field population densities in South Africa.

In their native ranges, *O. scabripennis* and *U. girardi* are under low parasitoid pressure (Krauss, 1964), and similarly in the introduced range very low levels of attack have been recorded, and include an unidentified hymenopteran parasitoid (Hill and Hulley, 1995) and eulopid ectoparasitoids (Cilliers, 1987a; Broughton, 2000). Other predators, like the mite *Pyemotes ventricosus* (Newport) have a minor impact on populations (Cilliers, 1987a). A few generalist predators have been recorded from field

populations of *T. scrupulosa* (Cilliers, 1987a), but these are generally insufficient to reduce their levels significantly. An egg parasitoid, *Erythmelus teleonemiae* Subha Rao (Hymenoptera: Mymaridae), has been recorded from India (Ganga Visalakshy, 1996), but equivalent species have not been recorded elsewhere.

3.5 CONCLUSIONS

The effective implementation of biological control against lantana will depend on the release of a suite of natural enemies that are able to cope with the factors that limit the impact of the agents already established. In particular, priority should be given to candidates with suitable biological attributes, which enable them to synchronize with the phenology of the weed and sustain high levels of damage throughout the growing season. This is especially appropriate for temperate regions, where agents need to recover early in the growing season to maintain continuous feeding pressure. More candidates are also required for subtropical regions to supplement the present levels of damage, and candidates with a high intrinsic rate of increase and high impact levels per individual should be given priority.

The importance of pre-adaptation to different lantana varieties may not be as critical as previously thought, but should still be incorporated in the host-specificity evaluation of new candidate agents on lantana. Because lantana occurs over a wide range of climatic conditions in South Africa, areas in ecoclimatically similar regions in the weed's native range are likely to provide candidates that are well suited to the 'new' conditions. Candidates with a wide geographic distribution in their native range may be better pre-adapted (Sands and Harley, 1981; Wapshere, 1985), although species with restricted distributions should not be discounted. New biocontrol candidates with a high risk of recruiting native parasitoids should be considered with caution, as the effectiveness of *C. lantanae* has been severely reduced by native parasitoids. The herringbone leaf-miner, *Ophiomyia camarae* Spencer (Diptera: Agromyzidae) is expected to recruit generalist parasitoids (Simelane, 2001), but its ability to attain large population levels in Florida (Baars, personal observations) where *L. camara* is introduced should encourage its release in South Africa.

Chapter 4

The role of climate in the geographic distribution of biocontrol agents on *Lantana camara*

4.1 INTRODUCTION

Although indigenous parasitoids have been shown to reduce the efficacy of some biocontrol agents on *L. camara* (Broughton, 2000; Chapters 2, 3), climate is considered to be the principal limiting factor to their distribution in South Africa and Australia (Day and Neser, 2000; Chapters 2, 3). Despite attempts to release and redistribute most of the agents into the major infestations of *L. camara* in South Africa, some have failed to establish in certain climate areas (Cilliers and Neser, 1991; Baars and Neser, 1999; Chapters 2, 3). Similarly in Australia, despite widespread releases, the tingid *Leptobyrsa decora* and the leaf-mining beetle *Uroplata fulvopustalata* Baly have only established at a few sites in the northern, more tropical areas of Queensland (Day and Neser, 2000). In an attempt to increase the ranges of such agents, biotypes that originated from areas in the native range that match the areas where agents failed to persist or performed poorly have been imported. These agent climatypes, defined as related populations that have differentiated mainly in response to differing climatic factors (Lincoln *et al*., 1982), were believed to be 'pre-adapted' to the environmental conditions in the target area and thus have a better chance of establishing and controlling the weed (Sands and Harley, 1981; Winder and Harley, 1982; Wapshere, 1985; Neser and Cilliers, 1990). In addition, these populations may be better synchronized with the weed phenology in the exotic environment (Oosthuizen, 1964). Indeed, importation of climatypes of the leaf-mining beetle, *Uroplata girardi,* is reported to have increased their distribution in cooler areas in Australia and South Africa (Winder and Harley, 1983; Cilliers and Neser, 1991). These ideas are not new, and were appreciated in the first attempts at the biocontrol of *L. camara* in the early 1900s when Koebele matched collection sites in Mexico to the infestations in Hawaii (Perkins and Swezey, 1924).

Several modelling techniques are available and complex climate analyses have provided useful predictions in the potential establishment and spread of invasive species.

These techniques have been applied in biological control to predict the potential weed (Panetta and Mitchell, 1991; Sindel and Michael, 1992; Julien and Maywald, 1995), and biocontrol agent distributions (Scott, 1992; Palmer and Pullen, 1996; Samways *et al*., 1999; Palmer *et al*., 2000), by comparing the climatic conditions in the native and introduced ranges using the CLIMEX model (Sutherst, 1991). As these modelling techniques are being developed, alternatives become available that use fundamentally different techniques, such as BIOCLIM (Busby, 1991) and a more recent technique using principal components analysis (PCA; Robertson *et al*., 2001).

In this chapter I determine the influence of climate on the distribution of agents established on *L. camara* in southern Africa by using the principal components analysis modelling technique. This technique uses the climate variables of sites where an agent is established (presence records) and compares them to unsampled sites both within and outside the range of *L. camara* in southern Africa to identify areas that are potentially climatically suitable. Consideration is given to: i) the potential for the redistribution of established agents, ii) the use of agent climatypes from their native range, and iii) the implications for the selection of new candidate agents, including the life history attributes that may be important and where in the native region surveys should be conducted. Although this climate analysis is restricted to the agents in southern Africa, these techniques can be applied elsewhere.

4.2 MATERIALS AND METHODS

4.2.1 *Insect distribution survey*

The 141 sampled sites that were used to determine the present distribution of the biocontrol agents established on *L. camara* in South Africa, which are described in Chapter 2, were used as the presence records in the distribution model.

4.2.2 *Predictive model*

The principal components analysis (PCA) modelling technique was used to predict the biogeographic distribution of the biocontrol agents on *L. camara* in southern Africa. The PCA technique is described in detail by Robertson *et al*. (2001), but in essence generates an interpretable probability distribution map based on the presence localities of the organism and the values of environmental variables at these localities. The technique relies on a strong correlation between the climatic predictor variables and the species' presence localities. A PCA is performed on the values of the climatic predictor variables associated with the presence localities to construct a hyperspace in which each axis is defined by an uncorrelated PCA axis. The origin of the hyperspace defines the fundamental niche of the organism. The values of predictor variables associated with the unsampled cells in a map region are mapped into the hyperspace, and the distance to the origin (fundamental niche) is assumed to represent their degree of suitability (in terms of the predictor variables). The PCA technique therefore generates quantitative output data for each grid-cell in a map region, expressed as probability values. The larger the probability value assigned to a grid-cell, the higher the bioclimatic suitability of that grid-cell for the organism, in this case the weed or biocontrol agent.

To make predictions using the PCA technique certain assumptions were made from the data. As many of the agents have either been established since the weed was introduced, or were released throughout the weed's range over twenty years ago they have had the opportunity to disperse to the areas where they are likely to establish. Although other factors such as parasitism and varietal preferences may have some influence, the variation in climatic conditions is considered to be the most important factor limiting the agents' distributions.

4.2.3 *Predictor variables*

The predictor variables used in the model include climate variables and altitude. The climate variables are interpolated from point data obtained from a network of weather stations in South Africa to produce a continuous data source for the map region

(Robertson *et al*., 2001). Principal components analyses are performed on the available climatic variable data as a pre-analytical data reduction technique. Ten predictor variables were selected (Table 4.1), and represent a summary of the climate.

When a linear relationship exists among one or more predictor variables used to build the model they are said to be multicollinear, and produce unreliable principal components (Robertson *et al*., 2001). Multi-collinearity was detected by means of the condition number (cn; see Robertson *et al*., 2001), which should be below 20, as numbers that range between 20 and 30 indicate serious multi-collinearity (Johnston, 1984). The condition number tends to be smaller when larger numbers of presence localities are used to build the model.

Table 4.1
The environmental predictor variables used in the principal components analysis modelling technique.[a]

No.	Predictor variable
1	Digital elevation model
2	Number of days with frost
3	Component axis 1 of a PCA on 12-monthly potential evaporation surfaces
4	Component axis 2 of a PCA on 12-monthly potential evaporation surfaces
5	Component axis 1 of a PCA on 12-monthly maximum temperature surfaces
6	Component axis 2 of a PCA on 12-monthly maximum temperature surfaces
7	Component axis 1 of a PCA on 12-monthly minimum temperature surfaces
8	Component axis 2 of a PCA on 12-monthly minimum temperature surfaces
9	Component axis 1 of a PCA on 12-monthly rainfall surfaces
10	Component axis 2 of a PCA on 12-monthly rainfall surfaces

[a] Variables used to build distribution models (Robertson *et al*., 2001).

4.2.4 *Distribution maps*

The map region (in this case South Africa, Lesotho and Swaziland) was subdivided into regular grid-cells and the probability values in each cell produced by the PCA modelling

technique were mapped back to the geographical coordinates to produce the suitability maps (using IDRISI32: a raster-based GIS software package). To facilitate the interpretation of the bioclimatic suitability maps, the probability values were classified into 'suitable', 'marginal' or 'unsuitable'. This was done by assigning the probability values for the presence grid-cells to one of the three classes according to a subjective assessment of the performance of the agent. The agent performance is defined as the long-term viability and potential to persist at abundant population levels. The classes would therefore correspond to where the biocontrol agent performed well ('suitable'), was present but performed poorly ('marginal') or where the climate was unsuitable for the establishment of the agent ('unsuitable'). Where the performance of agents could not be differentiated between 'suitable' or 'marginal', only two classes were used; i.e. 'suitable' or 'unsuitable'.

Maps were produced for *L. camara* and the 10 biocontrol agents established on this weed using the PCA-modelling technique to predict the bioclimatic suitability of areas in South Africa, Lesotho and Swaziland. The map for *L. camara* was produced using the total number of presence localities sampled during the insect survey (Chapter 2). To determine the most suitable climate areas for the establishment of agents, the distribution maps for the agents were superimposed, producing a map with the number of agents present in the different areas.

4.3 RESULTS

The predicted distribution map for *L. camara* indicates that areas with high bioclimatic suitability include parts of Gauteng, Northern, Mpumalanga, KwaZulu-Natal and the Eastern Cape provinces, and Swaziland (Fig 4.1). These predictions correspond well with the distribution records of *L. camara* in southern Africa (Henderson, 2001), and with distribution maps produced using the PCA-modelling technique from a larger sample size (Robertson *et al*., 2001).

A number of the presence localities for each agent were not included in the distribution models, because some samples occurred in the same grid-cell (Table 4.2). Of particular concern is the small number of sample sites used to predict the distribution of

the two leaf-mining beetles, *U. girardi* and *Octotoma scabripennis*, and the flower-feeding moth, *Lantanophaga pusillidactyla* (Table 4.2). Furthermore, the condition numbers for these three species indicate serious multi-collinearity, whereas the condition numbers for the other seven species were acceptable and ranged from 15 to 17. The continuous probability maps of four of the biocontrol agents were reclassified into only 2 classes (Table 4.2). This was done, because the agent damage was not immediately obvious during the sampling procedure (*e.g. Epinotia lantana* and *Ophiomyia lantanae*), or the insect populations were predominantly low and the levels of performance could not be differentiated (*e.g. Aristaea onychote* and *L. pusillidactyla*).

As different guilds on the plant might be subject to differing microclimatic conditions, the biological control agents on the weed were divided up into three main feeding guilds (Table 4.2). These guilds are dealt with separately below.

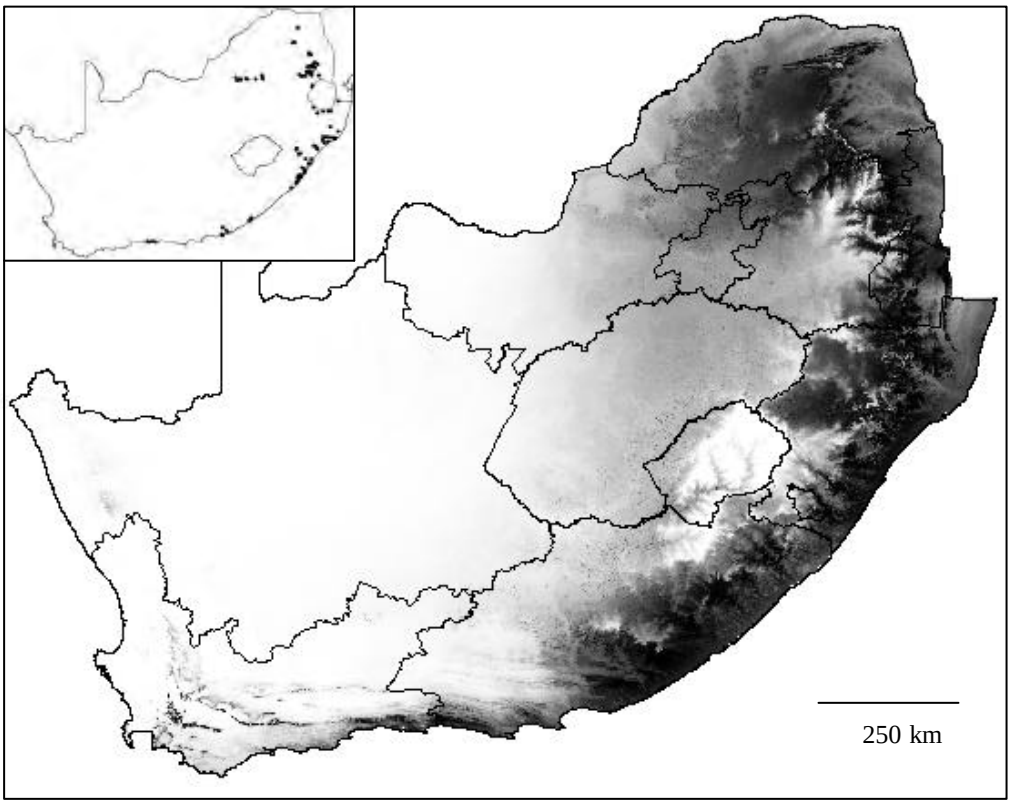


Fig. 4.1 Bioclimatic suitability map for *Lantana camara* in South Africa, Lesotho and Swaziland produced using 121 presence localities (see inset)(condition number = 16.90). Darker shades indicate higher probabilities.

Table 4.2
Biocontrol agents on *Lantana camara* and the data used in the PCA-modelling technique, condition numbers and the number of classes used in the bioclimatic suitability maps.

Feeding guild [a]/ Biocontrol agent	Sampled Sites	Presence Localities[b]	cn[c]	No. Classes
Leaf				
Hypena laceratalis	133	121	16.8	3
Aristaea onychote	98	92	16.6	2
Calycomyza lantanae	133	122	15.9	3
Salbia haemorrhoidalis	55	49	16.9	3
Octotoma scabripennis	49	47	37.5	3
Uroplata girardi	44	37	31.9	3
Flower, fruit and shoot-tip				
Ophiomyia lantanae	132	120	16.4	2
Epinotia lantana	86	80	15.5	2
Lantanophaga pusillidactyla	27	26	27.8	2
Other (leaf, inflorescence and axillary bud)				
Teleonemia scrupulosa	123	113	16.5	3

[a] Species grouped according to their plant resource requirements.
[b] The number of sampled sites that occur in separate grid-cells and are used to build the model.
[c] Condition number (cn) used to detect multi-collinearity of the predictor variables.

4.3.1 *Leaf chewers and miners*

Two of the agents, *Hypena laceratalis* and *Calycomyza lantanae*, are predicted to have a widespread 'suitable' distribution (Fig. 4.2 a, c) that corresponds with the predicted distribution of *L. camara* (Fig. 4.1). The 'marginal' range of *H. laceratalis* and 'suitable' range of *A. onychote* are also predicted to occur over large parts of the Free State Province where *L. camara* does not grow naturally (Fig. 4.2 a, b). The three other leaf feeders, *Salbia haemorrhoidalis*, *O. scabripennis* and *U. girardi* are predicted to have a restricted distribution (Fig. 4.2 d, e, f). Areas that are predicted to be bioclimatically

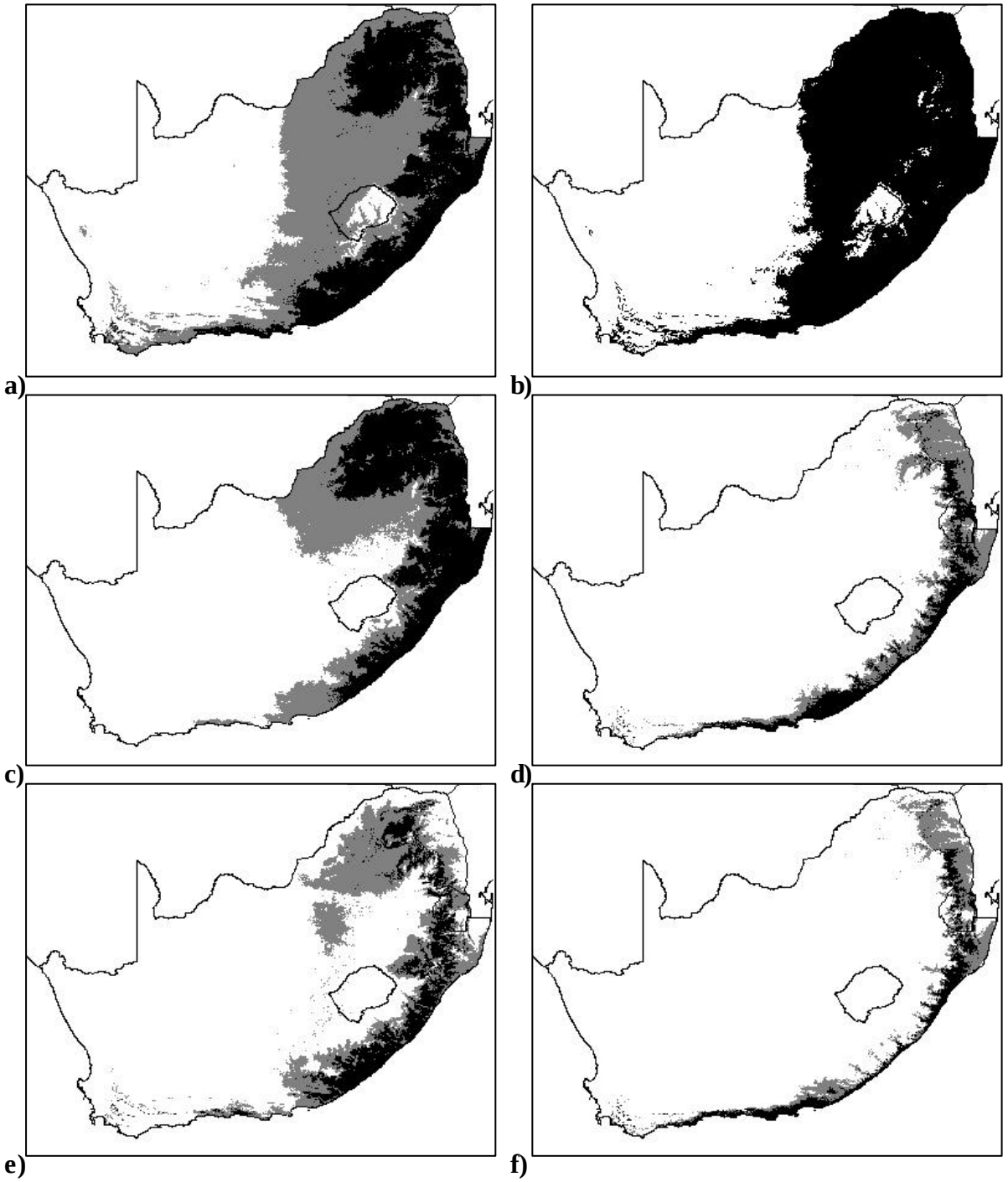


Fig. 4.2 Bioclimatic suitability maps for the leaf-feeding biocontrol agents on *Lantana camara* in South Africa, Lesotho and Swaziland. a) *Hypena laceratalis*; b) *Aristaea onychote*; (c) *Calycomyza lantanae*; (d) *Salbia haemorrhoidalis*; (e) *Octotoma scabripennis*; (f) *Uroplata girardi.* Key to shading: black = 'suitable'; grey = 'marginal'; no shading = 'unsuitable'.

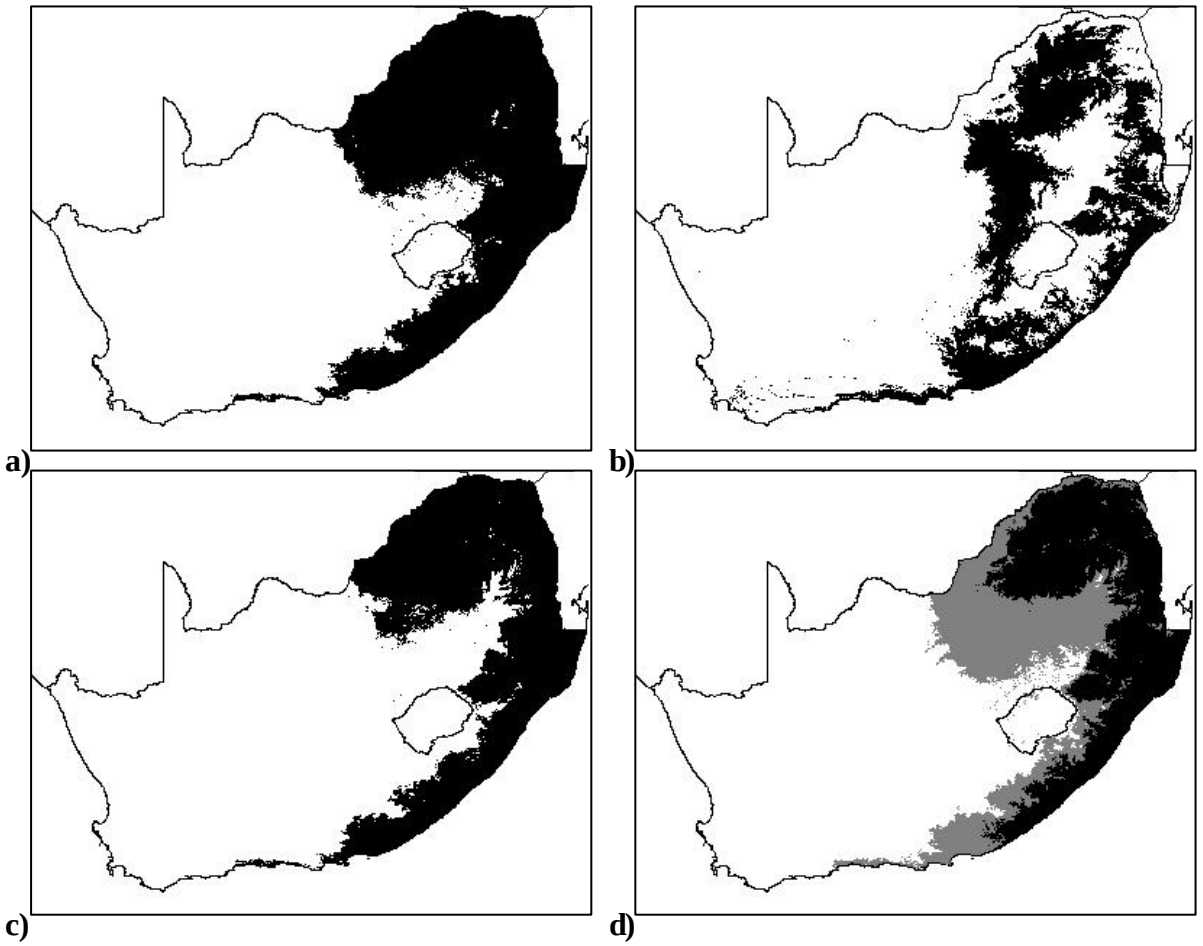


Fig. 4.3 Bioclimatic suitability maps for the flower-, fruit- and shoot-feeding and other biocontrol agents on *Lantana camara* in South Africa, Lesotho and Swaziland. a) *Ophiomyia lantanae*; (b) *Lantanophaga pusillidactyla*; (c) *Epinotia lantana*; (d) *Teleonemia scrupulosa*. Key to shading: black = 'suitable'; grey = 'marginal'; no shading = 'unsuitable'.

suitable for these agents include the east and coastal areas of the Eastern and KwaZulu-Natal provinces, and parts of Mpumalanga Province and Swaziland. Small suitable areas also occur in restricted areas in the Northern Province. The broader 'suitable' range of *O. scabripennis*, which includes the higher altitude areas of the Mpumalanga and Northern provinces (Fig. 4.2 e), may be the result of a less reliable prediction because of the high condition numbers indicating that the predictor variables used were multicollinear. Based on the observations during the field survey, the range of *O. scabripennis* is more likely to be similar to that of *S. haemorrhoidalis* (Fig. 4.2 d).

4.3.2 *Flower, fruit and shoot-tip feeders*

The bioclimatic suitability maps suggest that *O. lantanae* and *E. lantana* have wide climate tolerances (Fig. 4.3 a, c), and occur throughout the distribution of *L. camara*. The potential distribution map of *L. pusillidactyla* should be interpreted with caution because the prediction was based on predictor variables that were multicollinear. However, the general indication from the map suggests that it has a wide climatic tolerance (Fig. 4.3 b).

4.3.3 *Other (leaf, inflorescence and axillary bud sucker)*

The predicted 'suitable' range of *Teleonemia scrupulosa* (Fig. 4.3 d) corresponds with the potential distribution of *L. camara* (Fig. 4.1). The 'marginal' areas are predicted to extend beyond the naturalized areas of *L. camara*, suggesting that it has a wide climate tolerance and is suited to the range of climatic conditions over the weed's geographic distribution in southern Africa.

4.3.4 *Climatic areas suitable for biocontrol agents*

The two species *H. laceratalis* and *A. onychote* were excluded from this analysis and only the bioclimatic suitability maps of eight of the exotic biocontrol agents were superimposed (Fig. 4.4). The combined map suggests that in a large proportion of the areas that were predicted to be suitable for the weed (Fig. 4.1), at least 5 to 6 of the biocontrol agents are predicted to occur (Fig. 4.4). The most suitable areas, where most or all of the biocontrol agents are predicted to occur, include the eastern coastal areas of the Eastern Cape and KwaZulu-Natal provinces and the eastern parts of Mpumalanga province and the central and north-eastern parts of Swaziland (Fig. 4.4).

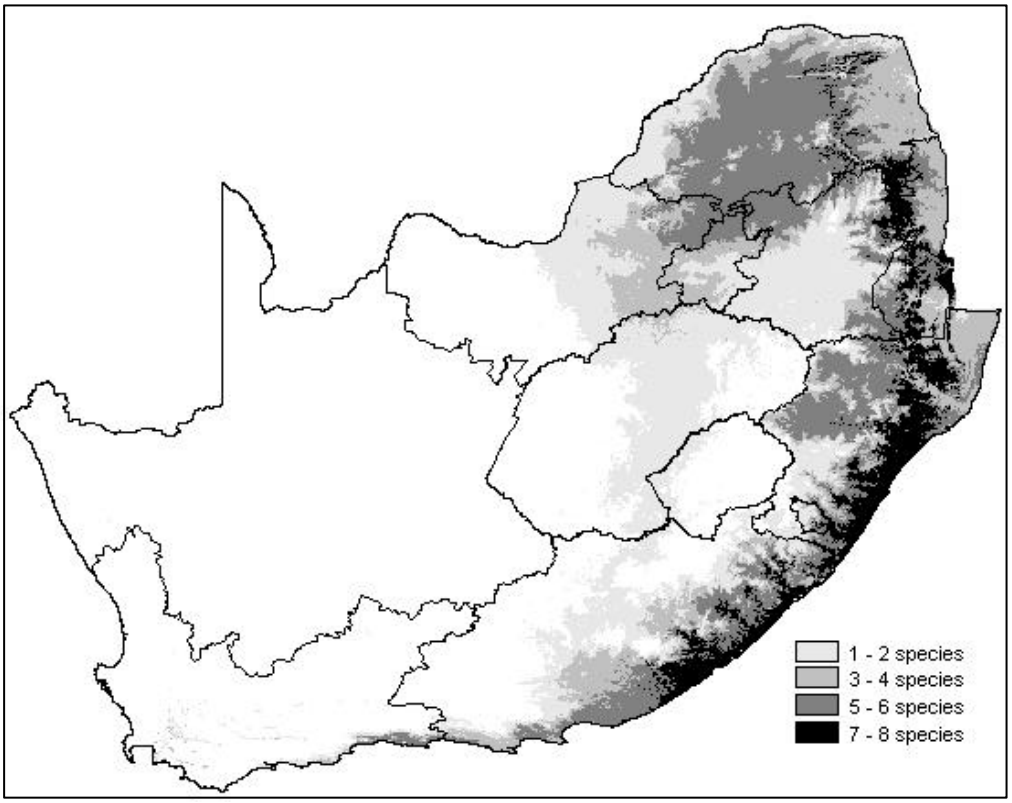


Fig. 4.4 A bioclimatic suitability map showing the number of biocontrol agents predicted to be present on *Lantana camara* in South Africa, Lesotho and Swaziland. The numbers of biocontrol agents are grouped into four classes described in the key.

4.4 DISCUSSION

4.4.1 *Weed characteristics in response to climate*

Long-term weather conditions in an area may act directly on the phenology of insects, or indirectly by changing the plant-herbivore relationships. The spatial variation in climatic factors cause differences in the plant's resources and thus changes their carrying capacity for insect populations. This variation in the availability of host resources can change an insect's survivorship, fecundity and development rate, or otherwise referred to as its intrinsic rate of increase (Gassmann, 1996). Indeed, the cold and dry winters in South Africa and Australia are reported to reduce the survivorship of insect populations on *L. camara* (Harley *et al*., 1979; Winder and Harley, 1983; Cilliers and Neser, 1991; Chapter

3). In response to the seasonal changes in the weed's growth pattern populations of the biocontrol agents experience a decline in numbers during the dry and cold winter conditions. Plants recover from their dormant and predominantly leafless state during spring and insect populations only increase to sufficient numbers to impact on plants by the middle of the growing season. The biocontrol agents that are dependant on the availability of leaves for survival, such as the two leaf-mining beetles, are unable to cope with the lack of plant resources and only persist where the weed maintains its leaf resource. Not all the leaf-chewers and miners are dependant on a continual supply of resources and probably survive the dormant period through a resistant life stage, permitting them to establish in the temperate parts of the weed's range in South Africa.

4.4.2 *Agent redistribution and the introduction of climatypes*

Two of the biocontrol agents, *H. laceratalis* and *A. onychote* are indigenous to South Africa (Cilliers and Neser 1991; Chapter 2). The distribution maps suggest that they are potentially able to occur throughout the range of *L. camara*, but also occur in parts of the Free State Province. Therefore, attempts to redistribute these agents are probably futile, as their populations do not appear to be restricted by climate. The predicted distribution maps of the exotic agents suggest that most are widespread and the redistribution of agents and importation of climatypes will probably not extend their distribution ranges. It may be more useful to introduce climatypes to extend the limited ranges of *S. haemorrhoidalis*, *O. scabripennis* and *U. girardi*. However, the present distribution of *S. haemorrhoidalis* may be the result of the gradual natural dispersal from releases made in KwaZulu-Natal, rather than climate limitations. After this agent was introduced in the 1960s (Oosthuizen, 1964) and found to have become established in the 1980s (Cilliers and Neser, 1991) no documented attempts have been made to redistribute it to the temperate areas (Chapter 2). Therefore, it may be more effective to field-collect this agent and introduce it to the higher elevation sites where it does not occur, before attempts are made to introduce a climatype that is matched to these conditions. On the other hand, the two leaf-mining beetles *O. scabripennis* and *U. girardi* were released throughout the major infestations of *L. camara* in South Africa (Cilliers, 1977, 1983;

Cilliers and Neser, 1991), but occur in restricted parts of the weed's range. Although these two agents are reported to be poor dispersers (Waterhouse and Norris, 1987), their limited ranges are likely to be in response to climate conditions. Indeed, introductions of a 'cold-adapted' strain of *U. girardi* were made to extend its range in Australia (Winder and Harley, 1983), but the results of these introductions are not available.

New agent climatypes may also be better adapted to climatic factors in certain areas of the introduced range than the original agent population, and thus improve its efficacy in the introduced range. The assumption here is that the original climatype or gene pool of the agent has naturally dispersed into its present range without gradually adapting to site-specific climatic conditions. Therefore, introducing an agent climatype matched to the 'marginal' areas may improve the agent's performance. However, it has been argued that the spread of agents on *L. camara* over time is the result of their adaptation to different climatic conditions in South Afirca (Cilliers and Neser, 1991). Alternatively the slow spread of agents may in part be explained by the 'lag-phase' often associated with the establishment and spread of introduced species, rather than an adaptation to climatic conditions. A combination of these scenarios is probably true, in which case introducing a climatype may offer a potential method of increasing the levels of damage on *L. camara*, but monitoring such introductions may prove very difficult (Neser and Cilliers, 1990). If climatypes are to be introduced it would be more effective to introduce agents that are not under other environmental pressures. This excludes *C. lantanae* and *E. lantana* due to high parasitoid pressures and *O. lantanae* and *L. pusillidactyla* because the nature of their damage is ineffective in reducing the invasive status of the weed (Chapters 2, 3).

4.4.3 *Implications for release strategies*

The distribution maps of the agents suggest that the climate over the range of the weed is suitable for the establishment of the majority of the agents. Three of the agents seem to be restricted to the sub-tropical range of the weed, and may benefit from either the redistribution of established populations or importation of climatypes to extend their range or improve their efficacy. The survey of the impact of all these agents suggests that

only five of the agents contribute significantly to reducing the growth rate and seed production of *L. camara* (Chapters 2, 3). However, only two *T. scrupulosa* and *H. laceratalis* seem to occur throughout the weed's range. Furthermore, in areas where the weed supports the entire compliment of agents, the combined levels of damage are insufficient to reduce the invasive status of lantana.

It is generally accepted that the control of *L. camara* will depend on a suite of biocontrol agents, and additional agents need to be released to improve its biocontrol (Cilliers and Neser, 1991; Baars and Neser, 1999; Day and Neser, 2000; Chapters 2, 3). Introducing an agent that has the capability to establish throughout the range of the weed is highly desirable. However, due to the lack of sufficient insect pressure in both the sub-tropical and temperate areas new agents that are specialized to each of these climatic conditions warrant consideration (Chapter 3). New initiatives have focused on collecting agents in areas in the native range that match the temperate climatic conditions, or that posses biological attributes which improve their synchrony with the seasonal changes in the weed. Indeed, surveys in Brazil suggest that natural enemies cause more intense damage to the weed in temperate areas as opposed to warmer tropical sites (Winder and Harley, 1982). Surveys have also been conducted in the high elevation and drier climates of Mexico (Urban and Neser, personal communication), but the results of these collections still need to be implemented. To improve the agent-weed synchrony agents that attack plant resources that remain apparent year round, and which posses the potential to diapause have been targeted (*e.g. A. championi*) or have been introduced and are being evaluated (Baars and Neser, 1999; Palmer *et al*., 2000). In addition, biocontrol agents more suited to the sub-tropical conditions are being considered (Baars and Neser, 1999; Simelane, 2001; Baars, 2002).

Due to the low establishment success of biocontrol agents on *L. camara* the release techniques used to introduce new agents have been scrutinized (Winder and Harley, 1982; Cilliers and Neser, 1991; Broughton, 2000; Day and Neser, 2000). Many factors play a role, including the use of bw release numbers, the release of agents onto unsuitable varieties, the accumulation of parasitoids and predators and the release of agents into areas with unsuitable climatic conditions. The combined distribution map of agents on *L. camara* (Fig. 4.4) suggests that the most suitable climatic conditions for the

establishment of the biocontrol agents include the warm, wetter parts of the Eastern Cape, KwaZulu-Natal and Mpumalanga provinces, and first releases of new agents are more likely to establish in these areas.

4.4.4 *Accuracy of model prediction*

It is important that the quality of the data used in the technique to produce the prediction is reliable. The PCA technique has an advantage over other techniques that it does not use absence data, which can be less reliable (see Robertson *et al*., 2001). However, the quality of the presence data is dependent on the sampling techniques used, and sampling bias must be minimized. In this survey the samples were only taken from *L. camara*, and as a result the distribution models of agents with alternative hosts, such as *H. laceratalis* and *A. onychote* are potentially biased. The known alternative host plants of these two indigenous agents include *Lippia* species (Baars and Neser, 1999; Kroon, 1999), which also occur in the same habitat as *L. camara* and the prediction may therefore still be reliable. On the other hand, the indigenous moth, *Characoma submediana*, feeds on *L. camara* (Chapter 3) and was omitted from the model because it also feeds on *Acacia karoo* (McGeoch and Krüger, 1994), which grows in a different habitat, causing a bias in the sampling technique. Furthermore, samples of *S. haemorrhoidalis* may be biased because its absence in the high elevation areas may be the result of the geographical barrier (Drakensberg Mountains) to colonization rather than its response to climatic conditions. The PCA-modelling technique is also unlikely to perform well when small samples of locality records of less than 40 are used (Robertson *et al*., 2001). Therefore, the suitability maps produced for *U. girardi* and *L. pusillidactyla* may be unreliable, but from field observations (Chapters 2, 3), the restricted distribution of *U. girardi* and the widespread distribution of *L. pusillidactyla* are likely to be reasonably accurate.

4.5 CONCLUSION

Understanding the impact of plant resources on the intrinsic rate of increase of agents is an important part in determining the reason for the success or failure of biocontrol agents.

Climate comparison or modelling techniques provide a useful tool to predict the agent-weed interaction in response to environmental factors in the introduced range. These techniques, like CLIMEX have been successful in selecting suitable release sites of *A. compressa* (Palmer *et al*., 1996; Day and Neser, 2000) and showing that the extensive releases of *Perapion antiquum* (Gyllenhal) (Coleoptera: Brentidae) on *Emex* spp. in Australia were made at climatically unsuitable sites (Scott, 1992). The PCA technique in this study provides useful information on the importance of redistributing established agents, improving future release techniques and identifying the biological attributes of agents that may improve the levels of biocontrol. Furthermore, this technique will prove valuable in predicting the potential distribution of new candidates in the early stages of release programmes.

Although useful, these techniques generate believable maps that must be interpreted cautiously, bearing in mind the underlying assumptions made to produce them (Cruttwell MacFadyen, 1991, 1998). Furthermore, even the best climate match provides no guarantee of success, as biocontrol agents have established outside their predicted range (Gassmann and Schroeder, 1995). Due to the complexity of *L. camara*, the advantages of matching climates, agent redistribution and introducing more effective climatypes may be lost through other factors such as the incompatibility of agents with the varieties of *L. camara* or the accumulation of native parasitoids or predators.

Chapter 5

The selection of candidate agents for the biological control of *Lantana camara* based on the phytophagous organisms associated with *Lantana* species in Jamaica

5.1 INTRODUCTION

The genus *Lantana* occurs naturally in South, Central and parts of tropical North America, where surveys for potential biocontrol agents for *L. camara* have been confined to species in the section *camara.* Surveys conducted on the closely related species, namely *L. tiliaefolia* and *L. glutinosa* Poepp. from Brazil (Winder and Harley, 1983), and *L. camara, L. hirsuta* Mart. and Gal., *L. urticifolia* Mill. and *L. urticoides* Hayek from tropical Central and North American countries (Palmer and Pullen, 1995; Krauss, 1962), have largely been the source of natural enemies released as biocontrol agents on the weed *L. camara*.

The West Indies falls within the natural range of *Lantana* species (Palmer and Pullen, 1995), and should therefore be a source of potential natural enemies. However, with the exception of a few natural enemies collected in Cuba (Krauss, 1962) and Trinidad (Stegmaier, 1966; Harley and Kassulke, 1973, 1974), there is no comprehensive list of the phytophagous organisms associated with *Lantana* species from this region. Furthermore, the collection of promising candidate agents during an opportunistic survey in Jamaica in 1994 (S. Neser, personal communication) has stimulated interest in more detailed searches in this region.

The selection of the first biocontrol candidates for release on *L. camara* was largely directed by the experience and scientific intuition of Albert Koebele in the early 1900s, and then by John Mann, and Noel Krauss in the 1950s. On average, 3 scientist-years are required for the evaluation and release of each biocontrol agent (Harris, 1991). The selection of the most effective natural enemies before host range evaluations are initiated could therefore constitute a considerable saving in both time and resources (Cruttwell McFadyen, 1998). Harris (1973) and Goeden (1983) developed prioritization systems, which scored biological control candidates according to various criteria. A modification of Harris's system, developed by Winder and Harley (1983), placed

emphasis on different feeding guilds, which were weighted according to the intensity of feeding damage over the range surveyed. Wapshere (1985) proposed an alternative approach, which placed no emphasis on the type of agent or the mode of attack, but regarded agents which reduce and maintain weed populations at low levels, in ecoclimatically similar situations in the country of origin as the priority candidates. Crawley (1986, 1989) argued that certain demographic parameters of biocontrol agents are associated with successful establishment and control. However, the application of these methods to different weed systems (Müller, 1990; Blossey, 1995) led to some concerns as to the validity of the parameters used, and Cullen (1995) emphasised caution when agent-weed interactions were simplified to develop these systems because it led to generalisations in the selection of candidate biocontrol agents.

The retrospective analysis of specific biological control programmes (Müller, 1990; Blossey, 1995; Chapters 2, 3, 4), current selection procedures (Harris, 1973; Zwölfer *et al*., 1976; Sands and Harley, 1981; Winder and Harley, 1982; Goeden, 1983; Wapshere, 1985; Crawley, 1986, 1989; Harris, 1991; Blossey, 1995; Cullen, 1995) and the methods used in field surveys (Scott and Adair, 1995; Causton *et al*., 2000) have yielded numerous parameters that potentially direct the successful selection of candidate biocontrol agents. Most of these studies emphasised the mode of attack by the natural enemies and their subsequent impact on the ecology of the weed, while their intrinsic rate of increase, geographic range, host specificity, synchrony with the weed, dispersal ability and susceptibility to predation and parasitism were considered less important in the selection of candidate agents. Notable parameters related to the weed that should also be considered include the acceptance of different varieties (biotypes) of the weed by candidates.

This chapter reports on a survey of the phytophagous organisms associated with *Lantana* species (chiefly *Lantana urticifolia*) in Jamaica. To prioritise the list of potential candidates collected, I ranked them using the selection systems developed by Harris (1973), Goeden (1983), Winder and Harley (1983) and Crawley (1986, 1989). These candidates were also ranked using a revised selection system that was developed to try and incorporate the parameters important in selecting the most promising agents for the biological control of *L. camara* in South Africa.

5.2 MATERIALS AND METHODS

5.2.1 *Field Survey*

The phytophagous organisms associated with *Lantana* species were sampled throughout Jamaica (Fig. 5.1). Plants were sampled in midsummer (July 1999) at 46 sites, which included roadsides, riparian zones, arable land borders and natural vegetation. Sites were selected where *L. urticifolia*, the dominant species of *Lantana*, was common, with at least 10 plants in close proximity. The surveying methods used were as described in Chapter 2 and plants were assessed for insect abundance using the five-category scoring system (Table 2.1). The samples (see Chapter 2) were imported into the quarantine facilities of the Plant Protection Research Institute (Pretoria, South Africa) for detailed analysis.

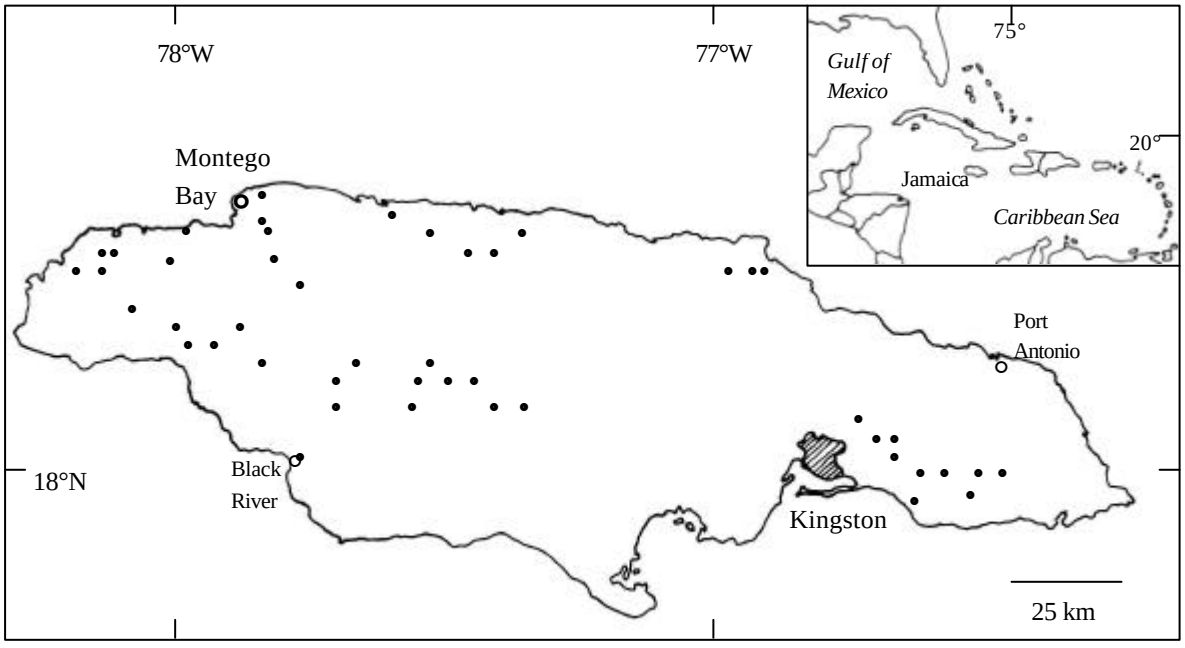


Fig. 5.1 The sites sampled (•) during a survey of the phytophagous organisms associated with *Lantana* species (chiefly *Lantana urticifolia*) in Jamaica.

5.2.2 *Host range analysis*

At sites where congeneric and other related species (Verbenaceae) co-existed with *L. urticifolia*, these species were also surveyed to determine the presence or absence of the phytophagous natural enemies and their feeding intensity. Assessments that were made on related plants included only the natural enemy species that were 'frequent' or 'abundant' on *L. urticifolia* at the same site.

5.2.3 *Sampling assessment*

A species-accumulation curve was plotted to determine whether the number of species collected was representative of the regional species pool on *L. urticifolia* in Jamaica. The sampled sites were ranked in ascending order, from those having the lowest to those having the highest number of natural enemies present per site, and the cumulative number of species collected was plotted against the cumulative number of sites sampled.

5.2.4 *Selection of biocontrol candidates*

The results of the field survey and information from earlier laboratory studies was used to determine the suitability of the phytophagous organisms as biocontrol candidates for further host-specificity evaluation in South Africa. Specific criteria for each species were used and applied to four ranking systems proposed by Harris (1973), Goeden (1983), Winder and Harley (1982), and Crawley (1986, 1989). The systems of Harris (1973) and Goeden (1983) assigned scores to aspects of the candidate's life history, which included host specificity, impact on the plant, phenology, fecundity, and distribution. Winder and Harley (1982) used differential weightings for five modes of feeding damage, which were further weighted by the candidate's relative abundance over the plant's range. Crawley (1986, 1989) used several demographic parameters to rank the probability of establishment and success of a candidate, including fecundity, egg aggregation, voltinism, size, damage, longevity, distribution, abundance and susceptibility to predation and parasitism (Blossey, 1995). The priorities assigned (rank order) to the natural

enemies by the four systems were compared to determine the consistency between the systems.

5.2.5 *Revised selection protocol*

A protocol for prioritising and therefore selecting new natural enemies for *L. camara*, was developed. The parameters that promote the selection of priority candidates were differentially weighted according to the emphasis placed upon each in the biocontrol literature (Harris, 1973; Zwölfer *et al*., 1976; Sands and Harley, 1981; Winder and Harley, 1982; Goeden, 1983; Wapshere, 1985; Crawley, 1986, 1989; Müller, 1990; Harris, 1991; Blossey, 1995; Cullen, 1995; Scott and Adair, 1995; Causton *et al*., 2000; Chapters 2, 3, 4). The agent's damage and impact on the ecology of the weed are considered twice as important as the candidate's intrinsic rate of increase and geographic and climatic range. These two parameters are in turn twice as important as the other parameters identified, including host range, synchrony with host, dispersal ability, predation and parasitism risk and susceptibility to weed varieties. These three groups of parameters therefore received a weighting of 4, 2, and 1 respectively. In addition, each parameter can either have a positive or negative impact on the suitability of a candidate on a specific weed. The agent's suitability regarding each parameter was assigned a value in a three-fold range, which ranged from –2 to 0, or –1 to +1, or 0 to +2. A negative value is assigned when the parameter reduces the biocontrol potential of a candidate, e.g. an agent that is susceptible to high levels of predation or parasitism, which enhances its chances of accumulating parasitoids in the introduced range. Similarly, a candidate that is unable to feed on the entire range of host plant varieties is unlikely to accept the complex of *L. camara* varieties in the introduced range and is also assigned a negative value. The values assigned to the parameters are totalled, and the candidates with the highest scores are prioritised. The revised system therefore scores each candidate according to its attributes and is not prioritised according to the regional species pool.

5.3 RESULTS

5.3.1 *Phytophagous organisms in Jamaica*

Twenty one species of phytophagous insects and mites were collected on *L. urticifolia* in Jamaica (Table 5.1). Of these eight were considered to be damaging to plant growth and/ or flower and fruit production (Table 5.1).

Aceria lantanae (Cook)(Acari: Eriophyidae) and *Falconia intermedia* (Heteroptera: Miridae) were consistently the most abundant species (Table 5.1), with the flower-galling mite, *A. lantanae*, occurring at more than 80% of the sites. Mite infestations varied considerably between sites, from a few infested flowers with numerous undamaged flowers and seeds present, to almost all of the flowers heavily galled. At most of the sites old galls were still attached to plants, indicating that infestations persisted on individual plants over time. Heavily galled plants had notably fewer leaves and were sparsely stemmed, suggesting that the galls act as nutrient sinks.

High population levels of the leaf-sucking lantana mirid, *F. intermedia*, occurred at approximately half of the sites and mostly in high numbers, causing severe leaf chlorosis.

The adults and larvae of the leaf-chewing flea beetle, *Omophoita albicollis* Fabricius (Coleoptera: Chrysomelidae), caused typically large 'shot holes' in the leaves. Larvae were seldom collected and adult feeding on the shoot tips was usually limited, with damage on only a small proportion of the leaves.

The populations of the leaf-tying moth, *Salbia haemorrhoidalis* were relatively low (Table 5.1) and although characteristic damage was regularly encountered, the impact on the leaves was limited. Where larvae were abundant, the damage to leaves was considerable and the plants were visibly stressed.

The two sap-sucking lace bugs, *Teleonemia* prob. *scrupulosa* and *T.* prob. *harleyi* Froeschner (Heteroptera: Tingidae), fed predominantly on the leaves, but also on the flowers. These two species were consistently associated with *L. urticifolia* at the sites sampled (Table 5.1) and where population levels were high, the leaf damage was severe.

Table 5.1
Phytophagous organisms associated with *Lantana urticifolia* in Jamaica, including their frequency of occurrence and relative abundance over the plant's range.

Order/ Family	Natural enemy species [a]	Mode of attack	Sites [b]	Abundance [c]
Acari				
Eriophyidae	*Aceria lantanae* (Cook)	Flower gall former	38*	2.57 (1-4)
Coleoptera				
Chrysomelidae	*Omophoita albicollis* Fabricius	Leaf chewer	18*	2.00 (1-4)
	Longitarsus sp. (2170; 2156)	Root feeder/ Leaf chewer	24*	1.96 (1-4)
Heteroptera				
Miridae	*Falconia intermedia* (Distant)	Leaf sucker	23*	2.78 (1-4)
Tingidae	*Teleonemia* prob. *scrupulosa* Stål (2167)	Flower and leaf sucker	36*	1.81 (1-3)
	Teleonemia prob. *harleyi* Froeschner (2174)	Flower and leaf sucker	43*	1.70 (1-4)
	Teleonemia sp. (2165)	Flower sucker	14	1.08 (1-2)
	prob. *Corythaica* sp. (2180)	Leaf sucker	4	1.00 (-)
Ortheziidae	*Orthezia insignis* Browne	Stem sucker	5	1.20 (1-2)
Homoptera				
Flatidae	Unidentified sp. (2176)	Stem sucker	9	2.22 (1-4)
Cicadellidae	Unidentified sp. 1 (2139)	Stem sucker	4	1.00 (-)
	Unidentified sp. 2 (2137)	Stem sucker	2	1.00 (-)
Diptera				
Agromyzidae	*Ophiomyia lantanae* (Froggatt)	Fruit borer	26	1.07 (1-2)
	Ophiomyia camarae Spencer	Leaf miner	15*	1.07 (1-2)
	Calycomyza lantanae (Frick)	Leaf miner	24	1.02 (1-2)
Lepidoptera				
Crambidae (= Pyralidae)	*Salbia haemorrhoidalis* Guenée	Leaf chewer and binder	18*	1.83 (1-4)
Tortricidae	*Epinotia lantana* (Busck) (= *Crocidosema lantana* Busck)	Fruit and flower receptacle feeder, and shoot tip borer	8	1.00 (-)
	Platynota rostrana Walker	Unknown	1	1.00 (-)
Pterophoridae	*Oxyptilus* sp. (Walker) (2188)	Flower, fruit and seed chewer	1	1.00 (-)
Geometridae	*Leptostalis* sp. (2182; 2184; 2193; 2194; 2200)	Leaf chewer	9	1.11 (1-2)
Lycaenidae	*Strymon bazochii* Godart	Flower and fruit chewer	2	1.00 (-)

[a] Accession numbers (AcSN) of the undetermined species are given in parentheses.
[b] Sites where the organism was present; frequency of association out of 46 sites sampled.
[c] Average relative abundance of the organism over the plant's distribution in Jamaica; range in parentheses (values defined in Chapter 2: Table 2.1).
* The damage caused by the organism is considered to be damaging to plant growth and or flower and fruit production of *Lantana urticifolia*.

The small 'shot-holes' which typify the feeding damage caused by adults of the root-boring flea beetle, *Longitarsus* sp. (Coleoptera: Chrysomelidae), was regularly encountered at several sites (Table 5.1). Where adult feeding damage was severe, plants were uprooted and the crown and roots were dissected, but no larvae or internal damage was observed. The larvae of *Longitarsus* sp. are therefore suspected to feed externally on the rootlets.

Three endophagous flies, *Ophiomyia lantanae*, *O. camarae*, and *Calycomyza lantanae* (Diptera: Agromyzidae), were considered to be 'rare' in abundance. The larvae of the seed fly, *O. lantanae*, fed on the fleshy ectocarp of the seeds leaving the embryo intact, and also occasionally bored into and pupated in the flower receptacles. The larvae of the leaf-miner, *O. camarae*, tunnelled into the mesophyll tissue and main veins of leaves, causing characteristic 'herring-bone' leaf mines. There was usually one larva per leaf and the damage caused the leaves to abscise. Larvae of the second leaf miner, *C. lantanae*, caused 'blotch' mines on leaves that usually damaged less than 25% of the leaf surface.

Another two species of lace bug, *Teleonemia* sp. and *Corythaica* sp. (Heteroptera: Tingidae), and the scale insect, *Orthezia insignis* Browne (Homoptera: Ortheziidae), were collected at several sites (Table 5.1), but the damage caused to the flowers, leaves and shoots respectively was considered negligible.

Five other species of Lepidoptera were collected (Table 5.1), of which the larvae of *Epinotia lantana* caused the most damage to plants. Larvae of *Oxyptilus* sp. (Pterophoridae) only damaged a few flowers per inflorescence, leaving the undamaged flowers to mature and fruit normally.

Three stem-sucking homopteran species were collected at relatively few of the sites (Table 5.1) and with the exception of the flatid, which was rated as 'frequent' at some sites, these species had a low abundance. Only adults of these three species were observed feeding on the stems of the plants, with no signs of the immature stages.

Table 5.2
Phytophagous natural enemies on *Lantana urticifolia*, and their presence on related plant species (Verbenaceae) occurring at the same sites.

Natural enemies on *L. urticifolia*[a]	Related plant species	*n*[b]	Natural enemies on related plant[c]	Damage intensity
F. intermedia *A. lantanae*	*Lantana camara* L. (native species)	1	*F. intermedia* *A. lantanae*	Damage similar to that on *L. urticifolia*
F. intermedia *O. albicollis* *T.* prob. *scrupulosa* *T.* prob. *harleyi*	*Lantana camara* L. (ornamental variety)	2	*F. intermedia* *O. albicollis* *T.* prob. *scrupulosa* *T.* prob. *harleyi*	Damage similar to that on *L. urticifolia*
A. lantanae *F. intermedia* *O. albicollis* *Longitarsus* sp. *T.* prob. *scrupulosa* *T.* prob. *harleyi* *S. haemorrhoidalis*	*Lantana trifolia* L.	14	*O. albicollis* (1) *S. haemorrhoidalis* (1)	Limited damage on nearby plants Damage on isolated leaves; plant entwined with *L. urticifolia*
A. lantanae *F. intermedia* *T.* prob. *harleyi*	*Lantana reticulata* Pers.	2	None	-
F. intermedia *T.* prob. *scrupulosa* *T.* prob. *harleyi*	*Lantana angustifolia* Mill.	2	None	-
F. intermedia *O. albicollis* *E. lantana*	*Verbena* prob. *Bonariensis*	2	None	-
F. intermedia *T.* prob. *scrupulosa* *T.* prob. *harleyi*	*Priva* sp.	1	None	-

[a] Natural enemies on *Lantana urticifolia* at the same site, at an abundance of either 'frequent' or 'abundant' (definition in Chapter 2: Table 2.1).
[b] The number of sampled sites where the related plant species was present.
[c] The value in parentheses indicates the number of sites at which the observation was made.

5.3.2 *Host range analysis*

In addition to surveying *L. urticifolia*, several other species of *Lantana* and Verbenaceae were surveyed (Table 5.2). *Aceria lantanae* and *Falconia intermedia* were abundant on

the *L. camara* variety native to Jamaica, while the ornamental *L. camara* variety supported a phytophagous fauna that was similar to that on *L. urticifolia*. *Lantana trifolia* showed minimal feeding damage by adults of *O. albicolis* and larvae of *S. haemorrhoidalis*. None of the natural enemies recorded on *L. urticifolia* were collected on any of the other related species occurring in the vicinity, including *L. reticulata* Pers., *L. angustifolia* Mill., *Verbena* prob. *bonariensis* and *Priva* sp..

5.3.3 *Sampling assessment*

The species-accumulation curve indicated that when half of the 46 sites were sampled, 90% of the natural enemy species had been collected (Fig. 5.2). The curve is initially steep with a rapid increase in the number of species collected, with an asymptotic decline in the accumulation of species with the cumulative number of sampled sites (Fig. 5.2). There was no further increase in the number of species collected after 32 of the 46 sites had been sampled (Fig. 5.2). The three unidentified homopteran species were not included in the analysis.

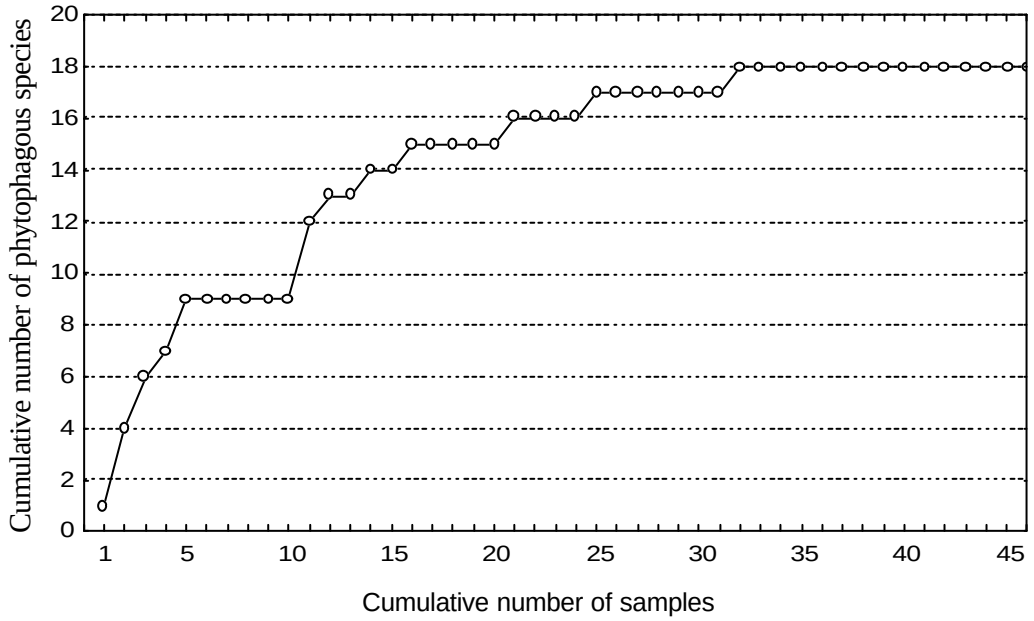


Fig. 5.2 Species-accumulation curve of the phytophagous organisms collected on *Lantana urticifolia* in Jamaica.

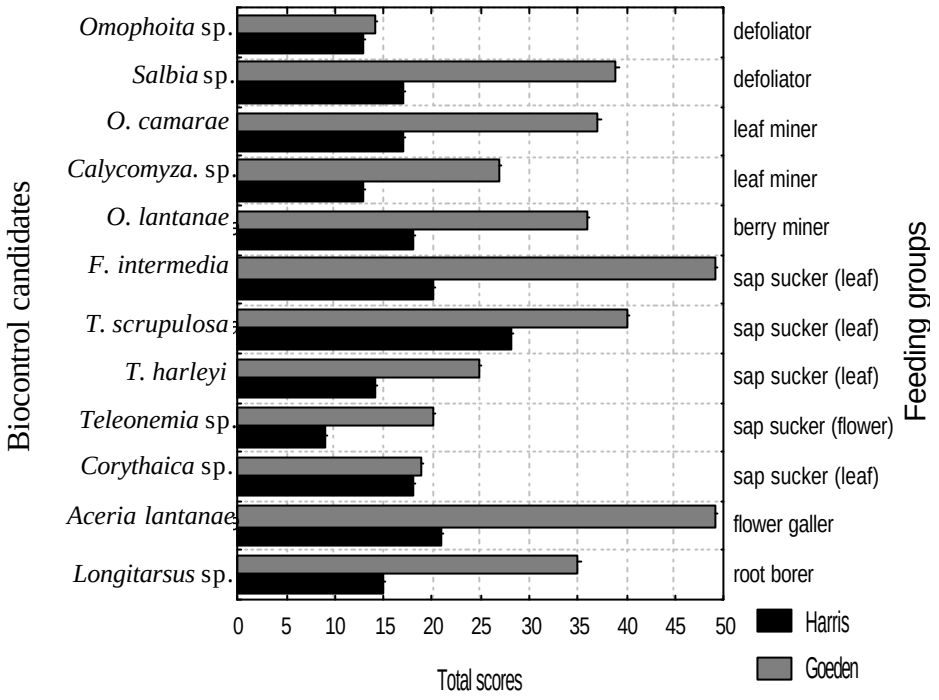


Fig. 5.3 Total scores assigned to the biocontrol candidates on *Lantana urticifolia* in Jamaica according to the scoring systems of Harris (1973) and Goeden (1983).

5.3.4 *Selection of biocontrol candidates*

Due to the lack of sufficient information on certain natural enemies collected during the survey, twelve of the twenty one species were ranked according to the different selection systems. Using Harris' (1973) system, priority was given to *T.* prob. *scrupulosa*, *A. lantanae* and *F. intermedia* (Fig. 5.3). Although the order of precedence changes, these species were also prioritised using Goeden's (1983) system (Fig. 5.3). With few exceptions, both systems placed a low priority on *O. albicolis*, *C. lantanae*, *T.* prob. *harleyi* and *Teleonemia* sp. (Fig. 5.3). The feeding group of the biocontrol candidates had no influence on the rank order in these two systems. Using Winder and Harley's (1982) system, *A. lantanae* and *F. intermedia* are also the two highest ranking candidates, but *Longitarsus* sp., which had a low rank in the other two systems, is also prioritised (Table 5.3). Using Crawley's (1986, 1989) system, modified by Blossey (1995), *A. lantanae*, *F. intermedia*, *T.* prob. *scrupulosa* and *Longitarsus* sp. were ranked as candidates with a high potential for successful establishment (Table 5.4). By contrast, *Ophiomyia lantanae*

Table 5.3
Weighted scores assigned to mode of feeding and abundance of the biocontrol candidates on *Lantana urticifolia* in Jamaica, according to the system used by Winder and Harley (1982).

Biocontrol candidates	Type of feeding	Score	Intensity of feeding damage	Score	Total score[a]
Omophoita albicollis	Leaf chewer	1	Medium	2	2
Salbia haemorrhoidalis	Leaf chewer	1	Medium	2	2
Ophiomyia camarae	Leaf-miner	2	Light	1	2
Calycomyza lantanae	Leaf-miner	2	Light	1	2
Ophiomyia lantanae	Berry-miner	2	Light	1	2
Falconia intermedia	Sap sucker	3	Heavy	5	**15**
T. prob. *scrupulosa*	Sap sucker	3	Medium	2	6
T. prob. *harleyi*	Sap sucker	3	Medium	2	6
Teleonemia sp.	Sap sucker	3	Light	1	3
Corythaica sp.	Sap sucker	3	Light	1	3
Aceria lantanae	Flower-galler	4	Heavy	5	**20**
Longitarsus sp.	Root-borer	5	medium	2	**10**

[a] Product of the scores assigned for the type and intensity of feeding damage, with the three highest ranking scores highlighted.

and *S. haemorrhoidalis* were ranked as low priority candidates (Table 5.4). Similarly, *A. lantanae*, *O. camarae*, *S. haemorrhoidalis* and *F. intermedia* were ranked as the most likely agents to provide successful control (Table 5.5). Although there was some consensus on the three priority candidates, and some of the lower ranked candidates, species downgraded in one system were prioritised in another (Table 5.6), creating an element of inconsistency.

5.3.5 *Revised selection protocol*

In the revised protocol, *F. intermedia, A. lantanae, O. camarae, T.* prob. *scrupulosa* and *T.* prob. *harleyi* all had relatively high scores (Table 5.7), and were considered to be the priority biocontrol candidates. Five other candidates were rated as lower priority, but should these be considered for evaluation, then *Longitarsus* sp. and *S. haemorrhoidalis* should be evaluated first (Table 5.7). Neither *Teleonemia* sp. nor *Corythaica* sp. should be considered as candidate biocontrol agents (Table 5.7).

Table 5.4

The ranking of natural enemies on *Lantana urticifolia* in Jamaica for establishment success, according to demographic parameters identified by Crawley (1986, 1989).[a]

Natural enemy species	Fecundity	Egg aggregation	Voltinism	Size	Damage per head	Adult longevity	Distribution	Abundance	Vulnerabil-ity to general predators	High parasitoid risk	Total[b]
Response to increase	Higher	Lower	Higher	Lower	Lower	Higher	Higher	Higher	Lower	Lower	
Omophoita albicollis	12	7	10	11	-	1	9	3	1	1	55
Salbia haemorrhoidalis	4	1	4	11	-	9	1	3	8	9	50
Ophiomyia camarae	4	1	4	2	-	9	1	8	8	9	46
Calycomyza lantanae	4	1	1	2	-	9	1	8	8	9	43
Ophiomyia lantanae	4	1	1	2	-	9	1	8	8	9	43
Falconia intermedia	4	1	4	2	-	7	6	1	1	1	**27**
T. prob. *scrupulosa*	2	7	4	8	-	2	1	3	1	1	**29**
T. prob. *harleyi*	2	7	4	8	-	2	9	3	1	1	37
Teleonemia sp.	4	7	10	10	-	2	9	8	1	1	52
Corythaica sp.	4	7	4	2	-	2	9	8	1	1	38
Aceria lantanae	1	1	1	1	-	7	6	1	8	1	**27**
Longitarsus sp.	4	7	10	2	-	2	6	3	1	1	36

[a] Ranking system modified by Blossey (1995).

[b] The sum of the rankings for the demographic parameters; lower scores indicate a higher priority. The three highest priority scores are highlighted.

- = Rank not assigned to demographic parameter.

Table 5.5
The ranking of natural enemies on *Lantana urticifolia* in Jamaica for success in controlling the target weed, according to demographic parameters identified by Crawley (1986, 1989).[a]

Natural enemy species	Fecundity	Egg aggregation	Voltinism	Damage per head	Adult longevity	Taxonomy	Total[b]
Response to increase	Higher	Higher	Higher	Higher	Lower		
Omophoita albicollis	12	1	10	1	12	1	36
Salbia haemorrhoidalis	4	7	4	1	1	3	**17**
Ophiomyia camarae	4	7	4	1	1	4	**17**
Calycomyza lantanae	4	7	1	11	1	4	24
Ophiomyia lantanae	4	7	1	11	1	4	24
Falconia intermedia	4	7	4	5	5	-	25
T. prob. *scrupulosa*	2	1	4	5	7	-	19
T. prob. *harleyi*	2	1	4	5	7	-	19
Teleonemia sp.	4	1	10	5	7	-	27
Corythaica sp.	4	1	4	5	7	-	21
Aceria lantanae	1	7	1	1	5	-	**15**
Longitarsus sp.	4	1	10	5	7	1	27

[a] Ranking system modified by Blossey (1995).
[b] The sum of the rankings for the demographic parameters; lower scores indicate a higher priority. The three highest priority scores are highlighted.
- = Rank not assigned; parameter unknown.

Table 5.6
A comparison of the order of priority assigned to natural enemies on *Lantana urticifolia* in Jamaica, using the four ranking systems developed to select potential biocontrol candidates.

Biocontrol Candidates	Harris (1973)	Goeden (1983)	Winder and Harley (1982)	Crawley (1986, 1989) Success of Establishment	Crawley (1986, 1989) Control success
Omophoita albicollis	10	12	8	12	12
Salbia haemorrhoidalis	6	4	8	10	**2**
Ophiomyia camarae	6	5	8	9	**2**
Calycomyza lantanae	10	8	8	7	7
Ophiomyia lantanae	4	6	8	7	7
Falconia intermedia	**3**	**1**	**2**	**1**	9
T. prob. *scrupulosa*	**1**	**3**	4	**3**	4
T. prob. *harleyi*	9	9	4	5	4
Teleonemia sp.	12	10	6	11	10
Corythaica sp.	4	11	6	6	6
Aceria lantanae	**2**	**1**	**1**	**1**	**1**
Longitarsus sp.	8	7	**3**	4	10

The three highest rankings in each selection system are highlighted.

Table 5.7

The ranking of natural enemies on *Lantana urticifolia* in Jamaica, using parameters important in the selection and prioritisation of potential candidate biocontrol agents.[a]

	Parameters related to biocontrol candidate							Parameters related to weed			
Natural enemy species	Agent damage/ individual	Intrinsic rate of increase	Range geographic/ climate	Host range	Synchrony with weed aspects	Dispersal ability	Predation/ parasitism Risk	Impact on weed ecology (growth and reproduction)	Suitability of host varieties		
Weighting of parameter[b] (Range of scores)[c]	x4 (0 to 2)	x2 (0 to 2)	x2 (0 to 2)	x1 (-1 to 1)	x1 (0 to 2)	x1 (0 to 2)	x1 (-2 to 0)	x4 (0 to 2)	x1 (-1 to 1)	Total[d]	Rank[d]
Omophoita albicollis	2	1	1	-1	0	1	0	0	1	13	9
Salbia haemorrhoidalis	1	1	2	1	0	2	-2	1	0	15	7
Ophiomyia camarae	2	2	2	1	0	2	-2	1	1	**22**	**3**
Calycomyza lantanae	1	2	2	1	0	2	-2	0	1	14	8
Ophiomyia lantanae	0	2	2	1	2	2	-2	0	1	12	10
Falconia intermedia	2	2	1	1	2	1	0	2	1	**27**	**1**
T. prob. *scrupulosa*	2	2	2	-1	0	1	0	1	1	21	4
T. prob. *harleyi*	2	2	1	0	0	1	0	1	1	20	5
Teleonemia sp.	0	1	1	0	0	0	0	0	0	4	11
Corythaica sp.	0	1	1	0	0	1	0	0	0	5	12
Aceria lantanae	2	2	1	1	2	1	-1	2	0	**25**	**2**
Longitarsus sp.	2	1	1	1	1	1	0	1	0	19	6

[a] Parameters identified as important are consistently emphasised in the weed biocontrol literature.

[b] Scores assigned to the parameter are multiplied by the weighting; parameters are weighted according to the emphasis placed on them in the weed biocontrol literature.

[c] Scores assigned have a three fold range, and either have a positive or negative influence on the selection of the candidate.

[d] Three highest scores/rankings highlighted.

5.4 DISCUSSION

The two most damaging and abundant natural enemies collected in Jamaica, were the flower-galling mite, *A. lantanae*, and the lantana mirid, *F. intermedia.* The large flower-galls induced by the mite may act as metabolic sinks (Baars and Neser, 1999), but primarily reduce seeding. The large populations of the lantana mirid, and the severe damage observed in the field indicate its potential as a biocontrol agent. Other natural enemies that were damaging include the two tingids, *T.* prob. *scrupulosa* and *T.* prob. *harleyi*, and the leaf-tying moth, *S. haemorrhoidalis.* Although population levels of *O. camarae* were relatively low during the survey, the larvae promote leaf abscission and are thus suitably damaging. However, being leaf miners, they are likely to recruit native parasitoids in South Africa (Chapters 2, 3). The impact of the larvae of the root-boring flea beetle, *Longitarsus* sp., on field plants was difficult to assess, but adult field populations were relatively abundant. Several unidentified *Longitarsus* species have been imported from Central America (Baars and Neser, 1999), and laboratory studies on *L. columbicus columbicus* Harold and *Longitarsus* sp. indicate that the larvae can cause severe damage to rootlets (Baars, 2001; Simelane, 2001).

The mode of attack of *C. lantanae* and *O. albicollis* is considered to be ineffective, and any significant impact caused by these candidates would depend on extremely high population levels. The negligible impact and low occurrence of the two tingids, *Teleonemia* sp. and *Corythaica* sp., excluded them from further consideration. The host-specificity of the unidentified homopteran species is questionable, as no immature stages were observed on any plants, suggesting that these species may require alternative hosts to complete their life cycle.

The spatial distribution of the sampling sites over the range of *L. urticifolia* in Jamaica (Fig. 5.1), and the decline in the accumulation of species as sample numbers increase (Fig. 5.2) suggested that the list of phytophagous species (Table 5.1) is representative of the regional species pool. Due to the short time span of the survey (7 days), and possible seasonal differences in the phytophage assemblage, new species may still await discovery. However, the relatively mild seasonal climate in Jamaica supports the idea that large seasonal changes in phytophage assemblages are unlikely. Therefore,

further detailed phenological studies on the natural enemies and their host in Jamaica are probably unjustified, and further surveying efforts would be better invested in other countries within the native range of *L. camara*.

Of the twenty one species recorded on *L. urticifolia* in Jamaica, fourteen also occur in North America (Palmer and Pullen, 1995), and six occur in Brazil (Winder and Harley, 1983). On the continents adjacent to Jamaica there are also several other congeneric natural enemy species, and the species pool associated with *Lantana* species is considerably more diverse. The relatively small number of endemic natural enemy species in Jamaica suggested that it was an unlikely centre of endemism for *Lantana* species. The theoretical model rarefraction curve described by Müller-Schärer *et al*. (1991: Fig. 2a) is comparable to the species-accumulation curve obtained during the survey in Jamaica (Fig. 5.2). Müller-Schärer *et al*. (1991) argues that such a species-accumulation curve suggests that the regional species pool consists of natural enemies that are common and widespread.

It is now widely accepted that host specificity screening of natural enemies under laboratory conditions can result in candidate agents displaying artificially wide host ranges (Baars and Neser, 1999; Baars, 2000a). Therefore, surveys and open-field trials in the native range of candidate biocontrol agents, provide additional insight into the range of plant species that are likely to be accepted under natural conditions. These observations have proven to be useful in several biocontrol programmes (Maddox and Sobhian, 1987; Clement and Cristofaro, 1995; Balciunas *et al*., 1996). The absence of severe levels of feeding damage on related verbenaceous plant species in Jamaica (Table 5.3) suggests that the most important natural enemies collected have a restricted host range. However, the host specificity evaluation of one of the species, *O. albicollis*, under laboratory conditions indicated an unacceptably wide host range, and this species was rejected as a biocontrol agent on these grounds (Baars and Neser, 1999). Evidence from the field in Jamaica, on the other hand, suggested that it had a restricted host range and that there might thus be grounds for reconsideration.

The dissimilarity between the selection systems and the generalisations resulting from the use of subjective selection parameters in such systems, have rendered them unreliable as predictive tools (Wapshere, 1985, 1992; Blossey, 1995; Cullen, 1995).

Although the mode and intensity of attack by natural enemies is considered to be important in the selection of biocontrol candidates (Zwölfer *et al*., 1976; Sands and Harley, 1981; Harris, 1991; Blossey, 1995; Cullen, 1995), the weighting system of Winder and Harley (1982) neglects the varying contribution that a certain mode of feeding can have on the ecology of a weed, particularly when different plant parts are damaged in combination. In comparing the order of candidates according to their potential for establishment and bringing about successful control, the demographic parameters identified by Crawley (1986, 1989) suggest that species with a high probability of establishment may have a low probability of control success. For example, *S. haemorrhoidalis* is ranked as having a comparatively low probability of establishment, but a high probability of control success (Table 5.6). However, this species established in South Africa after a small release of only 114 adults, and contributes significantly to the impact on lantana (Chapter 2). The contradiction between the probability of establishment and control, results in the unreliable ranking of candidate biocontrol agents (Blossey, 1995). A comparison of the ranking of candidates according to the four systems produced some similarities in the most promising species, but many contradictions in the ordering of others (Table 5.6). This emphasises the need for a revised protocol.

A selection system was developed to provide a predictive guideline that assigned priority to potentially effective natural enemies and discarded logically unimportant ones. Although the general nature of such systems is argued to have little predictive value (Schroeder and Goeden, 1986), they can potentially provide valuable insight into the selection of agents (Harris, 1991; Cullen, 1995). The revised protocol uses parameters which are generally considered to be associated with effective biocontrol candidates. The parameter that considers the agent's damage includes the type and severity of the damage caused per individual or population unit. The impact of different species varies according to the type of damage caused. For example, the two agromyzid flies, *O. camarae* and *C. lantanae*, are both internal leaf-miners, but larvae of *O. camarae* damage the leaf-petiole and can cause the premature abscission of leaves, whereas *C. lantanae* larvae only cause partial damage to a leaf. Individuals of *O. camarae* are thus more damaging than individuals of *C. lantanae*, which would require far higher population levels to induce comparable levels of damage.

Other parameters considered important in the revised selection system include the impact of agents on the vulnerable phenostage or critical life stage of weeds (Müller, 1990; Causton *et al*., 2000), a high intrinsic rate of increase, the similarity of climatic conditions between the native and introduced range and the ability of agents to sustain herbivory throughout the growing season. Furthermore, the dispersal ability of agents may increase their establishment success and subsequent spread. Certain insect groups are more prone to recruiting native parasitoids or predators (Chapters 2, 3) and are considered to be at a disadvantage to the other candidates. As weeds like *L. camara* persist as different forms, the compatibility of agents to the different varieties is also important.

Several of the candidate biocontrol agents present in Jamaica have either been established, or are being considered for release, in South Africa. The agents established include *T. scrupulosa*, *O. lantanae*, *C. lantanae*, *S. haemorrhoidalis*, *L. pusillidactyla*, *E. lantana* and *O. insignis*. Those being evaluated include *F. intermedia*, *A. lantanae*, *Longitarsus* sp., *O. camarae* and a species of *Leptostalis*. As mentioned, the leaf-feeding beetle, *O. albicollis*, has been rejected as a biocontrol agent. The tingid, *T. harleyi*, was released in Australia (Harley and Kassulke, 1973), but although it became established has little impact on lantana. However, the use of additional tingid species is still considered to be a viable option in South Africa (Baars, 2002). It is encouraging to note that certain species with proven effectiveness (*e.g. T. scrupulosa*) were rated as priority candidates in the revised selection protocol, and that agents that have failed to cause significant damage in South Africa (*e.g. C. lantanae*) were given a low priority. Several agents that have either recently been released (*e.g. O. camarae*, *F. intermedia*) or that are currently under consideration (*e.g. A. lantanae*) were also prioritized under the revised selection protocol, and it is thus predicted that they will be effective in South Africa.

Chapter 6

Life history and impact studies of the petiole-galling weevil *Coelocephalapion camarae* Kissinger (Coleoptera: Brentidae), a promising candidate agent for the biological control of *Lantana camara*

6.1 INTRODUCTION

The majority of agents that have been released on *L. camara* in South Africa have been leaf-, flower- and seed-feeding insects (Cilliers and Neser, 1991; Chapters 2, 3), and recently there has been a change to consider stem- and root-feeding species in an attempt to improve the level of biological control. As a result a stem-boring beetle, *Aerenicopsis championi* Bates (Palmer *et al*., 2000) a stem galling fly, *Eutreta xanthochaeta* (Baars and Neser, 1999), and the root-feeding flea beetles *Longitarsus columbicus columbicus* Harold (Baars, 2001) and *Longitarsus* sp. (Simelane, 2001) have been considered.

The success of endophagous insect agents on some other South African weeds (Hoffmann and Moran, 1999) supports the selection of endophagous species for *L. camara.* In particular, gall-inducing agents have been successful in several biocontrol programmes, particularly when the gall acts as a metabolic sink (Dennill, 1988; Harris and Shorthouse, 1996). The close association of gall inducers with their hosts implies a narrow host range (Forno *et al*., 1994; Harris and Shorthouse, 1996), reducing the risk of non-target effects.

Coelocephalapion camarae (Coleoptera: Brentidae), a recently described species native to Mexico (Kissinger, 2000), is a leaf-petiole galling weevil that is under consideration for release on *L. camara* in South Africa. In this chapter, I describe the life history, gall characteristics and damage caused by the weevil in an attempt to predict the contribution that *C. camarae* may make to the biological control of *L. camara*.

6.2 MATERIALS AND METHODS

Coelocephalapion camarae (2000) was collected from *Lantana* sp., probably *L. camara* (S. Neser, personal communication) at Cárdenas (Tabasco Province, Mexico) during a

survey in October 1997. This apionine weevil was cultured in the quarantine laboratories and glasshouses of the Plant Protection Research Institute (Pretoria, South Africa), for life history and impact studies. Laboratory cultures were maintained at 21°C (night) and 28°C (day), with 60- 70% RH and a 13 h photoperiod supplied by overhead fluorescent light-banks. The glasshouse studies were subject to temperatures of 20°C (night) and 30°C (day) and a natural summer photoperiod of about 14 h.

Reference cuttings were made from a single plant in a homogenous stand of each of eight different *L. camara* varieties naturalised in South Africa. These varieties were selected to represent the range of morphological features that are present in South Africa, notably growth form, leaf characteristics, leaf-petiole sizes, shoot tip characteristics and flower colour (Table 6.1). Plants used in subsequent trials were derived from cuttings taken from the reference plants, propagated under 50% shade net, with overhead irrigation, and grown in a standard growing medium of loam, coarse river sand and compost (1:1:1 ratio).

The results from trials described below were analysed using an ANOVA Fisher's Protected LSD at the 5% level (Genstat 5, 1993), unless otherwise specified.

6.2.1 *Native distribution and field status*

A field survey was conducted in Mexico to determine the extent of the distribution range of *C. camarae*. Thirty six sites were sampled along the gulf coast, from Merida (Yucatan Province) to Tampico (Veracruz Province) in October 1998. Sites were selected where *L. camara* plants were relatively common, with at least 10 plants occurring in close proximity to each other. The habitats sampled included roadsides, stream banks, boundaries of farmlands and natural vegetation. At each site, at least 10 plants were sampled, and two to three sections of each plant were shaken above a beating tray to dislodge the weevils for collection. In an attempt to quantify gall development, where field populations of *C. camarae* were relatively large, the available leaf-petiole galls were collected and their status of development was subsequently determined by dissection.

Table 6.1
Origin and key characteristic descriptions of the *Lantana camara* varieties from South Africa used as reference plants during trials.

L. camara Variety	Origin: town/ province	Grid reference	Distinguishing morphological characteristics	Flower colour[a]
009	Sycamore, Mpumalanga	25°35'13.7"S 30°27'08.5"E	Spiny and hairy shoot tips; leaves hairy; main stem with few spines	Light-pink
012	Sycamore, Mpumalanga	25°35'13.7"S 30°27'08.5"E	Shoot tip very spiny; leaves small and hairy; main stem very spiny	Light-Pink
015	Kiepersol, Mpumalanga	25°02'21.6"S 31°02'19.8"E	Shoot tip spiny; large broad dark hairy leaves; main stem spiny	White
017	Sabie east, Mpumalanga	25°03'17.1"S 30°57'03.6"E	Shoot tip hairy, spiny and reddish in colour; leaves hairy and small; hairy main stem with few spines	Orange-Reddish Pink
018	Sabie, Mpumalanga	25°07'04.9"S 30°45'39.2"E	Shoot tip spiny; leaves large, thick and tough; main stem spiny	Dark-pink
029	Hazyview, Mpumalanga	25°08'10.6"S 30°00'09.0"E	Shoot tip spiny; large broad dark hairy leaves; main stem spiny	White-Pink
150	La Mercy, KwaZulu-Natal	29°38'45.9"S 31°07'39.5"E	Scrambling shrub; shoot tips hairy, spiny and reddish in colour; leaves small and hairy	Orange
163	Scottburgh, KwaZulu-Natal	30°09'08.4"S 30°49'39.7"E	Spiny and hairy shoot tips; leaves hairy; main stem with few spines	Light-pink

[a] Colour of mature flowers.

6.2.2 *Rearing technique*

A culture was initiated from a group of up to 100 adults, confined for 5-7 days on a single *L. camara* plant (growing in a 11.25*l* plant bag) in a gauze cage (90 x 45 x 45cm). The weevils were then removed and mixed in equal proportions with weevils from other cultures to maintain a diverse genetic pool, before new cultures were initiated on fresh plants. Infested plants were kept separate on trolleys until the progeny emerged. New adults were collected and confined to fresh plants for two weeks, before being combined with other progeny to initiate new cultures.

6.2.3 *Life history*

Observations were made on cultures of *C. camarae* maintained in quarantine over a two and a half year period. The number of larval instars and duration of the life stages were determined by dissecting infected leaves progressively during trials. One hundred adults were exposed to *L. camara* (var. 163) plants in a gauze cage for 2 days and then removed. The same adults were exposed to a total of eight plants using the same procedure. Three to five leaves of the infected plants were dissected every 1 to 2 days, the life stages recorded, and the larval head-capsule widths were measured using an ocular micrometer.

To determine the pre-oviposition period, 10 newly emerged adults, collected from the culture stock, were exposed to 20cm *L. camara* (var. 009) shoot-tip cuttings that were placed in 'oasis', isolated in a 3 litre plastic container. The cuttings were examined and leaves dissected daily to record the initiation of oviposition. The plant material was replaced daily, and the experiment was repeated with six groups of adults.

6.2.4 *Resource preferences*

The oviposition and feeding behaviour of *C. camarae* was observed during both field and laboratory trials. The utilization of available leaf-petioles for oviposition, was observed at a field site in Palma Sola (Veracruz Province, Mexico), where adult *C. camarae* and symptoms of damage were relatively common. At this site, comprising a vacant plot near a stream, *L. camara* plants were abundant and ranged from small seedlings to 1m tall multi-stemmed shrubs. The top 30cm of five branches were collected from each of three plants. Leaves with galls and characteristic larval damage were collected randomly from the remaining plants at the site. Measurements were made of the leaf-petiole width (maximum width at the stem) and length (length from stem to where the first leaf-vein branches off) as well as the leaf width and length.

During glasshouse studies, three *L. camara* (var. 163) plants were exposed to 10 pairs of adults for 5 days, in gauze cages. The plants were then removed, and each leaf was examined for eggs and feeding marks. The width and length of the leaf-petioles and width and length of the leaves were measured. The adults were then re-exposed to the

same plants for a further 5 days, and the same measurements recorded, to examine oviposition behaviour when oviposition sites were limited. Results were subjected to linear regression analysis to determine the relationship between feeding and oviposition and the utilization of available plant resources.

6.2.5 *Effect of galling on leaf longevity*

The effect of leaf-petiole galls on leaf longevity was examined using five *L. camara* varieties, namely varieties 012,015,029, 150 and 163 (Table 6.1). Some 20-30 leaf-pairs were selected on three plants of each variety, and included leaves of varying age, position on the stem and leaf-petiole size. The leaf-petioles were measured and tagged and one from each pair was covered with a paper straw to prevent oviposition by *C. camarae*. The straws were cut to the sizes of the leaf-petioles, from the stem to the first leaf-vein. Each straw was split along the length, with the end covering the base of the leaf, and was cut at an angle to taper and cover the leaf-veins. The straws were rolled before placement to ensure a tight fit over the leaf-petioles. Each plant was exposed to 15 pairs of adults for 2 days in gauze cages. Adults were then removed and the positions of the eggs on the marked leaves were recorded. The status of the tagged leaves were recorded daily and trials were terminated 2 weeks after the adult progeny emerged from the leaf-petiole galls.

6.2.6 *Effect of galling on plant growth and biomass*

The impact of leaf-galling on the plant's growth rate was examined by exposing adults to two *L. camara* varieties, namely varieties 018 and 017 (Table 6.1). The trial plants were propagated for five months, using the methods described above, and fertilized twice with 5g of LAN. Twelve equally sized plants of variety 018 and nine of variety 017 were selected. Three control plants of each variety were sacrificed at the start of the trial (Control T_0) and the number of leaves and flowers and the wet and dry masses of the leaves, flowers, stems and roots were recorded. The plants were dried at 80°C for 48 hours, and weighed on an electronic scale (Mettler PM400).

The numbers of leaves and flowers on the remaining plants were counted, and the plants were placed into gauze cages (93 x 45 x 45cm) and kept in the glasshouse. Of these, a further three plants of each variety served as controls and were not exposed to the weevils (Control T_1), while three were exposed to 10 pairs of experienced adults that were about 3 weeks old. Three plants of *L. camara* 018 were exposed to 20 pairs of experienced adults. Adults were exposed to the plants for 5 days, after which the plants were removed from the cages. The plants were spaced apart to avoid overlapping of the foliage, and were watered when required. The plants were maintained for a further 30 days and were then cut down and the same parameters (see above) measured. The numbers of leaf-petiole galls per plant were also recorded.

6.3 RESULTS

6.3.1 *Native distribution and field status*

In addition to the original collection site at Cárdenas (Tabasco Province), adults and larvae of *C. camarae* were collected at six sites along the gulf coast of Mexico, during the 1998 survey (Fig. 6.1). When these records are combined with other locality records (Kissinger, 2000; A.J. Urban and S. Neser, personal observations), the distribution of *C. camarae* includes sites at altitudes between sea level and 1500 m and covers a wide geographic area (Fig. 6.1). The 1998 collections were confined to *L. camara*, but since the host plants relating to previous collections are unknown (Kissinger, 2000), these may include other *Lantana* species.

At Palma Sola (Veracruz Province), *C. camarae* populations were relatively high. At this site, 46 galled leaves were collected, of which 17% were parasitized (Fig. 6.2). Both larval and pupal hymenopteran parasitoids were observed, but were not reared for identification. Additional mortality was observed in some 20% of the galls, where the galls either aborted or the developing larvae or pupae had become squashed by excessive callus tissue (Fig. 6.2). Over 31% of the larvae were in their final instar and appeared healthy.

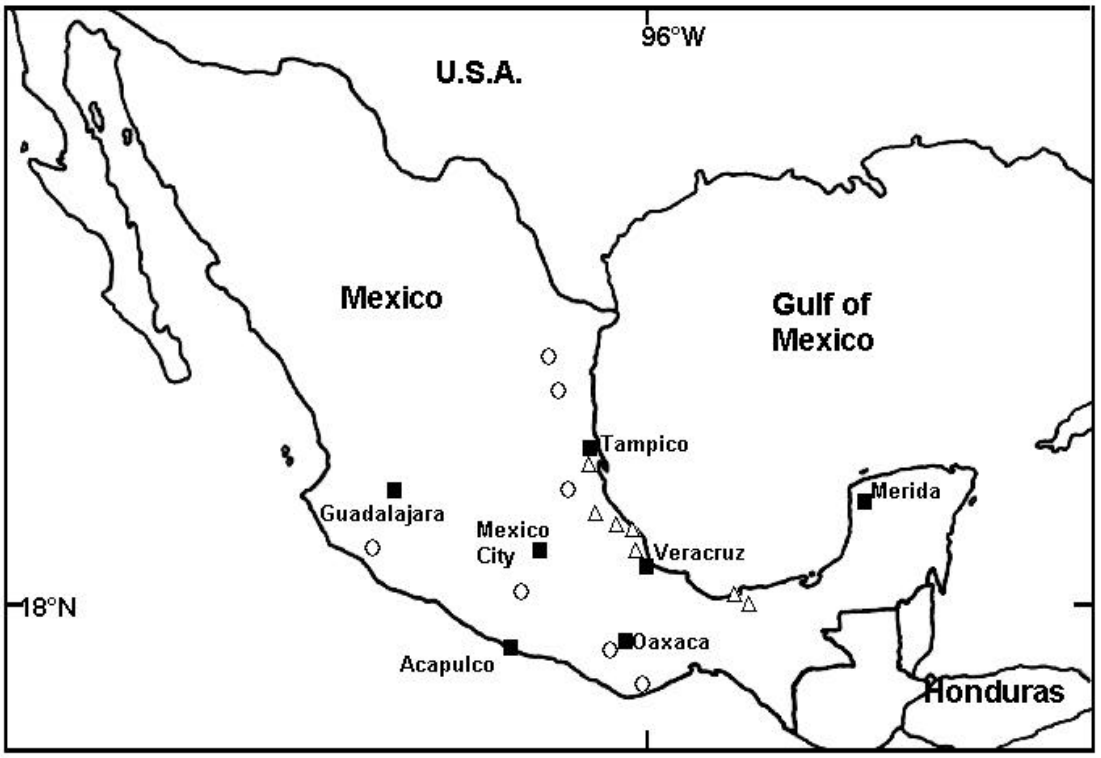


Fig. 6.1 The distribution of *Coelocephalapion camarae* in Mexico in relation to some large cities (■). Observations were made during a survey of *Lantana* species in 1998 (Δ), and from locality records (Kissinger, 2000; A.J. Urban and S. Neser, personal observations) (O).

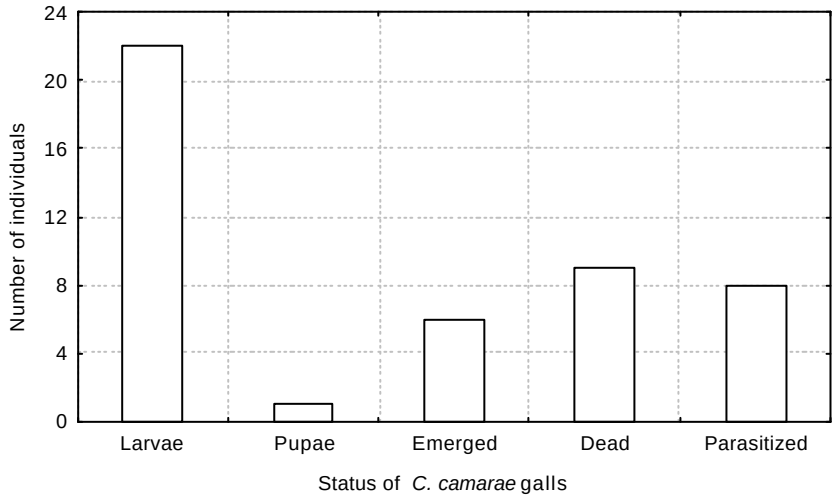


Fig. 6.2 The status of leaf-petiole galls of *Coelocephalapion camarae* collected in the field at Palma Sola (Veracruz Province, Mexico)(Total number of galls = 46).

6.3.2 *Life history*

Eggs of *C. camarae* were usually inserted into the leaf-petiole, but also into the flower-stalk at the base of the receptacle. When oviposition occurred in the leaf-petiole, the female chewed out a cavity in the strengthened parenchyma tissue and cambium layer, leaving the vascular tissue intact. The egg was then deposited and secured in position near the vascular tissue, with a pale yellow secretion. The entrance of the cavity was then plugged with a thin layer of frass, which then dried and sealed the cavity. The female finally deposited a secretion onto the surface of the petiole, some 2mm (mean ± SE = 1.86 ± 0.01; n=94) from the plugged egg cavity towards the stem, which served as an oviposition marker. The oviposition site was clearly visible on the undersurface of the leaf-petiole and usually appeared as two spots signifying the faecal plug and marker (Fig. 6.3b). Eggs were deposited from near the abscission layer close to the stem, up to the junction of the first leaf-vein where the available tissue tapered off. Leaf-petioles of all ages were suitable for oviposition and larval development. Eggs laid in the flower-stalks were inserted in a similar manner, but no oviposition marker was deposited. The eggs were pale white and ovoid (Fig. 6.3a), approximately 0.5mm in length, and took 6 days to develop (Table 6.2).

The hatching first instar larvae fed on the cambium and parenchyma tissue, tunnelling towards the stem. After tunnelling a short distance, usually less than 3mm, the larvae fed on the vascular tissue, which stimulated the plants to produce proliferated tissue and initiate the galls. The larvae remained relatively sedentary and fed predominantly on the vascular and proliferated tissue. During larval development most of the leaf-petiole tissue (in excess of 90%) was damaged, which disrupted the vascular system, preventing the transport of solutes to and from the leaf, occasionally causing the leaf to desiccate (Fig. 6.3d). The galls continued to grow until the larvae pupated. Pupation occurred inside a capsule, constructed from plant material and frass, within the gall. The galls were relatively small, not exceeding twice the width of undamaged petioles, and were not lignified. The larvae were small and white with a light brown head-capsule, and were C-shaped (Fig. 6.3c). The larvae passed through three instars and development from egg to adult took about 35 days (Table 6.2).

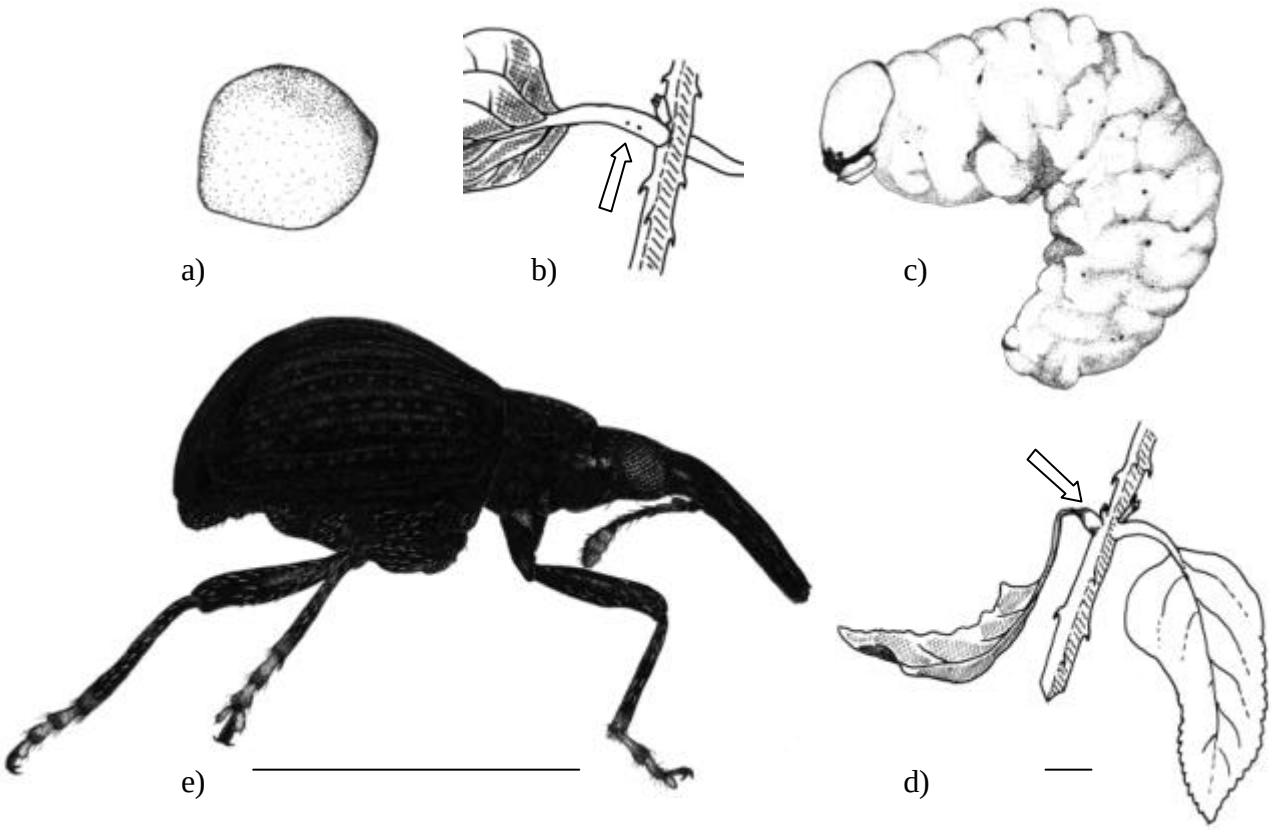


Fig. 6.3 Life stages and characteristics of *Coelocephalapion camarae*: a) egg; b) egg deposited in a leaf-petiole with the oviposition marker; c) final larval instar (third); d) leaf-petiole gall induced by the larva, causing the leaf to desiccate (scale bar 10mm); e) female beetle (scale bar 2mm).

Emerging adults remained in the gall for a day, for the exoskeleton to harden, before chewing through an epidermal 'window' prepared by the larva. The adults were small, mostly black, and highly active (Fig. 6.3e). There were no distinct external morphological difference between males and females, although females generally had wider abdomens and longer rostrums (Table 6.2). Adults often occurred in pairs, in close proximity and positioned almost at right angles to one another, on the undersurfaces of leaves. The males appeared to guard the female in this position. Mating occurred throughout the adult life span. Adults fed on the lower layers of the leaves, leaving the upper epidermal layers intact, and feeding damage resembled small 'shot holes'. The adults also occurred on the flowers and occasionally fed on the corolla. The pre-oviposition period was about 10 days (mean ± SE = 10.0 ± 0.2; *n*=6 groups of 10 adults),

after which eggs were laid at a rate of approximately one per day. The adults were long lived, with laboratory cultures surviving for at least 4-6 months. With the onset of winter, adults kept in the quarantine glasshouses aggregated at the base of plants, in dry curled-up leaves and in sheltered crevices. During this period, feeding and oviposition were markedly reduced.

Table 6.2
Details of the life stages of *Coelocephalapion camarae*.

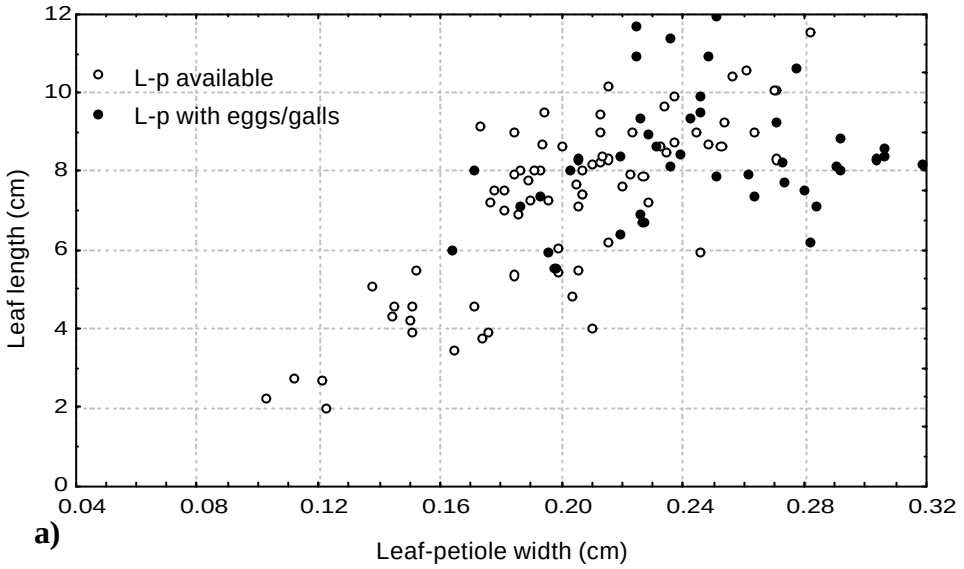
Life stage	Duration	n^a	Measurements (mm)[b]	n^a
Egg	6 days	35	0.46 ± 0.01 (length)	46
			0.35 ± 0.01 (width)	46
1st instar	5– 6 days	32	0.23 ± 0.002[c]	50
2nd instar	5– 7 days	26	0.28 ± 0.001[c]	50
3rd instar	10– 12 days	31	0.38 ± 0.001[c]	50
Pupa	6– 7 days	35		
Adult	10 d pre-oviposition	30	0.73 ± 0.01[d]	36
	4– 6 months		0.63 ± 0.01[d]	36

[a] *n*= number of individuals on which observations were made.
[b] Means ± standard error.
[c] Head capsule width of larvae.
[d] Length of the rostrum; as a sexually dimorphic character.

6.3.3 *Resource preferences*

The gradient in the architecture of *L. camara* plants is modular, typically resulting in strong correlations between leaf-petiole widths and leaf length (R^2= 0.87, *n*= 286) and leaf-petiole width and leaf-petiole length (R^2= 0.75, *n*= 317), as well as between leaf length and leaf width (R^2= 0.92, *n*= 254) and leaf-length and leaf-petiole length (R^2= 0.79, *n*= 286). The feeding patterns of adults were shown to be random during the trials; for example the number of feeding scars was not correlated with leaf size (R^2= 0.08, *n*= 286).

With the range of leaf-petiole widths available to *C. camarae* adults in the field, oviposition was restricted to leaf-petioles ≥ 0.15cm in width and leaves ≥ 5.52cm in length (Fig. 6.4a). Under controlled laboratory conditions, the patterns of resource



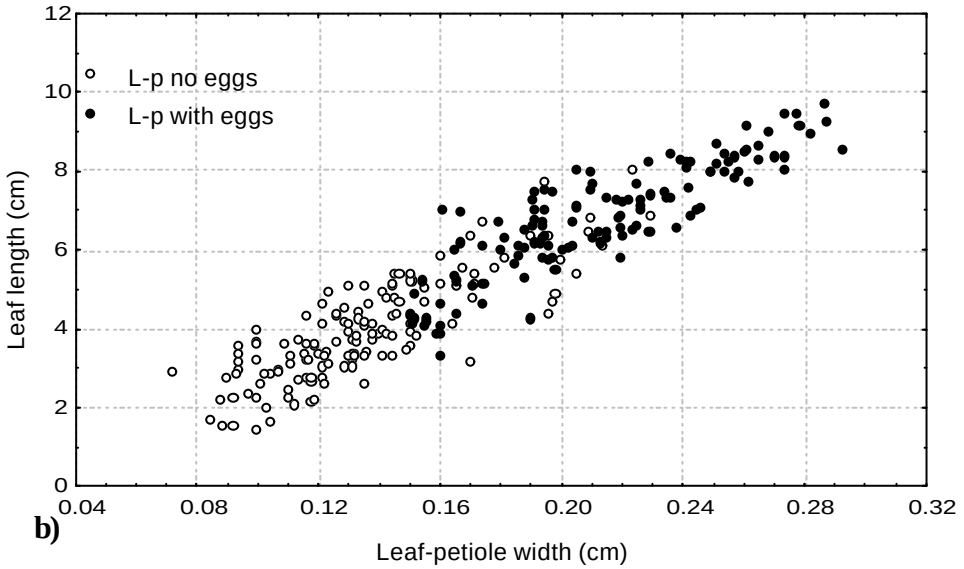


Fig. 6.4 The leaf-petioles (L-p) utilized by *Coelocephalapion camarae* amongst the leaf petioles on: a) field *Lantana camara* plants at Palma Sola, Mexico; b) a South African variety of *Lantana camara* (var. 163) exposed to the weevils in quarantine.

utilization between replicates were not significantly different (P>0.05) and the results were thus pooled for analysis. The patterns of oviposition in the laboratory were similar to those observed in the field, and egg laying was limited to leaf-petioles ≥ 0.15cm in width (range: 0.15- 0.30cm), and leaves ≥ 3.27cm in length (range: 3.27- 9.80cm)(Fig. 6.4b). Since plant growth is modular, these measurements equate to a minimum leaf-petiole length of 0.65cm.

When adults were re-exposed to previously exposed plants, the patterns of resource utilization remained the same despite the shortage of oviposition sites. Leaf-petioles < 0.15cm in width were not utilized as oviposition sites, while the remaining petioles were over-utilized with 1-4 eggs per petiole (Fig. 6.5). The intensity of multiple oviposition was not correlated with an increase in the diameter of the leaf-petiole (R^2 0.20, *n*= 320 leaves).

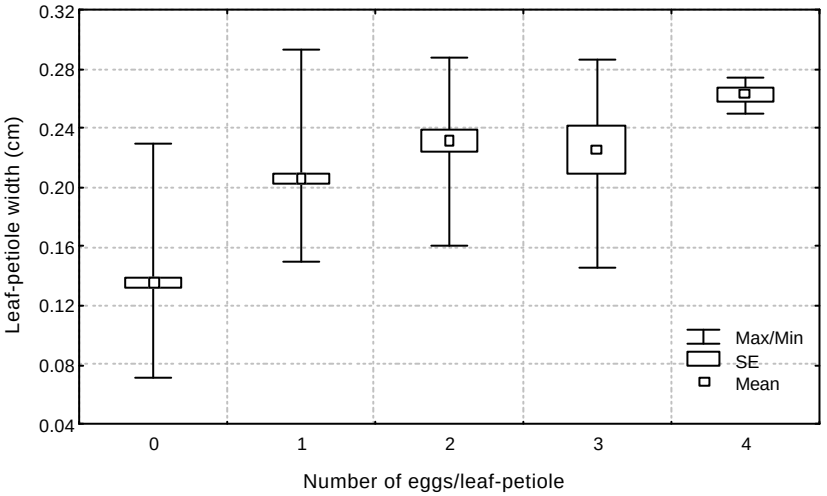


Fig. 6.5 The mean, standard error and range of widths of leaf-petioles that contained 0 - 4 eggs, deposited by *Coelocephalapion camarae* adults that were exposed to a limited number of oviposition sites.

6.3.4 *Effect of galling on leaf longevity*

There were no significant differences in the longevity of galled leaves between the six *L. camara* varieties that were exposed (P > 0.05), and the results were pooled for further analysis. Although a relatively low proportion of the galled leaves (37%) became

desiccated during the experiment, none of the opposite leaves that were the controls of the pairs became desiccated. Leaves took between 11 and 31 days to desiccate (n= 47), and there was no significant correlation between the leaf-petiole widths and the times taken for leaves to desiccate (Fig. 6.6). This suggests that the variation in larval damage to the vascular tissue of the leaf-petioles is not influenced by petiole width, plant variety or age of the leaf.

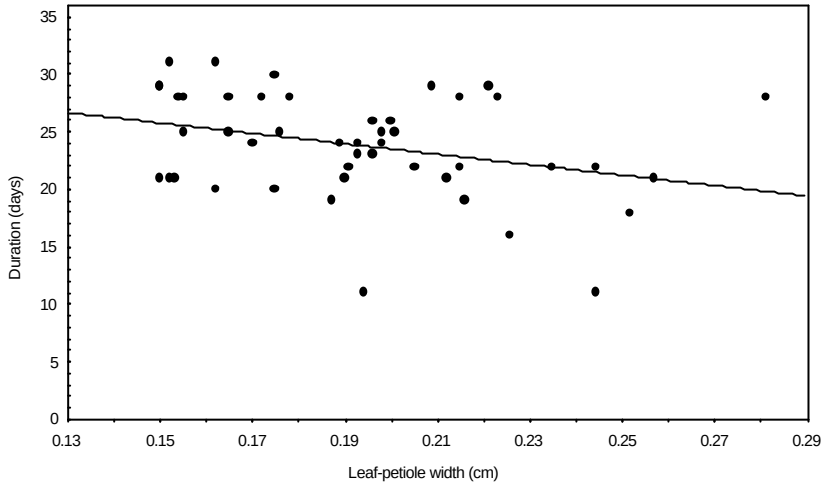


Fig. 6.6 The time taken for leaves to desiccate, on five South African *Lantana camara* varieties containing leaf-petiole galls of *Coelocephalapion camarae* (R^2= 0.10, n= 47). There were no significant between-variety differences in the rate of leaf desiccation ($P>0.05$).

6.3.5 *Effect of galling on plant growth and biomass*

The variation in biomass within the leaves, stems, roots and flowers was low, indicating that the control groups (Control T_0) for both lantana varieties were reliable in representing the state of the plants prior to the initiation of the experiments (Fig. 6.7). The two varieties used, despite being exposed to exactly the same planting and growing conditions, were different in size and varied in the size of the different plant parts (Fig. 6.7). During the course of the trial, all of the plant material in the control groups (Control T_1) increased significantly in dry mass (Fig. 6.7).

Plants that were exposed to 20 *C. camarae* adults and that suffered 14-18% galling of the leaf-petioles (Table 6.3), had slightly more leaf material (Fig. 6.7a, b) but similar masses of stem and floral material, compared with the controls. However, these plants suffered significant reductions in root biomass, with a 56% decrease relative to the control for *L. camara* var. 018 and a 35% decrease relative to the control for *L. camara* var. 017 (Fig. 6.7a, b). These reductions are noteworthy considering that the increases in root biomass between the control plants sacrificed at the start of the trial (Control T_0) and those harvested at its conclusion (Control T_1) amounted to 100% in the case of *L. camara* var. 018 and 27% in the case of *L. camara* var. 017. All in all, the average root biomass of plants exposed to 20 *C. camarae* adults was lower than that of the control plants sacrificed at the start.

The *L. camara* var. 018 plants that were exposed to 40 *C. camarae* adults and that suffered 44% galling of the leaf-petioles (Table 6.3), had considerably less leaf material, and 60% less root biomass relative to the control group (Control T_1)(Fig. 6.7a). The biomass of stem material was very similar to that of the controls while the floral biomass showed a slight, but non-significant increase.

Table 6.3
Intensity of leaf-petiole galling by *Coelocephalapion camarae* during impact studies on two South African *Lantana camara* varieties.

L. camara variety	Experimental treatment [a]	No. Leaves [b]	No. Galls [b,c]	Percentage leaves galled
018	Control T_0	150.0 ± 16.5	0.0 (-)	0
	Control T_1	167.0 ± 9.5	0.0 (-)	0
	20 Adults	181.0 ± 9.1	33.3 ± 4.3	18.4
	40 Adults	125.7 ± 17.6	55.3 ± 4.2	44.0
017	Control T_0	151.3 ± 30.2	0.0 (-)	0
	Control T_1	205.0 ± 10.2	0.0 (-)	0
	20 Adults	208.7 ± 35.7	29.7 ± 1.3	14.2

[a] Plants sacrificed as controls: at the start of the trial (T_0), and at the conclusion of the trial (T_1).
[b] Mean ± standard error.
[c] Number of galls recorded 35 days after adult exposure; (-)= No value applicable.

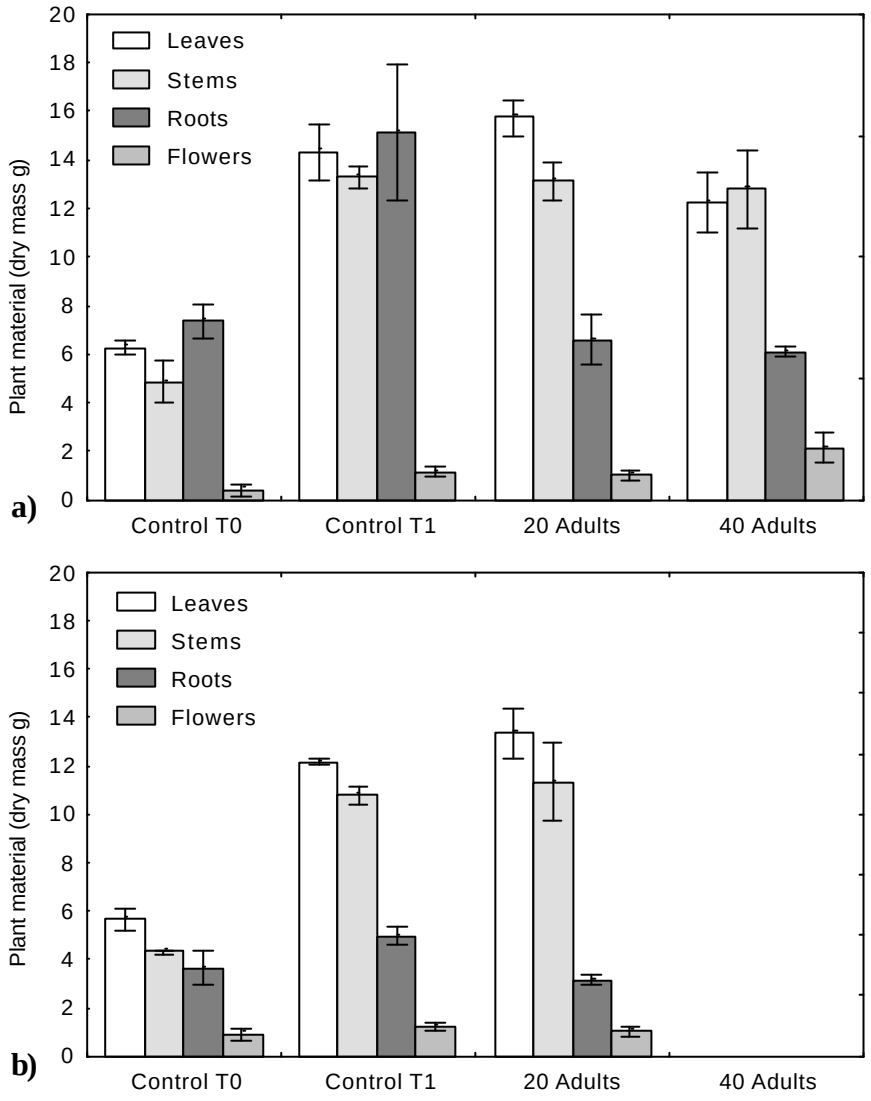


Fig. 6.7 The impact of leaf-petiole galls of *Coelocephalapion camarae* on tissues of two South African *Lantana camara* varieties, 018 (a) and 017 (b). The dry mass of plant material is grouped into leaves, stems, roots (material below where the first root arises from the stem) and flowers (including flower-stalks and seeds) for analysis. Control groups include the plants sacrificed at the beginning of trials (Control T0) and at the conclusion of trials (Control T1).

6.4 DISCUSSION

Current distribution records of *Coelocephalapion camarae* indicate that this species occurs over a wide geographic area in Mexico (Fig. 6.1), including sites from sea level to 1500 m. Because of the variation in climatic conditions over its native distribution, *C. camarae* may be preadapted to cope with the range of climatic conditions that occur over the distribution of *L. camara* in South Africa. However, the laboratory cultures of *C. camarae* used during this study originated from six individuals collected at a single locality. This suggests that the laboratory population might not be representative of the field population, and may thus need to be supplemented with individuals collected over a wide geographic range (Sands and Harley, 1981; Wapshere, 1985; Hopper *et al*., 1993). This has been considered necessary to not only increase the distribution of established agents on *L. camara* in South Africa, but also to improve the compatibility of the agents with the naturalized varieties of *L. camara* (Neser and Cilliers, 1990).

However, evidence of variation in behaviour and host range between biotypes of the cochineal bug *Dactylopius opuntia* (Cockerell) (Hoffmann *et al*., 1999) raises concerns that such differences may exist between populations of *C. camarae* that originate from geographically distinct areas within its native range. Furthermore, the highly successful apionine weevil *Trichapion lativentre* (Béguin-Billecocq) has established throughout the range of its target host, *Sesbania punicea* (Cav.) Benth. (Papilionaceae), in South Africa, despite the inadvertent introduction of a very small founder population (Hoffmann and Moran, 1999). Due to a lack of evidence, the importance of adaptation following introduction and the procedures to improve adaptability through genetic diversity are still unclear (Hopper *et al*., 1993). In addition, the deliberate combination of genetically isolated populations may result in an ill-adapted population, and attempts at matching populations from the country of origin to climate areas in the introduced range separately may be more effective. As a result, it has been decided that the current laboratory population of *C. camarae* should be further investigated for possible release and that additional introductions should only be considered if the releases fail to establish in South Africa.

The larvae of *C. camarae* inflict the most damage and reduce the transport of solutes to and from the leaves, by disrupting the vascular tissue in the leaf-petioles. This leaf damage stimulates the plants to compensate by altering the allocation of resources. The most noticeable short-term effect, at low galling densities, is a reduction in root biomass of up to 56% relative to healthy plants (Fig. 6.7a, b). Plants in response compensate through a flush of new leaves, which is prevented at higher galling densities. The use of realistic population levels during damage assessments in cage experiments is essential for extrapolating the results to field conditions (Briese, 1996). Since it is difficult to predict the size of field populations that will persist after release, low population densities provide an indication of the minimum potential impact. The effect of *C. camarae* on *L. camara* is density related, but even low densities of galling are expected to augment the stress induced by other established biocontrol agents.

In the drier areas within the range of *L. camara* in South Africa, populations of many biocontrol agents tend to only recover from the high levels of winter mortality by midsummer (Cilliers, 1987b; Chapters 2, 3). As a result, *L. camara* populations are able to recover early in the new season, and compensate for cumulative agent damage from the previous growing season. The changes in the activity of adult *C. camarae* in glasshouses with the onset of winter conditions, suggested that field populations may well diapause to survive the dry periods typical in Mexico. If this is true, then *C. camarae* field populations could be expected to recover early in the growing season of *L. camara* and sustain feeding pressure.

The ability of biocontrol agents to assess and avoid oviposition sites used by conspecifics reduces the possibility of intraspecific competition between immatures for a limited resource (Heard, 1995). The use of oviposition markers allows *C. camarae* to effectively utilize the available leaf resources, thereby maximizing offspring survival and enhancing its potential as a biocontrol agent. Although the mechanism behind the weevil's preference for larger leaf-petioles is still poorly understood, this oviposition requirement should influence the design and interpretation of host-specificity tests (Baars, 2000a). Indeed, the rejection of leaf-petioles below a threshold diameter has positive implications for host-specificity, since certain closely related non-target plant species may thereby be immune to oviposition by *C. camarae*.

Coelocephalapion camarae is not the first apionine weevil to be considered for release against *L. camara*. Two *Apion* species were released against *L. camara* in Hawaii (Perkins and Swezey, 1924), but both failed to become established (Julien and Griffiths, 1998). Two *Coelocephalapion* species were evaluated and released against *Mimosa pigra* L. in Australia (Forno *et al*., 1994; Heard and Forno, 1996). Another species, *Coelocephalapion aduncirostre* (Gerstaecker) was recorded on *L. camara* during surveys in Mexico (Palmer and Pullen, 1995), but host records suggest a casual association because it has been reared from *Aeschynomene villosa* Poir. and *A. americana* L. (Fabaceae) (Kissinger, 1988). The effectiveness of gall-inducing agents in biocontrol programmes (Harris and Shorthouse, 1996), and the potential of *C. camarae* as suggested by the results reported here, should encourage further investigations into similar endophagous species (Palmer and Pullen, 1995). Furthermore, since the long-lived adults have the potential to diapause, *C. camarae* may be able to persist in the drier areas of South Africa, survive winter defoliation and sustain impact levels from the start of the growing season. *Coelocephalapion camarae* is a potentially effective biocontrol agent that, should it prove to be host specific (Chapter 8), could be considered for release in South Africa and other countries where *L. camara* is problematic.

Chapter 7

Preference and performance of the biocontrol candidate, *Coelocephalapion camarae,* on varieties of *Lantana camara*

7.1 INTRODUCTION

Lantana camara is an extremely variable species that persists in many forms, sometimes referred to as cultivars, varieties, biotypes or phenotypes (Chapter 1). This weed was presumably derived through the deliberate hybridisation of species within the genus *Lantana* (Stirton, 1977), but vaguely resembles the parent species in its native range (Smith and Smith, 1982). Natural enemies introduced are therefore presented with a diversity of 'new' varieties and may be poorly adapted to cope with them in the new environment (Baars and Neser, 1999). Indeed, field studies showed that one of the agents *Teleonemia scrupulosa* had distinct varietal preferences in Australia (Harley *et al*., 1979). In addition, variatal preferences have been reported to occur in other biocontrol agents, such as *Uroplata girardi*, *Uroplata* sp., *Octotoma scabripennis* and *Calycomyza lantanae* (Harley and Kussulke, 1974; Sands and Harley, 1981; Cilliers, 1987a; Cilliers and Neser, 1991; Denton *et al*., 1991). Furthermore, the laboratory trials on four new biocontrol agents, the fungus species *Mycovellosiella lantanae* (Chupp) Deighton var. *lantanae* and *Prospodium tuberculatum* (Speg.) Arthur, the tortoise beetle *Charidotis pygmaea* Buzzi and the lantana mirid *Falconia intermedia* showed that they performed better on certain *L. camara* varieties (Morris *et al*., 1999; Day *et al*., 1999; Urban and Simelane, 1999; Den Breeÿen, 2000; Thomas and Ellison, 2000). However, field surveys and laboratory trials in South Africa (Baars, 2001; Chapter 3) and two recent studies in Australia (Broughton, 2000; Day and Neser, 2000) suggest that varietal preferences in lantana biocontrol agents have been over-estimated. This suggests that additional quantitative laboratory and field studies are needed to clarify the importance of this phenomenon in the biocontrol programme against *L. camara*.

This chapter investigates the adult reproductive and nymphal performance and adult preference of the petiole-galling weevil, *C. camarae,* on a selected sample of South African *L. camara* varieties. The preference and performance results on these varieties

are compared to that occurring on the natural host from Mexico, in an attempt to predict the weevil's potential to establish and persist on the *L. camara* varieties in South Africa.

7.2 MATERIALS AND METHODS

The origin of the *C. camarae* culture and culturing techniques are described in Chapter 6. The trials were conducted at the quarantine facilities of the Plant Protection Research Institute (Pretoria, South Africa). Six *L. camara* varieties (Table 7.1) were selected to represent the range of morphological features that are present over the geographic range of the weed complex naturalized in South Africa, notably growth form, leaf-petiole sizes, leaf and shoot tip characteristics and flower colour. The procedures used to collect and propagate the reference plants are described in Chapter 6. Plants were only used once in trials.

The adult weevils used in trials were either newly emerged (1-3 days old), or mature adults (3-4 weeks old) which were actively reproducing. These adults were collected from cultures kept on lantana reference varieties other than those used during the trial, in an attempt to avoid insect conditioning. The glasshouse studies were conducted in steel frame cages covered with psylla screen gauze (Climatex, South Africa), subject to temperatures of 20°C (night) and 30°C (day), and a natural photoperiod of 14 h.

Present distribution records show that *C. camarae* occurs over a wide geographic area in Mexico (Chapter 6). At Palma Sola (Veracruz Province, Mexico) *C. camarae* populations were relatively high, and rooted specimens of its natural host plant *L. camara* (R. Sanders personal communication, Texas Botanical Research Institute) were collected at this site and propagated as described in Chapter 6, before being used in subsequent trials.

The statistical analyses of the results were conducted using the programme Genstat (Genstat 5, 1993).

Table 7.1
Origin and key characteristic descriptions of the natural host and *Lantana camara* varieties from South Africa used as reference plants during the trials.

L. camara test plants	Origin: town/ province	Grid reference	Distinguishing morphological characteristics	Flower colour[a]
Mx	Palma Sola, Veracruz, Mexico	19°46'08.0"N 96°36'53.6"W	Shoot tips hairy and reddish in colour; leaves smooth and tapering; main stem smooth	Orange-Red
012[1]	Sycamore, Mpumalanga	25°35'13.7"S 30°27'08.5"E	Shoot tips very spiny; leaves small and hairy; main stem very spiny	Light-Pink
015[1]	Kiepersol, Mpumalanga	25°02'21.6"S 31°02'19.8"E	Shoot tip spiny; large broad dark hairy leaves; main stem spiny	White
017[1]	Sabie east, Mpumalanga	25°03'17.1"S 30°57'03.6"E	Shoot tips hairy, spiny and reddish in colour; leaves hairy and small; hairy main stem with few spines	Orange-Reddish Pink
029[1]	Hazyview, Mpumalanga	25°08'10.6"S 30°00'09.0"E	Shoot tip spiny; large broad dark hairy leaves; main stem spiny	White-Pink
150[1]	La Mercy, KwaZulu-Natal	29°38'45.9"S 31°07'39.5"E	Scrambling shrub; shoot tips hairy, spiny and reddish in colour; leaves small and hairy	Orange
163[1]	Scottburgh, KwaZulu-Natal	30°09'08.4"S 30°49'39.7"E	Shoot tips hairy and spiny; leaves woolly; main stem with few spines	Light-pink

[a] Colour of mature flower.
[1] South African *Lantana camara* varieties.

7.2.1 *Performance on South African varieties relative to the natural host*

The suitability of South African lantana varieties relative to the natural host was examined using three varieties, namely 029, 150 and 163 naturalized in South Africa, and *L. camara* imported from Mexico (Table 7.1). Newly emerged *C. camarae* adults, 15 pairs, were isolated on a single plant of each of these test plants in cages (93 x 40 x 40cm), for three consecutive 2-week periods. After each 2-week period the adults were removed and placed onto fresh plants. These trials were replicated three times on each variety tested, and concluded when all the progeny emerged.

On each plant the number of eggs on leaf-petioles and flower-peduncles were recorded, and plants were kept in the glasshouse until larval development was completed.

The gall development on leaf-petioles was monitored 3 weeks after isolation, and emerging adult progeny were collected daily. In an attempt to quantify emergence rates and mortality, the leaf-petiole and flower-peduncle galls were dissected at the conclusion of the trial. To determine the relationship between the availability of suitable resources and insect performance, the diameter of 120 leaf-petioles per plant was measured after the last 2-week exposure period. Results were subjected to analysis of variance (ANOVA), and means were separated using Fisher's Protected LSD at the 5% level.

7.2.2 *Preference for South African varieties with natural host present*

The preference between a South African lantana variety and the natural Mexican host was examined by exposing a pair of each in opposite corners of a square cage (55 x 55 x 55cm). These plants were equally spaced apart with minimal overlapping of foliage. Experienced *C. camarae* adults, 10 pairs, were introduced into the centre of the cage in an open plastic vial. The positions of these adults were recorded after 2 days and the beetles removed, then the plants were rotated clockwise by one position and the adults were reintroduced into the cage. This procedure was repeated until each plant was exposed in each position for a period of 2 days, resulting in a total exposure period of 8 days. At the conclusion of the trial the number and position of eggs were recorded on the plants. Each plant was then removed and kept separate until the larval development was complete. Two South African lantana varieties were exposed during these trials, 150 and 163 (Table 7.1), and each variety was tested three times.

To determine the influence of available resources on adult preferences, the diameter of 80 leaf-petioles from each plant exposed was measured 2 weeks after isolation, and the number of leaf-petioles with developing galls in the sample was recorded. Gall development was assessed after 30 days, and the subsequent emerging adult progeny were collected daily. To determine the influence of plant position on adult choice during trials, the position of adults on each plant in each position was analysed using a Chi-squared test ($P<0.05$). The other results were analysed using an ANOVA, means separated using Fischer's Protected LSD at the 5% level.

7.2.3 *Relative preference for South African varieties under choice conditions*

The preferences of *C. camarae* for the different South African lantana varieties was examined by exposing four varieties, 012, 017, 015 and 150 (Table 7.1) and a non-target plant simultaneously in a large walk-in cage. The related indigenous plant, *Lippia* sp. B was selected as the non-target plant because it was the most suitable species during preliminary host-specificity trials (Baars, 2000a). The plants were arranged in a 5x5-Latin square (five plants per species giving a total of 25 plants per trial) and the position of plants in columns and rows were randomised using Random Tables (Murdock and Barnes, 1986). The trial was conducted in a gauze cage (4 x 4 x 2m) placed in a fibreglass tunnel serviced by an extractor fan on one side drawing clean air through a wet wall in the opposite side. Plants were equally spaced, 60cm apart and 30cm from the side of the cage, with no overlapping of foliage. Temperatures in the tunnel ranged between 20°C at night and 30°C in the day, and the trial was replicated twice in late summer, with a natural photoperiod of 13 h.

A population of 250 experienced adults were released from vials (six containing 15, and 10 containing 16 adults each) placed on the floor, in the centre of each of four plants within the Latin square. The adults were exposed for 9 days to avoid the over-exploitation of oviposition sites during the course of the trial. At the conclusion of the trial the number of adults and eggs on each plant were counted, and the length of each leaf and height of the plants recorded.

Results were subjected to a five by five-square analysis, and means separated by Fisher's Protected LSD at the 5% level. The relationship between the number of eggs and the availability of resources was examined through correlation analysis, including factors such as total number of eggs, number of females, plant height and total number of leaves per plant. Further correlations were obtained for leaf area, leaf-petiole diameter and leaf length by measuring the leaves on ten branches exceeding a length of 60cm of each variety, selected from the stock of reference plants. Measurements taken at the conclusion of the trials could thus be converted to leaf area and numbers of available leaves using the correlation coefficient, and then correlated with the position of adults and eggs. The

leaves were considered available for oviposition when the leaf-petioles were ≥0.15cm in diameter (see Chapter 6).

7.3 RESULTS

7.3.1 *Performance on South African varieties relative to the natural host*

The reproductive performance of *C. camarae* over a 6-week period was not significantly different between the three South African lantana varieties and *L. camara* from Mexico (Table 7.2). Female survival was consistent between the varieties tested but decreased during the three exposure periods, with female survival rates of 100%, 92% and 86% for the periods one to three respectively. The numbers of eggs on leaf-petioles were not significantly different between varieties, whereas eggs on flower peduncles varied significantly (Table 7.2). The total numbers of eggs were not significantly different between varieties, but increased slightly from exposure period one to two, and decreased significantly during exposure period three (Table 7.2). Subsequent gall development and overall progeny emergence did not vary significantly between the plants tested. Rates of survival were notably higher on galls developing on leaf-petioles compared to flower-peduncles (Table 7.2). Survival on leaf-petioles was relatively consistent between the plants tested, with mean survival rates of over 92%, with the lowest rates of 89.5% occurring on *L. camara* var. 029. This small difference in larval survival is attributed to the larger average leaf-petiole diameter of *L.camara* var. 029, which had more undamaged tissue around the gall chamber that occasionally squashed the pupae. The reason for the low rates of survival on flower-peduncles is unknown, large differences occurred between the plants tested with significantly lower rates on *L. camara* from Mexico. The galls on flower-peduncles contained fully formed adults that died before emerging. There were no pollinating agents present in the glasshouses, which may have contributed to the variable development of berries and thus affected the state of the flower-peduncle.

Table 7.2

The reproductive performance of *Coelocephalapion camarae* during three consecutive two-week isolation exposures, on three South African *Lantana camara* varieties and the natural host *Lantana camara* from Mexico.

L. camara tested [a]	*n*	1st 2 weeks No. Eggs	1st 2 weeks No. Progeny	2nd 2 weeks No. Eggs	2nd 2 weeks No. Progeny	3rd 2 weeks No. Eggs	3rd 2 weeks No. Progeny	Ratio of eggs L-p:F-p [b]	% survival on L-p [c]	% survival on F-p [c]	L-p diameter (cm) [d]	L-p diameter with eggs (cm) [d]
Mx	3	97.7	76.0	116.7	78.3	80.7	57.0	2.8 : 1	92.4 a	29.1 c	0.168	0.190
029	3	96.0	79.7	102.0	72.7	82.3	60.0	4.9 : 1	89.5 b	61.6 a	0.181	0.209
163	3	88.3	60.7	105.0	77.3	82.0	62.3	4.6 : 1	93.0 a	43.2 bc	0.179	0.199
150	3	108.0	73.0	104.0	84.0	71.3	58.3	3.9 : 1	93.6 a	49.2 ab	0.164	0.177
Mean		94.5	69.9	94.5	69.9	94.5	69.9	-	92.1	45.8	-	-
S.E.M.		5.13	4.81	5.13	4.81	5.13	4.81	-	0.82	5.05	-	-
L.S.D.		ns	ns	ns	ns	ns	ns	-	2.39	14.73	-	-

[a] Plants tested, including the natural host *Lantana camara* from Mexico (Mx), and three South Africa *Lantana camara* varieties (Table 7.1).

[b] Ratios for leaf-petioles (L-p) and flower peduncles (F-p) were calculated using the total number of eggs for the three periods.

[c] The mean survival was calculated by comparing the total number of developing galls per plant and galls which led to the successful emergence of progeny.

[d] The mean leaf-petiole diameter (n= 120 per plant) of the plants in the third exposure. Standard error values = 0.002cm.

Means within a column followed by the same letter are not significantly different ($P<0.05$, Fisher's Protected LSD). ns = Result statistically not significant; – = Statistics not appropriate.

7.3.2 *Preference for South African varieties with natural host present*

The adults of *C. camarae* did not show a preference between the South African varieties and *L. camara* from Mexico (Fig. 7.1a, b). During the trial the position of plants did not significantly influence the position of adults (Chi-squared test: χ^2 <2.82, df =2, P = 0.008). At the conclusion of the adult exposure period there was no significant difference in the number of eggs between the varieties tested (Table 7.3). There were sufficient oviposition sites available during the trials because unutilized leaf-petioles and flower-peduncles were still available to weevils at the conclusion of the trials. Ovipostion occurred two to four times more frequently on the leaf-petioles than on the flower-peduncles. An oviposition preference for larger leaf-petioles was observed during the trial (Table 7.3).

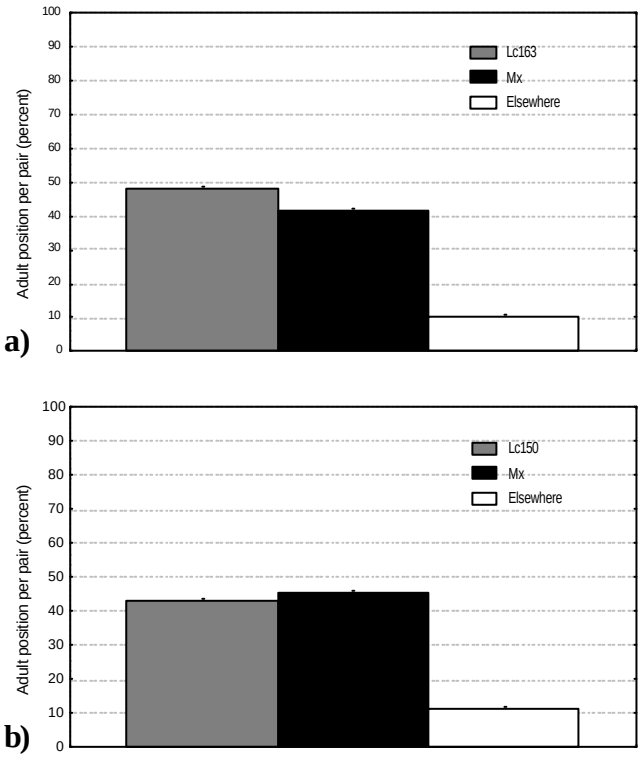


Fig. 7.1 Position of adult *Coelocephalapion camarae* in paired-choice trials, using two South African *Lantana camara* varieties, 163 (a) and 150 (b), compared to the natural host, *Lantana camara* from Mexico (Mx). Adults not on test plants during trials were noted as 'elsewhere'. The position of plants during the trials did not significantly influence the position of adults (Chi-squared test: χ^2 <2.82, df =2, P = 0.008).

Table 7.3
The oviposition preference and progeny development of *Coelocephalapion camarae* in paired-choice trials, using two South African *Lantana camara* varieties and *Lantana camara* from Mexico.

L. camara test plants	*n*	No. Eggs /plant[ad]	Ratio of eggs L-p:F-p[b]	No. Progeny /plant[ad]	L-p diameter (cm) [ac]	L-p diameter with egg (cm)
163	3	22.5 (2.4)a	4.2:1	18.0 (2.3)a	0.169 (0.002)	0.202 (0.002)
Mx	3	23.0 (2.5)a	3.0:1	20.7 (2.1)a	0.165 (0.001)	0.191 (0.002)
150	3	19.5 (3.0)a	3.3:1	17.2 (2.5)a	0.154 (0.001)	0.181 (0.002)
Mx	3	21.7 (2.4)a	2.1:1	20.2 (3.0)a	0.164 (0.001)	0.189 (0.002)

[a] The means were calculated per plant using two plants per replicate.
[b] The ratio of eggs on leaf-petioles (L-p) and flower-peduncles (F-p).
[c] The leaf-petiole diameter of 80 leaves per plant per replicate measured.
[d] The means within columns (for each pair) followed by the same letter are not significantly different (P<0.05, ANOVA Fisher's Protected LSD).

7.3.3 *Relative preference for South African varieties under choice conditions*

The number of adult females on each plant varied significantly amongst the lantana varieties, and there was a significantly lower number of females on *Lippia* sp.B than on any of the lantana varieties (Table 7.4). The position of males in the trials were similarly placed to the females. The total number of eggs oviposited per plant was not significantly different between the lantana varieties, but was significantly lower on *Lippia* sp.B than on lantana (Table 7.4). Although adults were unequally distributed between lantana plants, eggs deposited were equally dispersed, indicating that adults were mobile during the trials. Eggs on leaf-petioles were equally dispersed among the varieties, but there was a significant difference in the number of eggs on flower-peduncles on the varieties tested. Despite plants being of equal age, the flowering physiology was notably different between the varieties, and the number of flowers available varied between plants and varieties.

The correlation coefficients for leaf-petiole diameter and leaf area with leaf length show strong positive linear relationships between these morphological characteristics (R^2 values in Table 7.5). This permits the leaf length measurements of plants taken at the

Table 7.4

The relative preference of *Coelocephalapion camarae* adults for four South African *Lantana camara* varieties and a related indigenous plant, *Lippia* sp.B, that were presented simultaneously in two five by five Latin-squares in walk-in cages.

Plants tested	No. Females	No. Males	Eggs on L-p[a]	Eggs on F-p[a]	Total No. of Eggs	Plant Height (cm)	Leaf Area (cm^2)[b]	Total No. of leaves	L-p diameter (cm)[b]	L-p diameter: Min-Max	Leaves suitable/ plant[c]
L. camara 017	6.1 ab	3.4 ab	19.8 a	8.1 a	27.9 a	81.7 bc	2346 b	193 ab	0.157	0.042-0.254	112 b
L. camara 015	6.6 a	4.1 a	26.3 a	3.4 b	29.7 a	77.9 c	3106 a	164 b	0.183	0.062-0.283	132 a
L. camara 150	4.5 b	3.2 ab	22.8 a	3.0 b	25.8 a	93.7 a	2756 b	177 ab	0.154	0.070-0.221	127 ab
L. camara 012	4.6 b	2.5 b	24.6 a	1.8 bc	26.4 a	90.9 ab	2377 a	200 b	0.163	0.070-0.231	116 ab
Lippia sp.B	1.0 c	0.9 c	2.7 b	0.0 c	2.7 b	86.6 abc	1193 c	218 a	0.122	0.015-0.291	52 c
Mean	4.6	2.82	19.2	3.26	22.5	86.2	2355	190	-	-	108
S.E.M.	0.65	0.43	2.40	0.74	2.36	3.44	123.8	12.62	-	-	6.52
L.S.D.	1.87	1.23	6.96	2.13	6.84	9.98	358.7	36.56	-	-	18.89

[a] The eggs recorded on leaf-petioles (L-p) and flower peduncles (F-p).

[b] The leaf area and leaf-petiole diameters were calculated by converting leaf length measurements using the regression lines for each plant tested (Table 7.5).

[c] Leaves were considered suitable for oviposition when the leaf-petiole diameter ≥0.150cm. Standard errors = 0.001.

Means within a column followed by the same letter are not significantly different ($P<0.05$, Fisher's Protected LSD). – = statistical analysis not appropriate.

conclusion of trials to be converted to leaf-petiole diameters and leaf area measurements (Table 7.4). The plant architecture varied significantly between lantana varieties and *Lippia* sp.B, including characteristics such as plant height, leaf area, mean leaf-petiole sizes, total number of leaves and the number of leaves suitable for oviposition (Table 7.4). The correlation coefficient for the characters mentioned above show a poor linear relationship between these and the total number of eggs on plants. This suggests that the adults of *C. camarae* show no preference for the different lantana varieties even though the resources available varied between the plants in the trial. On the other hand, *Lippia* sp.B is considered an unsuitable host plant. Although the overall mean leaf-petiole diameter on *Lippia* sp.B was below the oviposition threshold, on average, 52 leaves per plant were considered suitable for oviposition and were not utilized during trials (Table 7.4). During the trials only 20% of the leaves suitable for oviposition on the lantana varieties were utilized, which precludes the possibility that limited resources influenced the results.

Table 7.5
Correlation coefficient values for leaf-petiole diameter and leaf area in relation to leaf length, used to convert measurements recorded in multi-choice trials.

Test plants	L-p and L-lgth[a]	*n*	L-area and L-lgth[b]	*n*
L. camara 017	0.85	94	0.95	218
L. camara 015	0.82	102	0.97	229
L. camara 150	0.86	119	0.93	189
L. camara 012	0.86	51	0.95	190
Lippia sp.B	0.66	110	0.96	210

[a] R^2 values for leaf-petiole diameter (L-p) and leaf length (L-lgth)(values significant at $P<0.001$).
[b] R^2 values for leaf area (L-area) and leaf length (L-lgth) (values significant at $P<0.001$).
n = Number of leaves measured.

7.4 DISCUSSION

The adult survival and reproductive performance of the petiole-galling weevil is similar on the South African *L. camara* varieties tested. These varieties varied significantly in growth morphology (Table 7.1) and despite the range of leaf-petioles available for oviposition (Table 7.2), larval development and survival were relatively consistent. The progeny survival rates on the lantana varieties are comparable with those occurring on the natural Mexican host. This indicates that *C. camarae* displays a good level of compatibility with the range of South African lantana varieties tested. However, the host compatibility should be interpreted cautiously because the extent of the host range of *C. camarae* in the country of origin is largely unknown. Studies have shown that biocontrol agents collected from other species of *Lantana,* like *L. fucata* Lindley, are incompatible with the introduced varieties of lantana (Day *et al*., 1999). Furthermore, at least one candidate agent performs better on plant species in the related genus *Lippia* than on *L. camara* (Heystek and Baars, 2001), although it was collected from *Lantana* species in Mexico (Palmer *et al*., 1996). Winder *et al*. (1984) note that comparative studies with the native host may explain why *Uroplata lantanae* Buzzi and Winder failed to establish in Australia.

Host location and selection by *C. camarae* is dependent on the adult, since the immature stages are sedentary. The results from paired-choice trials indicate that South African *L. camara* varieties are equally accepted as hosts relative to *L. camara* from Mexico. This suggests that the varieties naturalized in South Africa posses suitable characteristics to stimulate host acceptance behaviour. Although the morphology of plants varied significantly in the large walk-in multi-choice trials, adults showed no preference between the different *L. camara* varieties, while they consistently avoided the indigenous *Lippia* species.

The ability of natural enemies to utilize most or all *L. camara* varieties, is important in ensuring the successful establishment and subsequent impact in the country of introduction (Sands and Harley, 1981; Winder *et al*., 1984; Neser and Cilliers, 1990; Cilliers and Neser, 1991; Palmer *et al*., 2000; Baars, 2001). Based on the preference and performance results, *C. camarae* is expected to establish and impact equally on different

L. camara varieties in the field. However, the climate suitability and influence of native parasitoids on field populations are important factors influencing the success of biocontrol agents (Neser and Cilliers, 1990; Day and Neser, 2000; Chapters 2, 3, 4). It is therefore more likely that these factors will influence the biocontrol potential of *C. camarae* rather than varietal preferences, and these aspects warrant further research.

Chapter 8

Host range studies of *Coelocephalapion camarae* for the biological control of *Lantana camara* in South Africa

8.1 INTRODUCTION

The petiole-galling weevil, *Coelocephalapion camarae* was imported from Mexico in 1997 for screening as a potential biocontrol agent of *L. camara* (Baars and Neser, 1999; Baars and Heystek, 2001; Chapter 6). The attributes of *C. camarae* suggested that it has exceptional potential as a biocontrol agent (Chapter 6). This species occurs over a wide geographic range in Mexico and can thus potentially cope with the variation in climatic conditions over the range of the weed in South Africa (Chapter 4). The adults are subject to facultative diapause, and are likely to survive seasonal leaflessness and colonize plants early in the growing season. The larvae cause extensive damage per individual, and at low galling densities the nutrient supply to plants is significantly disrupted (Chapter 6). The use of oviposition markers increases the adult's potential for optimal resource utilization by avoiding conspecific eggs. In addition, the adult and immature stages of the weevil are predicted to perform equally well on the different lantana varieties in South Africa (Chapter 7).

Relatively small and robust weevils with attributes comparable to that of *C. camarae* are popular biocontrol candidates. The release of similar species has led to the successful control of some weed species (Cilliers, 1991a, 1991b; Hoffmann and Moran, 1999) and contributed to reducing the weed status of another species (Hill and Cilliers, 1999). In addition, other weevil species are currently considered to be potentially damaging in the early phases of other release programmes (Heard and Forno, 1996; Hill, 1999).

During laboratory trials many biocontrol candidates evaluated for *L. camara* accepted several indigenous *Lantana* and *Lippia* species as alternative host plants (Baars, 2000a; Baars, 2002). This phenomenon potentially reduces the pool of candidate agents that may be suitable for the biocontrol of *L. camara* (Baars and Neser, 1999). However, the specific oviposition requirements of *C. camarae* suggest that the resources of several

non-target indigenous species in South Africa are unsuitable. This chapter reports on the host specificity of *C. camarae*, and its suitability for release as a biocontrol agent for *L. camara* in South Africa.

8.2 MATERIALS AND METHODS

A culture of *C. camarae* imported from Mexico in November 1997, was collected at Cárdenas, Veracruz Province. The life history, native distribution and insect-plant interactions of *C. camarae* are described in Chapter 6. The adult reproductive performance and host preference studies suggest that several South African lantana varieties are equally suitable for *C. camarae* (Chapter 7).

Host-specificity trials were conducted in quarantine glasshouses at the Plant Protection Research Institute (Pretoria, South Africa). Glasshouse temperatures averaged around 20°C (night) and 30°C (day), with a photoperiod of about 13 h. Insect cultures were maintained on lantana plants kept in 15cm pots or 11.25*l* plant bags, isolated in gauze cages. Either newly emerged (1-3 days old), or experienced adult *C. camarae* (about 3 weeks old) were used during host-specificity trials. These beetles originated from cultures kept on lantana reference varieties other than those used during the trials. Test plant species were chosen on the basis of their taxonomic relatedness to *L. camara* (Cantino *et al*., 1992; Arnold and De Wet, 1993), using the centrifugal phylogenetic method (Wapshere, 1974, 1989). The status of the four species in the related genus *Lippia* (further abbreviated as *Li.*), *Li. javanica* , *Li. wilmsii* H. Pearson, *Li. scaberrima* Sond. and *Li. rehmanii* H. Pearson is known (Arnold and De Wet, 1993), but the other species in this genus are in need of study (E. Retief, National Botanical Institute, Pretoria). Two morphologically distinct forms, that belong to the genus *Lippia*, were collected near the town of Ngodwana in Mpumalanga Province, and were treated as separate species in this study and referred to as *Lippia* sp.A and *Lippia* sp.B (PPRI Reference no. IR37 and IR39: specimens lodged at National Botanical Institute, Pretoria). Plants were propagated and grown under the same conditions as described in Chapter 6.

Host-specificity results were subjected to analysis of variance (ANOVA) using Genstat regression (Genstat 5, 1993).

8.2.1 *Adult feeding and oviposition on test plants*

Five pairs of experienced *C. camarae* adults were isolated on 55 test plant species in 14 plant families, which included 13 species grown for commercial purposes. Plants were kept in 15-22cm pots with inverted 40*l* plastic containers with gauze panels, covering the above ground plant material. Adults were isolated on the plants for two weeks and then removed, and the feeding intensity and number of eggs laid on each plant was recorded. Test plants were kept separate for another two weeks, and the development of galls were recorded.

8.2.2 *Adult performance on suitable plants*

These no-choice trials were conducted to quantify the performance of adults on the plant species that proved suitable for feeding and oviposition. Newly-emerged adults, 25 pairs, were exposed to 11 test species and South African *L. camara* varieties. The adults were isolated on a single plant species placed in gauze cages (93 x 40 x 40cm), and exposed for two consecutive periods of 16 and 14 days. Adults were removed after each exposure period, and plants were kept separate. With one exception, each plant species was tested three to six times. Gall development on leaf-petioles was monitored 3 weeks after isolation, and emerging adult progeny were collected daily. Adult progeny were classed as males or females based on differences indicated in Chapter 6. Developmental time was recorded as the time taken for progeny to emerge following the end of the second exposure period. Results were subjected to an ANOVA with an unbalanced design, on log (base e) transformed data.

8.2.3 *Preference testing between* Lantana camara *and selected test species in paired-choice trials*

The adult preference between *L. camara* and the test plant supporting adult feeding and reproductive development were examined by exposing a pair of each plant species in opposite corners of a square cage (55 x 55 x 55cm). These plants were spaced equally

apart with minimal overlapping of foliage. Experienced adult *C. camarae*, 10 pairs, were introduced into the centre of the cage in an open plastic vial. The positions of these adults were recorded after 2 days and the beetles removed, then the plants were rotated clockwise by one position and the adults were reintroduced into the cage. This procedure was repeated until each plant was exposed in each position for a period of 2 days, resulting in a total exposure period of 8 days. At the conclusion of the trial the number and position of the eggs on the plants were recorded. Each plant was then removed and kept separate until the larval development of the beetles was complete. The gall development was assessed after 30 days, and emerging adult progeny were collected daily.

Two sets of control trials were conducted, one with two *L. camara* pairs and another with a pair of *L. camara* and a pair of an unrelated plant *Ageratina adenophora* (Sprengel) King and Robinson (Asteraceae). Each plant species was tested three to six times during two summer seasons (1998 and 1999). The influence of plant position during the trials was tested on accumulated adult numbers per species using Chi-squared tests. Oviposition and progeny development was subjected to an ANOVA, with means separated using Students t-test (selected species were analysed using log (base e) transformed data because of large differences in the data values between the test species).

8.2.4 *Adult preference in simultaneous-choice trials*

These trials were conducted to determine the oviposition preference of adults when presented with a choice of several plant species simultaneously in a large gauze cage (122 x 140 x 92cm). Experienced adults, 50 pairs, were exposed to plant species that had supported adult feeding and oviposition during no-choice trials. Plants were each assigned to one of ten positions in the cage using Random Tables (Murdock and Barnes, 1986). Plants were of similar size with minimal overlapping of foliage. Adults were introduced from suspended platforms, placed equidistant from plants. Two exposure periods were used; adults were either exposed for seven days (7-d exposure) or for twenty one days (21-d exposure). The 7-d exposure was used to prevent the over-utilization of host plant resources, and included 10 related plant species, and the trial was repeated four

times. The position of adults was recorded after 7 days, and the number of eggs on leaf-petioles and flower-peduncles were recorded. Plants were kept separate, and gall development was recorded after 30 days. Emerging adults were then recorded daily.

The 21-d exposure was conducted to induce a state of limited host plant resources and to determine the relative preference of adults on the related plants in close proximity. These trials included 14 plant species, each tested between five and eleven times, and the cage conditions were the same as described in the 7-d trials. Each replicate included only three *Lippia* species to limit the source of plant volatiles in the cage. Plants were separated after the exposure period and the adult progeny per plant was recorded daily. Both of the exposure periods included three unsuitable host plants to promote adult choice, including *Priva meyeri* var. *meyeri* Jaub. and Spach. and *Duranta erecta* L. (Both Verbenaceae) and *Clerodendrum glabrum* E. Mey. (Lamiaceae). Results for the two exposures were subjected to an ANOVA, with means separated using Fisher's Protected LSD at the 5% level. The results from the 21-d exposure were analyzed on log (base e) transformed data.

8.3 RESULTS

8.3.1 *Adult feeding and oviposition on test plants*

Adult feeding and larval development occurred on five *Lantana* species, including *L. camara*, six *Lippia* species and *Aloysia citrodora* Palau (Table 8.1). Although adults survived on most of the other 44 species, there was no feeding or oviposition suggesting that none of these species would be suitable as alternative host plants. However, 11 non-target species appeared to be suitable as hosts.

8.3.2 *Adult performance on suitable plants*

The number of females and males surviving the two exposure periods was not significantly different between the test species (Table 8.2). However, there was a

Table 8.1
List of plant species exposed to the adult stages of *Coelocephalapion camarae* in no-choice trials, to determine their suitability to support feeding and oviposition.

Plant family	Plant species	Common Name	*n*	Rating[c]	Eggs[d]
A. Native and Exotic test species					
BORAGINACEAE	*Heliotropium amplexicaula* Vahl.[a]	Heliotrope	3	-	-
VERBENACEAE	*Verbena brasiliensis* Vell.[a]	Brazilian vervain	4	-	-
	V. bonariensis L.[a]	Purpletop	4	-	-
	V. tenuisecta Briq.[a]	Moss verbena	4	-	-
	Lantana camara L.[a]	Lantana	16	+++	+
	L. rugosa Thunb.		3	++	+
	L. mearnsii Moldenke		3	+++	+
	L. montevidensis (Spreng.) Briq.[a]	Creeping lantana	4	++	+
	L. trifolia L.[a]		3	+++	+
	Lippia javanica (Burm.f.) Spreng.		3	+++	+
	Li. rehmannii H. Pearson		3	+++	+
	Li. wilmsii H. Pearson		3	+++	+
	Li. scaberrima Sond.		3	+++	+
	Lippia sp.A		3	+++	+
	Lippia sp.B		3	+++	+
	Aloysia citrodora Palau[ab]	Lemon-verbena	3	+++	+
	Phyla nodiflora (L.) Greene	Fogfruit	4	-	-
	Priva meyeri var. *meyeri* Jaub. and Spach.		3	-	-
	Duranta erecta L.[ab]	Golden dewdrop	4	-	-
	D. erecta L.[ab] (variegated)		4	-	-
LAMIACEAE	*Karomia speciosa* R. Fernandes		3	-	-
	Clerodendrum glabrum E. Mey.		3	-	-
	Teucrium trifidum Retz.		6	-	-
	Tinnea rhodesiensis S. Moore		3	-	-
	Lavandula angustifolia Ehrh.[ab]	English lavender	5	-	-
	Salvia africana-caerulea L.		6	-	-
	S. officianis L.[ab]	Sage	4	-	-
	S. elegans Vahl.[ab]	Pineapple sage	5	-	-
	S. greggi A.Gray[ab]		5	-	-
	Mentha piperita L.[ab]	Peppermint	3	-	-
	M. spicata L.[ab]	Spearmint	4	-	-
	Nepeta cataria L.[ab]	Catnip	4	-	-
	Origanum sp.[ab]	Origanum	7	-	-
	Plectranthus saccatus Benth. Codd.		3	-	-
	Plectranthus sp.1		4	-	-
	Plectranthus sp.2		3	-	-
	Tetradenia ripera Benth.		3	-	-
	Hemizygia obermeyerae Ashby		4	-	-
	Ocimum basilicum L.[ab]	Sweet basil	3	-	-
	Thymus vulgaris L.[ab]	Common thyme	5	-	-
SCROPHULARIACEAE	*Buchnera reducta* Hiern		3	-	-
	Mazus reptans N.B.Br.[ab]		5	-	-
BIGNONIACEAE	*Jacaranda mimosifolia* D. Don[ab]	Jacaranda	4	-	-
ASTERACEAE	*Ageratina adenophora* (Sprengel) King and Robinson[a]	Crofton weed	3	-	-

Table 8.1 continued

B**. Cultivated test species**					
LILIACEAE	*Allium cepa* L.[ab]	Onion	3	-	-
	A. porrum L.[ab]	Leek	4	-	-
CHENOPODIACEAE	*Spinacia oleracea* L.[ab]	Spinach beet	4	-	-
BRASSICACEAE	*Brassica oleracea* L.[ab]	Cabbage	3	-	-
FABACEAE	*Pisum sativum* L.[ab]	Pea	3	-	-
	Phaseolus coccineus L.[ab]	Runner-bean	3	-	-
	P. vulgaris L.[ab]	Bush-bean	3	-	-
APIACEAE	*Daucus carota* L.[ab]	Carrot	7	-	-
SOLANACEAE	*Lycopersicon esculentum* Mill.[ab]	Tomato	3	-	-
	Solanum melongena L.[ab]	Egg plant	4	-	-
PEDALIACEAE	*Sesamum indicum* L.[ab]	Sesame	3	-	-
ASTERACEAE	*Lactuca sativa* L.[ab]	Lettuce	3	-	-
POACEAE	*Zea mays* L.[ab]	Sweetcorn	3	-	-

[a] Plant species introduced to South Africa (Arnold and De Wet, 1993).
[b] Plant species of ornamental and/or economic value in South Africa.
[c] Feeding intensity compared to that on *L. camara*; - = no feeding; + = exploratory feeding; ++ = low feeding intensity; +++ = normal feeding intensity.
[d] Plant species suitable for oviposition; - = no eggs, + = eggs deposited.
n = Number of replicates.

significant drop in the overall mean for adults surviving from the first (mean ± SE: female: 23.4 ± 0.3; male: 21.4 ± 0.4) to the second exposure period (female: 20.0 ± 0.3; male: 17.8 ± 0.4). High survival rates on *P. meyeri* suggest that adult survival during the exposure period was independent of the plant species. The high female survival rates allow the direct comparison of the fertility ratio (progeny per number of adult females) between the test species. The number of adult progeny was significantly higher on *L. camara* than on the related plants tested (Table 8.2). Beetles on seven species, including the six indigenous *Lippia* species, produced 10 to 30% of the progeny produced on *L. camara.* The number of progeny increased notably on *L.camara*, *Lippia rehmannii*, *Li. scaberrima* and *Lippia* sp.B, from the first to the second exposure period, whereas similar numbers or fewer progeny emerged on the other test species. The low number of progeny on *L. rugosa*, *L. mearnsii* Moldenke, *L. montevidensis* (Spreng.) Briq., *Aloysia citrodora* and *P. meyeri* indicate that these are unsuitable to sustain a viable population of *C. camarae*. Small adult progeny were classed as males, and male biased ratios occurring on *L. rugosa*, *Li. javanica* and *Li. wilmsii* suggested that resources available on these species are limiting normal progeny development (See Honìk, 1993). The time taken for progeny to emerge was similar on most of the plants tested, with the shortest time taken on *L.*

Table 8.2

The adult survival, reproductive performance and the development of progeny of *Coelocephalapion camarae* kept on plant species suitable for adult feeding and oviposition in no-choice trials.

		0 to 16 days		17 to 30 days		Total progeny				
Plant species[a]	n[b]	No. Females[c]	No. Males[c]	No. Females[c]	No. Males[c]	0 to 16 days[cd]	17 to 30 days[cd]	F:M[e]	Duration (days)[cf]	n[g]
Lantana camara	6	23.6 (0.6)	21.8 (1.0)	21.8 (1.3)	18.6 (0.8)	107.8 (10.2)a	186.4 (16.3)a	1:1	32.4 (0.2)	849
L. rugosa	3	23.7 (0.3)	22.0 (1.2)	17.7 (2.9)	17.0 (2.1)	0.7 (0.7)ef	5.0 (3.6)ef	1:3.3	37.4 (1.8)	16
L. mearnsii	2	24.5 (0.3)	21.5 (0.3)	24.0 (0.0)	18.5 (0.3)	7.0 (0.5)	5.0 (1.0)	1:1	42.0 (1.0)	24
L. montevidensis	3	20.3 (2.7)	18.3 (2.2)	19.7 (2.6)	19.7 (2.9)	2.0 (0.6)de	7.3 (2.6)de	1:0.7	34.1 (0.9)	19
L. trifolia	3	23.7 (0.7)	22.0 (1.7)	20.7 (1.5)	18.3 (0.7)	10.7 (1.2)cd	10.0 (3.6)cd	1:0.7	33.7 (0.9)	27
Lippia javanica	5	23.6 (0.8)	22.0 (0.8)	21.0 (0.6)	18.8 (0.5)	12.4 (6.3)cd	11.6 (2.8)cd	1:1.8	36.2 (0.8)	39
Li. wilmsii	3	24.7 (0.3)	23.0 (1.5)	21.7 (0.9)	21.3 (1.7)	12.3 (5.9)cd	13.0 (5.3)cd	1:1.5	42.1 (1.2)	23
Li.rehmannii	6	22.7 (0.7)	20.5 (1.2)	17.8 (0.7)	14.5 (1.4)	22.3 (10.5)bc	50.7 (21.7)bc	1:1.2	39.6 (0.5)	159
Li. scaberrima	4	23.8 (0.8)	21.0 (1.7)	20.8 (1.3)	18.3 (1.3)	27.3 (12.4)bc	37.3 (26.7)bc	1:1.1	39.2 (0.4)	149
Lippia sp.A	4	24.3 (0.3)	22.5 (1.0)	19.3 (0.6)	19.0 (0.8)	23.5 (6.5)bc	27.3 (12.0)bc	1:0.9	36.1 (0.6)	109
Lippia sp.B	4	23.3 (0.6)	21.0 (1.3)	20.5 (0.5)	18.3 (1.2)	18.0 (7.2)b	55.3 (17.0)b	1:1	37.6 (0.4)	222
Aloysia citrodora	4	23.3 (0.3)	20.5 (1.3)	18.5 (2.5)	16.5 (1.4)	9.0 (1.2)d	4.5 (1.0)d	1:0.9	39.0 (1.7)	13
Priva meyeri	3	23.7 (0.3)	22.7 (0.3)	20.7 (0.9)	17.3 (0.7)	0.0 (-)f	0.0 (-)f	-:-	-	-

[a] Plants tested were flowering during the period of the trials.

[b] Number of replicates.

[c] Means within a column followed by no letter or the same letter are not significantly different (ANOVA, Fisher's Protected LSD, $P<0.05$). The standard errors are given in parentheses.

[d] Log (base e) transformed data used for analysis, *Lantana mearnsii* not included in analysis due to low replication.

[e] Ratio of male to female progeny, calculated from accumulated numbers for both exposure periods.

[f] Developmental time in days calculated from second exposure, i.e. time taken from the end of the exposure to adult progeny emergence.

[g] Numbers of individuals used to calculate the duration of development.

camara. The relatively longer developmental times on *L. mearnsii*, *Li. wilmsii*, *Li. rehmannii*, *Li. scaberrima* and *A. citrodora* suggest that these plants are less suitable for the immature stages of *C. camarae*.

8.3.3 *Preference testing between* Lantana camara *and selected test species in paired-choice trials*

During these trials adults showed a significant preference to locate and occupy *L. camara* when paired with the related test species (Fig. 8.1). Adults occurred in equal proportions when presented with two pairs of *L. camara*, whereas 95 % of the adults occurred on *L. camara* when paired with the unrelated *Ageratina adenophora*. Relatively small proportions of less than 13% of adults occurred on related *Lantana* species, *Li. scaberrima* and *P. meyeri*, suggesting that these plants are non-preferred and unsuitable hosts. These low proportions were equivalent to the proportions of adults that occurred elsewhere in the cage (i.e. that did not display host selection). A relatively higher proportion ranging between 17 and 27% of adults occurred on five of the *Lippia* species and *A. citrodora* (Fig. 8.1). These results suggest that in close proximity, these species, in particular *Li. rehmannii* and *Lippia* sp. A are the most favourable of the potential alternative host plants.

The numbers of eggs deposited during the trials were proportional to the distribution of adults (Table 8.3). The eggs were equally distributed when adults were exposed to two *L. camara* pairs, and were only laid on *L. camara* when paired with *Ageratina adenophora* (Table 8.3). Less than 13% of the eggs in each trial were deposited on *L. rugosa*, *L. mearnsii*, *L. montevidensis*, *A. citrodora* and *P. meyeri*. Relatively higher proportions of eggs, between 18 and 43%, were deposited on the remaining *Lippia* species. The highest number of progeny emerged on *L. camara* in all of the pairs, with egg to adult survival usually in excess of 78%. Very low numbers of adult progeny emerged from the test species, with notably high numbers on *Li. rehmannii*, *Lippia* sp. A and *Lippia* sp. B, which ranged between 2 and 8 progeny per plant (Table 8.3). The survival rates on these test species were significantly lower than on *L. camara*, with less than 38% of the eggs deposited developing into adult progeny.

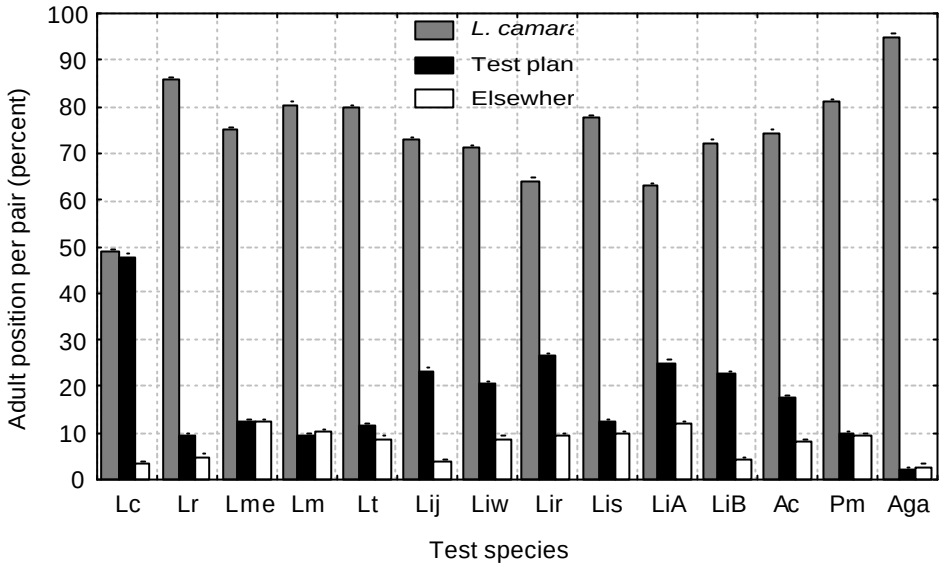


Fig. 8.1 The position of adult *Coelocephalapion camarae* in paired choice trials with *Lantana camara* as the control and 14 test species: *L. camara* (Lc); *L. rugosa* (Lr); *L. mearnsii* (Lme); *L. montevidensis* (Lm); *L. trifolia* (Lt); *Lippia javanica* (Lij); *Li. wilmsii* (Liw); *Li. rehmannii* (Lir); *Li. scaberrima* (Lis); *Lippia* sp.A (LiA); *Lippia* sp.B (LiB); *Aloysia citrodora* (Ac); *P. meyeri* (Pm); *Ageratina adenophora* (Aga). Adults not on test plants during trials were noted as 'elsewhere'. The plant positions in the cages did not significantly influence the adult positions in the trials (Chi-squared analysis: $\chi^2 < 9.563$, P = 0.008, D.F. = 2).

8.3.4 *7-d simultaneous-choice trials*

During these trials significantly more adults and eggs were recorded on *L. camara* than on any of the test species (Table 8.4). A small number of adults were recorded on *L. trifolia*, *Li wilmsii*, *Lippia* sp.B and *A. citrodora.* In addition, a very small number of eggs were recorded on these related plants (Table 8.4). The eggs were predominantly oviposited on the leaf-petioles, with the exception of *L. montevidensis* and *Li. scaberrima*, where eggs were deposited on the flower-peduncles. Larval survival, assessed as the number of eggs that resulted in gall development, was high on *L. camara*, *L. trifolia* and *Li. scaberrima*, and very low on the other test species (Table 8.4). The low survival rate on *A. citrodora* is attributed to the short leaf-petioles, which are unable to support larval development and usually abscise prematurely. The highest number of progeny was recorded on *L. camara*, with survival rates from gall development to adult

Table 8.3
Mean oviposition and development of progeny of *Coelocephelapion camarae* in paired-choice trials on *Lantana camara* and 13 plant species.

Plants tested	*n*	No. Eggs[a]	No. Progeny[a]	Percentage survival[b]
Lantana camara	6	21.4 (2.8)a	17.3 (2.0)a	81
L. camara		23.3 (1.5)a	18.6 (1.3)a	80
L. camara[1,2]	3	31.5 (3.1)a	25.0 (1.8)a	79
L. rugosa		0.8 (0.4)b	0.2 (0.2)b	25
L. camara[2]	3	30.5 (1.6)a	22.5 (1.9)a	74
L. mearnsii		4.6 (1.1)b	0.7 (0.4)b	15
L. camara[2]	6	31.5 (1.5)a	21.4 (1.5)a	68
L. montevidensis		2.6 (0.5)b	0.7 (0.4)b	27
L. camara[2]	6	28.2 (2.2)a	23.3 (1.8)a	83
L. trifolia		6.2 (1.1)b	1.9 (0.5)b	31
L. camara[1,2]	5	26.5 (2.9)a	22.8 (3.2)a	86
Lippia javanica		6.7 (0.7)b	0.5 (0.2)b	8
L. camara[2]	6	22.0 (2.3)a	17.3 (2.0)a	79
Li. wilmsii		8.0 (0.9)b	1.3 (0.3)b	16
L. camara	3	28.0 (1.4)a	21.0 (1.8)a	75
Li.rehmannii		13.0 (2.8)b	2.2 (0.5)b	17
L. camara	4	27.4 (3.0)a	19.1 (2.0)a	70
Li. scaberrima		5.0 (1.5)b	1.4 (0.5)b	28
L. camara	3	27.3 (4.1)a	19.5 (3.0)a	71
Lippia sp.A		20.7 (3.5)a	7.8 (1.2)b	38
L. camara	6	30.1 (1.6)a	25.7 (2.0)a	85
Lippia sp.B		11.2 (1.7)b	3.3 (0.9)b	30
L. camara[2]	5	25.1 (1.5)a	19.7 (1.2)a	79
Aloysia citrodora		1.9 (0.6)b	0.2 (0.1)b	11
L. camara[1,2]	3	27.2 (2.3)a	22.7 (3.4)a	84
Priva meyeri		0.2 (0.2)b	0.0 (-)b	0
L. camara	3	32.0 (1.7)a	23.7 (1.1)	74
Ageratina adenophora		0.0 (-)b	0.0 (-)	-

[a] Mean numbers of eggs and progeny per plant (2 plants/species), standard errors in parentheses.
[b] The percentage of eggs oviposited that survived to adulthood.
[1 or 2] Log (base e) transformed data used for 'No. Eggs' and 'No. Progeny' respectively. Means separated with a Student's t-test ($P<0.001$ for all species pairs, except for *Lippia* sp. A where $P<0.005$ for 'No. progeny').

progeny emergence of about 86%. In contrast, only a few adult progeny emerged from the related species. Adult progeny survival was significantly lower on all but one of the related plants, *Li. wilmsii*, than on *L. camara*.

8.3.5 *21-d simultaneous-choice trials*

These results are similar to the results of the 7-d exposure, with significantly more adults and progeny recorded on *L. camara* than on any of the test species (Table 8.5). However, a larger number of progeny were recorded on some of the test species, notably on *L. trifolia* and the *Lippia* species, than was the case in the 7-d exposure trial. These results suggest that when suitable oviposition sites on the preferred host plant are limited, *Li. rehmannii*, *Lippia* sp.A and *Lippia* sp.B could possibly serve as alternative host plants. A strong male bias recorded on *Li. javanica* and *Li. wilmsii* suggest that these species are unsuitable to sustain normal development of the immature stages.

8.4 DISCUSSION

No-choice host-specificity trials in classical weed biocontrol reliably determine the broader limits of susceptible plants, and form an essential part of the testing sequence (Wapshere, 1989; Cullen, 1990; Harris and McEvoy, 1995; Withers, 1997; Marohasy, 1998). During no-choice starvation trials on *C. camarae*, the adult feeding and reproductive development was restricted to 11 species in three genera in the family Verbenaceae. These include four congeneric species, two of which are indigenous, six indigenous species in the genus *Lippia* and the ornamental herb *Aloysia citrodora*. The performance of adults and immature stages varied significantly between the different species and the rank order of susceptible species was determined by comparing the fertility ratio of adults (progeny per number of adult females) (see Marohasy, 1998). No-choice trials were conducted over 30 days to create a state of deprivation, which can induce biocontrol candidates to accept lower ranked test species (Withers, 1997). The fertility ratios clearly indicate that *L. camara* is the most suitable host plant, and that the

Table 8.4

Host preference of *Coelocephalapion camarae* adults exposed to *Lantana camara* and nine related plants for 7 days in a simultaneous-choice trial, and the resultant development of progeny.

Plant species tested	*n*	No. Females	No. Males	No. Eggs	Ratio L-p:F-p[a]	No. Galls[b]	Total Progeny	% Survival: Galls to Adult[c]
Lantana camara	4	20.3 (2.4)a	11.5 (3.0)a	131.0 (11.9)a	1:0.2	108.5 (5.7)a	93.0 (2.3)a	86.1 a
L. montevidensis	4	0.5 (0.5)c	2.0 (0.9)b	0.8 (0.8)c	1:2.0	0.3 (0.3)c	0.0 (-)b	0.0 c
L. trifolia	4	3.3 (0.9)bc	1.8 (0.6)b	20.0 (5.2)bc	1:0.2	15.5 (5.5)b	4.5 (2.1)b	21.4 bc
Lippia wilmsii	4	6.8 (3.3)b	5.0 (2.2)b	9.8 (3.1)bc	1:0.1	4.5 (1.9)bc	2.3 (1.3)b	54.6 ab
Li. scaberrima	4	1.0 (0.4)c	0.8 (0.5)b	7.5 (4.1)bc	1:29	7.3 (4.1)bc	3.3 (2.3)b	28.2 bc
Lippia sp.B	4	4.3 (1.3)bc	3.0 (1.7)b	9.8 (1.8)bc	1:0.2	5.5 (1.7)bc	2.0 (1.4)b	25.0 bc
Aloysia citrodora	4	4.5 (1.3)bc	4.0 (1.6)b	22.5 (9.6)b	1:0.0	8.0 (3.9)bc	2.0 (0.9)b	33.2 b
Priva meyeri[d]	4	0.8 (0.3)	1.0 (0.4)	0.0 (-)	-	0.0 (-)	0.0 (-)	0.0
Duranta erecta[d]	4	0.3 (0.3)	0.0 (-)	0.0 (-)	-	0.0 (-)	0.0 (-)	0.0
Clerodendrum glabrum[d]	4	0.8 (0.5)	0.3 (0.3)	0.0 (-)	-	0.0 (-)	0.0 (-)	0.0

[a] Ratio of eggs oviposited on leaf-petioles and flower-peduncles.
[b] Number of galls induced after 30 days.
[c] Percentage survival was calculated from the number of galls to the number of adult progeny emerged (S.E.M. 14.5, L.S.D. 44.4).
[d] Means not included during analysis due to high frequency of zero values in the dataset.
Means within columns followed by the same letter are not significantly different (ANOVA, Fisher's protected LSD, $P<0.05$). The standard errors are given in parentheses.
n = Number of replicates.

Table 8.5
Host preference of *Coelocephalapion camarae* adults, exposed to *Lantana camara* and thirteen related plants for 21 days in a simultaneous-choice trial.

Plant species tested	*n*	Females[a]	Males[a]	Total progeny[a]	F:M[b]
Lantana camara	11	20.6 (1.7) a	15.3 (1.5) a	154.0 (23.2) a	1:1
L. rugosa	9	0.9 (0.2) f	0.7 (0.2) c	1.0 (0.4) g	1:0.8
L. montevidensis	11	2.5 (0.7) def	1.9 (0.8) c	1.6 (0.5) fg	1:0.8
L. trifolia	5	2.0 (0.3) cdef	0.8 (0.4) c	10.0 (6.8) cd	1:0.8
Lippia javanica	6	2.2 (0.3) bcde	2.2 (0.7) bc	7.8 (3.0) de	1:2.1
Li. wilmsii	5	3.6 (1.0) bcde	3.2 (1.8) bc	3.4 (0.7) de	1:2.4
Li. rehmannii	6	5.7 (1.7) b	4.5 (1.4) b	28.5 (12.5) bc	1:1.1
Li. scaberrima	6	2.3 (1.0) ef	2.7 (1.4) bc	11.2 (6.3) de	1:0.9
Lippia sp.A	6	4.3 (1.2) bc	3.2 (1.2) b	20.0 (8.4) b	1:0.8
Lippia sp.B	7	3.6 (0.8) bcd	1.9 (0.9) bc	28.3 (10.4) bc	1:1.3
Aloysia citrodora	7	4.1 (1.3) bc	2.7 (1.1) bc	4.0 (1.1) ef	1:1.3
Priva meyeri[c]	9	1.0 (0.3)	0.4 (0.2)	0.1 (0.1)	-
Duranta erecta[c]	11	0.5 (0.2)	0.2 (0.1)	0.0 (-)	-
Clerodendrum glabrum[c]	11	0.2 (0.1)	0.1 (0.1)	0.0 (-)	-

[a] Means within columns followed by the same letter are not significantly different ($P<0.05$, ANOVA, Fisher's protected LSD on log (base e) transformed data). The standard errors are given in parentheses.
[b] The ratio of female to male progeny, calculated from totals for replicates.
[c] Means not included during analysis due to high frequency of zero values in dataset.

four test species *Li. rehmannii*, *Li. scaberrima*, *Lippia* sp.A and *Lippia* sp.B are ranked much lower, but are potential alternative host plants. Very low fertility ratios on *L. rugosa*, *L. mearnsii*, *L. montevidensis*, *L. trifolia*, *Li. javanica*, *Li. wilmsii*, *A. citrodora* and *P. meyeri* indicate that these species in isolation would be unsuitable to sustain a viable population of *C. camarae.* The incidence of adult reproductive development on *Lippia* species was anticipated because many biocontrol candidates of *L. camara* accept species in this closely related genus during no-choice laboratory trials (Harley, 1971; Baars, 2000a; Baars, 2002). Furthermore, several natural enemies have been collected from *Lippia* species in their native range (Harley and Kassulke, 1971; Palmer *et al.*, 1996). Unless no-choice laboratory trial results are interpreted with care, the susceptibility of indigenous species may reduce the number of potential biocontrol

candidates that could be considered safe for release in South Africa (Baars and Neser, 1999; Baars, 2000a, 2002).

No-choice trials are effective in determining the fundamental host range of agents (Van Klinken, 2000), but these restrictive cage tests disrupt the host selection process of agents and often lead to ambiguous host range results (Cullen, 1990; Shepherd, 1990; Clement and Cristofaro, 1995; Harris and McEvoy, 1995). Test species accepted during laboratory trials are often not susceptible under field conditions (Baars and Neser, 1999; Baars, 2000a; Hill *et al*., 2000). These phenomena, known as 'false positive' results (Marohasy, 1998), highlight the difference between the fundamental and realised host range of candidates (Van Klinken, 2000). Although adult-choice trials provide the opportunity for preferences between plants to be determined, they also tend to provide conservative estimates of the host range due to restrictive cage conditions (Wapshere, 1989; Cullen, 1990).

The paired-choice trial results of *C. camarae* clearly indicated that *L. camara* was the most preferred plant, but in close proximity a relatively small number of adults and eggs were recorded on *Lippia* species, with the exception of *Li. scaberrima*, and *A. citrodora*. However, the survival rates of the immature stages on these non-target species suggested that all but three *Lippia* species were unsuitable substitute hosts. A small but appreciable number of progeny were recorded from *Li. rehmannii*, *Lippia* sp. A and *Lippia* sp. B. In simultaneous-choice trials, adults showed a very strong preference for *L. camara*, but a small proportion of eggs were recorded on other congenerics and several *Lippia* species. However, the eggs deposited on these test species typically suffered high mortality rates, and survival to adulthood was very low, whereas that on *L. camara* was in excess of 86%. In trials where the exposure period was extended to induce a state of deprivation (21-d exposure), a significant preference for *L. camara* was once again recorded. However, three of the species that proved susceptible in paired-choice trials, *Li. rehmannii*, *Lippia* sp. A and *Lippia* sp. B, were utilized as alternative host plants. Again, low numbers of progeny were recorded on these test species indicating that they are inferior host plants. Indeed, in large walk-in cage trials, in which the cage conditions mimicked the field situation, adults showed an almost exclusive preference for *L. camara* in the presence of *Lippia* sp. B (Chapter 7).

In the field, *C. camarae* will be presented with an unlimited source of *L. camara*. However, in the event of a sudden population explosion, such as that which resulted in *Teleonemia scrupulosa* feeding on sesame in Uganda (Greathead, 1968), it is likely that indigenous *Lippia* species in close proximity to the outbreak will sustain incidental damage. However, due to the weevil's low reproductive performance on these species it is unlikely that they will sustain a weevil population in the absence of *L. camara*. None of the South African *Lippia* species are endangered; indeed, several are regarded as minor weeds (Wells *et al.*, 1986). These species are largely pioneer plants and usually occur in grassland and bushveld communities where they are susceptible to being replaced by *L. camara.* Also, *Lippia* species have no special aesthetic value and are of minor economic importance, except for *Li. javanica* which is infrequently utilized as a tea (fever tea), and the extracts of which can be used as an inexpensive alternative to conventional mosquito repellents (Govere *et al.*, 2001). Incidental insect damage on *Lippia* species in close proximity to *L. camara*, albeit unlikely, is insufficient to cause any significant environmental damage, such as the extinction of a non-target species.

The biocontrol attributes of *C. camarae* indicate that it has the potential to significantly contribute to the control of *L. camara* in South Africa (Chapters 6, 7). As natural systems are complex, the release of a biocontrol agent always presents an element of risk, albeit minor, and the decision to release an agent must weigh up the benefits of potential control of the target weed against the possible risks of attack on non-target species (McFadyen and Marohasy, 1990; Olckers *et al.*, 1995; Cruttwell McFadyen, 1998; Hill *et al.*, 1999). *Lantana camara* causes extensive ecological damage in South Africa, and any minor damage that *C. camarae* may cause to the three most susceptible alternative hosts may well be considered as an appropriate 'trade-off' for the potential advantages of the control of this weed. The results and arguments presented here, in combination with those in Chapters 6 and 7 formed the basis of an application made to the regulatory authorities to release *C. camarae* on *L. camara* in South Africa.

Chapter 9

Biology and host-specificity of *Falconia intermedia,* a potential biological control agent of *Lantana camara* in South Africa

9.1 INTRODUCTION

Although it has been suggested that biological control agents that attack the structures of *L. camara* other than the leaves should be prioritized (Cilliers and Neser, 1991), leaf attacking agents like *C. camarae* (Chapter 6) and *U. girardi* (Muniappan *et al*., 1996) have a significant impact on the root resource and flower production of the weed respectively. Leaf-feeding biocontrol candidates therefore indirectly impact on the critical life stages of the weed, a factor important for effective biocontrol (Müller, 1990) and which should receive consideration when candidate agents are being selected (Chapter 5). In regions where the leaf resource is apparent throughout the season, particularly in the weed's subtropical range in South Africa, established leaf-feeding agents cause insufficient damage (Chapters 2, 3). In these regions, additional biocontrol agents are therefore necessary, but should possess biological attributes that favour a high intrinsic rate of increase and high levels of damage per individual (Chapters 3, 4).

During a survey of *Lantana* species in their native range, the leaf-feeding lantana mirid, *Falconia intermedia*, was identified as a potential biocontrol agent that warranted further evaluation (Palmer and Pullen, 1995). Recent field observations indicated that it is one of the most abundant and damaging natural enemies in Jamaica (Chapter 5). In this chapter, I present the results of life history studies and host-specificity tests on the lantana mirid, *F. intermedia*, and assess its suitability for release in South Africa through a 'natural' risk assessment.

9.2 MATERIALS AND METHODS

9.2.1 *Laboratory cultures and biology studies*

Falconia intermedia was imported into quarantine and cultured from a number of adults collected on a *Lantana* species near Guava Ridge in the foothills of the Blue Mountains

in Jamaica in 1994. Voucher specimens were deposited in the National Collection of Insects, Plant Protection Research Institute (Pretoria, South Africa), and the National Museum of Natural History (Washington DC, USA). The specimens were identified as *F. intermedia* (T.J. Henry, NMNH, Washington DC, USA), though previously placed in the genus *Adfalconia*. The taxonomic relationship between these two genera is unclear, and the generic placement of some of the species needs further study (Palmer and Pullen, 1998).

Populations of the lantana mirid were maintained on *L. camara* in gauze cages (90 x 45 x 45cm), in quarantine laboratories and glasshouses. Cages were illuminated by overhead daylight fluorescent light-banks in a controlled environment room with conditions fluctuating between 28°C during the day and 21°C at night, with a 13h photoperiod and 60-70% RH. Conditions in glasshouses were regulated, with temperatures averaging around 20°C (night) and 30°C (day), and subject to a natural summer photoperiod of about 13 h. Cultures were maintained by exposing adults to a plant for a period of 7-14 days, and then transferring them to new plants to avoid excessive leaf damage and oviposition. Plants were monitored for nymphal development, and observations were made on life cycle characteristics.

Potted lantana plants of the South African varieties dominant throughout the range in South Africa were used as culture plants. These reference varieties were collected and propagated as described in Chapter 6. Test plant species were chosen on the basis of their taxonomic relatedness to *L. camara* (Cantino *et al*., 1992; Arnold and De Wet, 1993), using the centrifugal phylogenetic method (Wapshere, 1974, 1989). The two undetermined species, *Lippia* sp.A and *Lippia* sp.B, were treated as described in Chapter 8.

Host-specificity results were subjected to analysis of variance (ANOVA, $P<0.05$) of an unbalanced design using Genstat regression (Genstat 5, 1993). Where large differences occurred between the means on different test species, an ANOVA on log-transformed data was conducted.

9.2.2 *Nymphal performance on test plants in no-choice trials*

These trials determined the range of plant species that could support the development of the immature stages of *F. intermedia*, and thus indicate the extent of the fundamental host range. Newly-emerged nymphs (10 per replicate), from eggs deposited on culture plants, were transferred to actively growing, potted test plants in 16 families. A total of 67 species was tested, including 17 species of economic importance, and each species was tested 3 to 11 times. Plants were observed daily for nymphal mortality, and nymphs reaching the adult stage were collected and the duration of development recorded. Trials were conducted over two summer seasons.

9.2.3 *Adult performance on suitable plants in no-choice trials*

These trials were conducted to investigate the suitability of the test plants that had supported nymphal development, for supporting the survival and reproduction of *F. intermedia* adults. Ten pairs of newly-emerged adults, from a culture cage, were confined on each test plant species for three consecutive periods of time. Adults were released in a cage (90 x 45 x 45cm) containing a single plant for 14 days and then collected and transferred to a new individual plant for a further 17 days. This was repeated for another 17 days, giving a total of 3 individual plants per *F. intermedia* group during the 48 days. Plants were isolated after each exposure, and the number of eggs deposited on each plant recorded. The number of eggs produced per female-day, i.e. oviposition rate, was determined by dividing the total number of eggs deposited per exposure period by the sum of the number of days that each female survived. Each plant species was tested between 2 and 6 times.

9.2.4 *Oviposition preference in multi-choice trials*

These trials were conducted to determine the oviposition preference of *F. intermedia* adults when presented with the simultaneous choice of several plant species in a large gauze cage. Twenty five pairs of adults (about 2 weeks old) from a culture cage were

released in a large cage (60 x 140 x 80cm) with about 13 plant species per replicate. The ratio of plants in the target genus and related species was adjusted to simulate natural conditions, with a maximum of three *Lippia* species per replicate. Five species that were unsuitable for nymphal development, including *L. montevidensis*, were included to stimulate adults into making a choice between plants and provide a 'non-preference control'.

To calculate the oviposition preference of adult females in this choice situation, a coefficient of discrimination (CD) (Heard, 1995) was used. The formula was: CD = (x-y)/(x+y); where x is the mean number of eggs deposited on the target plant and y is the mean number of eggs deposited on the test plant. The calculated values could range from +1 to -1, where +1 indicates the maximum preference for the *L. camara* target plant, 0 indicates no preference, and -1 indicates the maximum preference for the non-target test plant.

9.2.5 *'Natural' risk assessment*

The risk to non-target plant species in the event of the release of *F. intermedia* was quantified (*sensu* Wan and Harris, 1997; Olckers, 2000) as the product of the insect's oviposition preference and nymphal survival on test plants relative to that on *L. camara*. The formula used was: R = o.s; where 'R' is the relative 'natural' risk of attack; 'o' is the relative oviposition preference in multi-choice trials; and 's' is the relative nymphal survival in no-choice trials. The product of the values from these criteria gave the overall relative suitability of the test plants and hence their risk of attack.

9.3 RESULTS

9.3.1 *Life history of* F. intermedia

The eggs of *F. intermedia* were normally deposited singly, but on occasion were found in small groups of up to 5 eggs, on the underside of leaves. The egg was attached to the leaf by a posterior, stalk-like process (Fig. 9.1a) that was inserted into a leaf vein or laminar epidermal tissue, and probably also functioned to absorb moisture from the leaf tissue.

Eggs were usually cemented to the leaf with dark excrement, which covered most of the egg. The eggs were usually placed on the margin of the leaf, adjoining the leaf veins (Fig. 9.1b), and predominantly on the proximal half of the leaf. The eggs were small (length (mean ± SE) 0.53 ± 0.03mm; width 0.23 ± 0.02mm; *n*= 100), and embryonic development took 10-14 days under laboratory conditions (Table 9.1).

Nymphs and adults were highly mobile, spending most of the time feeding on the undersides of the leaves. The nymphs passed through five developmental instars (Table 9.1), and took about 13 days to reach the adult stage. The adult (Fig. 9.1e) was very active and highly mobile, and when disturbed moved to the opposite side of the leaf, and after repeated disturbance took flight. Adults had a pre-oviposition period of about 3 to 5 days, during which mating occurred repeatedly (Table 9.1). Adults lived for approximately 60 days in the laboratory, and produced large numbers of eggs. Nymphs and adults were leaf-suckers and feeding resulted in chlorotic spots on the lamina, which caused characteristic stippling of the upper leaf surface. Associated with the presence of adults and nymphs were small, dark, spherical faecal droplets deposited on the lower leaf surfaces. Extensive feeding damage caused leaves to appear silvery-white and to desiccate and abscise prematurely.

TABLE 9.1

Duration of life stages of *Falconia intermedia* on *Lantana camara* in the laboratory. [a]

Life stage	Duration (days) [b]	*n* [c]
Egg	10 - 14	-
1st instar	2.29 ± 0.45	56
2nd instar	2.04 ± 0.41	47
3rd instar	2.28 ± 0.45	43
4th instar	2.60 ± 0.55	40
5th instar	3.80 ± 0.69	40
Adult	3 - 5 pre-oviposition *ca.* 60 survival	-

[a] Day/night temperatures 28/21°C.
[b] Means ± standard deviation, or range.
[c] Number of individual observations.
- = Observations made from laboratory cultures.

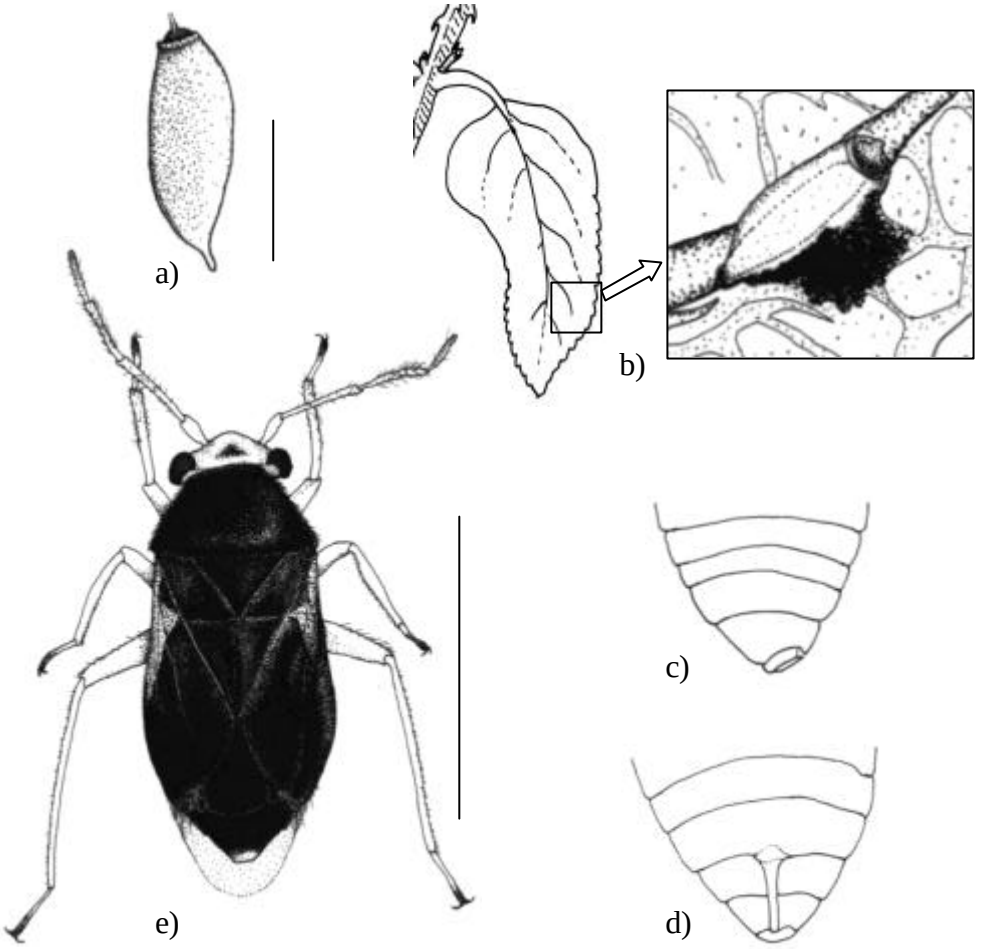


Fig. 9.1 Life stages and characteristics of *Falconia intermedia*: a) egg (scale bar 0.3mm); b) egg deposited on leaf under-surface; c) ventral view of male abdomen; d) ventral view of female abdomen; e) female adult (scale bar 2mm).

9.3.2 *Nymphal performance on test plants in no-choice trials*

The successful development of the nymphal stages of *F. intermedia* was restricted to *L. camara* and 11 closely related species, which belong mainly to the two genera, *Lantana* and *Lippia* (Table 9.2). The other plant species tested, including the indigenous *Lantana rugosa*, the introduced ornamental *Lantana montevidensis* and the 17 food crop species, proved to be non-hosts. Nymphal survival rates on *L. camara* were in excess of 80%,

TABLE 9.2

Host range of *Falconia intermedia*, as indicated by nymphal survival and development on test plant species in no-choice trials.

Plant Family	Plant species	Common name	*n*	Survival[c]
A. Phylogenetically related conservation, ornamental and flavouring species				
AMARANTHACEAE	*Amaranthus* sp. [ab]		4	-
BORAGINACEAE	*Heliotropium amplexicaule* Vahl.[a]		3	-
VERBENACEAE	*Verbena brasiliensis* Vell. [a]	Brazilian vervain	5	+
	V. bonariensis L. [a]	Purpletop	5	-
	V. tenuisecta Briq. [a]	Moss verbena	5	-
	Verbena x *hybrida* [ab]	Verbena mix	3	-
	Lantana camara L. [a]	Lantana	15	++++
	L. rugosa Thunb.		5	-
	L. montevidensis (Spreng.) Briq. [a]	Creeping lantana	5	-
	L. trifolia L. [a]		8	+
	Lippia javanica (Burm.f.) Spreng.		11	++++
	Li. rehmannii H. Pearson		11	++++
	Li. wilmsii H. Pearson		7	++++
	Li. scaberrima Sond.		9	+++
	Lippia sp.A		5	++++
	Lippia sp.B		5	++++
	Aloysia citrodora Palau [ab]	Lemon-verbena	3	+++
	Phyla nodiflora (L.) Greene	Fogfruit	5	-
	Cascanum hederaceum (Sond.) Moldenke		3	-
	Stachytarpheta mutabilis Vahl. [ab]		5	-
	Priva meyeri var. *meyeri* Jaub. and Spach.		10	+
	Duranta erecta L. [ab]	Golden dewdrop	5	-
	D. erecta L. [ab] (variegated)		5	-
LAMIACEAE	*Karomia speciosa* R. Fernandes		5	-
	Clerodendrum glabrum E. Mey.		5	-
	Teucrium trifidum Retz.		4	-
	Tinnea rhodesiensis S. Moore		3	-
	Lavandula angustifolia Ehrh [ab]	English lavender	3	-
	Nepeta calteria L. [ab]	Catnip	3	-
	N. musinii K. Spreng. Ex Henckel [ab]		3	-
	Lamium amplexicaule L. [ab]		3	-
	Salvia africana-caerulea L.		3	-
	S. elegans Vahl. [ab]	Pineapple sage	4	-
	S. patens L. [ab]	Origanum	3	-
	S. officinalis L. [ab]	Sage	3	-
	S. greggii A.Gray [ab]		4	-
	Thymus vulgaris L. [ab]	Common thyme	3	-
	Mentha piperita L. [ab]	Peppermint	3	-
	M. spicata L. [ab]	Spearmint	3	-
	Plectranthus saccatus Benth. Codd.		3	-
	Plectranthus sp. 2		3	-
	Hemizygia canescens Ashby		3	-
	H. obermeyerae Ashby		3	-
	Ocimum basilicum L. [ab]	Sweet basil	3	-
	Becium grandiflorum Sebald		3	-
SOLANACEAE	*Petunia* x *hybrida* Vilm.-Andr. [ab]	Petunia	3	-
SCROPHULARIACEAE	*Sutera burkeana* Hiern		3	-

Table 9.2 continued

	Sutera sp.		3	-
	Mazus reptans N.B.Br. [ab]		5	+
	Buchnera reducta Hiern		3	-
BIGNONIACEAE	*Jacaranda mimosifolia* D. Don [ab]	Jacaranda	3	-
B. Unrelated food crop species				
LILIACEAE	*Allium cepa* L. [ab]	Onion	4	-
	A. porrum L. [ab]	Leek	4	-
CHENOPODIACEAE	*Spinacia oleracea* L. [ab]	Spinach beet	4	-
CURCUBITACEAE	*Curcurbita pepo* L. [ab]	Squash	4	-
BRASSICACEAE	*Brassica oleracea* L. [ab]	Cabbage	4	-
	B. oleracea var. *botrytis* L. [ab]	Cauliflower	4	-
	Raphanus sativus L. [ab]	Radish	4	-
FABACEAE	*Pisum sativum* L. [ab]	Pea	4	-
	Phaseolus coccineus L. [ab]	Runner-bean	6	-
APIACEAE	*Daucus carota* L. [ab]	Carrot	4	-
SOLANACEAE	*Capsicum frutescens* L. [ab]	Green pepper	4	-
	Lycopersicon esculentum Mill. [ab]	Tomato	6	-
	Solanum melongena L. [ab]	Egg plant	3	-
	S. tuberosum L. [ab]	Potato	4	-
CURCUBITACEAE	*Curcurbita* sp. [ab]	Squash	4	-
ASTERACEAE	*Lactuca sativa* L. [ab]	Lettuce	4	-
POACEAE	*Zea mays* L. [ab]	Sweetcorn	4	-

[a] Plant species introduced into South Africa (Arnold and De Wet, 1993).
[b] Plant species of ornamental and/or economic value in South Africa.
[c] Nymphal survival rating in comparison to that on *Lantana camara*: + = ≤20%; ++ = 21 to 40%; +++ = 41 to 70%; ++++ = 71 to 100%; - = no nymphal survival and/or development.
n = Number of replicates.

with similarly high rates occurring on five *Lippia* species, including *Li. javanica*, *Li. rehmannii*, *Li. wilmsii*, *Lippia* sp.A and *Lippia* sp.B (Table 9.3). Significantly lower rates of survival occurred on *Lantana trifolia*, *Lippia scaberrima*, *Aloysia citrodora* and *Priva meyeri* var *meyeri*. Extremely low rates of survival and significantly slower rates of development were recorded on *Verbena brasiliensis* L. and *Mazus reptans* N.B.Br. (Table 9.3). The differences in the developmental periods on the remaining test species, although statistically significant, were not considered biologically meaningful. These trials suggest that the five abovementioned *Lippia* species may represent possible alternative hosts for nymphal development.

9.3.3 *Adult performance on suitable plants in no-choice trials*

Adult *F. intermedia* had the highest egg production over the 48 day exposure when

TABLE 9.3
Nymphal developmental period and survival rate of *Falconia intermedia*, from the first instar to the adult stage, on *Lantana camara* and related plant species during no-choice trials.

Plant species[a]	Developmental period (days) [b]	*n*	Survival rate (per 10 nymphs) [b]	*n*	Relative nymphal survival[c]
V. brasiliensis[a]	18.00 ± 0.00	2	0.40 ± 0.79c	5	0.05
L. camara	13.12 ± 0.13a	131	8.73 ± 0.45a	15	1.00
L. trifolia	13.64 ± 0.40ab	14	1.75 ± 0.62c	8	0.20
Li. javanica	13.58 ± 0.16ab	91	8.27 ± 0.53a	11	0.95
Li. rehmannii	13.38 ± 0.17ab	82	7.45 ± 0.53a	11	0.85
Li. scaberrima	15.92 ± 0.25d	38	4.11 ± 0.59b	9	0.47
Li. wilmsii	13.81 ± 0.20b	59	8.43 ± 0.67a	7	0.97
Lippia sp.A	14.05 ± 0.25b	40	7.60 ± 0.79a	5	0.92
Lippia sp.B	13.75 ± 0.24b	37	8.00 ± 0.79a	5	0.87
A. citrodora	14.67 ± 0.43bc	12	4.33 ± 1.02b	3	0.50
P. meyeri	15.31 ± 0.37cd	16	1.70 ± 0.56c	10	0.20
M. reptans[a]	24.00 ± 0.00	1	0.20 ± 0.79c	5	0.02

[a] Plant species not included in statistical analysis for developmental period, due to low number of records.
[b] Mean ± standard error. Means within columns followed by the same letter are not significantly different (P> 0.05, ANOVA, unbalanced design).
[c] Relative suitability determined using the mean survival on the test plants in proportion to that on *L. camara*.
n = Number of replicates.

isolated on *L. camara*, whereas significantly fewer eggs were produced by adults kept on *Lippia* species (Table 9.4). Adult female survival was high during the trials, with no significant differences between the number of 'female-days' on *Lippia* species and *L. camara* (Table 9.4). However, the oviposition rate was significantly lower on the *Lippia* species than on *L. camara*, ranging between 2.4 and 3.3 on *Lippia* spp. as opposed to 4.0 eggs per female-day on *L. camara* (Table 9.4). The oviposition curve over time for adults kept on the different species, had a peak in the first 2 weeks and then a gradual decrease over the following 5 weeks (Fig. 9.2). However, the most notable difference between the test species was the significantly higher oviposition rate over time for adults kept on *L. camara* than those on *Lippia* species (Fig. 9.2). The physiological suitability of the *Lippia* species for *F. intermedia* reproduction was 60-82% that of *L. camara* (Table 9.4). High

rates of adult mortality and low egg production were recorded for adults kept on *L. trifolia* and *P. meyeri* (Table 9.4), with adults only surviving and producing eggs for the first 2 weeks (Fig. 9.2).

TABLE 9.4
Oviposition rate of adult *Falconia intermedia*, exposed to *Lantana camara* and related plants in no-choice trials.

Plant species	*n*	Reproductive output (eggs)[ae]	Maternal effort (female-days)[be]	Oviposition rate (eggs/female-day)[ce]	Relative oviposition performance[d]
L. camara	6	1698.8 ± 30.0a	422.2 ± 9.6a	4.03 ± 0.09a	1.00
L. trifolia	3	9.8 ± 33.6e	137.8 ± 10.7c	0.13 ± 0.10f	0.03
Li. javanica	2	1028.0 ± 41.8cd	405.7 ± 13.3a	2.54 ± 0.13cd	0.63
Li. rehmannii	3	978.8 ± 33.6d	405.2 ± 10.7a	2.43 ± 0.10d	0.60
Li. scaberrima	3	1321.8 ± 33.6b	404.2 ± 10.7a	3.28 ± 0.10b	0.82
Li. wilmsii	3	944.1 ± 33.6d	371.2 ± 10.7a	2.56 ± 0.10cd	0.64
Lippia sp.A	3	1122.8 ± 33.6c	398.5 ± 10.7a	2.83 ± 0.10c	0.70
Lippia sp.B	3	1107.1 ± 33.6c	404.2 ± 10.7a	2.75 ± 0.10c	0.68
P. meyeri	3	73.8 ± 33.6e	178.5 ± 10.7b	0.47 ± 0.10e	0.12

[a] Mean ± standard error number of eggs deposited on plants exposed to 20 adults for 48 days, on 3 individual plants per replicate.
[b] Total number of female-days, calculated from the number of females surviving after each of three consecutive exposure periods.
[c] Number of eggs deposited per female-day, calculated from surviving adults after each of three exposures.
[d] Relative suitability determined by the mean number of eggs per female-day on test plants in proportion to that on *L. camara*.
[e] Means within a column followed by the same letter are not significantly different (P> 0.05, ANOVA, unbalanced design).
n = Number of replicates.

9.3.4 *Oviposition preference in multi-choice trials*

Despite an equal opportunity for adults to locate the test plants presented in a multi-choice situation, adult *F. intermedia* consistently preferred to deposit their eggs on *L. camara* (Table 9.5). Significantly fewer eggs were deposited on the other test plants, with a mean of 3-208 eggs per test plant accepted for oviposition, compared to a mean of over

870 eggs on *L. camara* (Table 9.5). Those plant species that were unsuitable for nymphal development (Table 9.2) and that were included in these trials, were consistently rejected for oviposition (Table 9.5). The coefficient of discrimination indicates that *L. camara* is the preferred species, and that most *Lippia* species are little (< 20%) accepted

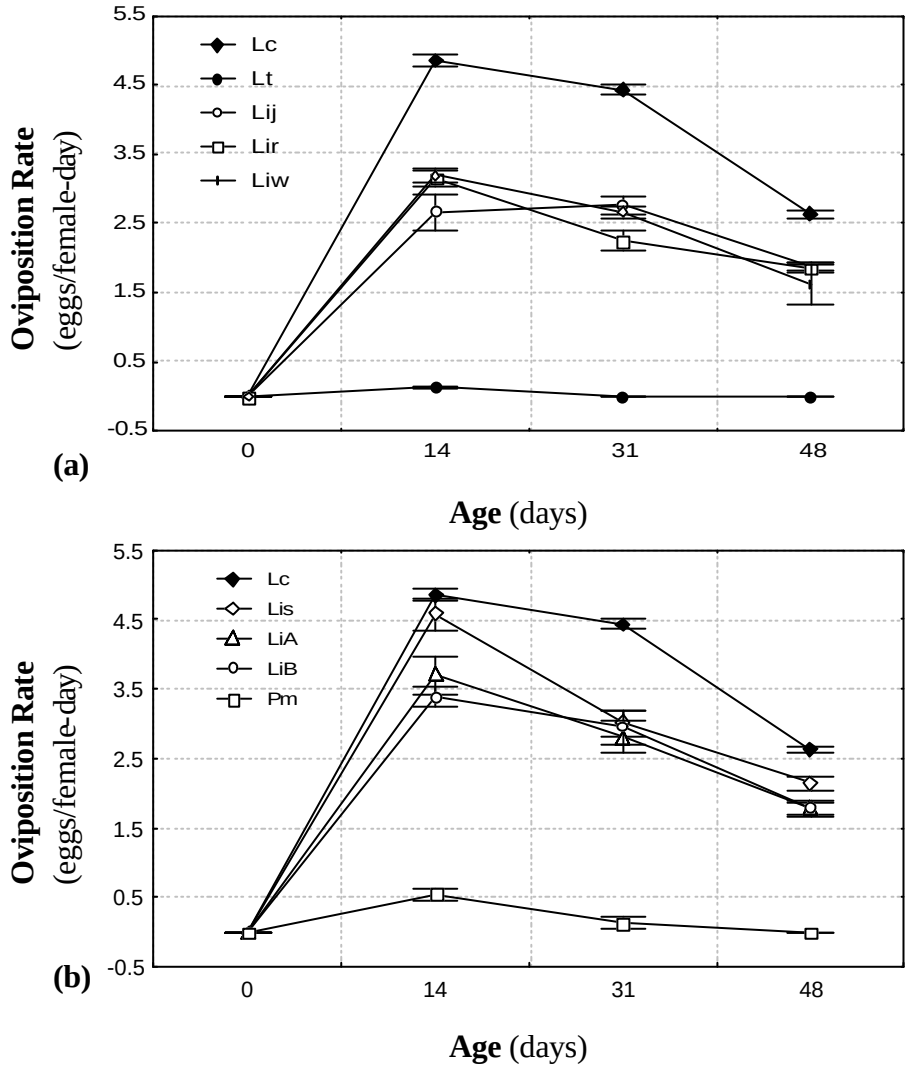


Fig. 9.2 Number of eggs (mean ± SE) deposited by *Falconia intermedia* per female-day, when isolated on *Lantana camara* and related test plants over 48 days, measured during three exposure periods: 0-14, 14-31 and 31-48 days. (a) *Lantana camara* (Lc); *Lantana trifolia* (Lt); *Lippia javanica* (Lij); *Lippia rehmannii* (Lir); *Lippia wilmsii* (Liw); and (b) *Lantana camara* (Lc); *Lippia scaberrima* (Lis); *Lippia* sp.A (LiA); *Lippia* sp.B (LiB); *Priva meyeri* (Pm).

TABLE 9.5
Oviposition preference of adult *Falconia intermedia* exposed to *Lantana camara* and related test plants in multi-choice trials.

Plant species[a]	*n*	Eggs deposited (mean)[ce]	(range)	Relative oviposition preference[d]
V. bonariensis[a]	4	0	0	0.00
L. camara	9	870.4 a	652 - 1042	1.00
L. montevidensis[a]	9	0	0	0.00
L. trifolia	9	2.5 e	0 - 8	0.01
Li. javanica	6	21.3 d	9 - 92	0.03
Li. rehmannii	8	15.6 d	0 - 108	0.02
Li. scaberrima	9	7.3 e	0 - 73	0.01
Li. wilmsii	3	48.4 cd	32 - 64	0.06
Lippia sp.A	3	92.9 bc	67 - 138	0.11
Lippia sp.B	3	208.4 b	178 - 235	0.24
A. citrodora	4	4.0 e	0 - 17	0.01
P. meyeri	8	4.8 e	0 - 47	0.01
D. erecta[a]	9	0	0	0.00
C. glabrum[a]	9	0	0	0.00
T. trifidum[a]	9	0	0	0.00
O. basilicum[a]	9	0	0	0.00

[a] Test plants not included in statistical analysis because of zero values.
[b] Transformed data from log values for number of eggs deposited on test plants.
[d] Relative suitability determined by the mean number of eggs deposited on test plants in proportion to that on *Lantana camara.*
[e] Means within a column followed by the same letter are not significantly different (P> 0.05, ANOVA, unbalanced design with log transformed data).
n = Number of replicates.

in the presence of *L. camara* (Fig. 9.3). *Lippia* sp.A and *Lippia* sp.B were the species most likely to be selected as alternatives (Fig. 9.3), but were significantly less preferred than *L. camara* (Table 9.5).

9.3.5 *'Natural' risk assessment*

The 'natural' risk of attack by *F. intermedia* on non-target plants (Table 9.6) is most in *Li. wilmsii, Lippia* sp.A and *Lippia* sp.B, which showed a 6%, 10% and 21% chance, respectively, of sustaining attack equivalent to that upon *L. camara* in the field. The risk

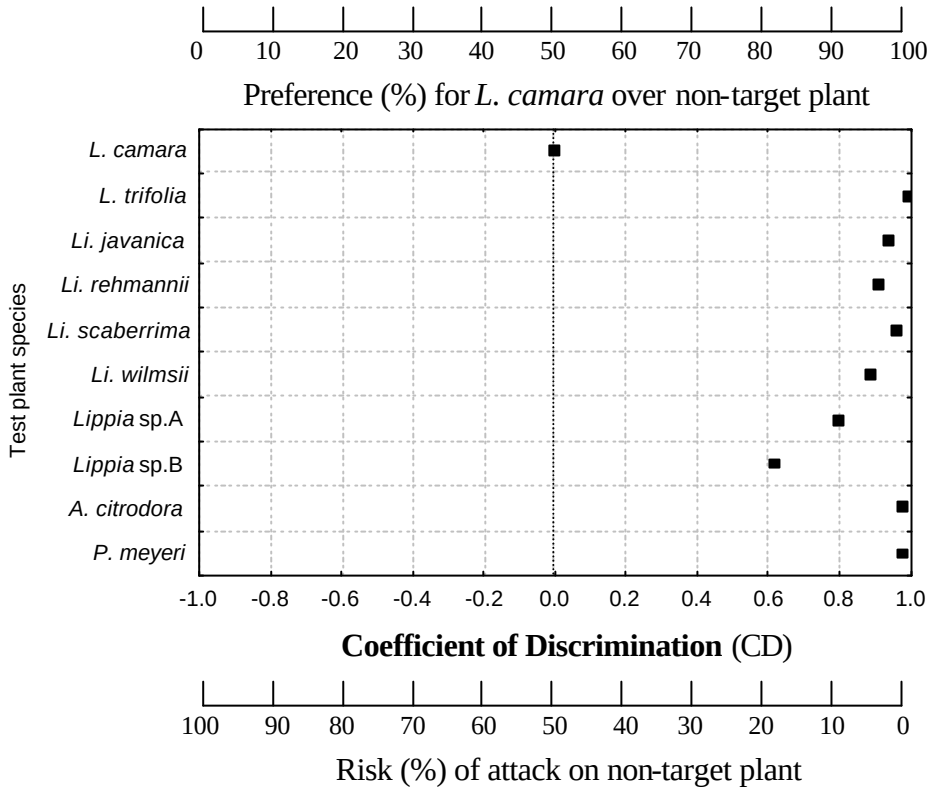


Fig. 9.3 Oviposition preference shown here as the coefficient of discrimination (CD) of adult *Falconia intermedia* amongst *Lantana camara* and related test plants in a multi-choice situation.

associated with the other six related plants, including three other indigenous *Lippia* species, was considerably lower and varied between 2.9% and 0.2% (Table 9.6).

9.4 DISCUSSION

The biological characteristics of the lantana mirid, *F. intermedia*, suggest that it has considerable potential as a biocontrol agent in that it has a high intrinsic rate of increase, the potential for multiple generations (up to 7 a year), continuous egg production and nymphal development, highly mobile adults, and high levels of damage per individual. Field observations indicate that the lantana mirid can reach high population levels and cause severe damage in Jamaica (Chapter 5) and also in Honduras, and occasionally in Mexico (Palmer and Pullen, 1998). All life stages, including the eggs that are partially

TABLE 9.6
Risk assessment of non-target attack by *Falconia intermedia*, using its preference for and performance on test plants in host specificity trials relative to that on *Lantana camara.*

Plant species	Relative oviposition preference[a]	Relative nymphal survival[a]	Relative 'natural' risk of attack[b]	
			(Proportion)	(%)
Lantana camara	1.00	1.00	1.0×10^{0}	100.0
L. trifolia	0.01	0.20	2.0×10^{-3}	0.2
Lippia javanica	0.03	0.95	2.9×10^{-2}	2.9
Li. rehmannii	0.02	0.85	1.7×10^{-2}	1.7
Li. scaberrima	0.01	0.47	4.7×10^{-3}	0.5
Li. wilmsii	0.06	0.97	5.8×10^{-2}	5.8
Lippia sp.A	0.11	0.92	1.0×10^{-1}	10.1
Lippia sp.B	0.24	0.87	2.1×10^{-1}	20.9
Aloysia citrodora	0.01	0.50	5.0×10^{-3}	0.5
Priva meyeri	0.01	0.20	2.0×10^{-3}	0.2

[a] Host suitability of test plant species determined by preference and performance criteria calculated from multi-choice and no-choice host-specificity trials (Tables 9.5 and 9.3).
[b] Product of suitability indices for preference and performance criteria.

inserted into the leaf, are dependent on the availability of the leaf resource in both space and time. However, *L. camara* plants in the dry and cold regions of South Africa typically persist as predominantly leafless shrubs during the winter. This suggests that, like the other folivorous agents established on the weed in South Africa, such as *O. scabripennis*, *U. girardi* and *S. haemorrhoidalis* (Chapters 2, 3, 4), the lantana mirid is likely to establish and be most effective in the warm, moist, eastern range of the weed. The lack of sufficient insect pressure in these areas suggests that the release of the lantana mirid will make a valuable contribution to biocontrol there.

The host plants of species in the genus *Falconia* are largely unknown, but none are known to be agricultural pests in their native range (Palmer and Pullen, 1998). Earlier host range trials on a representative sample of plants related to *L. camara*, suggested that *F. intermedia* has a narrow host range (Palmer and Pullen, 1998). During this study, no-choice nymphal and adult reproductive performance trials indicated that only *L. camara* and some species in the closely related genus *Lippia* are suitable as host plants. However,

the oviposition rate of adults was considerably higher on *L. camara* than on *Lippia* species, which suggests that these indigenous African species are relatively inferior alternative hosts. The results (Table 9.2) also clearly showed that *F. intermedia* poses no risk at all to other species in the Verbenaceae and Lamiaceae, or to 17 food crop species.

Several candidate biocontrol agents of *L. camara* have been recorded feeding on *Lippia* species in their country of origin (Harley and Kassulke, 1971; Palmer *et al*., 1996). Indeed, the congeneric *Falconia semirasa* (Distant) was collected from *Lippia myriocephala* Schlecht. and Cham. (Palmer and Pullen, 1998). In addition, no-choice laboratory trials have indicated that the majority of the candidate biocontrol agents evaluated accept at least three native South African *Lippia* species, but that these usually prove less suitable as hosts (Baars, 2000a; Baars, 2002; Chapter 8). The incidence of attack on this related genus, both in the field and in no-choice laboratory trials (see Harley, 1971), confirms the possibility that candidate agents may utilize these non-target plants as alternative hosts. Furthermore, certain indigenous *Lippia* species proved more suitable than *Lantana camara* as hosts for the stem-sucking membracid, *Aconophora compressa*, which caused its rejection as a biocontrol agent in South Africa (Heystek and Baars, 2001). Baars and Neser (1999) thus argued that unless limited feeding on *Lippia* species is accepted as an ecologically and environmentally justifiable 'trade-off' against the potential benefits to biocontrol of lantana, few candidate agents would ultimately be acceptable for release on *L. camara* in South Africa.

Adult no-choice tests under laboratory conditions are essential to determine the extent of the potential host range of candidates (Wapshere, 1989; Cullen, 1990; Harris and McEvoy, 1995; Withers, 1999). However, the acceptance of non-target species during these trials does not necessarily indicate an absence of field specificity (Zwölfer and Harris, 1971; Shepherd, 1990; Wapshere, 1989; Wan and Harris, 1997; Withers, 1997), and the laboratory host range is often not realised under field conditions (Harris and McEvoy, 1995; Marohasy, 1998; Hill *et al*., 2000). The lace-bug, *Teleonemia scrupulosa*, accepts several *Lippia* species in the laboratory (Baars, 2000a), but seldom attacks these plants in the field despite the fact that this agent has been established in South Africa for some 40 years (Baars and Neser, 1999).

The choice tests indicated that *F. intermedia* adults strongly preferred to oviposit on *L. camara*. However, in the presence of *L. camara*, a few related plants (mostly *Lippia* species) were marginally acceptable. *Lippia* populations in close proximity to lantana infestations, are thus likely to sustain some feeding damage. Since the nymphal stages of the lantana mirid are relatively immobile, host selection is dependent on the adults. New populations are initiated by dispersing adults that, according to the choice tests, are far more likely to select *L. camara*.

Risk assessments using relative suitability indices, provide numerical values that facilitate the decision on whether to release or reject a candidate biocontrol agent (Wan and Harris, 1997). Preference and performance criteria are considered in combination to predict host utilization (Cullen, 1990; Wan and Harris, 1997; Olckers, 2000).

The 'natural' risk of attack assessment used here was based on the rationale that the natural processes of (a) host plant selection, and (b) insect population growth are most simply simulated in the laboratory by (a) relative oviposition preference of mobile adults in a multi-choice arena, and (b) relative survival of sedentary juvenile progeny in a no-choice arena. Because these processes are both independent and sequential, it is valid that their product gives their joint effect. The 'natural' risk assessment (Table 9.6) indicated that *L. camara* is the optimum host, and that three indigenous plants, *Lippia wilmsii*, *Lippia* sp. A and *Lippia* sp. B, have a 6-21% chance of being used as alternative hosts. These species are thus at risk of some damage by *F. intermedia*, especially in the absence of *L. camara*. However, even highly successful biocontrol programmes do not eradicate their target weeds (Harris and Zwölfer, 1968; Cruttwell McFadyen, 1998), and *F. intermedia* thus seems likely to always be able to exert its strong preference for *L. camara* in the field. If *F. intermedia* populations become extremely abundant in the field, *L. camara* infestations may become locally defoliated. Under these conditions it is likely that *F. intermedia* populations may 'spill-over' onto the three indigenous *Lippia* species in close proximity to cause feeding damage, but only for a short-term. The low suitability of these *Lippia* species suggests that the lantana mirid is only likely to utilize them marginally and that none are threatened by extreme damage or extinction.

In assessing the risks involved with the release of *F. intermedia* in South Africa, due consideration was given to the weed status of *L. camara* (Chapter 1), the economic

and ecological implications of not controlling the weed (Simberloff and Stiling, 1996; Cruttwell McFadyen, 1998; Thomas and Willis, 1998), the importance of the biocontrol option (Baars and Neser, 1999; Chapters 2, 3, 4), the relatively minor anticipated impact on indigenous plants in Africa, and the fact that southern Asia, Indo-Malaysian region and the Pacific could benefit from this new biocontrol agent. The results of this study and the arguments presented above succeeded in convincing the regulatory authorities that *F. intermedia* should be released on *L. camara* in South Africa and field releases were initiated in April 1999 (Baars, 2000b).

Chapter 10

Preference and performance of *Falconia intermedia* on varieties of *Lantana camara* in South Africa

10.1 INTRODUCTION

The lantana mirid *Falconia intermedia* (Heteroptera: Miridae) is native to the Caribbean region, where it has been recorded on several *Lantana* species, including *L. urticifolia*, and *L. camara* (Palmer and Pullen, 1998; Chapter 5). In Jamaica, the mirid populations were found to be equally abundant on an ornamental variety of *L. camara* resembling some of the varieties in the countries where this plant has been introduced (Chapter 5). This suggested that the introduced varieties of *L. camara* in South Africa might be equally susceptible to the mirid. However, no-choice glasshouse trials on some Australian *L. camara* varieties, suggested that the varieties varied in their suitability to sustain the reproductive development of *F. intermedia* (Urban and Simelane, 1999). The ability of biocontrol candidates to utilize most or all of the *L. camara* varieties is considered to be important in ensuring their successful establishment and subsequent impact in the country of introduction (Sands and Harley, 1981; Winder *et al*., 1984; Neser and Cilliers, 1990; Cilliers and Neser, 1991; Palmer *et al*., 2000; Chapter 7).

The mirid had been found to have a satisfactorily narrow host range and was released in South Africa in 1999 for the control of *L. camara* (Baars, 2000b; Chapter 9). However, the variation in performance on Australian varieties suggests that the mirid may only be effective on a selection of the complex of varieties present in South Africa. This chapter thus reports on the performance and preference of *F. intermedia* on a number of South African *L. camara* varieties, to determine whether the range of varieties are equally susceptible.

10.2 MATERIALS AND METHODS

The culture of *F. intermedia* originates from adults and nymphs collected at Guava Ridge, Jamaica in 1994 (Chapter 9). The mirid cultures were kept in the glasshouses of

the Plant Protection Research Institute (Pretoria, South Africa) and maintained as described in Chapter 9. Ten *L. camara* varieties (Table 10.1; Chapter 6: Table 6.1) were selected to represent the range of morphological features that are present over the geographic range of the weed complex naturalized in South Africa, notably growth form, leaf and shoot tip characteristics and flower colour. The procedures used to collect and propagate the reference plants are described in Chapter 6.

The nymphs and adults used in trials were collected from cultures kept on lantana reference varieties other than those used during the trial, in an attempt to avoid insect conditioning. Trials were conducted in glasshouses and a fibreglass tunnel; the glasshouse conditions were as described in Chapter 7. The fibreglass tunnel was serviced by an extractor fan on one side drawing clean air through a wet wall in the opposite side, with temperatures of 20°C at night and 30°C in the day, and a natural photoperiod of 13 h.

The statistical analyses of the results were conducted using the programme Genstat (Genstat 5, 1993). Results were subjected to analysis of variance (ANOVA), and means were separated using Fisher's Protected LSD at the 5% level, unless otherwise stated.

Table 10.1
Origin and key characteristics of the *Lantana camara* varieties from South Africa used as test plants during trials.[a]

L. camara Variety	Origin: town/ province	Grid reference	Distinguishing morphological characteristics	Flower colour[b]
010	Sycamore, Mpumalanga	25°37'08.3"S 30°31'12.1"E	Shoot tip spiny; large broad dark hairy leaves; main stem spiny	Dark-pink
113	Nongoma, KwaZulu-Natal	27°53'37.0"S 31°38'27.6"E	Shoot tip spiny; leaves large, thick and tough; main stem very spiny	Dark-pink
165	Scottburgh, KwaZulu-Natal	30°09'08.4"S 30°49'39.7"E	Scrambling shrub; shoot tip spiny; large broad dark hairy leaves; main stem spiny	Orange-Red

[a] Other 7 varieties described in Chapter 6: Table 6.1.
[b] Colour of mature flowers.

10.2.1 *Nymphal performance on South African varieties*

No-choice trials were conducted to determine the suitability of different lantana varieties for supporting the development and survival of the nymphs of *F. intermedia*. Twenty newly emerged nymphs were transferred from a mirid culture to each of ten South African lantana varieties. The survival and development of the immature stages was monitored daily, and the adults that emerged were collected. Each variety was tested three times.

10.2.2 *Adult performance on South African varieties*

The suitability of South African lantana varieties for adult performance was examined using four varieties, namely 012, 029, 150 and 163 (Chapter 6: Table 6.1). Newly emerged *F. intermedia* adults, 30 pairs, were isolated on a single plant of each lantana variety in cages (93 x 40 x 40cm), placed on tables in a fibreglass tunnel. Adults were exposed for two consecutive 15-day periods; after the first period the adults were removed and placed on a fresh plant. These trials were replicated three times for each variety tested. Plants were isolated after the adults were removed, and the number of nymphs on the leaves of each plant were counted after 10 days.

10.2.3 *Preference amongst a choice of South African varieties*

The preferences of *F. intermedia* for the different South African lantana varieties were examined by exposing four varieties, 012, 029, 150 and 163 (Chapter 6: Table 6.1) and a non-target plant simultaneously in a multi-choice trial. The related indigenous plant, *Lippia wilmsii* was selected as the non-target plant because it was one of the most suitable species during host-specificity trials (Baars, 2000a, 2000b; Chapter 9), and it is the dominant *Lippia* species growing amongst *L. camara* infestations over large parts of the weed's geographic range in South Africa (J-R. Baars, personal observation). The plants were arranged in a 5x5-Latin square (five plants per species giving a total of 25 plants per trial) and the positions of plants in the columns and rows were randomised

using Random Tables (Murdock and Barnes, 1986). Three replicates of this trial were arranged alongside each other in a row (East to West) across the width of a fibreglass tunnel. The blocks were separated by a 3m high gauze screen, and plants in each block were equally spaced (40cm apart, 30cm from the sides of the gauze screen), with no overlapping of foliage.

Fifteen pairs of adults were released from vials at the base of each plant, giving a total of 750 adults per block. Trials ran for 2 weeks, after which the plants were cut down and the intensity of adult feeding damage on each leaf was scored as a percentage, and the number of eggs were counted using a dissecting microscope. At the conclusion of the trial the length of each leaf, number of branches and plant height was recorded.

Results were subjected to a five by five-square analysis (ANOVA), and means were separated by Fisher's Protected LSD at the 5% level. Correlation analysis was used to determine the relationship between the number of eggs and leaf area damaged, plant height, number of main (≥30cm) and side branches (<30cm), total leaf area, and the number of leaves per plant. The correlation between leaf area and leaf length was examined by measuring the leaves on ten branches of each test plant, each branch exceeding a length of 60 cm and all selected from the stock of reference plants. Measurements of the length taken at the conclusion of the trials could thus be converted to leaf area using the correlation coefficient, and then were correlated with the position of the eggs.

10.3 RESULTS

10.3.1 *Nymphal performance on South African varieties*

The developmental time and survival rate of the nymphs of *F. intermedia* were not significantly different between the ten South African lantana varieties (Table 10.2). Development from the first instar to adulthood took 13 to 14 days, and the nymphal survival rate was approximately 78% (Table 10.2). By contrast, the developmental time and survival rate was similar on the indigenous related plant *Li. wilmsii*, suggesting that this species is equally acceptable for nymphal development (Table 10.2).

TABLE 10.2
A comparison of the performance of the nymphal stages of *Falconia intermedia* confined on ten South African *Lantana camara* varieties and a related indigenous plant *Lippia wilmsii.*

Plant variety/ species	Development (days)[ac]	*n*	Survival (out of 20)[bc]	*n*
L. camara 009	14.0 ± 0.5a	44	15.7 ± 0.9a	3
L. camara 010	13.7 ± 0.3a	42	15.0 ± 1.0a	3
L. camara 012	14.0 ± 0.5a	44	15.7 ± 0.8a	3
L. camara 015	13.9 ± 0.4a	39	15.3 ± 0.8a	3
L. camara 018	13.7 ± 0.4a	44	16.7 ± 1.0a	3
L. camara 029	13.3 ± 0.4a	35	14.3 ± 1.4a	3
L. camara 113	13.4 ± 0.5a	49	15.7 ± 0.6a	3
L. camara 150	13.7 ± 0.4a	54	17.3 ± 1.0a	3
L. camara 163	13.8 ± 0.4a	42	16.0 ± 0.9a	3
L. camara 165	13.9 ± 0.3a	32	14.7 ± 0.9a	3
Lippia wilmsii[d]	13.77	-	16.86	-

[a] The duration of development in days, from 1st instar nymph to adult stage.
[b] The mean number of nymphs surviving to adult stage from 20 individuals.
[c] Means compared by ANOVA; those within columns followed by the same letter are not significantly different (P>0.05, Fisher's Protected LSD).
[d] Results extracted from Chapter 9: Table 9.3 (survival rate multiplied by 2).

10.3.2 *Adult performance on South African varieties*

The reproductive performance of *F. intermedia* over a 30-day period was not significantly different between the four South African lantana varieties that were tested (Table 10.3). The female survival was high, with rates of 97% and 89% for the two 15-day periods respectively (Table 10.3). On the other hand, males suffered higher rates of mortality, with survival rates of 69% and 61% for the two periods respectively (Table 10.3). The total number of nymphs recorded was not significantly different between varieties, but decreased from an overall mean of 1454 to 1197 from exposure period one to two (Table 10.3). There was considerable variation in the number of progeny per plant within varieties, indicated by the large standard errors of the mean and the least significant differences (Table 10.3). The number of progeny per female per day, α

TABLE 10.3

The reproductive performance of *Falconia intermedia* adults confined on four South African *Lantana camara* varieties during two consecutive 15-day exposure periods.

Plant species/ variety	*n*	Exposure period 1 to 15 days				Exposure period 16 to 30 days			
		No. Females[a]	No. Males[a]	No. Nymphs[b]	Fertility ratio[c]	No. Females[a]	No. Males[a]	No. Nymphs[b]	Fertility ratio[c]
L. camara 012	3	29.3 (0.7)a	22.3 (1.3)a	1462.7 (204.8)a	3.3 (0.5)a	27.0 (1.5)a	15.7 (0.7)a	1282.7 (73.3)a	3.1 (0.3)a
L. camara 029	3	27.7 (0.9)a	20.0 (0.6)a	1330.0 (127.2)a	3.2 (0.4)a	25.7 (0.3)a	11.3 (0.3)b	1154.3 (145.2)a	3.0 (0.4)a
L. camara 150	3	29.0 (0.6)a	18.3 (1.7)a	1438.3 (122.4)a	3.3 (0.4)a	26.0 (1.7)a	14.7 (0.7)a	1213.0 (88.9)a	3.2 (0.4)a
L. camara 163	3	30.0 (0.0)a	22.3 (0.9)a	1583.0 (78.3)a	3.5 (0.2)a	24.7 (1.5)a	9.3 (0.9)b	1137.3 (49.6)a	3.1 (0.3)a
Grand Mean		29.0	20.8	1454.0	3.4	25.8	12.8	1197.0	3.1
S.E.M.		0.7	0.9	152.4	0.4	0.8	0.6	89.4	0.3
L.S.D.		ns	ns	ns	-	ns	2.0	ns	-

[a] Number of females and males surviving on the test plant at the end of the exposure period.

[b] Female reproductive performance expressed as fecundity ratio [the number of progeny produced per female during the exposure period].

[c] Fertility ratio [number of progeny per female per day, calculated for 15-day exposure periods].

S.E.M. = Standard error of mean; L.S.D. = Least significant difference; ns = Not significant.

Means within a column followed by the same letter are not significantly different (P>0.05, Fisher's Protected LSD).

fertility ratio, was not significantly different between the four lantana varieties (Table 10.3). This implies that on average each female gave rise to about 97 progeny during the 30 days. The fertility ratio was slightly higher during the first 15 days, but remained comparatively high during the second exposure period (Table 10.3).

10.3.3 *Preference amongst a choice of South African varieties*

The correlation coefficients for leaf length versus leaf area showed strong positive linear relationships for all the plants measured (Table 10.4). This ensures that the leaf length measurements of plants taken at the conclusion of the choice trials can be converted to leaf area measurements with a fair degree of accuracy (Table 10.5).

TABLE 10.4
Correlation coefficients and trend line equations of the relationships between leaf length and leaf area for four South African *Lantana camara* varieties and the related plant *Lippia wilmsii*.[a]

Plant variety/species	n^b	R	Trendline equation[c]
L. camara 018	187	0.95	$y = 0.3674 x^{2.0811}$
L. camara 029	206	0.91	$y = 0.4414 x^{2.0220}$
L. camara 150	188	0.93	$y = 0.5648 x^{1.9695}$
L. camara 163	219	0.95	$y = 0.4164 x^{2.1209}$
Li. wilmsii	254	0.96	$y = 0.2788 x^{1.9470}$

[a] Power regression used as the best fit for the change in leaf dimensions.
[b] Number of leaves on ten branches sampled.
[c] The equation of the trend line for leaf length (x) and leaf area (y).

There were significant differences in feeding damage and rates of oviposition of *F. intermedia* adults between the plants tested. Significantly higher numbers of eggs were deposited on two of the *L. camara* varieties, 150 and 163, whereas varieties 029 and 018 supported about 50% less oviposition (Table 10.5). The related plant *Li. wilmsii* was the least preferred plant during the trial, and supported less than 25% of the oviposition recorded on the preferred *L. camara* variety 163 (Table 10.5). The percentage leaf damage on the different varieties showed a similar trend to that observed with oviposition

(Table 10.5), and significantly less leaf area was damaged on *Li. wilmsii* in comparison to that recorded on all of the *L. camara* varieties.

Plant architecture varied significantly between the different lantana varieties and *Li. wilmsii,* including characteristics such as plant height, leaf area and the total number of leaves (Table 10.5), and the number of main and side branches. The correlation coefficients for the abovementioned parameters showed poor relationships between these and the total number of eggs on the plants (Table 10.6). However, the number of eggs on the plants was significantly correlated with the percentage damage (Table 10.6). This suggested that the plant characteristics measured during the trial were not significant in adult choice, and it is thus possible that other morphological or physiological characteristics specific to the varieties resulted in the preferences recorded. The lower number of eggs deposited and lower levels of damage recorded on *Li. wilmsii* supports the contention that it is a poor alternative host in the presence of *L. camara*.

TABLE 10.5

Feeding and oviposition preferences of *Falconia intermedia* adults in a multi-choice trial involving 4 South African *Lantana camara* varieties and a related indigenous plant *Lippia wilmsii*.

Plant species/variety	Plant Height [a]	No. Leaves	Leaf Area [b]	Leaf area damaged [c]	% Leaf damage	No. Eggs [d]
L. camara 018	79.7 b	111.8 c	1401 c	277 c	20.3 a	457 b
L. camara 029	84.1 b	121.7 c	2196 b	333 c	15.5 b	512 b
L. camara 150	93.7 a	171.9 b	2258 b	457 b	20.6 a	811 a
L. camara 163	94.7 a	163.4 b	2563 a	549 a	21.5 a	939 a
Li. wilmsii	72.7 c	199.9 a	791 d	78 d	9.9 c	206 c
Grand Mean	85.0	153.7	1842	339	17.6	586
S.E.M.	2.1	6.8	65	26	1.7	62
L.S.D.	5.8	19.3	186	73	4.8	175

[a] Height measured from the base of plant to the average height of the branches (cm).
[b] Leaf area calculated using the relationship between leaf length and leaf area (mm^2) (different for each test plant variety/species: see Table 10.4).
[c] Leaves individually assessed for percentage damage, with area calculated as above (mm^2).
S.E.M. = Standard error of mean; L.S.D. = Least significant difference; means within a column followed by the same letter are not significantly different ($P>0.05$, Fisher's Protected LSD).
[d] Numbers represent means, and fractions have been rounded off to the nearest egg.

TABLE 10.6
Correlation matrix between the number of eggs deposited by *Falconia intermedia* adults and the leaf area damaged, and various morphological characteristics of the test plants exposed in multi-choice trials.

Plant species/variety	Parameters used in correlation with the number of eggs deposited					
	Total No. Leaves[a]	Total Leaf area[a]	Feeding damage (area/plant)[a]	Plant Height[a]	No. Main branches[a]	No. Side branches[a]
L. camara 018	NS	NS	**	NS	NS	NS
L. camara 029	NS	NS	**	NS	NS	NS
L. camara 150	NS	NS	**	NS	NS	NS
L. camara 163	NS	NS	**	NS	NS	NS
Lippia wilmsii	NS	NS	**	NS	NS	NS

[a] NS = Correlation with the number of eggs deposited per test plant not significant.
** = Correlation with number of eggs deposited significant at $P<0.01$ [correlations: 13 (n-2)].

10.4 DISCUSSION

For practical purposes, a select number of *L. camara* varieties were chosen to represent the range in morphological characteristics present in the complex of varieties that are naturalised in South Africa (Chapters 1, 6). The no-choice trials that examined nymphal survival and development and adult reproductive performance suggested that the varieties tested are equally suitable for *F. intermedia*. However, it is possible that other South African varieties that were not included in these trials are less suitable because Australian lantana varieties varied in their suitability for *F. intermedia* (Urban and Simelane, 1999). However, the reproductive performance of *F. intermedia* on the South African lantana varieties during these trials and during host range trials (Chapter 9) was more than four times higher than that recorded on the most susceptible Australian varieties (Urban and Simelane, 1999). The origins of the varieties naturalised in the different countries are unknown, but it is probable that they are different. Furthermore, the *L. camara* varieties were introduced into various countries mainly in the mid 1800s and have been isolated since, and are also known to hybridise in the field (Spies, 1984; Spies and Du Plessis, 1987). This has been regarded as a possible reason for the variation in the performance of agents in different countries (Day and Neser, 2000), and may explain why some agents

failed to establish in certain countries, for example *Leptobyrsa decora* (Heteroptera: Tingidae) (Cilliers and Neser, 1991; Baars, 2002) and *O. championi* in South Africa (see Chapter 3).

The results of no-choice trials suggest that *F. intermedia* will perform equally well on the different varieties of *L. camara* in South Africa. However, since the nymphs are unable to move between plants host choice is dependant on the adults. The results of simultaneous-choice trials suggest that certain South African lantana varieties are more acceptable, with a higher proportion of eggs deposited on two of the varieties. The grouping of the different varieties of *L. camara* has been attempted using different morphological characteristics, in particular flower colour (Smith and Smith, 1982; Stirton and Erasmus, 1990). In an attempt to identify patterns in the variation of insect performance on the different varieties, comparative studies have grouped varieties into flower colours (Haseler, 1966; Harley *et al.*, 1979; Cilliers 1987b; Cilliers and Neser, 1991; Broughton, 1999, 2000; Day and Neser, 2000). However, categories based on such characteristics may be arbitrary with respect to host choice because the attributes that govern a particular variety's susceptibility to insect attack are unknown, and the effects that morphological characteristics have on insect preferences are also unknown. Hopefully, the range of varieties selected for the preference and performance studies on *F. intermedia* represent a large proportion of the attributes that govern susceptibility. The results of choice trials indicate that certain varieties are more acceptable to the lantana mirid, and it is noteworthy that the two most preferred South African lantana varieties are very different to each other in morphology. The mechanisms responsible for the variation in insect preference may lie in differences in plant morphology or physiology, or in the variation in the endogenous condition of the insect or an interaction of these factors. Due to the complex origin of the weed, an understanding of the exact attributes that govern the susceptibility of *L. camara* varieties to biocontrol agents may thus be extremely difficult to achieve.

The results of the choice trials suggest that in a mixed stand of lantana varieties, with the most preferred varieties present, lantana mirid populations should build up quicker on the preferred varieties. A similar trend was observed with *Teleonemia scrupulosa*, which took longer to build up on the 'common pink' variety of lantana in

Australia (Harley *et al*., 1979). However, field observations suggest that under extremely high mirid densities (e.g. as observed at Tzaneen, in the Northern Province, South Africa), there are no noticeable differences in the number of insects and levels of damage between the lantana varieties present at a location (Baars, 2000c). It is possible that the rank order of the different varieties, as indicated by the choice trial results, may influence the dispersal of mirid adults in the absence of the preferred variety. However, it is more likely that the motivational threshold that stimulates the mirid to feed and oviposit (as opposed to disperse) on the most preferred variety, may be lower than when it is presented with the least preferred lantana variety. Therefore, in the absence of the preferred varieties the lower ranked lantana varieties will be equally acceptable. In addition, it is possible that environmental factors may alter the preference rank of the *L. camara* varieties (Waddell and Mousseau, 1996).

The compatibility of biocontrol candidates with different forms of weed species has proved important in other weed systems (Blossey and Schat, 1997; Volchansky *et al*., 1999; Zachariades *et al*., 1999; Githure *et al*., 2001). In order to improve the success of the biocontrol campaign against *L. camara*, it is important to introduce biocontrol agents that accept and perform well on at least a selection of the range of lantana varieties. The results presented in this chapter suggest that *F. intermedia* will perform equally well on the different varieties, but is likely to build up more quickly on certain varieties in a mixed stand of *L. camara*. In the absence of the preferred varieties the mirid populations are likely to build up equally well on the other varieties, and are unlikely to be stimulated to disperse in search of the preferred varieties. However, these predictions are based on cage trials and preferences for certain varieties in the field may be influenced by habitat requirements or other environmental factors, which will require further research to be elucidated.

Chapter 11

Evaluation of the non-target effects of *Falconia intermedia* in South Africa

11.1 INTRODUCTION

The release of a biocontrol agent in a new environment invariably presents an element of risk, although carefully minimized through the accumulation of information on the potential extent of the host range through host-specificity testing (Wapshere, 1974, 1989; Cullen, 1990; Cory and Myers, 2000). Biological control of weeds has an excellent track record (Cruttwell McFadyen, 1998), and the phenomenon of 'host shifts' or host range extensions to include non-target species is very rare (Marohasy, 1996). There are only three incidences of unanticipated attack on non-target plants, two of which could have been avoided by conducting thorough host-specificity testing. The other five instances of non-target impacts were all anticipated, where the agents were released at a time when attack on related plants that had no economic value was not considered important (Cruttwell McFadyen, 1998). However, such attack on non-target species can lead to significant environmental damage by reducing the reproductive output of non-target plants (Diehl and McEvoy, 1990; Louda *et al*., 1997). The small number of known cases of non-target effects is arguably a misrepresentation of the extent of this phenomenon because there is a lack of post release evaluations (Simberloff and Stiling, 1996). An increased global awareness of the potentially irreversible effects of weed biocontrol on native biodiversity, justifies the evaluation of the possible harm produced by increased importation rates and frequent lack of effective post release surveys (Samways, 1997; McEvoy and Coombs, 1999).

The lantana mirid, *Falconia intermedia* is an agent native to the Caribbean region that was released in South Africa for the biological control of *L. camara* (Baars and Neser, 1999; Baars, 2000c; Chapter 9). The host-specificity test results suggested that the lantana mirid is oligophagous, and that some species of an indigenous genus, *Lippia*, may be utilized as substitute host plants (Chapter 9). Given the choice in the laboratory adults preferred to deposit their eggs on the target weed, which indicates that feeding on indigenous species in the field is unlikely. Although the potential for non-target effects is

minor it is important to refine estimates of the severity, probability and consequences of these non-target effects. Laboratory trials often indicate artificially extended host ranges (Wapshere, 1989; Cullen, 1990), and field trials have proven useful to validate the laboratory results on other weed systems (Briese *et al*., 1995; Clement and Cristofaro, 1995; Balciunas *et al*., 1996; Hill *et al*., 2000), and to improve the predictability of biological control (Zwölfer and Harris, 1971; Harris and McEvoy, 1995).

This chapter therefore, assesses the non-target effects of *F. intermedia* by conducting field based choice trials in mixed plots of *L. camara* and *Li. wilmsii*. Observations are also made in a natural field situation, where the lantana mirid has been released, to determine the relative preference for *Li. wilmsii* in the presence of *L. camara*.

11.2 MATERIALS AND METHODS

11.2.1 *Single-choice field plot*

These trials were conducted to determine the preference of adult *F. intermedia* dispersing amongst plants of *L. camara* and the indigenous species *Lippia wilmsii* under field conditions. Six plots (4.5 x 1.5m) were cleared in a grassy area at the Plant Protection Research Institute in Pretoria. In each plot, three plants were planted in the soil in a row (East to West), about 1.5m apart (Fig. 11.1a). In the middle of the row, *L. camara* 163 (Chapter 6: Table 6.1) was planted as the source plant. Two test species were planted on either side of the source plant, including *L. camara* (163) and *Li. wilmsii*, and each species was tested three times on either side of the source plant.

Five hundred adults from a culture cage were isolated on the source plant for 2 days under a field gauze cage, which was then removed to allow the adults to disperse naturally. After 10 days the number of adults on each test species was counted, and the source plant was cut down to stimulate adult dispersal. After an additional 12 days the number of adults on each plant was recorded. After another 14 days, which allowed the adults to feed and deposit their eggs, a sub sample was taken from each plant, comprising five main branches (≥30cm) and three side branches (<30cm). The parameters recorded

in the samples included the number of leaves, length of leaves, intensity of stippling damage and the number of eggs (counted using a dissection microscope).

11.2.2 *Simultaneous-choice field plot*

To investigate the relative preference of dispersing adult *F. intermedia* for *L. camara* and *Li. wilmsii* in a mixed stand of plants, four plants of each species were planted in each of two concentric circles of 3 and 6m diameters with *L. camara* as a source plant in the centre (Fig. 11.1b). Plants were planted in alternating positions in the circles, in the direction of the cardinal points from the centre plant. Plants in a radial line in the two circles were different. One thousand five hundred adults were confined to the source plant for 7 days under a gauze cage. The cage was removed and the adults were left to disperse naturally. After 3 days, each plant was searched for five minutes and the number of adults were recorded and the number of leaves with characteristic damage counted. Half of the branches of the source plant were cut off and the trial ran for another 3 days. The observations were then repeated, and the entire source plant cut down. Observations were again made on two other occasions, both with 3 day intervals in between. The trial was left for another 3 days and then 30 leaves were sampled from each plant in the inner circle. The feeding damage on each leaf was assessed as a percentage, and the numbers of eggs deposited were counted. Leaf length measurements were recorded and converted to leaf area measurements using trend line equations described in Chapter 10, Table 10.3. The trial was repeated three times.

11.2.3 *Natural field-choice*

At a site where *F. intermedia* was being mass-reared for field releases (Tzaneen, Northern Province), adults occasionally escaped from the rearing cages and dispersed into the surrounding area. One hundred meters from the breeding nets, a large *L. camara* shrub and two *Li. wilmsii* shrubs were located in close proximity to each other. Ten branches (30 cm shoot tips) from each plant were cut off and brought back to the laboratory where the length of each leaf was measured, and the intensity of stippling

damage assessed as a percentage and the number of eggs per leaf was counted. The leaf length measurements were converted to leaf area measurements using the trend line equations for *L. camara* 017 (Chapter 7: Table 7.5) and *Li. wilmsii* (Chapter 10: Table 10.3).

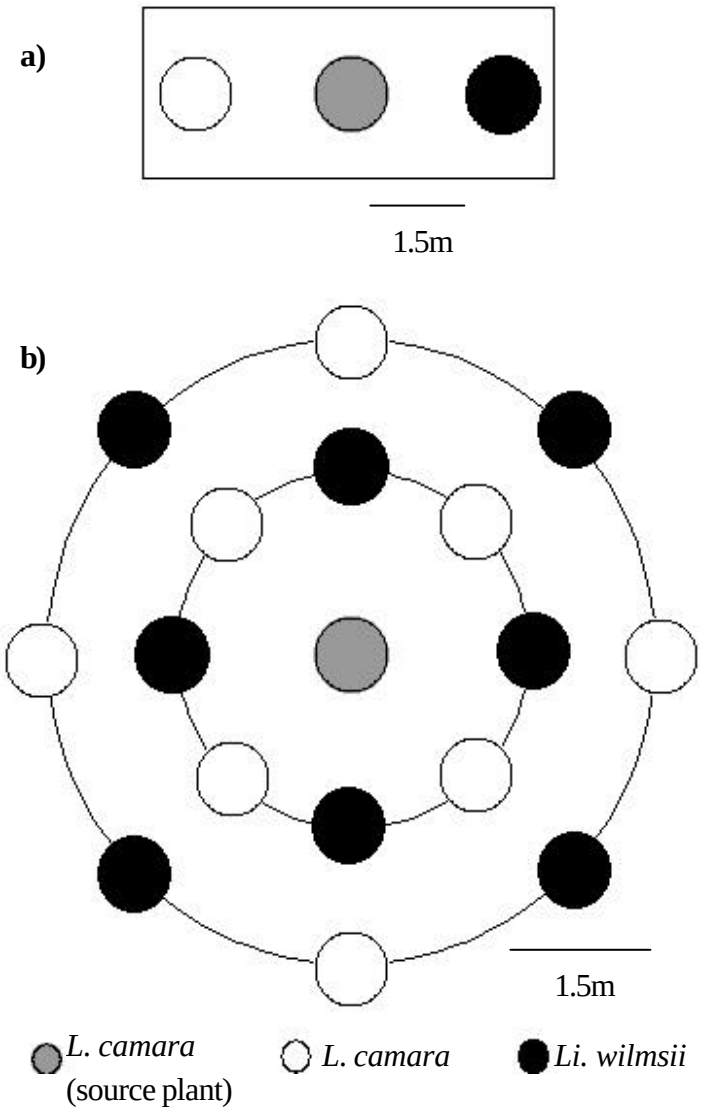


Fig. 11.1 Design of a) single-choice and b) simultaneous-choice field plot trials conducted on *Falconia intermedia*.

11.3 RESULTS

11.3.1 *Single-choice field plot*

After the gauze cage was removed most of the adults remained on the source plant, but a small number of adults were recorded on the *L. camara* test plant (Fig. 11.2). When the source plant was cut down to stimulate adult dispersal, significantly more adults were recorded on *L. camara* than on *Li. wilmsii* (Fig. 11.2), irrespective of the compass direction of the test plant (Chi-squared test not significant: χ^2 = 0.894, D.F. = 2). The populations of *F. intermedia* on the test species caused significantly more leaf damage to *L. camara* than to *Li. wilmsii* (Table 11.1). A mean of 680.5 eggs were deposited on *L. camara* compared to 28.8 eggs on *Li. wilmsii* plants (Table 11.1). These results suggest a strong preference for *L. camara*.

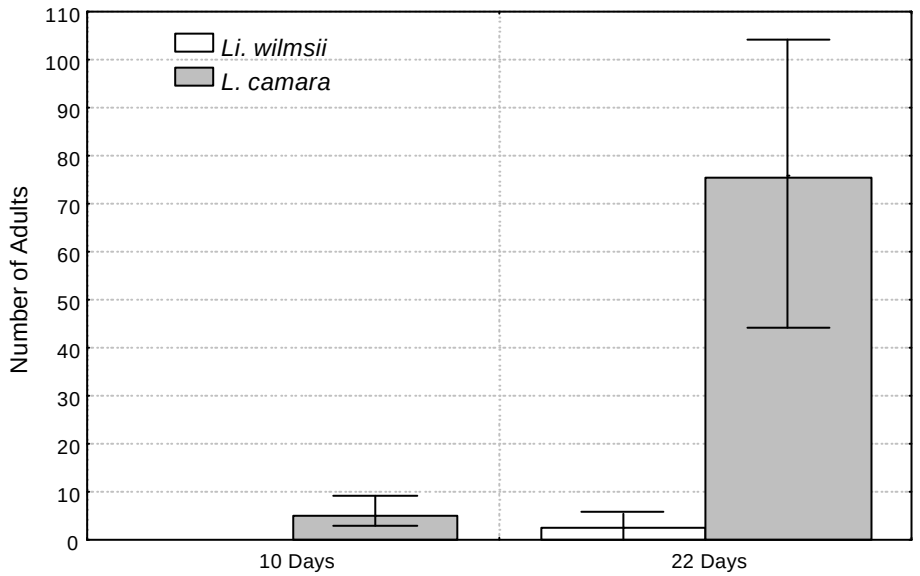


Fig. 11.2 Host location by dispersing adult *Falconia intermedia*, and the preference between *Lantana camara* and the indigenous related plant *Lippia wilmsii* in single-choice field trials.

11.3.2 *Simultaneous-choice field plot*

In the inner circle of plants, adults preferred to disperse to *L. camara* rather than to *Li. wilmsii* (Table 11.2). The mean number of adults on the plants increased as the centre

Table 11.1

The feeding and oviposition preferences of *Falconia intermedia* exposed to *Lantana camara* and the indigenous related plant *Lippia wilmsii* in single-choice field plot trials.[a]

Plant species	*n*	No. Leaves	Leaf area (cm^2)	Area damaged (cm^2)	Percentage damage	Total number of eggs	Number of eggs per leaf
L. camara (163)	6	90.8 (3.7)b	927.2 (62.1)a	245.9 (9.8)a	24.8 (0.8)a	680.5 (18.7)a	7.49
Li. wilmsii	6	159.5 (8.5)a	341.6 (19.4)b	6.1 (1.1)b	1.8 (0.3)b	28.8 (8.0)b	0.181

[a] Sub sample taken from test plants using 5 main branches (≥30cm) and 3 side branches (<30cm), standard errors given in parentheses.

Table 11.2

The numbers of *Falconia intermedia* adults on *Lantana camara* and *Lippia wilmsii* plants planted in two concentric circles with a *Lantana camara* plant in the centre as the source plant.

		INNNER CIRCLE[c]				**OUTER CIRCLE**[c]			
		Lantana camara		*Lippia wilmsii*		*Lantana camara*		*Lippia wilmsii*	
Dates monitored	*n*	Adults[d]	Leaves[d]	Adults[d]	Leaves[d]	Adults[d]	Leaves[d]	Adults[d]	Leaves[d]
3 Days[a]	3	3.3 (0.5)	5.5 (0.8)	0.2 (0.2)	0.2 (0.2)	0.9 (0.3)	1.8 (0.5)	0.0	0.0
6 Days[b]	3	20.8 (1.9)	32.5 (2.5)	1.9 (0.6)	3.5 (1.2)	2.5 (0.5)	5.3 (1.3)	0.8 (0.3)	1.1 (0.5)
9 Days	3	66.2 (3.0)	98.8 (7.6)	5.0 (0.8)	10.3 (1.7)	3.5 (0.5)	16.1 (3.1)	2.2 (0.6)	4.8 (1.2)
12 Days	3	65.8 (3.7)	194.6 (12.7)	7.2 (1.3)	21.8 (2.7)	3.0 (0.6)	24.3 (4.3)	1.3 (0.4)	8.7 (2.1)
Factors recorded		*Lantana camara*		*Lippia wilmsii*					
Leaf area damaged (cm^2 per 30 leaves)[e]	3	215.0 (8.5)		19.1 (1.7)		-		-	
No. eggs (per 30 leaves)[e]	3	275.0 (30.7)		40.1 (6.9)		-		-	

[a] Half the branches of the *Lantana camara* source plant in the centre cut down.

[b] The remaining branches of the *Lantana camara* source plant in the centre cut down.

[c] Total of eight plants, four of each species, planted in each of two concentric circles, 3m and 6m in diameter.

[d] The number of adults and leaves with characteristic feeding damage, observed in a five minute searching period.

[e] Sub sample of 30 leaves from each plant taken after 15 days, to determine the feeding and oviposition intensity; - = no sample taken.

plant was progressively cut down (Table 11.2, [a] and [b]), after which the number of adults on the plants remained relatively constant for the remainder of the trial (Table 11.2). The number of damaged leaves increased over time, and considerably more leaf area per 30 leaves was damaged at the conclusion of the trial on *L. camara* than on *Li. wilmsii* (Table 11.2). In addition, higher numbers of eggs were recorded on *L. camara* than on *Li. wilmsii* (Table 11.2).

Very few adults dispersed to the plants in the outer circle, with similarly low numbers of adults recorded on *L. camara* and *Li. wilmsii* (Table 11.2). However, the number of leaves damaged increased over time on *L. camara*, whereas fewer leaves were damaged on *Li. wilmsii* (Table 11.2). The low number of adults on the outer circle of plants suggests that adults that dispersed beyond the inner circle moved on elsewhere. The results show that *L. camara* is the preferred host plant, and suggest that in mixed stands of *L. camara* and the indigenous *Lippia* species, the insect population on *Lippia* plants would be small compared to that on *L. camara*.

11.3.3 *Natural field-choice*

During field observations, *L. camara* supported a considerably higher *F. intermedia* population than *Li. wilmsii* in the same area. Greater leaf damage (per 30cm shoots) was recorded on *L. camara* than on the two *Li. wilmsii* plants (Table 11.3). Almost nine times more eggs were recorded on *L. camara* than on *Li. wilmsii* (Table 11.3). Although *L. camara* had a greater leaf area per shoot tip than *Li. wilmsii* (Table 11.3), a higher number of eggs were deposited on *L. camara* per unit of leaf area.

11.4 DISCUSSION

Under field conditions, in mixed plots of *Li. wilmsii* and *L. camara* the dispersing *F. intermedia* adults preferred to feed and oviposit on *L. camara*. In close proximity the indigenous species, *Li. wilmsii*, sustained a small amount of feeding damage and a small

Table 11.3
The feeding damage and oviposition preference of naturally dispersing adult *Falconia intermedia* on *Lantana camara* and *Lippia wilmsii* plants in close proximity.[a]

Plant species	No. Leaves	Leaf area (cm^2)	Leaf area damaged (cm^2)	% Leaf damage	Total eggs	No. Eggs per leaf[b]
L. camara	111	1790.4	686.0	38.3	1359	12.2 (1.1)
Li. wilmsii	173	989.6	107.7	10.9	158	0.9 (0.1)
Li. wilmsii	176	1006.7	79.7	7.9	151	0.8 (0.1)

[a] Random sample of ten branches from each plant, 30cm in length from shoot tip.
[b] Standard error given in parentheses.

number of eggs were deposited. In a natural field situation the dispersing adults preferred to locate and oviposit on *L. camara*. Comparatively less feeding damage and a smaller number of eggs were recorded on the *Li. wilmsii* plants adjacent to *L. camara* plants. This suggests that under field conditions *Li. wilmsii* will function as a substitute host in the presence of *L. camara*, but will sustain negligible damage. Laboratory host-specificity tests (Chapters 9, 10) indicated that *Li. wilmsii*, and two other *Lippia* species may function as alternative hosts (Chapter 9). It is therefore likely that these two indigenous species may also sustain low levels of feeding damage and a small number of *F. intermedia* eggs in close proximity to *F. intermedia* populations on *L. camara* infestations.

Under conditions when increasing insect populations lead to a decrease in the availability of suitable feeding and oviposition sites on the preferred host, less preferred plants or plants possessing similar attributes necessary to stimulate host acceptance are theoretically at risk of attack. These plant species that share the same habitat become host substitutes (see Marohasy, 1996). Such conditions have been noted for *Teleonemia scrupulosa* (Heteroptera: Tingidae), when large populations dispersed onto fields of sesame after a sudden reduction in *L. camara* stands in close proximity (Greathead, 1968). Similarly, the beetle *Zygogramma bicolorata* Pallister (Coleoptera: Chrysomelidae) a biocontrol agent of *Parthenium hysterophorus* L. (Asteraceae) caused minor damage to sunflowers after insect populations defoliated the host plants in India (Jayanth *et al*., 1993). The extremely rapid build up of high mirid populations at release

sites in Tzaneen, South Africa (Baars, 2000c), suggests that 'spill-over' effects on *Lippia* species in close proximity to lantana stands are likely to occur. However, such high insect populations will probably persist over short periods of time until the host plants reach a lower state of equilibrium and mirid populations diminish and disperse.

The low incidence of attack on *Li. wilmsii* under field conditions shows that non-target impact occurs. Laboratory host-specificity testing indicated that limited damage was likely to occur, but it was decided that the limited damage by *F. intermedia* on a few *Lippia* species is an acceptable 'trade-off' for the potential impact on *L. camara* (Chapter 9). At some release sites mirid populations have become established and cause extensive damage to plants (Baars, 2000c; see Frontispiece), and the high impact achieved can be viewed as a justifiable 'trade-off' for the potential low levels of attack on *Lippia* species. To determine whether mirid populations will persist on *Lippia* plants in the field in the absence of *L. camara*, no-choice trials should be conducted in field plots and under natural situations.

Chapter 12

GENERAL DISCUSSION

12.1 INTRODUCTION

The success of the biocontrol programme against *L. camara* will depend on a suite of natural enemies that can cope with the extreme variability and wide distribution of the weed in South Africa, without compromising native Verbenaceae. It is apparent that the insect agents currently established on lantana in South Africa do not exert sufficient control and that additional natural enemies are required (Chapters 2, 3). However, almost certainly, without the suite of established agents lantana would present a more serious invasive threat in South Africa. Nevertheless, the project is faced with several challenges in order to improve the biocontrol programme as efficiently as possible by investing in the most promising biocontrol candidates. This study suggests that the lack of control in South Africa is attributed to a combination of factors including the poor selection of agents for release, the accumulation of native parasitoids, incompatibility of agents with the introduced varieties and variable climatic conditions over the weed's range. Consideration is given below to the contribution of each of these factors and how through addressing these factors the biocontrol of *L. camara* may be improved.

12.1.1 *Parasitism*

High rates of parasitism observed for *Calycomyza lantanae* and all but one Lepidoptera species appear to significantly reduce the efficacy of these agents in South Africa (Chapters 2, 3). This suggests that the evaluation of additional lepidopteran species should not be considered as a priority. Although the potential of an agent to recruit native parasitoids is difficult to predict, agents that possess a high risk of parasitism or predation (*e.g. Ophiomyia camarae*) should receive lower priority. However, the possibility of heavy parasitism by native parasitoids should not deter releases of new species or climatypes of natural enemies. Parasitized populations could still inflict significant

damage, provided that the populations are able to proliferate and survive seasonal and other adversities.

12.1.2 *Climate*

It is generally accepted that climate plays an important role in the distribution of weeds, and *L. camara* in South Africa seldom occurs where temperatures frequently fall below 5°C (Cilliers, 1983; Cilliers and Neser, 1991). Other environmental variables such as precipitation and altitude also affect the weed's range and invasiveness (Oosthuizen, 1964; Cilliers, 1983; Muniappan *et al*., 1996). In addition, indirectly climate changes the weed-agent relationships and thus in part dictate the ranges and efficacy of the biocontrol agents on *L. camara*. However, the distribution records (Chapters 2, 3) and suitability models (Chapter 4) suggested that most of the presently established agents tolerate a wide range of climatic conditions. Although most of these agents occurred in low abundance, the insect densities occasionally peaked in localized sites throughout their ranges. Therefore, with a few exceptions importing climatypes of the established agents is unlikely to be effective. A few agents, particularly those dependant on a continuous supply of the leaf resource, are restricted to the areas in the weed's range where the climatic conditions favour year round plant growth. Life-table analyses have suggested that variation in fecundity is an important factor regulating insect population dynamics (Preszler and Price, 1988). Therefore, importing agent climatypes that increase their intrinsic rate of increase (including survivorship and number of generations a year) in response to the climatic conditions may improve their efficacy. However, importing climatypes of the agents dependant on a continuous supply of the leaf resource are unlikely to result in their range extension to temperate conditions. In order to increase insect pressure in the temperate areas new candidates are required that possess attributes that improve their synchrony with the seasonal variability of the weed. The agents need to over winter without sustaining high mortality rates and colonize plants early in the new growing season to prevent plants recovering from the insect damage accrued in the previous season. The low impact levels even in climatic areas favourable for the

establishment of the entire compliment of agents established in South Africa suggest that new agents specifically targeted for the sub-tropical regions also need consideration.

12.1.3 *Overseas survey and candidate selection*

The long lists of natural enemies associated with *Lantana* species in the natural range (Winder and Harley, 1983; Palmer and Pullen, 1995) have encouraged the belief that more successful agents may still be available for consideration. Through isolation the islands in the Caribbean, which fall within the natural range of *Lantana* species are possible centres of speciation and may have endemic species that are worthwhile considering. In Jamaica eight of the natural enemies collected were considered damaging to the plant growth and or flower and fruit production of *L. urticifolia* (Chapter 5). However, the majority of the species collected in Jamaica also occur on the continents nearby, where a comparatively more diverse species pool exists. The small regional species pool in Jamaica suggests that future surveys for potential candidates may be more effective if they are conducted in South, Central and parts of North America as opposed to the Caribbean islands. As Cuba is the largest landmass it may offer a more diverse species pool and surveys on this island may still prove useful.

Selecting the most effective candidate agent may constitute a considerable saving in time and resources. Using the proposed selection system described in Chapter 5 six of the natural enemies collected in Jamaica were assigned a high priority, and *A. lantanae* and *F. intermedia* are the most promising of these candidates (Table 5.7). To determine what the priority of the agents already established in South Africa would be, they were ranked according to the proposed selection system (for Materials and Methods see Chapter 5). With one exception, the species indigenous to South Africa were omitted from this analysis. The indigenous moth *A. onychote* was substituted during this analysis by a comparable leaf miner *C. lantanella* from the native range (see Chapter 2) to determine its probable score. The majority of the established agents were assigned low scores using the selection system, and thus are rank as low priority candidates (Table 12.1). The agents, such as *T. scrupulosa*, *O. scabripennis* and *U. girardi* that cause

Table 12.1

The ranking of the established biocontrol agents on *Lantana camara* in South Africa and two new biocontrol candidates, using parameters important in the selection and prioritisation of candidate biocontrol agents.[a]

	Parameters related to biocontrol candidate							Parameters related to weed			
Natural enemy species	Agent damage/ individual	Intrinsic rate of increase	Range geographic/ climate	Host range	Syncrony with weed aspects	Dispersal ability	Predation/ Parasitism Risk	Impact on Weed Ecology (growth and repro.)	Suitability of host varieties		
Weighting of parameter[b] (Range of scores)[c]	x4 (0 to 2)	x2 (0 to 2)	x2 (0 to 2)	x1 (-1 to 1)	x1 (0 to 2)	x1 (0 to 2)	x1 (-2 to 0)	x4 (0 to 2)	x1 (-1 to 1)	Total[d]	Rank[d]
Epinotia lantana	1	1	2	1	0	1	-2	0	1	11	9
Salbia haemorrhoidalis	1	1	2	1	0	2	-2	1	0	15	6
Lantanophaga pusillidactyla	0	1	2	0	0	1	-1	0	1	7	10
Cremastobombycia lantanaella[e]	0	1	1	1	0	1	-2	0	1	5	11
Calycomyza lantanae	1	2	2	1	0	2	-2	0	1	14	7
Ophiomyia lantanae	0	2	2	1	2	2	-2	0	1	12	8
Teleonemia scrupulosa	2	2	2	-1	0	1	0	1	1	21	3
Octotoma scabripennis	2	1	1	1	0	0	0	1	1	18	4
Uroplata girardi	2	1	1	1	0	0	0	1	1	18	4
Coelocephalapion camarae	2	1	1	1	2	1	0	2	1	**31**	**1**
Falconia intermedia	2	2	1	1	2	1	0	2	1	**27**	**2**

[a] Parameters identified as important are consistently emphasised in the weed biocontrol literature (see Chapter 5).

[b] Scores assigned to the parameter are multiplied by the weighting, parameters weighted according to the emphasis received in the weed biocontrol literature (see Chapter 5).

[c] Scores assigned have a three fold range, and either have a positive or negative influence on the selection of the candidate.

[d] Scores/rankings highlighted for the two new biocontrol candidates.

[e] *Aristaea onychote* substituted by this serpentine leaf-miner from the weed's natural range.

considerable damage to *L. camara* in South Africa are assigned high scores and are thus ranked as priority candidates in the selection system. If the release programme were initiated again this analysis indicates that only a few of the presently established agents would be amongst the first agents to be considered. This suggests that, in hindsight, a poor selection of agents were released in South Africa and in part may explain the poor levels of control achieved, despite the large number of agents released. However, several candidate natural enemy species from the countries of origin are predicted to have the potential to improve the biocontrol of *L. camara* and bode well for the viability of the programme in South Africa (Palmer and Pullen, 1995; Chapter 5). The two new candidates, *C. camarae* and *F. intermedia* evaluated in this study are assigned the highest scores using the selection system (Table 12.1) and are predicted to considerably improve the levels of damage to the weed.

12.1.4 *Host compatibility and variety preferences*

Due to the extreme variability of the species *L. camara* presents a formidable target for biological weed control. The varieties in the introduced range, although they share many similar characteristics, were derived from the hybridization of an unknown source of *Lantana* species from the native range (Stirton, 1977) and are not comparable to any one of the parent species. The natural enemies used as biocontrol agents were therefore collected from a number of parent species and released against the complex of 'new' varieties in the introduced range. The mismatch between the agent from the natural range and the weed in the introduced range has been used to explain the poor establishment and control success of agents. Surprisingly, there is a complete lack of comparative studies between the performance of agents on the parent species and the introduced varieties of the weed. An analysis of the source of biocontrol agent introductions suggests that high success rates were achieved when collections were made from certain parent species, like *L. urticifolia* (Day and Neser, 2000). This implies that the varieties in the introduced range possess attributes that are more similar to certain parent species, and agents collected from these are more compatible with the introduced varieties of the weed. However, *U. girardi* is an agent that was collected from *L. tiliaefolia*, a parent species

considered dissimilar to the introduced varieties, and is one of the most successful of the biocontrol agents. Furthermore, the results of this study show that despite the close association between *C. camarae* and *L. camara* (*i.e.* gall former) this agent performs equally well on the natural host *L. camara* from Mexico as it does on the introduced varieties from South Africa (Chapter 7). The results from the preference studies on *C. camarae* also suggest that the introduced varieties possess similar host recognition stimuli to that of the natural host.

It is likely however that the agent-weed compatibility varies between agents and may be different between countries, as the weed varieties in different countries have probably originated from different sources. Indeed, performance studies on *F. intermedia* showed that there is a large difference between the Australian and South African varieties (Chapter 10). This is further supported by the variability between countries in the rearing success of agents (Palmer and Pullen, 1998), differences in the performance of agents (Day *et al.*, 1999; Urban and Simelane, 1999) and variation in floral and vegetative characters (Smith and Smith, 1984; Stirton and Erasmus, 1990). Until we understand the factors that influence the compatibility between the agent and weed variety it is important in the short-term that new candidate agents are collected from a variety of parent species. However, compatibility studies need to become a standard procedure in the screening process of new candidate agents for the biocontrol of *L. camara*. Furthermore, host range surveys in the country of origin may provide useful information on the association of the natural enemies with the various *Lantana* species. In addition, by exposing the introduced varieties to the suite of natural enemies in the country of origin, agents that are more compatible with the weed varieties may be pre-selected before screening (Neser and Cilliers, 1990). This coupled with performance and preference studies on the parent species under laboratory conditions may improve our understanding of the interactions between the agent and weed complex.

The large variation in the general morphology, cytology and genetic composition of the introduced lantana varieties raises the possibility that the biocontrol agents are better adapted to some varieties than others. Indeed, earlier reports of the variation in the performance of agents supported this contention. However, this study indicated that varietal resistance may have been over-estimated and that its relative importance in

explaining the poor performance of agents should be reconsidered (Chapter 3). This study showed that *C. camarae* and *F. intermedia* performed equally well on the South African varieties and *C. camarae* showed no preference between varieties in a multiple choice situation (Chapters 7, 10). Although *F. intermedia* adults preferred some varieties to others, its preferences were not expected to reduce its biocontrol potential in South Africa. Varietal performance and preference studies in the laboratory and in field plots using the established agents provide the opportunity to clarify the importance of this phenomenon. It is important that candidate agents utilize most or all of the lantana varieties in South Africa to ensure its establishment success and impact. Therefore the performance and preference studies will form an important part of the screening process of future candidate biocontrol agents.

12.1.5 *Non-target impact*

Recent host-specificity tests have indicated that most of the natural enemies currently under evaluation for the biocontrol of *L. camara* accepted related native plant species to varying degrees under laboratory conditions (Baars, 2000a, 2001). Ironically, natural enemies selected for the biocontrol of lantana are required to cope with the variability of the weed, but are also required to not accept closely related species. Two species in the genus *Lantana* and six in the genus *Lippia* native to South Africa (Arnold and De Wet, 1993; Chapter 8) are generally accepted as hosts by the candidate agents under laboratory conditions. Some insect species *(e.g. A. compressa* and *C. pygmae*a), which were acceptable for release in Australia (Julien and Griffiths, 1998), may accept and marginally affect these non-target indigenous species in South Africa (Baars and Neser, 1999), and has led to their rejection as agents (Heystek and Baars, 2001).

To reconcile fundamental and realized host ranges (see Van Klinken, 2000), laboratory and field studies were conducted on species that have been established in South Africa for decades. The tingid, *T. scrupulosa,* displayed non-host specific behaviour in the laboratory, feeding and developing on a wide range of species within the Verbenaceae (Baars, 2000a). However, preliminary studies provided no evidence of an extended host range in the field where *T. scrupulosa* was observed to display only limited

feeding on some native *Lippia* species (Baars and Neser, 1999). These studies provided further evidence of the conservative nature of laboratory tests and suggest that extended laboratory host ranges are often, as in many biocontrol programmes worldwide, not realized under field conditions. To understand the progression in the severity of attack on related species from confined to natural conditions its important that established species are brought back into the laboratory and no-choice, multi-choice and walk-in cage tests are conducted.

This study indicated that although the indigenous *Lippia* species were acceptable to *C. camarae* and *F. intermedia* during laboratory trials, performance was poor relative to that on *L. camara* (Chapters 8, 9). Risk assessment using the insect's oviposition preference and nymphal survival on test plants relative to that on *L. camara*, can provide a useful tool to facilitate a decision on whether to release or reject a biocontrol candidate (Chapter 9). However, the release of an agent invariably presents an element of risk (Chapter 10). One solution to determining the acceptability of expanded laboratory host ranges would be to accept possible limited feeding on such non-target species in the field, as an ecologically justifiable 'trade-off ' against the benefits of releasing agents that have the potential to suppress such an environmentally damaging target weed. However, the data required to show the ecological impact of *L. camara* is mostly lacking. Alternatively, the number of new natural enemies that will ultimately be considered acceptable for release in South Africa will be limited, with obvious constraints on the biocontrol programme.

12.1.6 *Biocontrol candidates*

The attributes of both *C. camarae* and *F. intermedia* suggest that they have exceptional potential as biocontrol agents. The petiole-galling weevil is likely to persist throughout the weed's range in South Africa and through facultative diapause is well suited to survive the periods of leaflessness and colonize plants early in the growing season (Chapter 6). The lantana mirid relies on a continuous supply of the leaf resource to survive and therefore will probably, like the other folivores only establish in the sub-tropical range of the weed (Chapter 9). Even in this potentially restricted range, the mirid

is predicted to make a significant contribution by increasing the levels of damage in areas where the weed maintains its leaf resource. Both of these new candidate agents cause high levels of impact by reducing the photosynthetic capacity of the weed, but achieve this in different ways. *Coelocephalapion camarae* larvae feed on the vascular tissue of the leaf-petioles which disrupt the transport of solutes, and the adults and nymphs of *F. intermedia* aggregate on leaves to cause chlorotic speckling. Therefore, *C. camarae* achieves the high levels of damage through its high impact per individual, whereas *F. intermedia*, although each individual can be relatively damaging, achieves the high levels of damage by its high intrinsic rate of increase. Furthermore, the adults of *C. camarae* use oviposition markers, which improves their potential for optimal resource utilization by avoiding conspecific eggs. The adults, although selective in their choice of suitable leaf-petioles utilized lantana leaves of all ages, which suggests that the entire compliment of leaves on branches on field plants are potentially at risk of attack. This study also showed that the South African lantana varieties were equally suitable to both species, and although the lantana mirid preferred certain varieties under multi-choice conditions, both of the agents were predicted to cope with the complex of introduced lantana varieties.

12.1.7 *Implementing biocontrol of* Lantana camara

The results of this study suggest that there are three main factors that largely determine the potential complexity of a biocontrol programme on *L. camara* in a country of introduction. These include (a) the number of native Verbenaceae present in the country, (b) the amount of variation in the climatic conditions over the geographic range of the weed, and (c) the diversity of the complex of weed varieties naturalized in the country. These factors were used to build a predictive model (Fig. 12.1) to determine the prospects of achieving successful biocontrol of lantana in different countries and thus identify the strategy to be employed to implement an effective programme. *Lantana camara* is a serious invasive weed in most countries in Africa, the Indo-Malaysian region, the South Pacific Islands and in the eastern parts of Australia and the considerations regarding each of the three factors are different in each country.

The model is based on the rationale that as the complexity of each factor reduces, the prospects of implementing and achieving successful biocontrol improves (Fig. 12.1). For example, host range trials in this study (Chapters 8, 9) and in studies on other agents (Baars 2000a; Simelane, 2001; Baars, 2002) indicate that the natural enemies imported into South Africa for the biocontrol of *L. camara* usually accept related indigenous non-target species during confined laboratory trials. Detailed host range trials are then required to determine the probability of such non-target impacts occurring under field conditions. This severely delays the project, and reduces the number of candidate agents that would ultimately prove acceptable for release (Baars and Neser, 1999; Heystek and Baars, 2001). Countries with a few or no native Verbenaceae would therefore benefit form a larger selection of potentially safe candidate biocontrol agents. Also, the time taken to screen the candidate agents in these countries may be relatively short. Indeed, two of the biocontrol agents released in Australia (Palmer *et al*., 1996; Day *et al*., 1999) severely damage species native to South Africa and may be rejected for introduction onto the African continent. Furthermore, the host screening of each of these two agents required several scientist's years before ultimately being rejected. Therefore, the prospects of implementing a biocontrol programme in countries such as St. Helena, an Atlantic Ocean island that has no native Verbenaceae (S. Neser, personal communication), is greatly improved in comparison to countries in Africa.

The variation in climate can also influence the prospects of biocontrol, because in countries with a large variation in the climatic conditions over the range of the weed, the biocontrol agents are required to adapt to the seasonal changes (Chapters 2, 3, 4). In South Africa some of the agents are unable to establish in the temperate conditions and new agents with the potential to cope with the seasonal leaflessness of plants are prioritized. On islands where the climatic conditions are moderate the biocontrol of *L. camara* has been rated to be substantial (Muniappan *et al*., 1996) and in some instances complete (Denton *et al*., 1991) and the control is attributed largely to folivores. The model (Fig. 12.1) thus predicts that the prospect of biocontrol improve in countries where the variation in climatic conditions is small.

Due to the disparity between the parent species in the native range and the weed varieties of *L. camara* the biocontrol agents introduced may be poorly adapted to survive

and reproduce on the 'new' host. The model (Fig. 12.1) predicts that in countries where the source of weed varieties is more diverse agents are presented with a large difference in plant characters, which increase the chances of incompatibility between the agent and the weed. On islands, the introduction of weed varieties is arguably less diverse and the agents are more likely to establish and impact on the small number of varieties equally. However, the importance of this factor is still under question as the two new agents in this study show good compatibility with the large number of weed varieties from South Africa.

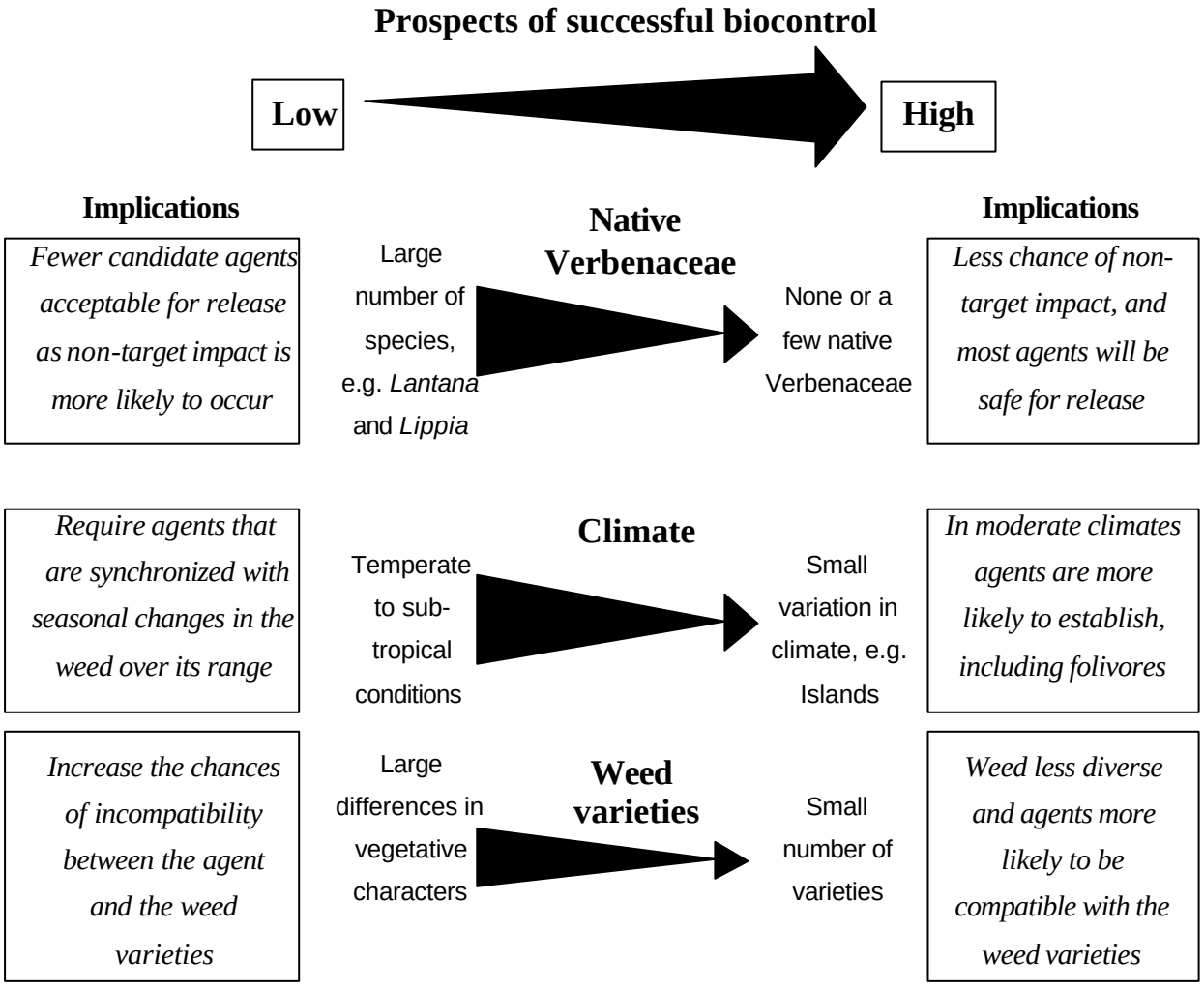


Fig. 12.1 Diagram showing the important factors that influence the prospects of successful biocontrol of *Lantana camara*.

The model (Fig. 12.1) predicts that in a country with a small number of native Verbenaceae, a small variation in climatic conditions and a few weed varieties, like Rodrigues (Mauritius) (Fowler *et al*., 2000) the prospect of successful biocontrol of *L. camara* are very high. Whereas, in a country with a large number of native Verbenaceae, a large variation in climatic conditions and a number of weed varieties, like South Africa the prospects are low. Nevertheless, by selecting agents to address each of these factors as efficiently as possible the prospects of biocontrol, as this study has suggested, is likely to improve in countries such as South Africa.

12.2 CONCLUSION

Inevitably the success of the programme on lantana will be compared to other programmes that on average require 2 to 3 agents to achieve success (Anonymous, 1999; Cruttwell McFadyen, 2000) or weeds that are suppressed by only one agent (Cilliers, 1991a, 1991b; Hill, 1999). From this study its clear that the biocontrol of *L. camara* is extremely complex with a number of factors that reduce the prospect of successful biocontrol. Although biocontrol researchers are still in search for the 'silver bullet', early assessments of the programme suggested that the biocontrol of lantana would rely on a suite of agents and remains to be the case. The lack of success has been attributed to several factors in this study, and instead of searching for the perfect agent new candidates need to be evaluated to address each of these limiting factors as efficiently as possible. Additional research is needed to improve our understanding in many aspects, including the compatibility of the agent and weed complex, the importance of preferences between varieties, the role of climate in the establishment and spread of agents and particularly in South Africa and other African countries the acceptability of closely related species (*e.g. Lippia* species) under laboratory conditions and the realization of extended host ranges under field conditions. Despite the problems associated with the programme, *Lantana camara* remains a candidate for biological control in South Africa.

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