The suitability of *Alagoasa extrema* Jacoby (Coleoptera: Chrysomelidae: Alticinae), as a biological control agent for *Lantana camara* L. in South Africa

THESIS

Submitted in fulfilment of the requirements
for the Degree of
MASTER OF SCIENCE *Ad Eundem Gradum* (Science)
of Rhodes University

by

HESTER ELIZABETH WILLIAMS

Department of Zoology and Entomology
Rhodes University
Grahamstown, South Africa

February 2003

ABSTRACT

Lantana camara Linnaeus (Verbenaceae), commonly known as lantana, is a highly invasive weed in many parts of the world. In South Africa it is naturalized in several provinces where it invades pastures, riverbanks, mountain slopes and valleys and commercial and natural forests, forming dense, impenetrable thickets. Chemical and mechanical control methods are expensive, labour intensive and provide only temporary relief as cleared areas are rapidly reinfested by seedlings and coppice growth. A biological control programme was initiated in South Africa in the 1960s, but despite the establishment of 11 agent species, it was considered to have had limited success. Several factors are thought to restrict the impact of the biocontrol agents. Firstly, *L. camara* occurs in a range of climatic regions, some of which are unsuitable for the establishment of agent species of tropical and subtropical origin. Secondly, *L. camara* is the result of hybridization between several *Lantana* species, forming a complex of hybridized and hybridizing varieties in the field, which match none of the *Lantana* species in the region of origin. This causes partial insect-host incompatibility, displayed as varietal preference. Thirdly, parasitism appears to have significantly reduced the effectiveness of several natural enemies. In spite of all these constraints, biological control has reduced invasion by *L. camara* by 26%. However, the weed is still very damaging and additional natural enemies are required to reduce infestations further.

A flea-beetle species, *Alagoasa extrema* Jacoby (Coleoptera: Chrysomelidae), was collected from several sites in the humid subtropical and tropical regions of Mexico, and imported into quarantine in South Africa and studied as a potential biocontrol agent for *L.*

camara. Favourable biological characteristics of this beetle included long-lived adults, several overlapping generations per year, and high adult and larval feeding rates. Observations from the insect's native range and studies in South Africa suggest that *A. extrema* would probably be more suited to the subtropical, rather than the temperate areas in South Africa.

Laboratory impact studies indicated that feeding damage by *A. extrema* larvae, over a period spanning the larval stage (16 to 20 days), reduced the above-ground biomass of *L. camara* plants by up to 29%. Higher larval populations resulted in a higher reduction of biomass.

Varietal preference and suitability studies indicated that *A. extrema* exhibits a degree of varietal preference under laboratory conditions, with one of the white pink *L. camara* varieties proving the most suitable host. This variety is one of the most damaging varieties in South Africa and is particularly widespread in Mpumalanga Province.

Although *A. extrema* proved to be damaging to *L. camara*, laboratory host range trials showed it to be an oligophagous species, capable of feeding and developing on several non-target species, especially two native *Lippia* species (Verbenaceae). The host suitability of these species was marginally lower than that of *L. camara* and the potential risk to these indigenous species was deemed to be too high to warrant release. It was therefore recommended that *A. extrema* not be considered for release in South Africa.

ACKNOWLEDGEMENTS

I am grateful to H.R. Hepburn for accepting me as a Masters candidate, and to M.P. Hill for his patience, guidance and support as mentor. Also my thanks to T. Olckers, A. Urban and F. Heystek for useful suggestions and comments. Thanks are due to L. Morey and M. Smit of the ARC-Biometry Unit for assisting me with the statistical side of my work. Finally, I am grateful towards L. Madire for her technical assistance. Several organizations have provided financial support, including National Department of Agriculture, Department of Water Affairs and Forestry and the Agricultural Research Council of South Africa.

TABLE OF CONTENTS

CHAPTER 1

GENERAL INTRODUCTION

1.1 Distribution, weed status and taxonomy

Lantana camara Linnaeus (Verbenaceae), commonly known as lantana, is a highly invasive weed that originates from South and Central America (Stirton 1977; Spies 1984; Baars and Neser 1999). As a decorative ornamental, several varieties (cultivars) of lantana have been widely distributed throughout the tropics, subtropics and warm temperate regions of the world. Lantana has at present an almost cosmopolitan distribution and is rated as one of the world's worst weeds (Holm *et al*. 1977). In South Africa it is naturalized in regions of the Limpopo, Gauteng, Mpumalanga and KwaZulu-Natal provinces, as well as the southern coastal regions of the Eastern and Western Cape provinces (Baars and Neser 1999) (Fig 1.1).

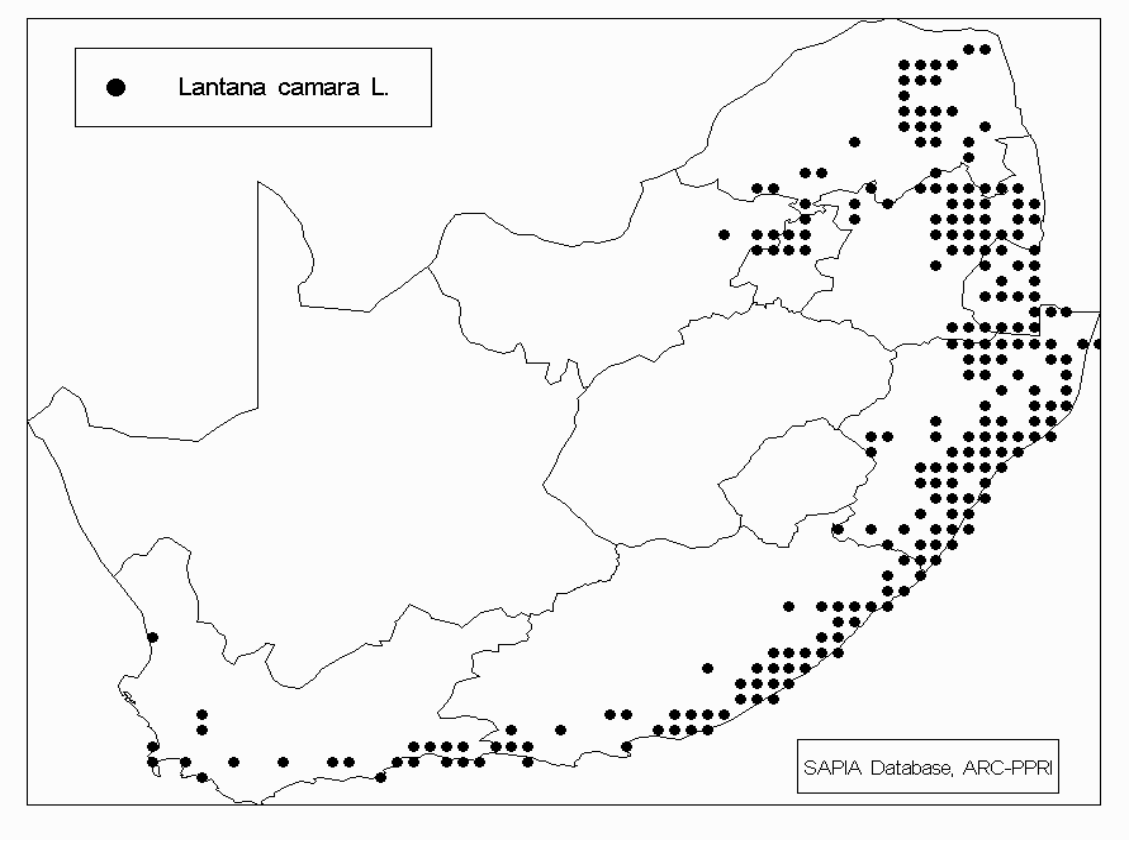

Fig. 1.1: Distribution of *Lantana camara* in South Africa.
(Map drawn by L. Henderson, Plant Protection Research Institute).

Lantana invades pastures, riverbanks, mountain slopes and valleys and commercial and natural forests, forming dense, impenetrable thickets (Cilliers and Neser 1991; Baars and Neser 1999). Together with this aggressive growth, lantana also suppresses surrounding plant species through allelopathy, and therefore interrupts regeneration processes (Gentle and Duggin 1997a) and reduces the biodiversity of natural ecosystems (Baars and Neser 1999). Lantana is poisonous to animals and, if consumed, can cause photosensitivity of mucous membranes, liver and kidney damage, paralysis of the gall bladder, intestinal haemorrhage and death within 1 to 4 days in cattle, sheep and horses (Kellerman *et al.* 1996). In 1996, the impact of cattle mortalities from lantana poisoning in South Africa was estimated to be in excess of US$ 160 000 (Kellerman *et al.* 1996).

Lantana camara presents a complex taxonomic problem owing to the considerable variation in the plant's morphological characteristics and physiological and genetic composition. Stirton (1977) and R. Sanders (unpublished data – in Day and Neser 2000) suggest that *L. camara* is a hybrid species and part of a complex, consisting of several species of *Lantana*, all morphologically similar, but with visible variations in flower colour, spininess of the stems and hairiness of the leaves. As a result of hybridization, there is no naturally occurring species that matches any of the lantana varieties occurring in South Africa, or elsewhere in the world (L.S. Smith, unpublished data – in Day and Neser 2000). Ongoing field hybridization results in the continuous production of new varieties (Spies 1984; Cilliers and Neser 1991). This has led to the description of hundreds of different varieties (cultivars) (Spies 1984). The varieties can be distinguished, either morphologically by differences in the flowers (size, shape and colour), leaves (size, colour and hairiness), and stems (degree of spinescence) (Smith and Smith 1982). Physiologically they can be distinguished by differences in rates of growth and general vigour (Spies and Stirton 1982a,b), chromosome numbers (Spies and Stirton, 1982a), degree of toxicity to livestock (Everist 1974, Hart *et al.* 1976, Kellerman and Coetzer 1984, Swarbrick 1986), and fertility and cytology (Spies and Stirton 1982a,b,c Spies 1984, Spies and du Plessis 1987). Wells and Stirton (1988) stated that no two individuals in hybrid colonies were the same and that each was unique. Day and Neser (2000) reported that over 650 recognised horticultural varieties are in existence

worldwide and Graaf (1986) estimated up to 40 occurring in South Africa. Table 1.1 lists the 10 most important and widespread varieties in South Africa (J-R. Baars and C.J. Cilliers, pers. comm.). These varieties are traditionally used during laboratory culturing and the screening of potential biological control agents in South Africa.

Table 1.1: A list of 10 of the most important widespread *Lantana camara* varieties in South Africa.

***Lantana camara* variety**	**Distinguishing morphological characteristics**	**Mature flower colour**	**Collection Site**	
			Grid reference	**Location**
163 LP*	Shoot tips hairy and spiny; leaves very hairy; main stem with few spines	Light Pink	30º 09’ 08.4”S 30º 49’ 39.7”E	nr. Scotsburgh, KwaZulu-Natal
150 O	Scrambling shrub, shoot tips hairy, spiny and reddish in colour; leaves small and hairy	Orange	29º 38’ 45.9”S 31º 07’ 39.5”E	nr. La Merci, KwaZulu-Natal
015 WY	Shoot tip spiny; large broad dark hairy leaves; main stem spiny	White (Yellow throat)	25º 02’ 21.6”S 31º 02’ 19.8”E	nr. Sabie, Mpumalanga
010 DP	Shoot tip spiny; leaves small, dark and hairy; main stem very spiny	Dark Pink	25º 37’ 08.3”S 30º 31’ 12.1”E	nr. Waterval-Boven, Mpumalanga
029 WP	Shoot tip spiny; large broad dark hairy leaves, main stem spiny	White Pink	25º 08’ 10.6”S 31º 00’ 09.0”E	nr. Hazyview, Mpumalanga
009 LP	Shoot tip spiny; leaves hairy; main stem spiny	Light Pink	25º 35’ 13.7”S 30º 27’ 08.5”E	nr. Sycamore, Mpumalanga
017 O	Shoot tip hairy, spiny and reddish in colour; leaves hairy and small; hairy main stem with few spines	Orange Red	25º 03’ 17.1”S 30º 57’ 03.6”E	24 km east of Sabie, Mpumalanga
021 LP	Shoot tip hairy; leaves broad and hairy; main stem spiny	Light Pink	24º 59’ 30.2”S 31º 14’ 34.8”E	8km east of Sabie, Mpumalanga
113 DP	Shoot tip hairy; dark hairy leaves; main stem spiny	Dark Pink	27º 53’ 37.0”S 31º 38’ 27.6”E	50km sth of Pongola, KwaZulu-Natal
018 DP	Very hairy shoot tip; woolly leaves; main stem hairy with few spines	Dark Pink	25º 07’ 04.9”S 30º 45’ 39.2”E	nr. Sabie, Mpumalanga

*Varieties are named according to their collection waypoint number and mature flower colour abbreviation.

1.2 Control Options

1.2.1 Chemical and Mechanical control

Herbicides and mechanical control methods are expensive, labour intensive and provide only temporary relief as cleared areas are rapidly reinfested by seedlings and coppice growth, and are therefore only effective with continued follow-up treatments (Baars and Neser 1999). Controlled low- to moderate-intensity fires appear to reduce invasions by *L. camara*, and can be an effective, preventative management strategy (Gentle and Duggin 1997b). However the use of fires might not always be a suitable option as infestations are often near to, or in, indigenous forests, grazing lands and plantations.

1.2.2 Biological Control

Since the initiation of the biological control programme against *L. camara* during 1961/62 (Baars and Neser 1999), 20 insect species have been released as biological control agents against this invader in South Africa (Table 1.2) (Julien and Griffiths 1998; Baars and Neser 1999). Despite the establishment of 11 of these species (3 of which were already present in South Africa prior to deliberate introduction) (Julien and Griffiths 1998), the biocontrol programme was considered to have had limited success (Cilliers and Neser 1991; Baars and Neser 1999). Almost from the onset of the programme it was realized that biological control of *L. camara* in South Africa would be hard to achieve (Neser and Annecke 1973; Cilliers and Neser 1991). This was proven to be the case, as not only did several of the introduced species fail to establish, but those species that did, were relatively ineffective (Cilliers and Neser 1991). The limited success of the biocontrol programme on the whole, can be attributed to a number of factors, some of which are discussed below.

Table 1.2: Natural enemies released on *Lantana camara* in South Africa and their current status.

Natural enemy species	Origin	Main releases	Mode of attack	Status	Damage inflicted
Coleoptera: Cerambycidae					
Plagiohammus spinipennis (Thompson)	Mexico via Hawaii via Australia	1973	Stem-borer	Not established	-
Coleoptera: Chrysomelidae					
Alagoasa parana (Samuelson)	Brazil via Australia	1985	Leaf chewer	Not established	-
Octotoma championi (Baly)	Costa Rica via Australia Central America via Australia	1978 1995	Leaf miner	Establishment unconfirmed	Unknown
Octotoma scabripennis (Guérin-Méneville)	Mexico via Hawaii via Australia	1971 and 1974 and 1981	Leaf miner	Established in the moist, warm eastern range of lantana. Abundant in localized inland areas	Extensive defoliation, but localized
Uroplata girardi (Pic)	Paraguay via Hawaii via Australia	1974 1983	Leaf miner	Established, abundant in KwaZulu-Natal coastal regions. Present in low numbers in warm, moist inland range of lantana	Extensive defoliation in coastal regions
Uroplata lantanae (Buzzi and Winder)	Brazil via Australia	1984	Leaf miner	Not established	-
Uroplata fulvopustulata (Baly)	Costa Rica via Australia	1978	Leaf miner	Not established	-
Diptera: Agromyzidae					
Calycomyza lantanae (Frick)	Trinidad via Australia Florida USA	1982 1989	Leaf miner	Widely established in low numbers, heavily parasitized	Unknown
*Ophiomyia lantanae** (Froggatt)	Mexico via Hawaii	1961	Fruit miner	Widely established and abundant but heavily parasitized	Low impact on seed viability
Diptera: Tephritidae					
Eutreta xanthochaeta (Aldrich)	Mexico via Hawaii	1983	Stem galler	Not established	-
Hemiptera: Miridae					
Falconia intermedia (Distant)	Jamaica	1999	Leaf sucker	Newly released	Unknown
Hemiptera: Tingidae					
Teleonemia elata (Drake)	Brazil via Australia	1972	Flower and leaf sucker	Not established	-

Table 1.2 continued

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

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

1.2.2.1 Effect of climate

Lantana camara varieties occur over a broad range of climatic regions in South Africa, ranging from the winter rainfall regions of the Western Cape, to the subtropical and tropical southern and eastern regions of KwaZulu-Natal and Mpumalanga, and to the dry, frosty winter highveld regions of Limpopo, Gauteng and North West provinces (Fig. 1.1) (Henderson 2001). The most severe infestations of *L. camara* are found in the subtropical and tropical areas, but the plant is able to cope very well in areas with cold winters and/or low rainfall by abscising its leaves. Most of the biological control agents originated in tropical to subtropical regions and establishment of these species in cold inland regions was not very successful. The periods of leaflessness have a substantial impact on the biocontrol agents' population numbers, especially leaf-feeders, when no food is available during this time. This is firstly due to an inability to cope with harsh winter temperatures, and secondly, these insect species, being of tropical origin appear to have no mechanisms, such as pupal or adult diapause, to overwinter without food. It seems that harsh winter conditions reduce populations either to extinction or to levels so low that they are unable to build up rapidly enough in spring and summer to have any real effect on the plant. Most agents established in South Africa are found in discrete areas, with climate considered as the principal contributing factor to their distribution, as many areas contain more than one *L. camara* variety, and varietal preference is therefore less likely to be the limiting factor (Day and Neser 2000).

There is some evidence that cold adapted 'strains' of these agents might have evolved in their native ranges. Several 'strains' of an insect species might evolve in its native country, each strain adapted to a specific set of environmental and physiological conditions. In Australia, *Calycomyza lantanae* (Frick) (Agromyzidae) was initially presumed to have established in Northern Queensland only, and it was suggested that the fly was unable to survive through the subtropical winters elsewhere (Willson 1979, Waterhouse and Norris 1987). However, in 1981, *C. lantanae* was found to be established in New South Wales and Taylor (1989) suggested that a climatically adapted strain may have arisen. Climatic maladaptation may also explain the delayed

establishment of *C. lantanae* in the inland areas of South Africa (Cilliers and Neser 1991).

1.2.2.2 Effect of original host species and varietal differences in *Lantana camara*

Lantana camara is the result of hybridization between several lantana species, forming a complex of hybridizing varieties in the field. None of the *Lantana* species in the center of origin resembles any of the lantana varieties occurring in South Africa. This means that the varieties that introduced insect biocontrol agents encounter in the field, could be genetically far removed from the naturally occurring *Lantana* species. Therefore incompatibility or partial incompatibility is to be expected (Baars and Neser 1999). Scott (1998) (in Day and Neser 2000) suggested that varieties of *L. camara* in different countries may have originated from different *Lantana* species, as DNA studies have shown that varieties found in Hawaii, for example, are different from those found in Australia. These differences in varieties between countries may partly explain why some agents have established in some countries but not in others (Day and Neser 2000). For many years entomologists have surveyed, sampled and collected potential agents from a number of lantana entities (including *Lantana urticifolia* Miller, *Lantana tiliifolia* Chamisso, *Lantana hirsuta* Mart.and Gal. and *Lantana fucata* Lindley) in Mexico, the Caribbean and Brazil (Day and Neser 2000). Through DNA studies, Scott (1998) indicated that *L. camara* varieties in Australia have the closest affinity to *L. urticifolia* and *L. tiliifolia*. Day and Neser (2000) state that agents collected from host species closely related to the relevant *L. camara* varieties are more successful in establishing and that future research should give priority to collecting potential agents from the closest related species. At this stage it is still unknown as to which *Lantana* species the South African varieties are the most closely related, and further research is still needed in this regard.

It has been reported that different lantana varieties have influenced the performance of several insect biocontrol agents and that preferences for certain varieties of lantana are displayed (e.g. *Teleonemia scrupulosa* and *Calycomyza lantanae*) (Radunz 1971; Harley and Kassulke 1974; Harley *et al*. 1979; Cilliers 1987; Cilliers and Neser 1991). To

overcome this problem a suite of insect natural enemy species, adapted to all the varieties will be needed to control *L. camara* (Cilliers and Neser 1991; Baars and Neser 1999). Insect agents currently established on lantana in South Africa do not exert sufficient control on all varieties and additional natural enemies are thus required.

1.2.2.3 Effect of parasitism

Parasitism appears to significantly reduce the effectiveness of several natural enemies. Oosthuizen (1964) reported that in Hawaii, numbers of the agromyzid seedfly *Ophiomyia lantanae* (Froggatt) were kept in check by two hymenopterous parasitoids. In South Africa, Cilliers (1987) found a number of parasitoid species belonging to four families, emerging from samples of *O. lantanae* infested fruits. Although the influence of these parasitoids was not quantified, they seemed likely to have had a considerable effect on *O. lantanae* populations. The leaf-mining agromyzid fly, *C. lantanae* is widely established in the subtropical and temperate regions of South Africa, but observations indicate that the insect's impact is reduced by extensive larval parasitism (Baars and Neser 1999). *Cremastobombycia lantanella* (Busck), a leaf-mining gracillariid, is also widely established in South Africa, but populations never reached outbreak populations as it suffers extensive parasitism (Baars and Neser 1999). Numbers of the noctuid moth, *Hypena laceratalis* (Walker), thought to be native to southern Africa, are kept in check in South Africa by pathogens and several parasitoid species that attack the larvae (Oosthuizen 1964). In contrast, this insect is a very successful biological control agent in Hawaii (Julien and Griffiths 1998), which is probably due to the absence of African natural enemies (Cilliers and Neser 1991). Extensive parasitism by native generalist parasitoids might be the reason why *Eutreta xanthochaeta* (Aldrich), a stem-galling fly, failed to establish in South Africa (Baars and Neser 1999), as high rates of parasitism have also been reported in other countries where it has become established (Daun and Messing 1996).

1.2.2.4 Other factors

Cilliers and Neser (1991) proposed several additional reasons, although unsubstantiated, for establishment failures. These are i) herbicidal or mechanical destruction of sites

before the newly released agents had a chance to become established and to disperse (e.g. *Octotoma championi* Baly, *Uroplata lantanae* Buzzi and Winder, *Neogalea esula* (Druce)); ii) numbers released were below a minimum threshold for populations to survive (e.g. *U. lantanae*, *E. xanthochaeta*, *Telenemia elata* Drake, *Lantanophaga pusillidactyla* (Walker)); iii) predation, especially of eggs by ants (e.g. *Alagoasa parana* Samuelson); and iv) unsuitable microhabitats (e.g. *A. parana*).

1.3 Discussion

Despite these several constraints, the biological control programme against *L. camara* has resulted in some reduction in the severity of the weed. Van Wilgen *et al* (2002) calculated the potential condensed area that is suitable for invasion by *L. camara* in South Africa, to be 44 663 km^2. Without biocontrol, 100% of this area will become invaded by the year 2095. Currently, lantana has already invaded 18 414 km^2 (41.3%) of this potential suitable area. Without the biocontrol agents released up until 2000, the invasion of lantana would have been 67.3% (van Wilgen *et al* 2002). Thus, biocontrol has reduced potential lantana invasions by 26%. This portrays a better than expected scenario, in that, in spite of only 11 of the 20 agents released, and that most of them are found only in discrete areas, a 26% reduction in invasion has nevertheless been achieved.

The biological control programme in South Africa was suspended in 1986 (Cilliers and Neser 1991) and revived in the early 1990s with renewed resources. Van Wilgen *et al* (2002) estimated the economic cost and benefits of biological control of weeds at a national scale in South Africa. It was calculated that the benefits (in terms of stream flow gain, land value and biodiversity) and costs (biocontrol research) between the initiation of research on the biological control programme against *L. camara* up to the year 2000 was 22:1. The benefit of preventing invasion by *L. camara* in South Africa (in terms of economic use of water, biodiversity and preservation of value of land) for the year 2000 was US$67/ha/yr (van Wilgen *et al*, 2002). The monetary impact of the released agents is therefore much greater than anticipated, and the motivation to preserve the value of land, provide compelling reasons to continue with the programme.

Leaves are the centre of resource production, and therefore an important target for biocontrol (Baars and Neser 1999). Cilliers (1987) reported that the natural enemies established in South Africa, notably the three leaf-feeding species, *O. scabripennis*, *U. girardi* and *T. scrupulosa*, periodically do defoliate lantana stands, but fail to sustain these levels of damage. This niche is therefore in need of additional herbivore pressure and new leaf feeders, that might make use of this resource more successfully, should be considered. However, the leafless period that *L. camara* undergoes during winter is a major obstacle for leaf-feeding insects. Species that are able to overcome this period by either going into winter diapause or by seeking shelter and being able to withstand food-shortages, and then being able to rapidly build up population numbers during the growing season, would make use of this niche more successfully. Several insect species are currently being studied and screened in quarantine as potential biocontrol agents for *L. camara*, among these are the petiole-boring weevil, *Coelocephalapion camarae* Kissinger; and the leaf-feeding flea-beetle, *Alagoasa extrema* Jacoby. The adults of both of these species are long-lived and able to withstand periods of food-shortages, unlike the leaf sap-sucking mirid *Falconia intermedia* (Distant), where the adults and nymphs need to feed continuously on leaves. *Falconia intermedia*, in spite of spectacular damage and very high population numbers in its first summer of release, was only able to over-winter in sheltered areas where leaves were available throughout the winter months, while the numbers at many of the other release sites have dwindled (Heystek, unpublished report).

1.4

Aims of study

This project focused on the pre-release host specificity screening of *Alagoasa extrema* as a biocontrol agent for *L. camara* in South Africa. Another congeneric flea-beetle, *A. parana* Samuelson, was released in South Africa in 1985, but did not establish. Several reasons for this failure have been proposed, including ant predation on the eggs, unsuitable microhabitats (see above) (Cilliers and Neser 1991) and insufficient numbers released (Cilliers, pers com). *Alagoasa parana* is an univoltine species that overwinters as newly emerged adults and produces offspring the following spring/summer. This

overwintering phase was thought to be a good strategy to enable the insect to overcome the dry, leafless winter period in the inland areas of South Africa, but in spite of this it still failed. Observations on *A. extrema* in the laboratory indicated that this insect produces more than one generation per year, although no marked reduction in egg-laying and feeding was observed during winter in the temperature-controlled glass house. Photoperiod therefore doesn't seem to have an influence on oviposition, although in the field, low temperatures and leaflessness might cause the adults to seek shelter and reduce feeding and egg-laying. Should *A. extrema* be able to cope with unfavourable winter conditions, then it might prove to be a more successful biocontrol agent for *L. camara* than *A. parana*, as it would be able to rapidly build up population numbers with its successive generations during the summer. *Alagoasa parana* was also released in Australia in 1981 and persisted for 2 years until the site was destroyed by fire (Julien and Griffiths 1998). In recent times, the flea-beetle has been imported from Brazil and released in Australia for a few consecutive years in an attempt to achieve establishment, as mass-culturing of the insect proved too problematic. However, this was unsuccessful and due to a reduction in budget and a shift of focus towards stem attackers, *A. parana* became a low priority agent and the project was discontinued (M.D. Day, pers. comm.).

The overall aim of this research project was to determine the suitability and potential of *A. extrema* as an additional biocontrol agent against *L. camara* in South Africa. Chapter 2 describes the biology of *A. extrema*, reared on one of the more common varieties of *L. camara* (029 White Pink) (see Table 1.1). However, to justify the cost involved in introducing a particular biological control agent, it is important to demonstrate that the agent is capable of reducing the biomass and/or altering the pattern of resource allocation to lower the reproductive potential and/or the competitiveness of the target weed. The potential impact of *A. extrema* larval feeding on the growth of *L. camara*, at least under quarantine laboratory conditions, was investigated (Chapter 3). In order to avoid the problems that have plagued the success of previously released agents in the field, preference for, performance on and compatibility with field varieties of *L. camara* was studied under laboratory conditions (Chapter 4). Chapter 5 investigates the host specificity of *A. extrema* under quarantine laboratory condition and assesses the

possibility of releasing the flea-beetle as biocontrol agent against *L. camara* in South Africa. In the final discussion (Chapter 6), conclusions are made on the suitability and potential of *A. extrema* as a biocontrol agent for *L. camara* in South Africa.

CHAPTER 2

BIOLOGY OF *ALAGOASA EXTREMA*

2.1 Introduction

The noxious weed *Lantana camara* is, despite being the subject of biological control since the early 1960s, still an invader species of major importance in South Africa. Van Wilgen *et al.* (2002) calculated that *L. camara* has currently invaded 41.3% of the area that is suitable for potential invasion. Even with the impact of the 11 established biocontrol insects on *L. camara* stands, notably the periodic defoliation by the three leaf-feeding species, *O. scabripennis*, *U. girardi* and *T. scrupulosa*, these levels of damage are not maintained (Cilliers 1987) and *L. camara* stands are still expanding. Extra herbivore pressure, in the form of additional biocontrol agents, is needed to limit further spread.

Lantana camara has been the target of biocontrol in 29 countries, with variable success (Broughton 2000). Crawley (1986, 1989a, 1989b) rated *L. camara* as the most successful target of weed biocontrol in several countries, but also the most frequent unsuccessful target species because of failures in many other locations, with different insect species contributing to the success in different countries. This inability to predict success is attributed to high genetic variability of *L. camara* and the weed's ability to populate diverse habitats (Broughton 2000). In her review and evaluation of *L. camara* biocontrol programs, Broughton (2000) found leaf-, flower-, and fruit-feeding insects to be the most successful biocontrol agents. But this author also suggested that new defoliating species should not be considered, as artificial defoliation experiments by Winder (1980) and Broughton (1999), showed that lantana was able to survive continual defoliation for at least 1 to 2 consecutive years. Winder and van Emden (1980) found that attack by insects reduces plant growth more than an equivalent amount of artificial clipping. Broughton (2000) admitted that the effects of plant competition (intra- and interspecific), drought and frost on lantana in combination with insect defoliation were unknown. Continuous seasonal attack by leaf-feeding insects should eventually weaken lantana's ability to survive and reduce its reproductive output. Baars and Neser (1999) argued that leaves are

the centre of resource production, an important niche, and as the established biocontrol agents are unable to maintain their levels of attack, additional agents targeting this niche should be considered. Additional pressure on this niche might tip the scales in favour of biocontrol.

The success of established leaf-feeding biocontrol agents in South Africa has been greatly reduced by the leafless period that *L. camara* undergoes to survive winter. The leaf-sucking mirid *Falconia intermedia* (Distant) is a good example. Within its first season of release, impressive damage and population build-up were found at several release sites. After the following winter, no insects could be found at several of the sites, while survival was possible only in areas where leaves were present throughout winter which permitted population build-up during the following summer (F. Heystek, unpublished data). Leaf-feeding insects, which are able to endure *L. camara*'s leafless period, would be suitable candidates for release.

Currently several leaf-, stem-, and flower attacking insect species are being studied in quarantine as potential biocontrol agents for *L. camara* in South Africa. Among these are the polymorphic alticine flea-beetle *Alagoasa extrema* Jacoby. Several alticine species have proved to be valuable biological control agents, e.g. *Agasicles hygrophila* Selman and Vogt for the control of alligator weed (*Alternanthera philoxeroides* (Mart.) Griseb) in the USA (Vogt *et al*. 1979) and Australia (Julien and Griffiths 1998); *Longitarsis jacobaeae* (Waterhouse) for the control of tansy ragwort (*Senecio jacobaea* L.) in Canada (Harris *et al*, 1984); and *Lysathia* n. sp. for the control of parrot's feather (*Myriophyllum aquaticum* (Vell.) Verdc.) in South Africa (Cilliers 1999).

A literature survey revealed 10 *Alagoasa* species and their known host plant species (Table 2.1). The host ranges of these *Alagoasa* species include plant species from 4 families, but are mostly limited to the Verbenaceae and Lamiaceae; none of the plant species are economically important species. Begossi and Benson (1988) state that tropical American alticines, especially the subtribe Oedionychina (to which the genus *Alagoasa* belongs), feed mainly on a few genera of the families Verbenaceae and Lamiaceae, with

some also using Acanthaceae, Bignoniaceae, Boraginaceae, Cruciferae, Onagraceae and Solanaceae.

Table 2.1: Published host plant records of *Alagoasa* spp.

***Alagoasa* sp.**	**Host Plant Family**	**Host Plant**	**Locality**
A. parana	Verbenaceae	*Lantana tiliaefolia*[1]	SE Brazil
		L. glutinosa[1]	SE Brazil
		L. camara[1]	SE Brazil
A. bicolor	Verbenaceae	*Aegiphila matinicensis*[2]	SE Brazil
		Clerodendrum aculeatum[3]	SE Brazil
A. apicata	Verbenaceae	*Aegiphila sellowiana*[4]	SE Brazil
A. areata	Verbenaceae	*Duranta plumieri*[4]	SE Brazil
A. decemguttata	Bignoniaceae	*Tabebuia caraiba*[4]	SE Brazil
		T. impetiginosa[4]	SE Brazil
	Cruciferae	*Gochnatia barrossii*[4]	SE Brazil
		G. polimorpha[4]	SE Brazil
	Verbenaceae	*Callicarpa reveesii*[4]	SE Brazil
		Lantana camara[4]	SE Brazil
		L. lilacina[4]	SE Brazil
A. florigera	Verbenaceae	*Aegiphila lhotzkiana*[4]	SE Brazil
A. cf. *pantina*	Acanthaceae	*Justicia* aff. *Klenii*[4]	SE Brazil
		Thunbergia alata[4]	SE Brazil
		Lantana lilacina[4]	SE Brazil
A. scissa	Acanthaceae	*Justicia* aff. *klenii*[4]	SE Brazil
A. sexplagiata	Verbenaceae	*Lantana camara*[4]	Brazil
A. trifasciata	Verbenaceae	*Lantana camara*[4]	Brazil
		Stachytarpheta cayenensis[4]	

[1] Recorded by Winder, Sands and Kassulke (1988)

[2] Recorded by Virkki (1982)
[3] Recorded by Virkki (1980)
[4] Recorded by Begossi and Benson (1988)

During a survey and collection trip for natural enemies of *L. camara* in the tropical and subtropical parts of Mexico, notably the provinces of Yucatan, Tabasco and Veracruz, adults and larvae of *A. extrema* were observed feeding on leaves of plants of an orange-flowering *Lantana* species. A number of adults and larvae were collected and brought back to South Africa for screening as a potential biological control agent for *L. camara*.

A thorough knowledge and understanding of the biology of a potential biological control agent is essential. Under controlled quarantine glasshouse conditions, behaviour and performance of the insect on its natural host plant (the target species) are studied to enable comparisons with that on test plant species (non-target species). Knowledge of the biological characteristics also gives an indication of the potential of the insect species as a biological control agent, e.g. rate of increase, fecundity, longevity, mobility, generations per year and feeding rate.

In this chapter studies on the biology of *A. extrema* are discussed.

2.2 Materials and Methods

Collection of the beetle was achieved by hand collecting all adults and larvae on a lantana plant with minimum disturbance of the vegetation as the adults jump readily. Hidden larvae and adults were then collected by means of a beating tray.

A culture of *A. extrema* was established on potted plants in the quarantine glasshouse at the Rietondale Experimental Farm (ARC-PPRI) in Pretoria, South Africa. The captured adults were released onto caged potted plants, and the larvae reared to adulthood in petri-dishes on cut leaves of South African naturalized *L. camara* plants. Voucher specimens were lodged at the National Collection of Insects (Biosystematics Division, ARC-PPRI, Pretoria). Sample specimens were sent to Dr C. N. Duckett (University of Puerto Rico) for identification.

All biological studies were conducted in a quarantine laboratory with temperatures varying between 21ºC (night) to 30ºC (day) and relative humidity varying between 35%

and 65%. Natural daylight was supplemented with growth lights, resulting in a photoperiod of approximately 14 hours. All observations were made on potted plants of, and where mentioned, cut leaves of the *L. camara* variety 029 White Pink (see Chapter 1, Table 1.1).

Source plants of the various *L. camara* varieties and plant species for host specificity testing were collected from homogenous stands of varieties or plants in the field or bought from nurseries, and planted in a 'weed garden' on the grounds of PPRI. Cuttings of plants to be used in culturing and host specificity testing were made from these source plants and allowed to root in a medium of coarse river sand. The rooted cuttings were transplanted into pots with a standard soil mixture of equal parts of coarse river sand, loam and compost. Plants were held in a nursery under 50% shade net, with overhead irrigation, and pruned and fertilized as needed.

Aspects of the biology of *A. extrema* that were studied included: the biology and duration of the immature stages (egg stage, the larval instars and pupal stage), and the adult stage, which included the pre-oviposition period and female fertility. Studies on the immature and mature stages were undertaken by keeping egg clusters in small airtight containers on moist tissue paper and allowing them to hatch. Time to hatching was recorded as half way between the two observation periods per day. The emerging larvae were then separated into petri-dishes containing moist tissue paper and *L. camara* leaves. Leaves were replaced every second to third day until the larvae were ready to pupate. The number and duration of the larval stages were recorded by counting the number of moults. The petri-dishes were then filled with moist soil and the larvae allowed to pupate. The duration of the pupal stage (from the time that larvae burrowed into the soil to adult eclosion) was recorded. The newly-eclosed adults were separated into mating pairs and each pair was kept on a caged, potted plant until oviposition occurred. The pre-oviposition period was recorded. Twenty-eight newly emerged females were divided into 5 groups and kept on caged potted plants. The adults were transferred to new plants every 5 to 7 days and the number of eggs laid by the females counted. This process was

repeated 6 times and the average number of eggs laid per female per day was calculated from these data.

2.3 Results

2.3.1 Collection localities and identification

Alagoasa extrema was widespread and present at 31 sites visited in the provinces of Yucatan, Tabasco and Veracruz, although not very abundant per site (Fig. 2.1). On average between 1 and 7 larvae, and 1 to 2 adults were found per site, and in total about 97 larvae and 20 adults were collected. Typical “shot-hole” flea-beetle damage was observed at almost all of the sites.

The colour morphs of the flea beetle were found striped, spotted and black. Initially, two species of the genus *Alagoasa* Bechyné were identified. Striped specimens were similar to specimens of *A. quadrilineata* (Harold), while spotted specimens were comparative to specimens of *A. extrema* Jacoby. It was eventually realized that the two ‘species’ were actually polymorphic forms of a single species, and the earliest given name was used. Hence, the species was identified as *A. extrema* (C.N. Duckett, pers. comm.).

Figure 2.1: Localities where *Alagoasa extrema* was collected in Mexico in 1998. (Yellow squares indicate collection sites.)

2.3.2 Life stages

Egg. Females laid their eggs in groups (batches) of 17 to 22 eggs (Mean ± SE = 20.8 ± 0.4; n=12 females) in moist areas amongst the leaf litter (Fig. 2.2). The eggs were orange in colour when laid, becoming darker as they develop and were conically shaped and laid in an upright position. The incubation period ranged between 9 to 10 days (Mean ± SE = 9.5 ± 0.1; n=38 egg batches) (Table 2.2).

Larval instars. There were three larval instars. On emergence the larvae were orange in colour, but as they started feeding they became a darker orange-brown. All instars had lateral protrusions on both sides of each abdominal segment (Fig. 2.3). The newly emerged larvae move up the host plant along the stem and any leaves touching the soil surface, to feed on the leaves and occasionally the flowers. At high densities, feeding by the larvae skeletonized the leaves. In the glasshouse, abscission of badly damaged leaves was observed. Larvae usually moulted in the leaf litter. The duration of the first instar

was 4 to 6 days (Mean ± SE = 5.2 ± 0.1; n=34), with the second instar 4 to 9 days (Mean ± SE = 5.0 ± 0.2; n=41) and the third instar 3 to 6 days (Mean ± SE = 4.6 ± 0.1; n=43) (Table 2.2).

Pupa. When the larvae were ready to pupate, the late third instars moved down to the soil surface, burrowed into the soil to a depth of about 2 to 3cm and constructed a pupation chamber out of soil particles and saliva. The pupae of *A. extrema* were bright yellow in colour, but as they grew older, the hind-legs and eyes became darker. The pupal stage (taken from the time the larva burrowed into the soil to adult emergence) lasted 18 to 25 days (Mean ± SE = 21.1 ± 0.2; n=61) (Table 2.2).

Adult. On eclosion the adults remained in the pupal cell for about a day until their elytra hardened. Adults usually emerged in the early afternoon and started feeding on the leaves of their host plant by chewing holes of 3-6mm in diameter, but occasionally larger. Adults were also found at times to feed on the flowers. Females were generally larger in size with body length varying between 60 to 68.5mm (Mean ± SE = 63.8 ± 0.0; n=31) and antennae length between 28.5 to 39mm (Mean ± SE = 33.6 ± 0.0; n=31). The body length of males varied between 49 to 59mm (Mean ± SE = 55.1 ± 0.0; n=17) and antennae length between 29 to 35mm (Mean ± SE = 31.8 ± 0.0; n=17). After a pre-oviposition period of 11 to 26 days (Mean ± SE = 15.8 ± 0.8; n=21), during which copulation occurred, females started to oviposit. A female lay on average 7 eggs per day (n=28 females). Adult lifespan was observed to be longer than 10 months.

As adults, *A. extrema* exhibits three distinctive colour morphs (Fig. 2.4): a striped morph that is yellow with 2 black longitudinal stripes on each elytra; a black morph that is black with a pair of yellowish spots on the posterior tip of the elytra; and a spotted morph that is black with one pair of large and three smaller pairs of yellowish spots on the elytra. The pronotum and abdomen of all of the morphs are red. An egg packet laid by a single female can give rise to all three colour morphs.

Table 2.2: Comparison of the duration of the life stages of *Alagoasa parana* and *Alagoasa extrema* on *Lantana camara*.

Life stage	***Alagoasa parana*** (in Australia under quarantine insectary conditions)*	***Alagoasa extrema*** (in South Africa under quarantine laboratory conditions) (Mean ± SE)
Pre-oviposition	7 months (overwinter)	11-26 days (15.76 ± 0.84; n=21)
Egg	21-25 days	9-10 days (9.53 ± 0.08; n=38)
Larva: 1st instar	5-7 days } (x=16)	4-6 days (5.18 ± 0.11; n=34)
2nd instar	5-8 days	4-9 days (5.02 ± 0.16; n=41)
3rd instar	4-6 days	3-6 days (4.61 ± 0.11; n=43)
Prepupa + Pupa	21-24 days	18-25 days (21.1 ± 0.20; n=61)
Total: egg to adult	61-70 days	42-46 days (44.4 ± 0.24; n=29)
Adult lifespan	*ca* 10 months	*ca* 10 months

* Studies conducted in Brisbane, at 25.0 ± 0.5 ºC.

Fig. 2.2: *Alagoasa extrema* eggs laid in clusters at the base of the host plant stem as indicated by the arrows

Fig. 2.3: The three larval instars of *Alagoasa extrema*

Fig. 2.4: The three colour morphs displayed by the adults of *Alagoasa extrema*

2.4 Discussion

The biological characteristics of *A. extrema* indicate that it has potential as a biological control agent. The adults are long-lived; several overlapping generations are produced annually and both the adults and larvae have high feeding rates. *Alagoasa extrema* was found at several sites in the humid subtropical to tropical regions of Mexico, indicating a relatively wide distribution, although limited to higher rainfall areas.

Winder *et al*. (1988) suggested that *A. parana* showed a preference for moist conditions in Brazil, and that it would most likely be suited to the coastal rain forest fringes of Australia. *Alagoasa extrema*, having the same basic biological needs as *A. parana*, in that moist micro-climates are necessary for the survival and hatching of the eggs, would probably also be more suited to the subtropical and tropical areas of South Africa. Field studies done by Winder *et al.* (1988) over 2 years on the abundance of *A. parana* on lantana in Brazil, indicated that population levels reached a peak of 8 adults per 100 branches and 27 larvae per 100 branches during the growing season. Mean defoliation levels varied between 7 and 26%, while defoliation of up to 47% caused by larvae was observed on individual plants. Compared to *A. parana*, *A. extrema* with its shorter lifecycle (Table 2.2) and overlapping generations, might be able to build up to larger populations and have a potentially a better chance of establishing and supplementing the herbivore stress on the target weed. Although *A. extrema* would probably be limited to the subtropical regions of South Africa, these are also the more heavily lantana-infested areas.

Parasitism and predation have been linked to the failure of some of the agents released on lantana in South Africa (Chapter 1). The larvae and adults of *A. extrema* regurgitate enteric fluids, which are probably distasteful, and the adults display bright contrasting colour patterns that could also signify unpalatibility. These characteristics might provide some protection and increase the chances of establishment and population build-up. According to C. Duckett, University of Puerto Rico (pers. comm.) parasitism by tachinids on the genus *Alagoasa* is fairly common, and also predation by pentatomid adults, although the later is quite rare. No parasitoids were reared from the material collected in

Mexico. These observations suggest that lower rates of parasitism and predation might be found on *A. extrema*, once this insect is released into the field.

Begossi and Benson (1988) reasoned that the very similar colour patterns displayed by many of the Oedionychina flea beetles, including several *Alagoasa* species, suggested mimicry. The three very conspicuous, constant colour morphs of *A. extrema* might either be the unpalatable models in a Batesian mimicry situation or be part of a Müllerian mimicry circle. Begossi and Benson (1988) tested the palatability of Oedionychina species to chickens and the rejection rates observed during of tests suggested that if mimicry was involved, then it was of the Müllerian type. However, Begossi and Benson (1988) also stated that the contrasting and bold colour displays, the slow flight patterns which ensure recognition of colour-patterns, and the tendency to aggregate, could also suggest aposematism. If aposematic colouration and mimicry protect Oedionychina flea beetles from predators, it is not clear why all these beetle species have not converged to the same colour pattern (Begossi and Benson 1988), and especially why *A. extrema* invests in three very different colour forms. Information on the distribution, genetics and physiology, the mimetic species present in the distribution range of *A. extrema*, and the selection pressures to which the insect are exposed, is lacking. Therefore, few firm conclusions can be drawn concerning the biological significance of its colour morphs.

2.5 Conclusion

The above results and observations suggest that *A. extrema* would, once released, establish in subtropical areas where lantana infestations thrive. One of the most important characteristics of *A. extrema* is that the adults are long-lived, which might enable the insect to endure the leafless period of its host during winter. Its relatively short lifecycle, several generations produced per year and defense mechanisms could enable the insect to reach high population levels and contribute to the defoliation of lantana stands and possibly a further reduction in the competitiveness of the weed. In the next chapter the impact of larval feeding on the host plant was studied and is discussed.

CHAPTER 3

THE IMPACT OF *ALAGOASA EXTREMA* ON A SELECTED SOUTH AFRICAN *LANTANA CAMARA* VARIETY UNDER LABORATORY CONDITIONS

3.1 Introduction

A fundamental theory of plant-herbivore interactions is that herbivores impact on their host plants by reducing plant fitness (Strong *et al*. 1984, Crawley 1989c, Wise and Sacchi 1996). Debate concerning the impact of insect attack on natural populations of their host plants persists with some reports on injurious effects on host plants (Dirzo 1984, Crawley 1989c, Wise and Sacchi 1996, Briese 1996), while other argue that herbivory may increase the fitness of host plants by stimulating compensatory growth (Inouye 1982, McNaugton 1983, 1986, Maschinski and Whitham 1989). Briese (1996) found that the stem-boring weevil *Lixus cardui* Olivier reduced both the plant growth and reproductive capacity of *Onopordum* thistles (Asteraceae: Cardueae). Wise and Sacchi (1996) found that herbivory by the horse nettle beetle, *Leptinotarsa juncta* (Chrysomelidae: Chrysomelinae), and the eggplant flea beetle, *Epitrix fuscula* (Chrysomelidae: Alticinae), caused a decrease in sexual reproduction and a reduction in root biomass of *Solanum carolinense* L. On the other hand, Solomon (1983) found that *S. carolinense* plants were able to compensate for initial energy losses due to attack by the fruit-reducing moth *Frumenta nundinella* Zeller (Gelechiidae) and to become as productive as uninfested plants.

Artificial defoliation experiments by Winder (1980) and Broughton (1999) demonstrated that when 100% of *L. camara* leaves were removed every month over a 1- to 2-year period, the plant recovered. However, insect feeding is more damaging than artificial removal of leaves, but these experiments suggested that lantana is capable of compensating for insect defoliation (Winder 1980, Winder and van Emden 1980, Broughton 1999, 2000). None of the defoliating insects established on *L. camara* in South Africa, inflict damage throughout the year because of declining populations in autumn (*T. scrupulosa*) and winter (*U. girardi* and *O. scabripennis*) (Harley *et al.* 1979,

Cilliers 1982, 1987, Broughton 1999), creating a “lag period” in spring, when lantana plants recover from the previous season’s damage (Harley *et al*. 1979, Cilliers 1982, 1987, Broughton 1999). Van der Meijden (1989) stated that it is assumed that plants have a limited ‘energy budget’ against insect attack, because other functions such as growth, maintenance and reproduction cannot be stopped completely to allow for the continuous allocation of reserves to compensatory growth. Attack by leaf-feeding biocontrol agents must thus eventually reduce the fitness of lantana.

To justify the cost involved in introducing a particular biological control agent, it is important to demonstrate that the insect species will have a negative impact on its host plant. Laboratory experiments give an indication of the effect a biocontrol agent might have on the performance of the host plant, but do not *per se* demonstrate the effect on the plant’s population dynamics (Crawley, 1989c).

In this chapter the potential impact of the leaf-feeding flea-beetle, *A. extrema*, on the growth of one common and highly invasive variety of *L. camara* is quantified.

3.2 Materials and Methods

The potential impact of *A. extrema* larvae on plants of *L. camara*, variety 029 White Pink, was measured. Cuttings were made from the source plant of *L. camara* variety 029 White Pink (see chapter 1) and allowed to root in coarse river sand. The rooted cuttings were transplanted into vermiculite and allowed to grow for 2 months under glasshouse conditions and fertilized twice weekly with a water-soluble fertilizer (Nitrosol®). Twenty-five plants of similar architecture were chosen and divided into 5 groups. Group 1 was used as control plants to determine dry weight of the above- and below-ground biomass before the test, while groups 2 to 5 were exposed to larval densities of 0 larvae, 2 larvae, 5 larvae and 10 larvae respectively.

Newly-emerged larvae were transferred to potted plants, which were caged to prevent larvae from escaping. Larvae were allowed to complete their development and as soon as all larvae had moved down into the soil to pupate, the plants were cut down. After drying

the plant material in an oven, at 70ºC, for at least 24 hours, the following measurements were made: above-ground dry weight (leaves, flowers, stems) and below-ground dry weight (roots).

Data were analyzed using the statistical program GenStat (2000). The experiment was designed as a completely randomized design with 5 treatments and 5 plants per treatment. Differences between treatments were tested for in an analysis of variance. The data was acceptably normal with homogeneous treatment variances. Treatment means were compared using Fishers' Protected t-test Least Significant Difference (LSD) at the 5% level of significance (Snedecor and Cochran, 1980), if the F-probability from the ANOVA was significant at 5%.

3.3 Results

The above-ground dry weight of variety 029 WP was significantly reduced following attack by larvae from density levels of 5 and 10 larvae per plant (Fig. 3.1). Feeding by 5 larvae per plant reduced the above-ground dry weight by 19% and feeding by 10 larvae caused a reduction of 28%. Larval feeding reduced these plants' above-ground dry weight to such an extent that the weights did not differ significantly from the above-ground dry weight of the control plants cut down for measurement prior to the start of the test (Fig. 3.1). The above-ground dry weight of plants that were attacked by 2 larvae did not differ significantly from plants that were not attacked (Fig. 3.1), although a 16% reduction in the weight of above-ground dry material was achieved.

Attack by larvae over this short period had no significant impact on root growth, as there were no significant differences between the under-ground dry weights of plants attacked at the different levels of larval densities (Fig. 3.1). Since this study involved only a single replicate with 5 plants per larval density group, more replicates would have allowed a more reliable and sensitive analysis.

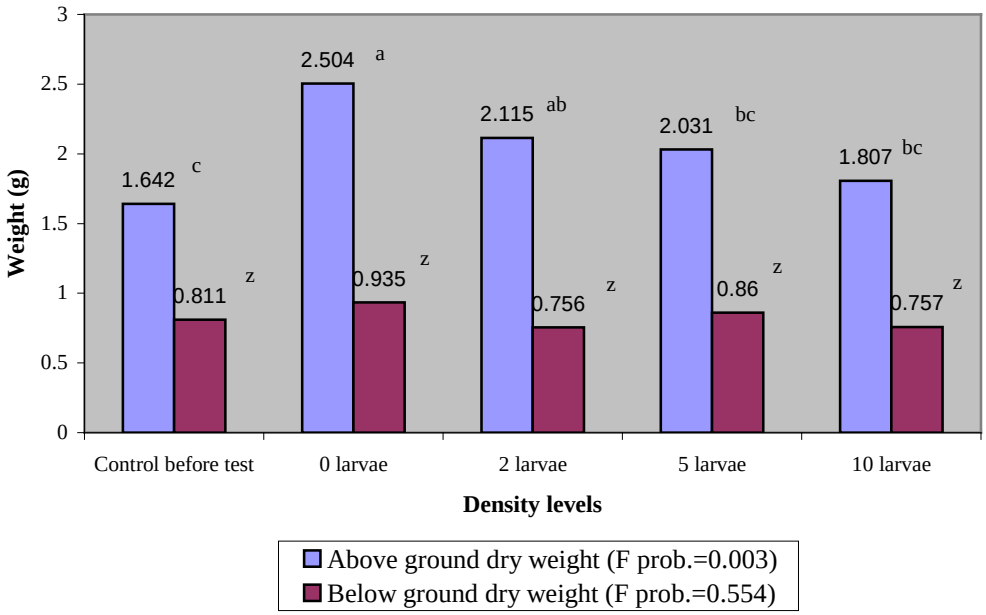

Fig. 3.1: Impact of different *Alagoasa extrema* larval densities on the growth of small *Lantana camara* (029 White Pink) plants under laboratory conditions. (Mean dry weights followed by the same letter do not differ significantly ($p < 0.05$; ANOVA).

3.4 Discussion

The above results show that under certain levels of attack, feeding damage by *A. extrema* larvae can significantly reduce the above-ground biomass of their host plant. Although the impact of adult feeding was not studied, it will certainly contribute towards a further reduction of biomass. In similar studies conducted by Winder and van Emden (1980) on *A. parana*, this insect significantly reduced plant dry weight and net assimilation rates. Field studies done by Winder *et al* (1988) over 2 years on the abundance of *A. parana* on lantana in Brazil, indicated that population levels reached a peak of 8 adults per 100 branches and 27 larvae per 100 branches during the growing season. These authors also found that mean defoliation levels varied between 7% and 26%, while defoliation of up to 47% caused by larvae was observed on individual plants. Winder and Harley (1982) also reported on the impact of alticine species, stating that extensive attack by larvae of a species of *Oedionychis* nr. *arcifer* (Harold) (later identified as *Alagoasa parana* sp. n. in

Samuelson (1985)), of up to 20 larvae per 1000 leaves, contributed towards the reduction of flowering and fruiting at Castro in Brazil. Extensive leaf damage by the same species (21-29 larvae and adults per 1000 leaves) contributed towards poor flowering and fruiting at Guarapuava Forest in the following season. Should *A. extrema* establish in the field in South Africa, these figures give some indications on the population levels the beetle could reach, as *A. parana* generally has the same environmental needs as *A. extrema*. The impact of *A. extrema* might be even greater as it is a multivoltine species capable of producing several overlapping generations during the growing season, while *A. parana* is a univoltine species.

Van der Meijden (1989) suggested that weed species with an effective regrowth capacity might not show spectacular population reductions after the successful introduction of biocontrol agents. These species will be vulnerable only to attack of the storage organs that enable regrowth, or to repeated attack of other plant parts through which reserves are exhausted, either by one or more herbivore species. Thus, the multivoltine *A. extrema* could, through attacking the leaves of its host plant, contribute towards depleting the reserves of *L. camara* plants. According to Harris (1971) the loss of mature leaves is normally the most damaging to the plant as these leaves represent a direct reduction in the photosynthetic capacity of the plant. Thus, attack by *A. extrema* larvae and adults is all the more meaningful, as no preferences based on the age of the leaves were shown and feeding on both younger and older leaves were observed. Damage caused by biocontrol agents often seem to not cause obvious stress to their host plant; nonetheless, if they are capable of reducing the biomass and/or of altering the pattern of resource allocation to lower the reproductive potential of the host plant, they could well contribute to a reduction in competitiveness of the target weed and influence its population dynamics (Briese 1996).

Alagoasa extrema, once established in climatically favourable areas, could well augment other established agents in defoliating *L. camara* infestations, depleting secondary reserves and reducing the competitiveness of the weed.

CHAPTER 4

THE PREFERENCE OF *ALAGOASA EXTREMA* FOR DIFFERENT *LANTANA CAMARA* VARIETIES

4.1 Introduction

One of the main factors contributing to the variable results of the biocontrol programme on *L. camara* in South Africa, has been the varietal complexity of the weed (Baars and Neser 1999, Chapter 1). However, there are conflicting reports in the literature about the extent to which failures in *L. camara* biocontrol can be attributed to varietal preferences of the established agents. Some authors (e.g. Harley *et al* 1979; Cilliers 1982; 1987; Neser and Cilliers 1989; Crawley 1989a;b) contend that varietal preferences are the main cause of failures in the lantana biocontrol programme. Cilliers and Neser (1991) found that *Hypena strigata* (F.), *Octotoma scabripennis* Guèrin-Mèneville, *Calcomyza lantanae* Frick and *Uroplata girardi* Pic, all displayed a preference for pink-flowering varieties in South Africa. Similarly, Haseler (1966) reported that *Neogalea sunia* Guenée preferred white and pink -flowering *L. camara*, while *Salbia haemorrhoidalis* Guenée preferred red flowering *L. camara* in Australia. Harley (1973) also found that *Teleonemia scrupulosa* Stål did not perform as well on common pink-flowering *L. camara* as on other varieties in Australia. However, other authors contend that certain agents are unaffected by the different varieties (Broughton 1999, 2000; Day and Neser 2000). Broughton (1999), using field studies in southeast Queensland, found that five species of leaf-feeding insects, including *T. scrupulosa*, *C. lantanae*, *U. girardi* and *O. scabripennis*, displayed no varietal preferences. Similarly, Day and Neser (2000) found *U. girardi*, *O. scabripennis* and *Ophiomyia lantanae* Froggatt to be present on all of the five major groups of *L. camara* varieties in Australia, but contrary to Broughton (1999), found that *T. scrupulosa* and *Aconophora compressa* Walker did display varietal preferences. In other studies, laboratory trials indicated clear varietal preferences in *Ectaga garcia* Becker, *Charidotis pygmaea* Klug, *Alagoasa parana* Samuelson, *Falconia intermedia* (Distant) and the fungus *Mycovellosiella lantanae* (Chupp) Deighton var. *lantanae*, with populations dying out on certain less preferred varieties (Morris *et al*. 1999, Urban and

Simelane 1999, Day and Neser 2000). These inconsistent reports of varietal preferences thus emphasize the need for additional quantitative laboratory and field studies to clarify the importance of this phenomenon in the biocontrol programme against *L. camara*.

Varietal preference could play an important role in the host specificity testing of agents for *L. camara*, as it is necessary to identify which variety will support the best performance of the insect, so as to allow host suitability comparisons with non-target species. To determine the variety that supports optimal performance and to avoid problems with host plant incompatibility as far as possible, it is necessary to test whether a potential agent shows any preferences for, and/or performs better on any of the most common *L. camara* varieties.

In this chapter I determine the adult preference and reproductive performance of *A. extrema* on a number of South African *L. camara* varieties.

4.2 Materials and Methods

Varietal preference of *A. extrema* was determined by adult choice trials, larval no-choice trials and multi-generation no-choice trials using five different *L. camara* varieties (Table 4.1). The varieties selected are regarded as of the most important and widespread in South Africa (C.J. Cilliers and J-R. Baars, pers. comm., Chapter 1), and are represented in major *L. camara* infestations in KwaZulu-Natal and Mpumalanga provinces.

Table 4.1: *Lantana camara* varieties used during preference and performance studies of *Alagoasa extrema*.

***Lantana camara* variety**	**Distinguishing morphological characteristics**	**Mature flower colour description**	**Collection Site**	
			Grid reference	**Location**
163 LP*	Shoot tips hairy and spiny; leaves very hairy; main stem with few spines	Light Pink	30º 09’ 08.4”S 30º 49’ 39.7”E	nr. Scotsburgh, KwaZulu-Natal
150 O	Scrambling shrub, shoot tips hairy, spiny and reddish in colour; leaves small and hairy	Orange	29º 38’ 45.9”S 31º 07’ 39.5”E	nr. La Merci, KwaZulu-Natal
015 WY	Shoot tip spiny; large broad dark hairy leaves; main stem spiny	White (Yellow throat)	25º 02’ 21.6”S 31º 02’ 19.8”E	nr. Sabie, Mpumalanga
010 DP	Shoot tip spiny; leaves small, dark and hairy; main stem very spiny	Dark Pink	25º 37’ 08.3”S 30º 31’ 12.1”E	nr. Waterval-Boven, Mpumalanga
029 WP	Shoot tip spiny; large broad dark hairy leaves, main stem spiny	White Pink	25º 08’ 10.6”S 31º 00’ 09.0”E	nr. Hazyview, Mpumalanga

*Varieties are named according to their collection GPS waypoint number and mature flower colour abbreviation.

The larval and multi-generation no-choice trials were conducted in a quarantine laboratory with temperatures varying between 21ºC (night) and 29ºC (day) and the relative humidity varying between 35% and 65%. Overhead plant growth lights supplemented natural daylight resulting in a 14h photoperiod. Adult choice trials were conducted in a quarantine tunnel with temperatures varying between 13ºC (night) and 30ºC (day) and the relative humidity varying between 35% and 65%.

4.2.1 Adult choice trial

The adult choice trial was conducted in a large walk-in cage (4m x 4m x 2m) in a ventilated quarantine tunnel. Older plants (up to 1m tall), grown in 10*l* pots, were used during the trials. Following a latin square design, six of each of the five *L. camara* varieties and the related *Lippia wilmsii* (Verbenaceae) were randomly arranged in the

cage without their foliage touching. *Lippia wilmsii* was included in the test to act as a control and ensure that a choice is made between the *L. camara* varieties and the *L. wilmsii* plants, should there be no definite indications of preference for specific *L. camara* varieties. In total, 180 gravid females and 30 males (calculated as 6 females and 1 male per *L. camara* plant) were released in the cage. Adults used in the trial were, prior to testing, allowed to feed on a variety of *L. camara* which was different to the five varieties tested, because some insects have been shown to be influenced by prior experience (Traynier 1979). The adults were removed after a week and the number of egg packets in the soil of each potted plant was recorded. Data were analyzed using the statistical program GenStat (2000). Differences between varieties were tested for in an analysis of variance. The data were acceptably normal and the treatment variances homogeneous. Variety means were separated using Fisher's Protected t-test with the Least Significant Difference (LSD) at the 5 % level (Snedecor and Cochran, 1980), and the F-probability from the ANOVA was taken as significant at 5 %.

4.2.2 Larval no-choice trials

Ten newly-emerged unfed larvae were placed onto the foliage of potted plants of the five *L. camara* varieties. To prevent larvae from escaping, ventilated cages made from 2*l* honey jars, were fitted over the plants with the mouths of the jars pushed about 1cm into the soil of the pots. Larvae that fell off could thus easily climb back onto the host plant. The number of larvae surviving was recorded. A minimum of 5 replicates were conducted for each variety. Data were analyzed using the statistical program GenStat (2000). A completely randomized design was used for the experiment. Differences between varieties were tested for by means of a One-way ANOVA. The data were acceptably normal and the treatment variances homogeneous. Variety means were separated using Fisher's Protected t-test with the Least Significant Difference (LSD) at the 5 % level (Snedecor and Cochran, 1980), and the F-probability from the ANOVA was taken as significant at 5 %.

4.2.3 Multi-generation trials

These trials determined a) whether the different *L. camara* varieties could support consecutive larval development to the third generation with comparable larval survival rates; and b) whether comparable ovarian development was supported by these varieties. The multi-generation trials were conducted by culturing each replicate of the original larval no-choice trial through to the third generation (where viable offspring were available). The newly-eclosed adults (F), obtained from the larval no-choice trials, were transferred to fresh plants of the same variety and allowed to feed and oviposit. The number of eggs laid per female during the first 45 days after eclosion was recorded. Where viable eggs were produced, 10 newly-emerged larvae were placed onto the foliage of fresh plants of the same variety. The number of larvae surviving to adulthood and developmental time to adult eclosion (F1) were recorded. Once again, the number of eggs laid per female during the first 45 days after eclosion was recorded. The same process was followed for the third generation (F2). Data were analyzed using the statistical program GenStat (2000). The experiment was designed as a completely randomized design with two factors, namely, the *L. camara* varieties and the 3 generations. Differences between varieties and generations and variety-by-generations interaction were tested for by means of an analysis of variance. The data were acceptably normal and the treatment variances homogeneous. Variety means were separated using Fisher's Protected t-test with the Least Significant Difference (LSD) at the 5 % level (Snedecor and Cochran, 1980), and the F-probability from the ANOVA was taken as significant at 5 %.

4.2.4 Host plant suitability analysis

The suitability of the 5 different *L. camara* varieties as host plants for *A. extrema* was compared. A risk analysis method, proposed by Wan and Harris (1997) for quantifying the safety of biocontrol agents, was employed. In this instance however, it was used to calculate the suitability of each *L. camara* variety as host plant for *A. extrema*. This method allows the comparison of the suitability of the different varieties in terms of numerical scores or percentages.

The calculation was done by measuring the insect's performance on each variety, at different stages in the host plant selection process, as a proportion of that of the most suitable variety. The performance criteria used were i) plant preference, ii) oviposition preference, iii) oviposition potential and iv) larval survival. Plant preference and oviposition preference were determined by the mean number of adults present on the plants of the different varieties, and the mean number of egg packets laid in the soil around the plants of the different varieties during the adult choice trials (Table 4.2). Oviposition potential was determined as the mean number of eggs laid per female during the first 45 days after eclosion, when reared on the different *L. camara* varieties during the adult multi-generation no-choice trials (Table 4.5). Larval survival was the mean number of larvae surviving to adulthood during the larval no-choice trials (Table 4.3). The product of the scores calculated for the above performance criteria assessed the potential of the 5 different *L. camara* varieties to support viable reproductive populations of *A. extrema*. For each criterion, R represents the insect's performance on the *L. camara* test variety relative to that on the most suitable variety (Table 4.6).

4.3 Results

4.3.1 Adult choice trial

Adults and egg packets were found on all the *L. camara* varieties and on *L. wilmsii* (Table 4.2). There were no significant differences between the mean numbers of adults found on *L. camara* varieties 029 WP, 163 LP, 015 WY and 010 DP. However, significantly less adults were found on *L. camara* variety 150 O and *L. wilmsii*. There were no significant differences between the numbers of egg packets found in the soil around *L. camara* varieties 029 WP, 163 LP, 015 WY and 150 O, while significantly less eggs were found on *L. camara* variety 010 DP and *L. wilmsii*.

Table 4.2: Host selection of *Alagaoasa extrema*, during adult multi-choice trials involving a 6x6 latin square design with 5 different *Lantana camara* varieties and *Lippia wilmsii.*

***Lantana camara* variety or test plant species**	**Number of adults per plant (Mean ± SEM)[Y]**	**Number of egg packets per plant (Mean ± SEM)[Y]**	**Times (out of 6 replicates/plants) chosen as oviposition site**
L. camara 163 LP	4.3 ± 0.9^{ab}	4.17 ± 0.9^{ab}	5
L. camara 029 WP	6.3 ± 0.9^{a}	6.17 ± 0.9^{a}	6
L. camara 015 WY	6.0 ± 0.9^{a}	4.17 ± 0.9^{ab}	6
L. camara 150 O	3.2 ± 0.9^{bc}	5.33 ± 0.9^{a}	6
L. camara 010 DP	4.0 ± 0.9^{ab}	1.83 ± 0.9^{bc}	6
L. wilmsii	0.5 ± 0.9^{c}	0.33 ± 0.9^{c}	2
F-probability	0.004	0.001	

[Y] – SEM is the standard error of the mean. Means in the same column followed by the same letter are not significantly different ($p < 0.05$; ANOVA; Fishers' protected t-test LSD.

4.3.2 Larval no-choice trials

Larval development was supported by all five *L. camara* varieties (Table 4.3). Percentage survival to adulthood was highest on *L. camara* 010 DP at 74%, but did not differ significantly from the survival recorded on the other *L. camara* varieties, which ranged from 57% to 72% (Table 4.3).

Table 4.3: Percentage survival to adulthood of neonate larvae of *Alagoasa extrema* on 5 different *Lantana camara* varieties during no-choice trials.

***Lantana camara* Variety**	**n**	**Number of larvae surviving/ replicate (Mean ± SEM)[Z]***	**Percentage survival to adulthood**
L. camara 163 LP	6	7.0 ± 1.0[a]	70
L. camara 029 WP	6	7.2 ± 1.0[a]	72
L. camara 015 WY	6	5.7 ± 1.0 [a]	57
L. camara 150 O	5	5.8 ± 1.0 [a]	58
L. camara 010 DP	5	7.4 ± 1.0 [a]	74
F-probability		0.625	

[Z] – SEM is the standard error of the mean. Means followed by the same letter are not significantly different ($p < 0.05$; ANOVA; Fishers' protected t-test LSD).

* - Out of 10 larvae placed on the plant.

4.3.3 Multi-generation trials

Both the larval survival rate and adult ovipositional performance did not differ significantly between the different lantana varieties during the three generations and did not increase or decrease significantly over the three generations (Tables 4.4 and 4.5). Although larval survival rates and adult ovipositional performances were lowest on *L. camara* 015 WY, this was not statistically significant.

Table 4.4: Survival of *Alagoasa extrema* larvae when reared on 5 different *Lantana camara* varieties for 3 consecutive generations during multi-generation trials.

Species	**n**	**Larval survival F (Mean ± SEM)***	**n**	**Larval survival F1 (Mean ± SEM)***	**n**	**Larval survival F2 (Mean ± SEM)***	**Varieties mean over 3 generations (Mean ± SEM)[X]**
L. camara 163 LP	5	7.0 ± 1.1	4	5.8 ± 1.3	4	5.3 ± 1.3	6.1 ± 0.7[a]
L. camara 029 WP	6	7.2 ± 1.1	5	8.2 ± 1.2	4	4.8 ± 1.3	6.8 ± 0.7[a]
L. camara 015 WY	6	5.7 ± 1.1	3	3.7 ± 1.5	1	3.0 ± 1.9	4.3 ± 0.8[a]
L. camara 150 O	5	5.8 ± 1.2	5	7.6 ± 1.2	5	5.6 ± 1.2	6.3 ± 0.7[a]
L. camara 010 DP	5	7.4 ± 0.5	5	7.0 ± 1.2	5	6.2 ± 1.2	6.9 ± 0.7[a]
Generation Mean [Y]		6.655 ± 0.51[a]		6.612 ± 0.56[a]		5.068 ± 0.60[a]	

F-probability: Varieties: P=0.229
Generations: P=0.087
Varieties x Generation Interaction: P=0.812

[X] – Means in this column followed by the same letter do not differ significantly at the 5% level.

[Y] – Means in this row followed by the same letter do not differ significantly at the 5% level .

* - SEM is the standard error of the mean. Out of 10 larvae placed on the plant.

Table 4.5: Ovipositional performance of *Alagoasa extrema* females when reared on 5 different *Lantana camara* varieties for 3 consecutive generations during multi-generation trials.

Species	**n**	**No of eggs laid per female during 1st 45 days F (Mean ± SEM)**	**n**	**No of eggs laid per female during 1st 45 days F1 (Mean ± SEM)**	**n**	**No of eggs laid per female during 1st 45 days F2 (Mean ± SEM)**	**Varieties mean over 3 generations (±SEM) [X]**
L. camara 163 LP	5	220.0 ± 34.7	4	102.9 ± 38.8	4	145.0 ± 38.8	161.6 ± 21.6[a]
L. camara 029 WP	6	234.6 ± 31.7	5	151.4 ± 34.7	3	196.3 ± 44.8	197.1 ± 20.9 [a]
L. camara 015 WY	5	134.4 ± 34.7	2	197.9 ± 54.9	1	131.9 ± 77.6	154.6 ± 30.8 [a]
L. camara 150 O	5	227.4 ± 34.7	5	172.4 ± 34.7	5	200.7 ± 34.7	202.2 ± 20.3 [a]
L. camara 010 DP	5	159.0 ± 34.7	5	219.0 ± 34.7	4	124.4 ± 38.8	169.5 ± 20.8 [a]
Generation Mean [Y]		200.8 ± 15.5[a]		167.1 ± 17.0[a]		163.2 ± 19.8[a]	

SEM is the standard error of the mean.

F-probability: Varieties: P=0.439
Generations: P=0.226
Varieties x Generation Interaction: P=0.224

[X] – Means in this column followed by the same letter do not differ significantly at the 5% level.

[Y] – Means in this row followed by the same letter do not differ significantly at the 5% level.

4.3.4 Host plant suitability analysis

Lantana camara variety 029 WP was the most successful host variety for *A. extrema* under laboratory conditions (Table 4.6). Performance and survival on this variety was consistent throughout the various trials. *Lantana camara* variety 163 LP was 42% as suitable as a host plant, followed by variety 150 O (34%), variety 015 WY (29%) and variety 010 DP being the least suitable at 13%. However, despite these findings, neither larval survival (Table 4.3), the viability of larvae reared on the varieties for 3 generations (Table 4.4) nor the number of eggs laid by the 3rd generation females, differed significantly between the 5 varieties. It was only during the adult multi-choice trials (Table 4.2) that significant differences were found between the mean number of adults on 029 WP and 150 O and between the mean number of egg packets on 029 WP and 010 DP. The analysis thus overestimates the non-significant differences in performance on the different varieties, but since the adults make the choice regarding the suitability of the different varieties as oviposition sites, the analysis does provide a practical numerical score to differentiate between the varieties.

Table 4.6: A comparison of the suitability of 5 different *Lantana camara* varieties as host plants for *Alagoasa extrema*

Variety	Plant preference (R^1)	Oviposition Preference (R^2)	Oviposition Potential (R^3)	Larval Survival (R^4)	Suitability Index ($R^1xR^2xR^3xR^4$)
L. camara 163 LP	0.684	0.676	0.938	0.976	0.42
L. camara 029 WP	1.000	1.000	1.000	1.000	1.00
L. camara 015 WY	0.948	0.676	0.573	0.791	0.29
L. camara 150 O	0.501	0.864	0.969	0.809	0.34
L. camara 010 DP	0.632	0.297	0.678	1.032	0.13

4.4 Discussion

Alagoasa extrema exhibits a degree of varietal preference under laboratory conditions. The host plant suitability analysis calculated 029 WP to be the most suitable host variety, although the other four tested varieties were able to support viable populations of *A. extrema* for three consecutive generations in the laboratory. Not taking other factors such as climate and predation into consideration, all five of the tested varieties should thus be able to support viable populations of *A. extrema* in the field. The five tested *L. camara* varieties are listed among the 11 most important varieties in South Africa and are widespread in Mpumalanga and KwaZulu-Natal provinces, where some of the most severe infestations of *L. camara* are found.

The importance of studies to determine varietal preferences is highlighted by cases such as *F. intermedia*, where varietal preference studies indicated that certain varieties were totally unsuitable, resulting in 100% mortality of the mirid (Urban and Simelane 1999, Day and Neser 2000). Another species that displayed varietal preferences was *A. parana*, which accepted the red and pink-edged red Australian *L. camara* varieties and only partially accepted the common pink, orange and white Australian *L. camara* varieties, with populations dying out on the less preferred varieties (Day and Neser 2000). Among the potential biocontrol agents currently being studied in quarantine, both the petiole-galling *Coelocephalapion camarae* Kissinger and the root-attacking *Longitarsus* sp. display no varietal preferences, with good compatibility with South African lantana varieties (Baars unpublished, Simelane unpublished).

Although *A. extrema* displayed some degree of varietal preferences, this phenomenon should not impact significantly on the insect's chances of establishment. If *A. extrema* is found to be sufficiently host specific to promote release, it could have an impact on a number of widespread *L. camara* varieties in areas where additional stress on the weed is urgently needed.

CHAPTER 5

LABORATORY HOST RANGE OF *ALAGOASA EXTREMA*, A POTENTIAL BIOLOGICAL CONTROL AGENT FOR *LANTANA CAMARA* IN SOUTH AFRICA

5.1 Introduction

All potential weed biological control agents need to undergo extensive host specificity testing to ensure that their release would not result in unacceptable non-target impact (van Klinken 2001). Host specificity studies are routinely undertaken to determine which plant species are included in a candidate's fundamental host range under laboratory conditions. The absolute limits to an insect's fundamental host range are determined by such factors as its metabolic and sensory capabilities, physical limitations and behavioural programming (van Klinken 2001). Host specificity testing can be divided into several steps: 1) identifying the fundamental host range of the potential agent; 2) identifying the life stage that makes the host choice; and 3) determining whether non-target species are included within the fundamental host range and thereby predicting whether, and to what extent, they will be attacked under field conditions. Host specificity testing encompasses choice and no-choice trials where representatives of either the immature or the mature stages of the potential agent are exposed to a series of test plant species in order to quantify certain parameters, usually mortality but also feeding damage and/or oviposition.

The realized host range, i.e. those plant species that are accepted as suitable hosts under field conditions, forms a subset of the fundamental host-range (van Klinken 2001). It is an accepted phenomenon that laboratory-based host specificity screening can lead to artificial host range extension, and over-estimate the range of plants suitable for survival under field conditions (Cullen 1990, Shepherd 1990, Hill and Hulley 1995). Under natural conditions, an insect follows a normal behavioural sequence based on appropriate cues, which lead to the selection of its correct host (Wapshere 1989). Usually, under restricted cage conditions, not all of the necessary cues are present, and the insect will

display behaviour characteristic of subsequent steps in its behavioural sequence and not the expected response. Thus, a species might be included in the fundamental host range of the larval stage, but not in that of the discerning ovipositing female, which represents an earlier step in the behavioural sequence of the host selection process. Thus, under field conditions the female would not recognize that plant species as a host, even though her offspring would have been able to develop on that species (Wapshere 1989). Consequently, such species should not be rejected as biocontrol agents because of unnatural larval feeding.

Recent host specificity tests have indicated that most of the natural enemies currently being evaluated as potential biocontrol agents for *L. camara* in South Africa, accept closely related native and introduced plant species to varying degrees under restricted cage conditions (Baars and Neser 1999; Baars 2000). Prior to 1990, biocontrol agents obtained via Australia were released in South Africa, with virtually no additional host specificity testing besides the studies conducted in Australia, as these tests were considered to be sufficient for South African requirements (Cilliers and Neser 1999). Subsequent studies have indicated that *Teleonemia scrupulosa* (Stål), a tingid that was released under these circumstances and that has been established in South Africa for decades, feeds and develops on a wide range of species of Verbenaceae under laboratory conditions, but in the field has displayed only limited feeding on some native *Lippia* species (Baars and Neser 1999; Baars 2000). These studies suggest that the extended host ranges determined under laboratory conditions are often not realized in the field and that closely related species, at most, qualify as marginal hosts under field conditions (Baars and Neser 1999).

In this chapter the host range of *A. extrema* under quarantine laboratory conditions is described and its suitability as a biocontrol agent discussed.

5.2 Materials and Methods

Studies to determine the host range of *A. extrema* included larval no-choice trials, adult choice trials and multi-generation trials. Larval no-choice trials test whether non-target

species can support larval development. Adult choice trials test whether non-target species are accepted as suitable feeding and oviposition sites. Multi-generation trials test whether non-target species can support successive generations of the agent under a no-choice situation. A plant species that supports successive generations of an agent is potentially an alternative host and is therefore at risk if the agent was to be released (Day 1999).

Larval no-choice trials and the multi-generation trials were conducted in a quarantine laboratory with temperatures varying between 21ºC (night) and 29ºC (day) and the relative humidity varying between 35% and 65%. Overhead plant growth lights supplemented natural daylight resulting in a 14h photoperiod. Adult choice trials were conducted in a quarantine tunnel with temperatures varying between 13ºC (night) and 30ºC (day) and the relative humidity varying between 35% and 65%.

5.2.1 Test plant species

The test plant species were selected according to Wapshere's (1974) centrifugal phylogenetic testing method. Test plants (Table 5.1) consisted of 33 representative species in the families Verbenaceae and Lamiaceae as well as some economically important families. *Lantana camara* variety 029 WP was used as the control plant during all trials as it proved to be the most suitable host variety for *A. extrema* (see Chapter 4). Culture and test plants were maintained in pots under drip and overhead irrigation, under 50% shadenet and were fertilized with LAN (Sasol Fertilizers®) and Super phosphate (All-Gro®) as needed. Although there are four native *Lippia* species described (Arnold and De Wet 1993), two additional taxa with different morphological characteristics and odours were treated as separate species and referred to as *Lippia* sp. A and B. Specimens of the latter have been lodged at the herbarium of the National Botanical Institute in Pretoria, South Africa (collector's accession numbers 11 and 28).

Table 5.1: Test plant species used in the various trials to determine the host specificity of *Alagoasa extrema*.

Plant species	Common Names	Trials conducted
Verbenaceae		
Verbena brasiliensis Vell.*		AC
Verbena bonariensis L.*		AC
Lantana angolensis Moldenke		AC
Lantana camara L.*	Lantana	LNC, AC, MGNC
Lantana dinteri Moldenke		LNC, AC, MGNC
Lantana mearnsii Moldenke		LNC, AC, MGNC
Lantana rugosa Thunb.		LNC, AC, MGNC
Lantana montevidensis (Spreng.) Briq.*#	Creeping lantana	LNC, AC, MGNC
Lantana trifolia L.*		LNC, AC, MGNC
Lippia javanica (Burm.f.) Spreng.		LNC, AC, MGNC
Lippia rehmanni H. Pearson		LNC, AC, MGNC
Lippia wilmsii H. Pearson		LNC, AC, MGNC
Lippia scaberimma Sond.		LNC, AC, MGNC
Lippia sp. A		LNC, AC, MGNC
Lippia sp. B		LNC, AC, MGNC
Phyla nodiflora (L.) Greene		LNC, AC, MGNC
Aloysia citriodora Palau*#	Lemon verbena	LNC, AC, MGNC
Priva meyeri var. *meyeri* Jaub. & Spach.		LNC, AC, MGNC
Duranta erecta L.*#		LNC, AC
Lamiaceae		
Clerodendrum glabrum E. Mey.		LNC
Karomia speciosa R. Fernandes		LNC, AC
Lavandula angustifolia Ehrh.*#	English lavender	LNC
Nepeta caltaria L.*#	Catnip	LNC
Salvia africana-caerulea L.		LNC
Salvia elegans Vahl.*#	Pineapple sage	LNC
Mentha piperita L.*#	Peppermint	LNC
Mentha spicata L.*#	Spearmint	LNC
Plectranthus sp.		LNC
Ocimum basilicum L.*#	Basil	LNC
Solanaceae		
Solanum melongena L.*#	Egg plant	LNC
Umbelliferae		
Daucus carota L.*#	Carrot	LNC
Chenopodiaceae		
Beta vulgaris L.*#	Beetroot	LNC
Cruciferae		
Brassica oleracea L.*#	Cabbage	LNC

* - Plant species introduced to South Africa (Arnold and De Wet 1993)
# - Plant species of economic and/or ornamental value in South Africa.
**LNC – Larval no-choice trials, AC – Adult choice trails, MGNC – Multi-generation no-choice trials.

5.2.2 Larval no-choice trials

Twenty-nine plant species were included in the larval no-choice trials. Ten newly emerged and unfed larvae were placed onto the foliage of potted plants of the test plant species and *L. camara* as control and confined in ventilated cages to prevent the larvae escaping. The number of larvae surviving to adulthood and the developmental time to adult eclosion were recorded. A minimum of three replications were conducted for each test plant species. Data were analyzed using the statistical program GenStat (2000). The experiment was designed as a completely randomized design with 29 species. Differences between species were tested for by means of One-way ANOVA. The data were acceptably normal and the treatment variances homogeneous. Species means were separated using Fisher's Protected t-test with the Least Significant Difference (LSD) at the 5 % level (Snedecor and Cochran 1980), and the F-probability from the ANOVA was taken as significant at 5 %.

5.2.3 Adult choice trial

The adult choice trial was conducted in a large walk-in cage (4m x 4m x 2m) in a ventilated quarantine tunnel. Older plants (up to 1m tall), grown in 10*l* pots, of the test plant species on which larvae were able to complete their development were used during these trials. *Lantana camara* was included as a control plant. In addition, *Verbena bonariensis*, *V. brasiliensis* and *Lantana angolensis* were also included, as these species were not available during the larval no-choice trials. Two Lamiaceae species completed the 20 test plant species and acted as additional 'controls' to check that females do not feed and oviposit randomly, but make actual choices between the test plants. The plants were arranged in the cage following a 4x5 rectangular lattice design, without their foliage touching. A total of 90 experienced females and 45 experienced males (calculated as 10 females and 5 males per plant species supporting more than 50% larval to adult survival) were released in the cage and removed after 10 days. The number of adults present on each test plant and the number of egg batches in each plant pot were recorded. Data were analyzed using the statistical program GenStat (2000). Differences between species were tested for by means of an analysis of variance. The data were acceptably normal and the treatment variances homogeneous. Species means were separated using Fisher's

Protected t-test with the Least Significant Difference (LSD) at the 5 % level (Snedecor and Cochran 1980), and the F-probability from the ANOVA was taken as significant at 5 %.

5.2.4 Multi-generation trials

These trials were done to determine whether the relevant test plant species were able to support consecutive larval development to the third generation, and to monitor any reduced fitness and ovipositional output, through being fed inferior quality food. The multi-generation trials were conducted by culturing each group of adults that originated from larvae that survived the no-choice trials, through to the third generation (where viable offspring were available). The newly-eclosed adults (F), obtained from the larval no-choice trials, were transferred to fresh plants of the same species and allowed to feed and oviposit. The number of eggs laid per female during the first 45 days after eclosion was recorded. Where viable eggs were produced, 10 newly-emerged larvae were placed onto foliage of the test plant species. The number of larvae surviving and developmental time to adult eclosion (F1) were recorded. Once again, the number of eggs laid per female during the first 45 days after eclosion was recorded. The same procedure was carried out for the third generation (F2). Data were analyzed using the statistical program GenStat (2000). The experiment was designed as a completely randomized design with two factors, namely, the test plant species and the 3 generations. Differences between species and generations, and the interactions between them, were tested for by means of an analysis of variance. The data were acceptably normal and the treatment variances homogeneous. Species means were separated using Fisher's Protected t-test with the Least Significant Difference (LSD) at the 5 % level (Snedecor and Cochran 1980), and the F-probability from the ANOVA was taken as significant at 5 %.

5.2.5 Risk Analysis

The risks to non-target plant species were analyzed and quantified by the method developed by Wan and Harris (1997), by measuring the insect's performance on each test plant, at different stages in the host plant selection process, as a proportion of that on *L. camara* (029 WP). The relative performance risk of *A. extrema* was determined against

15 Verbenaceae and 2 Lamiaceae non-target species that were fed on, or oviposited on, to varying degrees during the choice and no-choice trials (Table 5.6). The performance criteria used were i) plant preference, ii) oviposition preference, iii) oviposition potential and iv) larval survival. Plant preference and oviposition preference were determined by the mean number of adults present on the test plants, and the mean number of egg packets laid in the soil around the test plants during the adult choice trials (Table 5.3). Oviposition potential was determined as the mean number of eggs laid per female during the first 45 days after eclosion, when reared on different test plant species during the multi-generation no-choice trials (Table 5.5a, F-generation). Larval survival was the mean number of larvae surviving to adulthood during the larval no-choice trials (Table 5.2). The product of the calculated scores for each of the above performance criteria assessed the risk of *A. extrema* utilizing and establishing viable reproductive populations on a non-target plant species. For each criterion, R represents the insect's performance on the test plant relative to that on *L. camara* (029 WP) (Table 5.6). To facilitate calculation, zero values were recorded as 0.001 (sensu Wan and Harris 1997).

5.3 Results

5.3.1 Larval no-choice trials

All 15 Verbenaceae species tested, supported larval development (Table 5.2), while none of the Lamiaceae or the economically important species tested, were suitable for larval development. *Lantana camara* (029 WP) was the most suitable host with 72% of larvae developing to adulthood, although this was not significantly higher than survival on *L. mearnsii* (63%), *Lippia rehmanni* (66%), *Lippia* sp. A (53%), *Lippia* sp. B (67%), *Aloysia citriodora* (54%) and *Priva meyeri* var *meyeri* (66%). Several other indigenous *Lantana* and *Lippia* species proved to be less suitable hosts for *A. extrema* larvae, with survival rates varying between 15% and 33%.

Table 5.2: Mean number of *Alagoasa extrema* larvae developing to adulthood on different test plant species during larval no-choice trials.

Plant species	**n**	**Number of larvae surviving (Mean ± SEM)[Z]**
Verbenaceae		
L. camara 029 White Pink	6	7.2 ± 1.0[a]
L. dinterii	4	2.8 ± 1.3[bc]
Lantana trifolia	6	3.3 ± 1.0[bc]
Lantana mearnsii	4	6.8 ± 1.3[a]
Lantana rugosa	8	2.9 ± 0.9[bc]
Lantana montevidensis	6	2.7 ± 1.0[bc]
Lippia rehmanni	5	6.6 ± 1.1[a]
Lippia javanica	8	1.5 ± 0.9[c]
Lippia scaberimma	8	2.5 ± 0.9[c]
Lippia sp. A	6	5.3 ± 1.0[ab]
Lippia wilmsii	6	3.0 ± 1.0[bc]
Lippia sp. B	6	6.7 ± 1.0[a]
Phyla nodiflora	6	3.0 ± 1.0[bc]
Aloysia citriodora	7	5.4 ± 1.0[ab]
Priva meyeri var *meyeri*	5	6.6 ± 1.1[a]
Duranta erecta	5	0
Lamiaceae		
Clerodendrum glabrum	3	0
Karomia speciosa	3	0
Lavandula angustifolia	3	0
Salvia africana-caerulea	3	0
Salvia elegans	3	0
Mentha spicata	3	0
Mentha piperita	3	0
Plectranthus sp.	3	0
Nepeta caltaria	3	0
Ocimum basilicum	3	0
Solanaceae		
Solanum melongena	3	0
Umbelliferae		
Daucus carota	3	0
Chenopodiaceae		
Beta vulgaris	3	0
Cruciferae		
Brassica oleracea	3	0
F-probability		<0.001

SEM is the standard error of the mean.

[Z] – Means followed by the same letter are not significantly different ($p < 0.05$; ANOVA; Fishers' protected t-test LSD).

5.3.2 Adult choice trial

Most adults were found on *L. camara* (029 WP), *L. trifolia*, *Lippia rehmann*, *L. javanica* and *Lippia* sp. B (Table 5.3), although the numbers did not differ significantly between these four species and *L. camara*. Although there were no significant differences between the numbers of egg packets laid in the soil around the test plants, the highest numbers were found in the soil of the above four species. With the exception of *L. trifolia*, these species were also consistently chosen as suitable oviposition sites in all three replicates (Table 5.3).

Table 5.3: Host selection by adults of *Alagoasa extrema* during multi-choice trials, with test plant species arranged in a 4x5 rectangular lattice design.

Species	**n**	**Number of adults (Mean ± SEM)^{Z}***	**Number of egg packets (Mean ± SEM)**	**Times (out of 3) chosen as oviposition site**
Verbenaceae				
Verbena brasiliensis	3	1.3 ± 3.3	0	0
V. bonariensis	3	0.7 ± 3.3	0	0
Lantana camara 029 WP	3	15.7 ± 3.3^{a}	3.0 ± 1.2	3
L. angolensis	3	0.7 ± 3.3	0	0
L. dinterii	3	0.3 ± 3.3	0	0
L. trifolia	3	14.3 ± 3.3^{abc}	2.7 ± 1.2	2
L. mearnsii	3	2.0 ± 3.3^{e}	0.3 ± 1.2	1
L. rugosa	3	1.3 ± 3.3^{e}	0	0
L. montevidensis	3	1.0 ± 3.3	0.3 ± 1.2	1
Lippia rehmanni	3	14.7 ± 3.3^{ab}	2.3 ± 1.2	3
L. javanica	3	8.0 ± 3.3^{abcde}	1.0 ± 1.2	2
L. scaberimma	3	2.3 ± 3.3^{de}	0.3 ± 1.2	1
Lippia sp. A	3	5.0 ± 3.3^{cde}	1.3 ± 1.2	2
L. wilmsii	3	5.3 ± 3.3^{bcde}	0	0
Lippia sp. B	3	11.7 ± 3.3^{abcd}	2.7 ± 1.2	3
Phyla nodiflora	3	0	0	0
Aloysia citriodora	3	5.0 ± 3.3^{cde}	0	0
Priva meyeri var *meyeri*	3	1.3 ± 3.3	0	0
Duranta erecta	3	0.3 ± 3.3	0	0
Lamiaceae				
Karomia speciosa	3	0.3 ± 3.3	0	0
F-probability		0.026	0.818	

SEM is the standard error of the mean.

^{Z} – Means within column followed by the same letter are not significantly different ($p < 0.05$; ANOVA; Fishers' protected t-test LSD). Means without any letter was not included in statistical analysis because of too low a number of adults/egg packets.

* - Out of 135 adults.

5.3.3 Multi-generation trials

Eight test plant species out of the original 15 Verbenaceae species that supported larval development, including *L. camara* 029 WP, sustained oviposition and larval development up to and including the third generation (Table 5.4 and 5.5a). *Lippia scaberrima* supported larval development up to the third generation, but no eggs were laid by the F2-generation females (Table 5.5b). The mean number of larvae surviving over three generations on *L. camara* 029 WP did not differ significantly from the number surviving on *L. rehmanni, Lippia* sp. B and *Priva meyeri* var *meyeri*. A significantly lower survival rate was found on *Lantana mearnsii, L. rugosa, L. montevidensis* and *Lippia* sp. A. The mean number of eggs laid over three generations by females reared on *L. camara* 029 WP did not differ significantly from that laid by females reared on *Lantana mearnsii, L. rugosa, Lippia rehmanni, Lippia* sp. A, *Lippia* sp. B and *P.meyeri* var *meyeri*. The exception was *L. montevidensis* on which females produced a significantly lower mean number of eggs.

Table 5.4: Survival to adulthood of *Alagoasa extrema* larvae when reared on different test plant species that supported development for 3 consecutive generations during multi-generation no-choice trials

Species	**n**	**Larval survival F (Mean ± SEM)**	**n**	**Larval survival F1 (Mean ± SEM)**	**n**	**Larval survival F2 (Mean ± SEM)**	**Species (Mean ± SEM)[X]**
Lantana camara 029 WP	6	7.2 ± 1.2	5	8.2 ± 1.3	4	4.8 ± 1.4	6.8 ± 0.7[a]
L. mearnsii	4	6.8 ± 1.4	3	2.3 ± 1.6	2	4.0 ± 1.6	4.1 ± 0.9[bc]
L. rugosa	8	2.9 ± 1.0	3	2.3 ± 1.2	1	6.0 ± 1.2	1.8 ± 0.6[d]
L. montevidensis	6	2.7 ± 1.2	4	4.5 ± 1.2	3	0.7 ± 1.3	2.1 ± 0.7[cd]
Lippia rehmanni	5	6.6 ± 1.3	5	6.8 ± 1.3	4	3.0 ± 1.4	5.6 ± 0.8[ab]
Lippia sp. A	6	5.3 ± 1.2	4	5.8 ± 1.2	3	4.0 ± 1.2	3.9 ± 0.7[bc]
Lippia sp. B	6	6.7 ± 1.2	4	6.3 ± 1.3	3	7.3 ± 1.4	5.8 ± 0.7[ab]
Priva meyeri var *meyeri*	5	6.6 ± 1.3	5	6.0 ± 1.3	3	3.3 ± 1.4	5.2 ± 0.8[ab]
Generation Mean[Y]		5.4[a]		4.4[a]		2.6[b]	

SEM is the standard error of the mean.

F-probability: Species: P<0.001
Generation: P<0.001
Species x Generation Interaction: P=0.796

[X] - Means within this column followed by the same letter are not significantly different at the 5% level.

[Y] - Means within this row followed by the same letter are not significantly different at the 5% level.

Table 5.5a: Ovipositional performance of *Alagoasa extrema* females when reared on different test plant species that supported oviposition for 3 consecutive generations during multi-generation no-choice trials.

Species	**n**	**No of eggs per female during 1st 45 days F (Mean ± SEM)**	**n**	**No of eggs per female during 1st 45 days F1 (Mean ± SEM)**	**n**	**No of eggs per female during 1st 45 days F2 (Mean ± SEM)**	**Species Mean (±SEM)[X]**
Lantana camara 029 WP	6	234.6 ± 31.7	5	151.4 ± 34.8	3	196.3 ± 44.9	199.0 ± 20.8[a]
L. mearnsii	4	196.7 ± 38.9	2	166.4 ± 54.9	1	49.6 ± 77.7	156.4 ± 30.2[a]
L. rugosa	3	103.2 ± 44.9	1	261.7 ± 77.7	1	92.5 ± 77.7	153.8 ± 36.8[a]
L. montevidensis	4	56.7 ± 38.9	3	17.0 ± 44.9	1	3.1 ± 77.7	32.5 ± 28.3[b]
Lippia rehmanni	5	215.7 ± 34.8	4	112.9 ± 38.9	2	44.9 ± 54.9	146.4 ± 23.5[a]
Lippia sp. A	4	202.9 ± 38.9	3	254.8 ± 44.9	3	89.8 ± 44.9	197.0 ± 25.1[a]
Lippia sp. B	5	272.5 ± 34.8	4	217.3 ± 38.9	3	62.6 ± 44.9	211.1 ± 22.6[a]
Priva meyeri var *meyeri*	5	168.1 ± 34.8	4	201.9 ± 38.9	2	140.0 ± 54.9	173.6 ± 23.5[a]
Generation Mean [Y]		194.2 ± 13.0[a]		171.1 ± 15.4[a]		93.2 ± [b]	

SEM is the standard error of the mean.
F-probability: Species: P<0.001
Generation: P<0.001
Species x Generation Interaction: P=0.161
[X] – Means within this column followed by the same letter are not significantly different at the 5% level.
[Y] – Means within this row followed by the same letter are not significantly different at the 5% level.

Table 5.5b: Test plant species that were unable to support oviposition to the third generation during multi-generation no-choice trials

Species	**n**	**No of eggs per female during 1st 45 days F (Mean ± SE)**	**n**	**No of eggs per female during 1st 45 days F1 (Mean ± SE)**	**n**	**No of eggs per female during 1st 45 days F2 (Mean ± SE)**
L. dinterii	3	14.0 ± 11.1	0	0	0	0
L. trifolia	3	100.5 ± 12.9	1	0	0	0
Lippia javanica	2	88.2 ± 18.2	1	24.7 ± 0.0	0	0
Lippia scaberimma	4	145.6 ± 48.3	1	59	1	0
Lippia wilmsii	5	0.6 ± 0.6	0	0	0	0
Phyla nodiflora	4	9.4 ± 4.7	0	0	0	0
Aloysia citriodora	6	54.1 ± 17.5	0	0	0	0

5.3.4 Risk Analysis

Calculation of the risk of *A. extrema* utilizing and establishing viable reproductive populations on non-target plant species (Table 5.6), indicated that *Lippia* sp. B had a 72% probability of supporting such populations, compared with 62% in *L. rehmanni*, 16% in *L. trifolia* and 9% in *Lippia* sp. A a 9%. The likelihood of the remaining species serving as alternative hosts for *A. extrema*, varied between 1% and less than 0.001%.

Table 5.6: Risk analysis on the performance of *Alagoasa extrema* on non-target plant species relative to that on *L. camara* (variety 029 WP)

Species	**Plant Preference (R^1)**	**Oviposition Preference (R^2)**	**Oviposition Potential (R^3)**	**Larval Survival (R^4)**	**Risk of Attack ($R^1xR^2xR^3xR^4$)**
Verbenaceae					
Lantana camara 029WP	1.000	1.000	1.000	1.000	1.000
L. dinterii	0.021	0.001	0.060	0.384	4.8×10^{-7}
L. trifolia	0.914	0.890	0.428	0.464	0.16
L. mearnsii	0.128	0.110	0.838	0.941	0.01
L. rugosa	0.085	0.001	0.440	0.402	1.5×10^{-5}
L. montevidensis	0.064	0.110	0.242	0.372	6.3×10^{-4}
Lippia rehmanni	0.936	0.777	0.920	0.921	0.62
L. javanica	0.511	0.333	0.376	0.209	0.01
L. scaberimma	0.149	0.110	0.621	0.349	3.6×10^{-3}
Lippia sp. A	0.319	0.443	0.865	0.743	0.09
L. wilmsii	0.340	0.001	0.0026	0.418	3.7×10^{-7}
Lippia sp. B	0.745	0.890	1.162	0.930	0.72
Phyla nodiflora	0.001	0.001	0.040	0.418	1.7×10^{-8}
Aloysia citriodora	0.319	0.001	0.231	0.757	5.6×10^{-5}
Priva meyeri var *meyeri*	0.085	0.001	0.717	0.921	5.6×10^{-5}
Duranta erecta	0.021	0.001	0.001	0.001	2.1×10^{-11}
Lamiaceae					
Karomia speciosa	0.021	0.001	0.001	0.001	2.1×10^{-11}

5.4 Discussion

There are virtually no records of the host plants of *A. extrema*. Palmer and Pullen (1995), reporting on the phytophagous arthropods associated with *L. camara*, *L. hirsuta*, *L. urticifolia* and *L. urticoides*, mentioned a chrysomelid species, *Alagoasa* pr. *extrema*, found by Mann and Krauss during a previous survey in 1954. No further details were given other than the "association" with the four *Lantana* species.

This study showed *A. extrema* to be an oligophagous herbivore, capable of ovipositing and developing on a number of indigenous and exotic verbenaceous species. Table 5.7 gives the results of adult choice trials on four biocontrol agents currently being studied or that have been studied during the last 5 years. In adult choice trials *F. intermedia*, *Coelocephalapion camarae* Kissinger and *Leptostales ignifera* Warren fed on and/or laid eggs on other *Lantana* and several *Lippia* species, but at a much lower rate than on *L. camara*. These plant species under field conditions should not be able to support populations of the biocontrol agents, and might suffer limited feeding under periods of extremely high population densities, creating a "spill-over" effect. Thus, in spite of oviposition, feeding and development that took place on these species, all three the candidate biocontrol agents were or are to be released. On the other hand, during adult choice trials, a very promising stem-attacking insect, *Aconophora compressa* fed and oviposited on *Lippia* sp. B to such an extent that it was statistically comparable ($p>0.05$) to that on *L. camara* (Table 5.7). During no-choice multi-generation trials, comparable and sometimes superior performance was found on *Lippia* sp. B, and *A. compressa* thus had to be rejected because of these results (Heystek unpublished). Likewise, comparable performance by *A. extrema* was found on several verbenaceous species (Tables 5.4, 5.5a, 5.6).

Table 5.7: Results of adult choice trials with potential biocontrol agents to demonstrate the host range expansion onto *Lantana* and *Lippia* species under quarantine laboratory conditions (Baars 2000, Simelane 2002, Heystek unpublished, Williams unpublished).

Potential biocontrol agent	***Lantana* species accepted as feeding/oviposition sites**	***Lippia* species accepted as feeding/oviposition sites**	**Agent rejected /released**
Falconia intermedia (Hemiptera: Miridae)	*L. camara* *L. trifolia*+	*L. javanica*+ *L. rehmannii*+ *L. scaberrima*+ *L. wilmsii*+ *Lippia* sp. A+ *Lippia* sp. B++	Released
Coelocephalapion camarae (Coeloptera: Apionidae)	*L. camara* *L. rugosa*+ *L. montevidensis*+ *L. trifolia*+	*L. javanica*+ *L. rehmannii*+ *L. scaberrima*+ *L. wilmsii*+ *Lippia* sp. A+ *Lippia* sp. B+	To be released
Leptostales ignifera (Lepidoptera: Geometridae)	*L. camara*	*L. rehmanni*+ *Lippia* sp. A+ *Lippia* sp. B+	To be released
Aconophora compressa (Hemiptera: Membracidae)	*L. camara*	*L. javanica*+ *L. rehmannii*+ *L. wilmsii*++ *Lippia* sp. A+ *Lippia* sp. B+++	Rejected

\+ Feeding and/or oviposition on this species **much lower** than on *L. camara*; should not qualify as a marginal host plant under field conditions,
++ Feeding and/or oviposition on this species **lower** than on *L. camara*; could qualify as a marginal host plant under field conditions,
+++ Feeding and/or oviposition on this species **comparable** than on *L. camara*; should qualify as an alternative host plant under field conditions.

The analysis of the risks posed by *A. extrema* to field populations of the test plant species, indicated that *Lippia* sp. B and *L. rehmanni* are likely to serve as alternative hosts in the field. Several biocontrol practitioners in South Africa have made use of a risk analysis, including Olckers (2000) with the screening of *Gargaphia decoris* Drake for the biological control of *Solanum mauritianum*, and Baars (pers. comm.) for the screening of *F. intermedia*. Olckers (2000) found that the probability of non-target species sustaining

reproductive populations ranged between <1% to 19.5%, and based on these results, permission was granted for the release of *G. decoris*. Baars (pers. comm.) found that the species that came closest to *L. camara* in terms of host suitability was *Lippia* sp. B, with a suitability of 24%, and based on these results, permission was granted for the release of *F. intermedia*. The probability of non-target attack on *Lippia* sp. B (72%) and *L. rehmanni* (62%), when compared to *L. camara* 029 White Pink, by *A. extrema* under field conditions is thus much greater. Baars (2000) stresses that host-range extension by natural enemies under laboratory conditions should be interpreted with care, and that more emphasis should be placed on behavioural factors that influence host acceptance. Oviposition choice by females plays a more important role in the host recognition process than does larval survival, and in the presence of *L. camara,* females will consistently recognize *Lippia rehmannii* and *Lippia* sp. B as acceptable hosts. In spite of the decrease in egg production when development occurs on these species, *A. extrema* will still pose a threat to these two species. Unlike biocontrol agents such as *F. intermedia*, *G. decoris* and *Gratiana spadicea* (Klug), where non-target species were deemed to be unlikely to support populations of the biocontrol agent, and where the damage to these species would be no more than incidental (assuming a worst-case scenario) (Hill and Hulley 1995, Baars 2000, Olckers 2000), the multi-generation trials indicated that the two *Lippia* species would be able to support populations of *A. extrema* and that damage to these species could be considerable.

The above considerations suggest that, should *A. extrema* be released in South Africa, the target weed, *L. camara*, along with some indigenous *Lippia* species are likely to serve as host plants. The potential risk to these indigenous species appears to be too great and it thus seems prudent that *A. extrema* should not be released in South Africa.

CHAPTER 6

GENERAL DISCUSSION

6.1 Suitability of *A. extrema* as an additional agent for *L. camara*

The biological control programme against *Lantana camara* in South Africa has had limited success (Chapter 1) and the weed still poses a threat to agricultural production and biodiversity, despite attack from several biocontrol agents over a number of decades. Additional agents are needed to supplement the herbivore stress on the weed. Several factors have constrained or affected the success of the programme (Chapter 1). The most important factors constraining the agents are climatic incompatibility, varietal preferences, and parasitism.

Lantana camara occurs over a broad range of climatic regions in South Africa. Most of the biological control agents originate from tropical and subtropical areas and establishment of these species in cold inland regions was not successful. *Lantana camara* plants abscise their leaves during winter and this leaf-less period together with lethal cold temperatures, can be devastating for the introduced leaf-feeding insects in particular. *Alagoasa extrema* is such a leaf-feeding candidate agent, which was collected from the subtropical areas of Mexico. Thus, the introduction of yet another leaf-feeding insect of tropical origin seems to go against lessons learned from past experience. Baars and Neser (1999) argued that leaves are the center of resource production and since the established leaf-feeders currently do not maintain adequate defoliation levels (Cilliers and Neser 1991, Baars and Neser 1999), there is a need for additional leaf-feeders. Most of the established leaf-feeders are recognized by characteristics such as short-lived adults, adults and/or immatures that need to feed continuously and are thus poorly adapted to cope with leaf-less periods, e.g. *Falconia intermedia, Teleonemia scrupulosa*, and *Hypena laceratalis*. Species such as *F. intermedia* are able to overwinter only in areas where sheltered pockets allow *L. camara* plants to retain their leaves (Heystek, pers. comm.). From these areas, they build up their numbers in spring and cause severe but sporadic damage. Leaf-feeders with long-lived adults, e.g. *Uroplata girardi* and

Octotoma scabripennis, have been among the most successful biocontrol agents for *L. camara* (Cilliers and Neser 1991, Broughton 2000), causing extensive but also localized defoliation. Potential leaf-feeding biocontrol agents that have long-lived adults that can enable them to overcome leaf-less periods should thus be targeted. *Alagoasa extrema* with its long-lived adults could fulfill this role. However, it was acknowledged from the start that *A. extrema* would probably only be able to establish in the subtropical areas of South Africa, but since these are also the most heavily lantana-infested areas in South Africa (see Chapter 1, Fig. 1.1), and *A. extrema* could therefore contribute significantly to the biocontrol programme.

The second important factor that caused the apparent lack of success of the biological control programme against *L. camara*, is varietal preferences displayed by the biocontrol agents. Conflicting reports on just how much this factor has contributed to the variable levels of biocontrol success are found in the literature and are discussed in Chapter 4. Varietal preference studies (Chapter 4) indicated that *A. extrema* showed some degree of preference for certain varieties, but that all the tested varieties were able to support populations of this insect for several generations. The most suitable variety, 029 White Pink, is listed as one of the 11 most invasive lantana varieties in South Africa, and is particularly widespread in subtropical Mpumalanga, an area where some of the most severe infestations of *L. camara* are found, and where additional stress on the weed is still needed.

The third important factor negatively influencing the success of the biological control programme against *L. camara*, is parasitism. The population numbers, and consequently impact, of several established biocontrol agents are reduced by parasitism (see Chapter 1). In Chapter 2 it was shown that several characteristics of *A. extrema* suggest unpalatibility, a feature that could confer protection against potential predators and parasitoids and thus increase the chances of establishment and population build-up. However, it is not known if parasitoids from native flea beetle species might make use of *A. extrema* as food source.

However, despite these favourable attributes, host specificity tests indicated that *A. extrema* is able to oviposit and develop on a number of indigenous and exotic verbenaceous species (Chapter 5). An analysis of the risks posed by *A. extrema* to field populations of the more vulnerable test plant species, indicated that the target weed, *L. camara*, along with the indigenous *Lippia* sp. B and *L. rehmanni* are likely to serve as hosts in the field. The potential risk to these two indigenous *Lippia* species was deemed to be too great and it was thus decided that *A. extrema* should not be released in South Africa.

6.2 Influence of testing procedures on determining an agent's suitability

The rejection of *A. extrema* forces one to critically consider the selection of agents and means by which host specificity testing is conducted and what problems, and perhaps errors, can be addressed and avoided, and what possible improvements can be suggested for future testing procedures.

Standard host specificity tests and adaptations thereof were used to determine the physiological host range of *A. extrema* under quarantine laboratory conditions (Fig. 6.1). These included larval no-choice, adult choice and multi-generation no-choice trials. From the results of these tests, the risks posed to non-target species by the possible release of *A. extrema* were determined by means of a risk assessment and a recommendation on the suitability for releases of *A. extrema* was made.

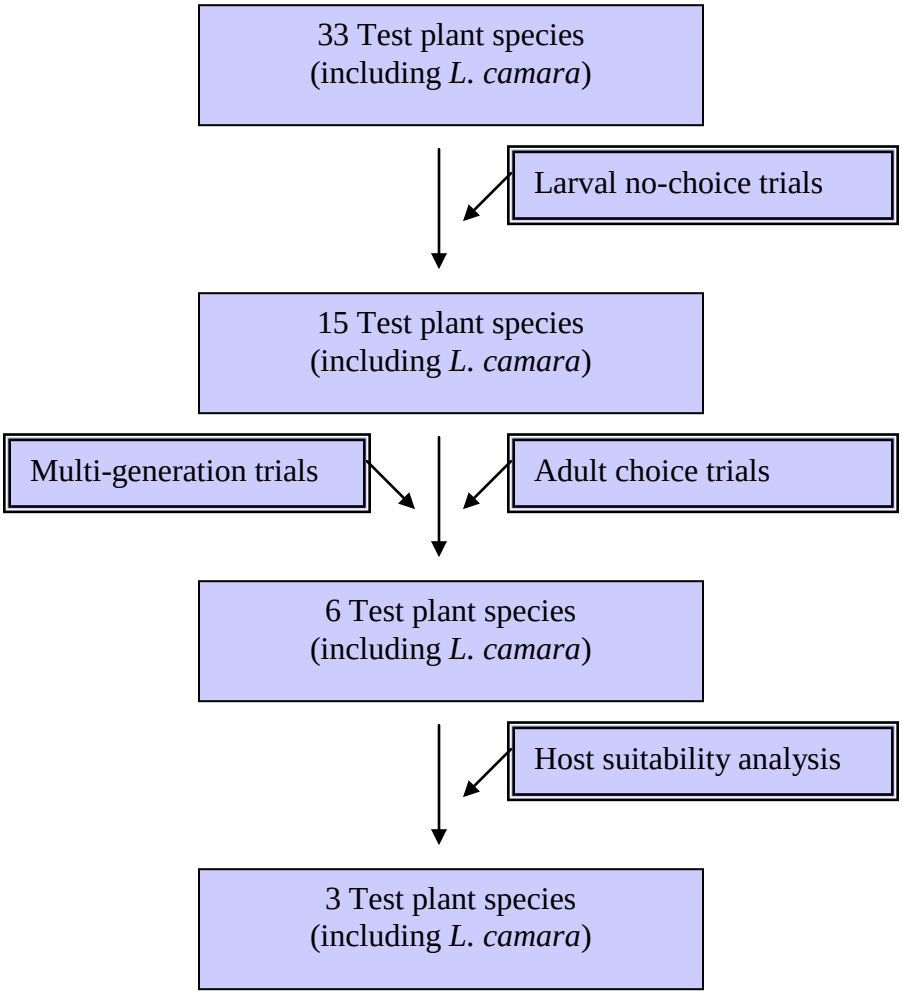

Fig. 6.1: The standard host specificity procedures and adaptations thereof used to determine the host range of *Alagoasa extrema* under quarantine laboratory conditions.

A fundamental question is thus whether any of the standard testing procedures could have been modified so as to provide more accurate results and thus theoretically a different outcome. In particular two factors are known to influence the outcome of host specificity testing and are discussed below. These factors are: a) the experimental design, and b) insect behavioural phenomena as affected by experience.

No-choice trials determine the candidate's fundamental host range, i.e. the absolute limits to an insect's host range that are determined by such factors such as its metabolic and sensory capabilities, physical limitations and behavioural programming (van Klinken

2000). In no-choice tests, an insect species is generally unable to exercise all of the discriminating behaviours that might cause it to reject a host in a more natural arena (Hill 1999). A negative test therefore provides very strong evidence that a particular plant species is not a potential host but, on the other hand, 'false positive' results can be generated. In such cases, plant species that would not qualify as hosts under field conditions, are accepted under the restricted conditions imposed by no-choice tests (Hill 1999). Under the conditions of larval no-choice trials, larvae of *A. extrema* were able to complete their development on 14 species, including the target weed (Chapter 5, Table 5.3). However, induced preferences in larvae can cause 'false negatives' in tests, if the insects have prior experience of the target weed, or any other plant that induces a strong preference for that plant (Traynier 1979, Heard 1999). Because unfed neonate larvae were used during the no-choice trials, induced preference could not have influenced the trial and the results (Chapter 5, Table 5.3) can be considered to accurately reflect the fundamental host range of *A. extrema*.

The larval no-choice trials were modified continued as multi-generation trials. Multi-generation trials test whether non-target species can support successive generations of potential biocontrol agents in no-choice situations (Day 1999). Under these circumstances, 8 species including the target weed were able to support populations of *A. extrema* for 3 consecutive generations (Chapter 5, Tables 5.5, 5.6a). During some of the trials, low numbers of adults completed their development, or sex-ratios were unbalanced, such that only a few pairs of adults were obtained to continue the trial. The ideal situation would have been to increase the insect population size on such plant species to obtain a more reliable mean of the insect's fertility but, unfortunately, logistics made this very difficult. The results of the multi-generation trials (Chapter 5, Table 5.5, 5.6a) should still be accepted, as it would be unreasonable to conclude that the plant is not at risk because only a few adults emerged. Because of population size differences, a low percentage survival in the laboratory could translate to a large number of adults in the field (Day 1999). Table 5.6a also indicated that there was a significant decrease in the average numbers of eggs laid by females reared on *Lippia* sp. B and *L. rehmanni* by the third generation, compared to oviposition by females reared on *L. camara* that remained

statistically constant. It could be argued that should any established populations on *Lippia* sp. B and *L. rehmanni* become extinct at any stage, the plants would still be subjected to periodic damage by *A. extrema*, or could become more acceptable as hosts, as adaptation could occur over time (Day 1999).

Choice trials involve the simultaneous presentation of two or more plant species to the insect in the same arena (Edwards 1999). These tests usually follow no-choice trials and involve those species on which feeding, oviposition or complete development had occurred during the no-choice tests. These tests detect an agent's preference when given a choice. If only the target host was attacked, or was attacked to a far greater extent than any of the test species, then it can be concluded that the agent in question is host specific (Edwards 1999). During adult choice trials *A. extrema* adults oviposited on 9 plant species, including the target species (Chapter 5, Table 5.4). Furthermore, the multi-generation no-choice trials indicated that only 6 of these species, including *L. camara*, were able to support consecutive generations of *A. extrema* (Chapter 5, Table 5.5, 5.6a) and it can thus be concluded that only these 6 species are likely to serve as hosts for *A. extrema* in the field.

Several mechanisms associated with previous experience of the test insects, could have influenced the results of the adult choice trials. The adults used during the choice trials had been reared on the target species during routine culturing, prior to their use in the trials, so preference for *L. camara* could thus have been artificially induced (Heard 1999). However, the results of the adult choice trials, during which feeding and oviposition occurred on several test species, suggested that induced preferences did not occur (Chapter 5, Table 5.4).

On the other hand the adults could, as a result of previous exposure, have been in a state of central excitation where contact with the target weed, a highly ranked host species, would have increased the responsiveness and readiness of the insects to feed and oviposit (Heard 1999). In this state, females of *A. extrema* would have searched for suitable oviposition sites (namely moist secluded areas in the soil) and could well have been

stimulated to oviposit randomly in the pots of any of the test plant species. However, the results of the adult choice trials indicated that the adults did discriminate between the test species, as eggs were mostly laid in the soil around plant species that supported higher percentages of larval development. Also, no oviposition occurred in the soil around the two 'control species', *Duranta erecta* and *Karomia speciosa*, on which no larval development had occurred and which were specifically included in the test arena to test whether the adults were indeed exercising a choice (Chapter 5, Table 5.4).

It could also have been expected that, if the adult females were in a state of central excitation when they entered the choice situation, they would initially have oviposited on whichever plant species was encountered first, but with time, feeding and oviposition would eventually have declined on the lower ranked species, while continuing on the highest ranked or preferred host plant species (Withers *et al.* 1999). In hindsight, a criticism of, or possible error in the execution of the adult choice trials, was that the trials were run for a period of only 10 days and that the results were based only on the results recorded at the end of the trial period. Should the trial have been run for a longer period, and should oviposition data have been recorded at different intervals during the trial, then the ranking of host plant species, could possibly have been more pronounced.

Another phenomenon typical of adult choice trials is 'spill-over' of oviposition onto lesser-ranked species because of overcrowding. Simelane (2002) found with the leaf-mining agromyzid fly, *Ophiomyia camarae*, another agent for *L. camara*, that as low as eight females confined onto two plants per paired-choice trial, caused oviposition on *Lippia* species, whereas no spill-over occurred when a single pair of adults were used. Overcrowding seems unlikely to influence the oviposition behaviour of *A. extrema*, since Oedionychina flea beetles are known to aggregate (Begossi and Benson 1988).

The results of the adult choice trials indicated that *L. camara* is the highest ranking host plant species for *A. extrema*, but that *Lippia* sp. B and *L. rehmanni* are so closely ranked below *L. camara*, that these species could serve as possible alternative hosts.

6.3 The importance of risk assessments

In the risk analysis (Chapter 5, Table 5.7), the number of non-target species that could be at risk, should *A. extrema* be released, was reduced to two species, namely *Lippia rehmanni* and *Lippia* sp. B. The analysis employed to quantitatively assess the risk that the release of *A. extrema* would pose to non-target species, was based on a method developed by Wan and Harris (1997) (Chapter 5). Several South African biocontrol practitioners used this risk analysis to promote the release of agents, notably Olckers (2000) with *Gargaphia decoris* Drake for *Solanum mauritianum*, and Baars (pers. comm.) with *F. intermedia* for *L. camara* (Chapter 5). Baars (2000) stated that behavioural mechanisms that limit the accepted (i.e. true) host range, in this case the females that select oviposition sites, should be incorporated and emphasized during the risk analysis. However, results obtained from the no-choice trials should be carefully considered, as the possible broadening of an agent's host range under deprived conditions (and consequent "spill-over" effects) can be foreseen and predicted (Withers 1997). The risk analysis used during the host specificity testing of *A. extrema* takes into account performance factors from results of both the no-choice as well as the choice trials, giving a well-balanced reflection of the risks posed to non-target species.

Baars and Neser (1999) stated that because of possible limited attack on some indigenous *Lantana* and *Lippia* species, the number of new natural enemies that will ultimately be considered acceptable for release on lantana in South Africa will be limited, thus constraining the biocontrol programme against this extremely invasive weed. Species such as the stem-sucking *A. compressa* that was likely to have made a valuable contribution, since it is able to kill stems of its host plant and is also able to survive dry winters on plants that are devoid of leaves, had to be rejected because of potential damage and possible population build-up on some native *Lippia* species. Unless regulatory authorities and other affected bodies can accept possible damage on 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 weed as *L. camara*, the potential impact of several very promising agents, such as *A. extrema* and *A. compressa* will be lost. Thus is seems prudent that an analysis of the risks

associated with the release of a 'questionable' agent like *A. extrema* should be considered against an analysis of the risks of not releasing it, i.e. the additional environmental damage that will accrue if *L. camara* is allowed to continue unchecked.

6.4 Other considerations

The taxonomic relationship between some of the genera in the family Verbenaceae should be reexamined. Testing has indicated that most of the insect species tend to accept closely related native plant species to varying degrees, under laboratory conditions (Baars 1999, Simelane 2002, Heystek, pers. comm.). These species included several indigenous and introduced *Lantana* species, as well as species in the closely related indigenous genus *Lippia* (Chapter 5, Table 5.7). What is remarkable is that, although some ovipostion and feeding occurred on one or two of the related *Lantana* species, more often the more distantly related *Lippia* species tended to be more acceptable for feeding and oviposition, often supporting higher feeding and oviposition rates than on the *Lantana* species. In particular, *Lippia* sp. B has proved to be a superior host for *A. compressa* relative to some of the other *Lantana* species as well as several of the *L. camara* varieties (Heystek, pers. comm.), while it has also proven to be very closely ranked beneath *L. camara* in terms of the host preferences of *A. extrema*. *Lippia* sp. B was also the second preferred host of *F. intermedia* (Baars, pers. comm.). These insect species suggest that the relationship between *L. camara* and some of the *Lippia* species may well be much closer than the relationship between *L. camara* and other congeneric species, at least where their secondary plant chemicals that serve as insect attractants or repellants are concerned. This raises the question as to whether these species should not all be included in the same genus. Wapshere (1989) stated that related plants species have similar morphological structures and secondary chemical constitutions and that only minor adjustments in the host selection sequence would facilitate the inclusion of such species as hosts. The host ranges of phytophagous insects should thus give an indication of how closely affiliated the related plant species are. In this instance, it is strongly suggested that some of the *Lippia* species are more closely related to *L. camara* than are some of the other *Lantana* species.

6.5 The potential of *A. extrema* for use in other countries

Although *A. extrema* is not suitable for release in South Africa, it could have considerable potential for release against *L. camara* in Australia. Indeed, Australia and South Africa have long been collaborative partners in the battle against *L. camara* and potential agents have often been exchanged between the two countries. Since no native *Lantana* or *Lippia* species are represented in the indigenous Australian flora, there are thus no possible alternative hosts for *A. extrema*. An exception could be the exotic *Lippia alba* (Mill.) N.E. Br. *ex* Britton & P. Wilson, on which non-target feeding would be of no concern. In any event, the release of *A. extrema* would pose no threat to the indigenous Australian flora.

Australian researchers have invested a substantial amount of money in trying to successfully establish *A. parana* in Australia. In spite of diligent efforts, which included the seasonal collection of large numbers of the insect in Brazil and releasing them in Australia, establishment was not achieved (M. Day, pers. comm.). *Alagoasa extrema*, as alternative biocontrol agent for the same niche, could prove to be a more successful agent. Compared to *A. parana*, it has a shorter lifecycle, is multivoltine with several generations produced annually and may display a degree of tolerance towards some natural enemies (Chapter 2). These characteristics could facilitate successful establishment and high population levels in the field. Winder *et al.* (1988) suggested that *A. parana* would be most suited to the coastal rain forest fringes in Australia, a habitat that is in need of additional biological control agents for *L. camara*. Since *A. extrema* is most suited to moist conditions, which are fundamental to the insects' survival, this species could thus fill this niche in Australia. In November 2002, adults of *A. extrema* were exported to Australia to undergo host specificity screening.

REFERENCES

Arnold, T.H., and B.C. de Wet. 1993. Plants of southern Africa: names and distribution. *Memoirs of the Botanical Survey of South Africa No. 62.* National Botanical Institute, Pretoria, South Africa.

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

Baars, J-R. and Neser, S. 1999. Past and present initiatives on the biological control of *Lantana camara* (Verbenaceae) in South Africa. In: Olckers, T and M.P. Hill (eds.), *Biological Control of Weeds in South Africa (1990-1998). African Entomology Memoir* No. 1: 21-33.

Begossi, A. and Benson W.W. 1988. Host plants and defense mechanisms in Oedionychina (Alticinae). In: Jolivet P., Petitpierre E. and Hsiao T.H. (eds.); *Biology of Chrysomelidae.* Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 57-71.

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

Broughton, S. 1999. "Evaluation of some insect species introduced for the biological control of *Lantana camara* L. in South-East Queensland, Australia," PhD dissertation, The University of Queensland, Brisbane, Australia.

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

Cilliers, C.J. 1982. "An evaluation of the effects of imported insects on the weed Lantana camara L. in South Africa," Ph.D. dissertation, Rhodes University, Grahamstown, South Africa.

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

Cilliers, C.J. 1999. *Lysathia* n. sp. (Coleoptera: Chrysomelidae), a host-specific beetle for the control of the aquatic weed *Myriophyllum aquaticum* (Haloragaceae) in South Africa. *Hydrobiologia* 415: 271-276.

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

Crawley, M.J. 1986. The population biology of invaders. *Philosophical Transactions of the Royal Society of London B.* 314: 711-31

Crawley, M.J. 1989a. Plant life-history and the success of weed biological control projects. In: E.S. Delfosse (ed.) *Proceedings of the VII International Symposium of Biological Control of Weeds*, Rome, Italy, 1988. pp. 17-26. Isituto Sperimentale per la Patologia Vegetale Ministero dell' Agricoltura e delle Foreste, Rome.

Crawley, M.J. 1989b. The successes and failures of weed biocontrol using insects. *Biocontrol News and Information* 10: 213-223.

Crawley, M.J. 1989c. Insect herbivores and plant population dynamics. *Annual Review of Entomology.* 31: 531-564.

Cullen, J.M. 1990. Current problems in host specificity screening. In: E.S. Delfosse (ed.) *Proceedings of the VII International Symposium of Biological Control of Weeds*, Rome, Italy, 1988. pp. 27-36. Isituto Sperimentale per la Patologia Vegetale Ministero dell' Agricoltura e delle Foreste, Rome.

Daun, J. J. and Messing, R. H. 1996. Response of two opiine fruit fly parasitoids (Hymenoptera: Braconidae) to the lantana gall fly (Diptera: Tephritidae). *Biological Control*. 25: 1428-1437.

Day, M.D. 1999. Multi-generation trials: their use in assessing the host range of a potential biological control agent. In: Withers, T.M., L. Barton Browne and J. Stanley (eds.) *Host specificity testing in Australasia: towards improved assays for biological control*. CRC for Tropical Pest Management, Brisbane, Australia. pp. 11 – 19.

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

Dirzo, R. 1984. Herbivory: a phytocentric overview. In: R. Dirzo, and J. Sarukhan (eds.) *Perspectives on plant population ecology*. Sinauer, Sunderland. pp. 141-165.

Edwards, P.B. 1999. The use of choice tests in host specificity testing of herbivorous insects. In: Withers, T.M., L. Barton Browne and J. Stanley (eds.) *Host specificity testing in Australasia: towards improved assays for biological control*. CRC for Tropical Pest Management, Brisbane, Australia. pp. 35-43.

Everist, S.L. 1974. *Poisonous plants of Australia*. Angus and Robertson, Sydney. 684 pp.

GenStat for Windows. 2000. *Release 4.2 Fifth Edition*. VSN International Ltd., Oxford.

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

Gentle, C. B. and Duggin, J. A. 1997b. *Lantana camara* L. invasions in dry rainforest-open ecotones: the role of disturbances associated with fire and cattle grazing. *Australian Journal of Ecology* 22: 298-233.

Graaf, J.L. 1986. *Lantana camara*, the plant and some methods for its control. *South African Forestry Journal.* March: 26-30.

Harley, K.L.S. 1973. Biological control of *Lantana* in Australia. In: A.J. Wapshere (ed) *Proceedings of the III International Symposium on Biological Control of Weeds*. Montpellier, France. Commonwealth Institute of Biological Control, Farnham Royal, UK. p. 23-29.

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

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

Harris, P. 1971. Weed vulnerability to damage by biological control agents. In: *Proceedings of the 2nd International Symposium of Biological Control of Weeds*. Rome, Italy. October 4 – 7. pp. 29 – 39.

Harris, P., A.T.S. Wilkinson and J.H. Myers. 1984. *Senecio jacobaea* L., Tansy Ragwort (Compositae). In: J.S. Kelleher and M.A. Hulme (eds.). *Biological control programmes against insects and weeds in Canada 1969-1980*. Commonwealth Agricultural Bureaux, England. pp.195 – 201.

Hart, N.K., J.A. Lamberton, A.A. Sioumis, H. Swares and A.A. Seawright. 1976. Triterpenes of toxic and no-toxic taxa of *Lantana camara*. *Experientia*. 32: 412-413.

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

Heard, T.A. 1999. Concepts in insect host-plant selection behaviour and their application to host specificity testing. In: R. Van Driesche, T. Heard, A. McClay and R. Reardon (eds.). *Proceedings of Session: Host specificity testing of exotic arthropod biological control agents – the biological basis for improvement in safety. X International Symposium on Biological Control of Weeds*, Bozeman Montana, USA, July 4-14. pp. 1-10.

Henderson, L. 2001. Alien weeds and invasive plants: A complete guide to declared weeds and invaders in South Africa. *Plant Protection Research Institute Handbook No. 12.* Paarl Printers, Cape Town. p. 118.

Hill, M.P. and Hulley, P.E. 1995. Biology and host range of *Gratiana spadicea* (Klug, 1829) (Coleoptera: Chrysomelidae: Cassidinae), a potential biological control agent for the weed *Solanum sisymbriifolium* Lamarck (Solanaceae) in South Africa. *Biological Control*, 5: 345-352.

Hill, R.L. 1999. Minimising uncertainty – in support of no-choice tests. In: Withers, T.M., L. Barton Browne and J. Stanley (eds.) *Host specificity testing in Australasia: towards improved assays for biological control*. CRC for Tropical Pest Management, Brisbane, Australia. pp. 1-10.

Holm, L. G., Plucknett, D. L., Pancho, J. V. and Herberger, J. P. 1977. *The world's worst weeds.* The University Press of Hawaii, Honolulu.

Inouye, D.W. 1982. The consequences of herbivory: a mixed blessing for *Jurinea mollis* (Asteraceae). *Oikos* 39: 269-272.

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

Kellerman, T.S. and J.A.W. Coetzer. 1984. Hepatogenous photosensitivity diseases in South Africa. *Technical Communication No. 193*, Department of Agriculture, Republic of South Africa, 23 pp.

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

Maschinski, J. and T.G. Whitham. 1989. The continuum of plant responses to herbivory: the influence of plant association, nutrient availability, and timing. *American Naturalist.* 134: 1-19.

McNaughton, S.J. 1983. Compensatory plant growth as a response to herbivory. *Oikos* 40:329-336.

McNaughton, S.J. 1986. On plants and herbivores. *American Naturalist*. 128: 765-770.

Morris, M.J., A.R. Wood and A. den Breeÿen. 1999. Plant pathogens and biological control of weeds in South Africa: a review of projects and progress during the last decade. In T. Olckers and M.P Hill (eds.). *Biological Control of Weeds in South Africa (1990-1998). African Entomology Memoir No. 1*: 129-137.

Neser, S. and Annecke, D. P. 1973. Biological control of weeds in South Africa. *Entomological Memoir No. 28*. Department of Agricultural Technical Services, Pretoria. 27pp.

Neser, S. and C.J. Cilliers, 1989. Work towards biological control of *Lantana camara*: Perspectives. In: E.S. Delfosse (ed). *Proceedings of the VII International Symposium on Biological Control of Weeds.* Rome, 1988. Instituto Sperimentale per la Patalogia Vegetale Ministero dell 'Agricoltura e delle Foreste, Tome, Italy. pp. 363-369.

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

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

Palmer, W.A. and K.R. Pullen. 1995. The phytophagous arthropods associated with *Lantana camara*, *L. hirsuta*, *L. urticifolia* and *L. urticoides* (Verbenacae) in North America. *Biological Control* 5: 54 – 72.

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

Samuelson, G.A. Description of a new species of Alagoasa (Coleoptera: Chrysomelidae) from southern Brazil associated with *Lantana* (Verbenaceae). *Revista Brasileira de Entomologia* 29(3/4): 579-585.

Scott, L. 1998. Identification of *Lantana* spp. taxa in Australia and the South Pacific. Final Report. Australian Centre for International Agricultural Research. Canberra, Australia.

Shepherd, R.C.H. 1990. Problems which arise with host specificity testing of insects. . In: E.S. Delfosse (ed.) *Proceedings of the VII International Symposium of Biological Control of Weeds*, Rome, Italy, 1988. pp. 85-92. Isituto Sperimentale per la Patologia Vegetale Ministero dell' Agricoltura e delle Foreste, Rome.

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

Smith, L.S. and D.A. Smith. 1982. The naturalized *Lantana camara* complex in eastern Australia. *Queensland Botanical Bulletin No. 1*. Queensland Department of Primary Industry, Brisbane, 26 pp.

Solomon, B.P. 1983. Compensatory production in *Solanum carolinense* following attack by a host specific herbivore. *Journal of Ecology*. 71: 681-690.

Spies, J. J. 1984. A cytotaxonomic study of *Lantana camara* (Verbenaceae) from South Africa. *South African Journal of Botany*. 3: 231-250.

Spies, J.J. and H. du Plessis. 1987. Sterile *Lantana camara*: Fact or theory. South African Journal of Plant Soil, 4: 171-174.

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

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

Spies, J.J. and C.H. Stirton. 1982c. Embryo sac development in some South African cultivars of *Lantana camara*. Bothalia, 14: 113-117.

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

Strong, D.R., J.H. Lawton and R. Southwood. 1984. *Insects on plants*. Harvard University Press, Cambridge.

Swarbrick, J.T. 1986. History of the lantanas in Australia and origins of the weedy biotypes. *Plant Protection Quarterly*. 1: 115-121.

Taylor, E.E. 1989. A history of biological control of *Lantana camara* in New South Wales. *Plant Protection Quarterly*. 4: 61 – 65.

Traynier, R.M.M. 1979. Long-term changes in the oviposition behaviour of the cabbage butterfly, *Pieris rapae*, induced by contact with plants. *Physiological Entomolgy* 4: 86 – 96.

Urban, A.J. and D.O. Simelane. 1999. Performance of a candidate biocontrol agent, *Falconia intermedia* (Hemiptera: Miridae), on selected biotypes of *Lantana camara* (Verbenaceae). In: J.B.J. van Rensburg and J. van den Berg (eds.). *Proceedings of the Twelfth Entomological Congress*. Entomological Society of Southern Africa. p. 120.

Van der Meijden, E. 1989. A plant's response to herbivory: The trade-off between defense and regrowth. In: Delfosse, E.S. (ed.), *Proceedings of the VII International Symposium on Biological Control of Weeds*, 6 – 11 March 1988, Rome, Italy. pp. 137 – 144.

Van Klinken, R.D. 2000. Host specificity testing: Why do we do it and how we can do it better. *In*: Van Driesche, R. T. Heard, A McClay and R. Reardon (eds.) *Proceedings of Session: Host specificity testing of exotic arthropod biological control agents - The biological basis for improvement in safety. The X International Symposium on Biological Control of Weeds*. Bozeman, Montana, USA. July 4-14, 1999. pp. 54 – 68

Van Wilgen, B.W., M.P. de Wit, H.J. Anderson, D.C. Le Maitre, I.M. Kotze, S. Ndala, B. Brown and M.B. Rapholo. 2002. Costs and benefits of biological control of invasive alien plants: case studies from South Africa. *Ecological Economics*. (In press)

Virkki, N., 1980. Flea beetles, especially Oedionychina, of a Puerto Rican Marshland in 1962-72. *Journal of Agriculture of University of Puerto Rico*. 64: 63-92.

Virkki, N., 1982. On the biology of Oedionychina (Chrysomelidae, Alticinae). *Tribolium Information Bulletin*, July: 172-173.

Vogt, G.B., J.U. McGuire Jr., and A.D. Cushman. 1979. Probable evolution and morphological variation in South America disonychine flea beetles (Coleoptera: Chrysomelidae) and their amaranthaceous hosts. *Technical Bulletin of the U.S. Department of Agriculture No 1593*, 148 pp.

Wan, F-H. and P. Harris. 1997. Use of risk analysis for screening weed biocontrol agents: *Altica carduorum* Guer. (Coleoptera: Chrysomelidae) from China as a biocontrol agent of *Cirsium arvense* (L.) Scop. in North America. *Biocontrol Science and Technology* 7: 299 – 308.

Wapshere, A. J. 1974. A strategy for evaluating the safety of organisms for biological weed control. *Annals of Applied Biology* 77: 201-211

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

Waterhouse, D.F. and K.R. Norris. 1987. Biological Control . Pacific Prospects. Australian Centre for International Agricultural Research. Inkata Press, Melbourne, 454 pp.

Wells, M. J. and Stirton, C. H. 1988. *Lantana camara* a poisonous declared weed. *Farming in South Africa. Weeds A.27.*

Willson, B.W. 1979. The current status of biological control of rangeland weed projects in Australia. In: *Invited Reviews and Situation Papers*, Australian Agricultural Council, 11-15 June 1979, Queensland Agricultural College. Lawes Australia, pp. 214 – 222.

Winder, J.A. 1980. “Factors affecting the growth of *Lantana* in Brazil,” Ph.D dissertation, University of Reading, England.

Winder, J.A. and K.L.S. Harley. 1982. The effects of natural enemies on the growth of *Lantana* in Brazil. *Bulletin of Entomological Research* 27: 599-616.

Winder, J.A., D.P.A. Sands and R.C. Kassulke. 1988. The life history, host specificity and potential of *Alagoasa parana* Samuelson (Coleoptera: Chrysomelidae) for biological control of *Lantana camara* in Australia. *Bulletin of Entomological Research* 78: 511 – 518.

Winder, J.A. and H.F. van Emden. 1980. Selection of effective biological control agents from artificial defoliation/insect cage experiments. *Proceedings of the 5th International Symposium on the Biological Control of Weeds*. Brisbane, Australia. pp. 415 – 439.

Wise, M.J. and C.F. Sacchi. 1996. Impact of two specialist insect herbivores on reproduction of horse nettle, *Solanum carolinense*. *Oecologia*. 108: 328-337.

Withers, T.M. 1997. Changes in plant attack over time in no-choice tests: an indicator of specificity. *Proceedings of the 50th New Zealand Plant Protection Conference* 214-217.

Withers, T.M., L Barton Browne and J. Stanley. 1999. How time-dependant processes can affect the outcome of assays. In: R. Van Driesche, T. Heard, A. McClay and R. Reardon (eds.). *Proceedings of Session: Host specificity testing of exotic arthropod biological control agents – the biological basis for improvement in safety. X International Symposium on biological control of weeds*. Bozeman Montana, USA, July 4-14. pp. 27-41.