The evaluation *cf Phenrica* sp.2 (Coleoptera: Chrysomelidae: Alticinae), as a possible biological control agent for Madeira vine, *Anredera cordifolia* (Ten.) Steenis in South Africa

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ABSTRACT

Anredera cordifolia (Basellaceae), Madeira vine, is a perennial, semi-succulent climber native from Paraguay to southern Brazil and northern Argentina. It has a history of weediness and difficulty of control once established. In South Africa Madeira vine has a wide range and distribution with altitudes ranging from 10-1800m above sea level. Described as a transformer species, its sheer weight is capable of breaking branches off trees, causing the potential collapse of forest canopies. Chemical and mechanical control methods are expensive, labour intensive and may provide only temporary relief. A biological control programme was therefore initiated in 2003.

Cf Phenrica sp. 2 (Coleoptera: Chrysomelidae: Alticinae), was field collected from *A. cordifolia* in Brazil, SSW of Cascavel in the Paraná Province during a survey in November 2003. Eggs are laid in groups of 16 with the average fertility rate being 89%. After going though three larval instars, the larvae pupate in the soil with the adults eclosing after a period of 17 days. The total developmental time for a generation from egg to egg ranges between 7-8 weeks. Biological traits that favour the flea beetle as a possible biological control agent include long-lived adults (up to 5 months) and multiple generations during the summer period. Both adults and larvae feed extensively on leaves and stems and although developmental rates will slow down during the winter period, no indication of a definite diapause was found under the prevailing laboratory conditions.

After completing the larval no-choice trials with twenty-six plant species from 14 plant families *Phenrica* sp. 2 proved to be adequately host specific, as larval development was only supported by 3 Basellaceae species (including the control *A. cordifolia*) and one Portulacaceae species. All of these are introduced species in South Africa. The only indigenous *Basella* species could not be tested as it has a very marginal distribution, and because it's inconspicuous nature, it is seldom seen or collected. Adult multi-choice trials were restricted to species that could sustain larval development to give some indication of the acceptability of these species for adult feeding and oviposition. Although adult feeding was initially concentrated on *B. alba*, the oviposition preference was clear-cut as females only oviposited on *A. cordifolia*.

In order to quantify the impact of *Phenrica* sp. 2 on plant biomass and to assess the incidence and intensity of foliar damage, a pair of adults was confined to the host plant, for 2 generations, with different levels of larval densities. The results indicated that the host plant, due to both larval and adult feeding, suffered leaf losses of up to 55%. *Anredera cordifolia* was however still capable of enlarging the root mass despite suffering huge leaf losses. This would imply that *A. cordifolia* has an effective re-growth capacity and it will only be vulnerable to attack of the storage organs that enable re-growth, or to repeated attack of other plant parts through which reserves are exhausted. Unfortunately the period of exposure (24 days) was too short to prove that *Phenrica* sp. 2 impacts on the below ground dry mass, but should

the plant be completely defoliated, as was observed in the field, the host plant would be forced to deplete stored resources.

Phenrica sp.2 has shown to be very host specific and although *A.cordifoia* loses its leaves during the winter period in most provinces in South Africa, the adults are long-lived and should be able to survive the leafless periods. Further more the relatively short life cycle, high fecundity and 3 generations per year should theoretically insure a strong population build-up that would improve the chances of establishment in the field. All indications are that *Phenrica* sp. 2 is an agent well worth considering for the biological control of *A. cordifolia*.

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Chapter 1

General introduction and literature review

1.1 Invasive alien plants

While biological migration is a natural process, biological invasions are a human-caused phenomenon, and one of the most important effects humans have had on the Earth's ecosystems. Humans move species beyond their native ranges both deliberately and inadvertently, and many of these species become established and spread in their new habitat (Moody *et al.* 1988; Rejmánek 1996a; Vitousek 1997).

Global reviews of the effects of plant invasions suggest that the most damaging species transform ecosystems by using excessive amounts of resources (e.g. water, light and oxygen), by adding resources (e.g. nitrogen), by promoting or suppressing fire, by stabilising sand movement and/or promoting erosion, by accumulating litter or by accumulating or redistributing salt. All of the above can have a significant impact on the ecosystem structure, composition and processes, the building blocks of biodiversity (Richardson 2004). This is however not the only threat that alien invasive plants pose. Even in areas where human activities have degraded or transformed habitats, goods and services provided by these ecosystems still contribute substantially to human well-being. By altering the functioning of ecosystems the capacity of the ecosystems to deliver goods and services is also affected (Richardson 2004).

There is probably only a 1% chance of any given introduced species becoming weedy or invasive through a single, intentional introduction, but this can have disastrous economic consequences. It is therefore important to understand which biological characteristics make a species a good invader so that potentially invasive species can be screened and the cost of invasion reduced or prevented, by preventing initial introduction (Goodwin *et al*. 1999). Rejmánek (1996b) researched numerous key theories that can help predict the risk of future invasions, and came to the conclusion that we still lack satisfactory answers to questions such as “what attributes make some species more invasive?” or “what makes some ecosystems more invadable than others?” The need to analyse invasive ability before invasions occur is undeniable and even though we do not yet have a perfect knowledge, we are gradually accumulating knowledge that is deemed necessary to assess risk (Reichard 2001). Unfortunately plant introductions have increased greatly in the last three decades especially in the forestry-, pasture- and nursery industry. This will inevitably lead to increased weed problems after a lag time that may be 50 years or longer. Characteristics such as ease of establishment, rapid growth, and high competitiveness are often factors highly desirable in these industries and these introduced species are more likely to become invasive weeds (Cruttwell McFadyen 1998).

The influx of alien plant species into South Africa began in the 1600s, when the Cape of Good Hope was a major stop for European ships, sailing to and from the Spice Islands. Hundreds of species of plants were brought in and cultivated. South Africa became a focal point in Africa for the establishment of

alien plants, especially from Australia and South and Central America (Zimmermann *et al*. 2004). According to the Southern African Plant Invaders Atlas (SAPIA), a project that collected basic information such as distribution, abundance and habitat types of alien plant invaders, the most invasive species have been recorded from savanna (294 species in 653 quarter-degree square (15 minute square scale)) and grassland biomes (293 species in 624 squares). Plant species were selected for inclusion in the atlas primarily on the basis of their importance or potential importance as invaders of natural or disturbed areas. The smallest biomes, fynbos and forest, stand out as having fewer recorded invasive species (156 in 153 squares and 191 in 165 squares, respectively), but many more invasive species in these biomes were recorded as abundant (Henderson 1998). In addition to the existing invasions, many invasive species have not fully occupied the potential, suitable habitat and many new invaders are regularly added to the list (Nel *et al*. 2004).

1.2 Control methods of alien invasive plant species

Human communities and natural ecosystems worldwide are under siege from a growing number of destructive invasive alien species (including disease organisms, agricultural and environmental weeds as well as insect pests). These species subsequently lead to the erosion of natural resources, they cause an upset in ecosystem stability and pose a threat to economic productivity (Richardson 2004). Environmental weeds are as ecologically damaging as land clearing, but their attack is subtle because the loss of native fauna and flora species will only be known once these effects are measured,

which they seldom are (Cruttwell McFadyen 1998). As global trade and travel accelerate, the problem grows in severity and geographic extent and besides their effect on agriculture, forestry and human health; biological invasions are widely recognized as the second-largest global threat to biodiversity (Wilcove *et al.* 1998). Therefore the challenges facing conservation biologists and managers in many parts of the world are those relating to the control of alien species, impact prevention and the repair of systems damaged by aliens (Byers *et al.* 2001).

Chemical -, mechanical - and biological control, as well as combinations of these (integrated management), has been used in an effort to control alien invasive plant species with varying degrees of success (Zimmermann *et al*. 2004). Mechanical or cultural control is generally not feasible in natural ecosystems, and widespread use of herbicides is economically unsustainable and unacceptable on environmental grounds and for health reasons (Cruttwell McFadyen 1998). As a management tool, biological control is a very attractive alternative. It is cost-effective and safe. Of the 352 biological control agents released until 1998, only 0.6% have been observed causing significant non-target population suppression in one or two countries where released, and 10% of releases have been observed feeding on anticipated closely related non-target plants (Cruttwell McFadyen 1998; Sheppard 2003; Zimmermann *et al*. 2004). In some instances biological control can also be successfully integrated with other management practices. For instance in Tasmania, Australia, wick-wiping of herbicides in summer to kill flowering ragwort (*Senecio jacobaea* L.) is currently being recommended, as it reduces

seed production without any damage to the rosettes. This in return provides a food source for the flea beetle, *Longitarsus flavicornis* (Stephens) (Potter *et al.* 2004). Most importantly, however, biological control is self-sustaining (Cruttwell McFadyen 1998; Zimmermann *et al*. 2004).

Plant immigrants often achieve an advantage in naturalisations having been introduced without their debilitating native enemies (predators, grazers, parasites and competitors). This principle is well established and forms the basis for biological control, because the introduction of missing natural enemies into a new range is the objective of a biological control programme (Mack 1996).

Intentional biological control has contributed to the suppression of invasive alien plants in many countries throughout the world, and more than 400 species of organisms have been used against approximately 280 weed species (Julien & Griffiths 1998).

Biological control started in South Africa with the importation of plant feeding cochineal insects, which were released against *Opuntia monacantha* Haw. (drooping prickly pear), in 1913 (Moran & Zimmermann 1991). Since then 95 species of biological control agents have been introduced into South Africa to control 48 weed species. In order to evaluate the success of the biological control programmes in South Africa, three categories of control were recognized. The categories were based on the amount that alternative control methods (chemical or mechanical) have been reduced since the introduction

of biological control agents on the weed. The degree of control is classified as: (i) complete, when no other control measures are needed to reduce the weed to acceptable levels, at least in areas where the agents are established; (ii) substantial, when other methods are still needed to reduce the weed to acceptable levels, but less effort is required because the extent or density of the weed infestations has declined or because there has been a reduction in the rate at which the weed disperses or reinvades cleared areas; and (iii) negligible, when control of the weed remains almost entirely reliant on other measures in spite of damage inflicted by the agents (Hoffmann 1995). Of all the introductions made in South Africa, 25% resulted in the complete control of the target weed species and 32% in substantial suppression. A further 25% of the introductions have been made fairly recently and can therefore not be evaluated in terms of these criteria (Macdonald 2004).

1.3 Emerging weeds

A 15 th century historian, Francesco Guicciardini once said “small beginnings, hardly worthy of notice, are often the cause of great misfortune” and although this was said within a political context, his statement applies perfectly to invasive plants as small, seemingly harmless infestations eventually spread over vast areas causing huge ecological- and economic losses (Dewey & Andersen 2004). Successful eradication depends almost entirely on early detection and timely control and if one or more early infestations of an invasive plant species are not detected, eradication is impossible for all practical purposes. Eradication is a very attractive management tool because it can be cost effective and efficient compared with the indefinite commitment

of resources necessary to contain an invasive plant species (Cunningham *et al.* 2004). There is no single strategy that will completely solve the invasive species problem, but a combination of strategies can still be useful to minimize it. Of all the options, early detection and rapid response to new invaders is seen as the most cost effective and environmentally sound approach. Early detection and rapid response (EDRR) does not restrict trade and movement of species, EDRR addresses only species that have established free-living, self –perpetuating populations, EDRR causes minimal and short effects on the invaded habitat regardless of the methods used for eradication of the population, and EDRR aims to restore the invaded habitat to a natural balance (Westbrooks 2004). This concept is one of the strong driving forces behind the so-called “sleeper weeds” (Australia) or “emerging weeds (South Africa).

Many countries have recognised the value of early detection especially in terms of the ecological and economical impact of invasive plants. In 1996 in the United States it was estimated that invasive weeds were spreading at a rate of approximately 1840 ha/day on western wild lands and an astounding 800 000 ha of land is being invaded in the Western United States each year (Dewey & Andersen 2004). In South Africa the SAPI database has shown that 10 million hectares of land have been invaded although most of it only sparsely, and in Australia in excess of 20 million hectares (Martin 2003).

The estimated cost of exotic invasive species to the American economy is in the order of $138 billion per annum (Westbrooks 2004); A$ 4 billion per

annum in Australia (Cunningham *et al.* 2004) and in South Africa R1.95 billion has been spent by the Working for Water Programme between 1995 and the end of the 2003 financial year (Marais *et al*. 2004). A study conducted by Smith *et al.* (1999), showed that the 20 year cost to eradicate a 10 ha infestation of weeds, is less than one–fifth of that required to eradicate a 100 ha infestation, and only 2% of the cost of eradicating a 1000 ha infestation of the same species. These losses are incurred despite having the necessary legislation in place. One of the possible explanations is that; except for situations where public welfare is at risk, there is often a tendency to ignore many invasive species because of the lack of environmentally acceptable, and affordable control methods. People therefore often prefer to tolerate the "problem" at the expense of the cure and this has led to an ecological crisis with no simple solution. There is so little awareness of this issue that it will take a large and concerted effort to develop public support and to marshal the necessary resources that will be needed to effectively address this new environmental threat (Westbrooks 2004).

In Australia and the United States of America, a national effort has been made to develop and implement an early detection and response system for invasive plants. These systems rely heavily on public participation and interest and a great deal of money and effort is being put into awareness campaigns, training and the development and maintenance of the infrastructure necessary to drive the system. In South Africa early surveys and later the SAPIA project were amongst others, initiated to identify the early stages of invasions. Apart from projects undertaken by PPRI, originally

funded by the Department of Agriculture, or undertaken opportunistically. The Working for Water Programme co-ordinated by the Department of Water Affairs and Forestry has been the first initiative to consistently fund research on early detection and rapid response with biological control being the main thrust of control. We are however still facing the mammoth task of creating public awareness and interest. The widespread acceptance and support from authorities and the public for biological control specifically, has eventually encouraged the targeting of incipient weeds, which will in return considerably enhance the prospects for success (Zimmermann & Neser 1999; Olckers 2004).

The topic of this thesis is the proposed biological control of one such incipient weed, *Anredera cordifolia*.

1.4 *Anredera cordifolia*

Anredera cordifolia (Ten.) Steenis (Basellaceae, syn. *Boussingaultia cordifolia* Ten.; *B. gracillis* Miers) in various countries also known as Madeira vine, lamb's tail, mignonette vine or white shroud is a perennial, semi- succulent South American climber (from Paraguay to southern Brazil and northern Argentina) (WESSA 2002; Starr *et al*. 2003). This fast-growing climber has heart- shaped, glossy green leaves and spikes of small fragrant, white flowers. The succulent stems produce numerous irregular, fleshy, thickened stem tubers in axils, both on aerial and prostrate stems, and these serve as long-lived propagules (Blood 2002). *Anredera cordifolia* may be deciduous,

and growth commences in spring and flowering is from February to May (WESSA 2002).

1.4.1 Native range and global distribution

In its native range *A. cordifolia* occurs mostly in forest, grassland, cropland, woodland and scrub with annual temperatures and rainfall ranging from 10-30°C and 500-2000mm respectively (Starr *et al*. 2003). With invasive characteristics such as dispersal via rhizomes and aerial tubers, as well as an aggressive, smothering, and vining nature, *A. cordifolia* has become a major pest in Australia, New Zealand, South Africa, Hawai'i and other Pacific Islands (PIER 2000; Weeds Australia 2000). The earliest record in South Africa comes from a herbarium specimen of a plant that was 'an escape from cultivation" in King Williams Town (Eastern Cape Province) in 1894 (Henderson pers. comm.). Madeira vine now has a wide range and distribution from the Western Cape, through the southern- & Eastern Cape, Lesotho, Swaziland, KwaZulu-Natal, Mpumalanga and Gauteng at altitudes ranging from 10-1800m (Fig. 1.1) (Henderson 2001; Jordaan 2003).

When a new biological control programme is launched, a comparison with another similar weed is a practise often used. For the present study it seemed only fitting to compare the similarities that exists between *A. cordifolia* and *Pereskia aculeata* Mill. (Family Cactaceae). *Pereskia aculeata*, another climber with fleshy leaves, was listed as an active invader with the ability to grow, reproduce and disperse widely within forest margins and gaps within closed forest. *Pereskia aculeata,* commonly known as Barbados gooseberry

or leaf cactus has a similar distribution pattern to that of *A. cordifolia* (Fig. 1.2). Both *A. cordifolia* and *P. aculeata* rely heavily on vegetative means for spread and reproduction, and both are considered to be forest invaders, smothering natural vegetation, sharing a very similar niche, and being very difficult to control, chemically and mechanically.

Although *P. aculeata* is in an early stage of establishment in Australia, *A. cordifolia* has already become a major problem and is targeted as a priority for biological control in Queensland. In South Africa we are faced with the inverse. *Pereskia aculeata* was declared a noxious weed in the late 1970's, and has been blamed in part for the degradation of some conservation areas and economic losses suffered by the forest industry. On the other side *A. cordifolia*'s invasive potential is only now being realised, especially in the Southern Cape region and early intervention to curb the spread of *A. cordifolia* should receive urgent attention. The Australian government is already aware of the potential threat of *P. aculeata* to eucalypt communities in subtropical, northern Australia, and methods to keep further infestations at bay through early intervention, have been put in place (Weed Management Guide 2003).

The *P. aculeata* invasions have prompted the Working for Water Programme, to make available funding for the collection and screening of biological control candidates. Earlier opportunistic work by PPRI resulted in the release of the flea beetle *Phenrica guérini* (Bechyné) in 1991. Adults were released at nine sites throughout KwaZulu-Natal and the Eastern Cape. Unfortunately the work had to be truncated permanently. *Phenrica guérini* was assumed not to

have established at any of the KwaZulu-Natal sites, and no further surveys for establishment at or near the release sites were undertaken. Despite apparent early failures, a new culture was provided to Rhodes University, and as a result *P. guérini* did establish in the Eastern Cape Province, at the Port Alfred site. More recently it was also released, and successfully established at Pennington on the Natal south coast, but it is still too early to determine its impact on *P. aculeata* (Klein 1999; Hill pers. comm.).

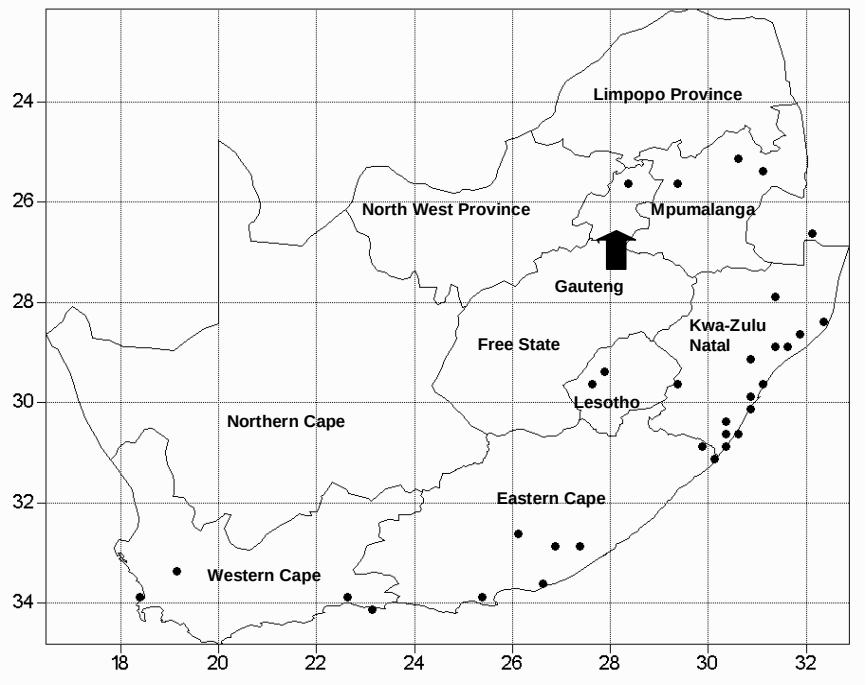

Figure 1.1 Recorded occurrence of *Anredera cordifolia* in South Africa from information supplied by the SAPIA database

(Map drawn by L. Henderson, Plant Protection Research Institute, Pretoria)

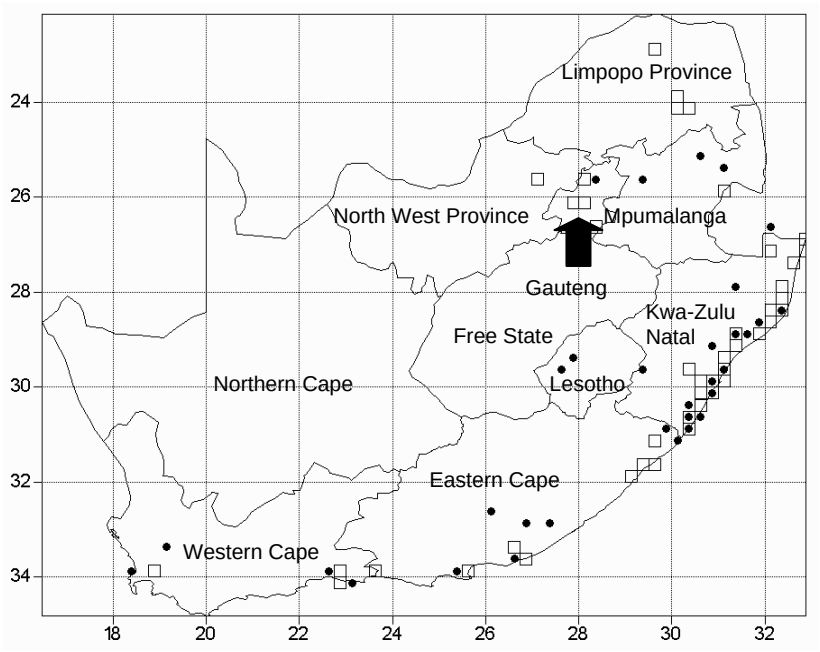

Figure 1.2 Recorded occurrence of *Anredera cordifolia* (•) and *Pereskia aculeata* (□) from information supplied by the SAPIA database
(Map drawn by L. Henderson, Plant Protection Research Institute, Pretoria)

1.4.2 Relatedness of *Anredera cordifolia* to local and introduced plants

The family Basellaceae consists of 4 genera with more or less 20 species occurring mainly in tropical and subtropical South America but also in Africa and Asia (Jordaan 2000). In southern Africa there are only 2 genera: *Basella* and *Anredera*. Within the *Basella*, 5 species have been identified of which *B. alba* is widespread in Africa and Asia, and locally naturalised in tropical and subtropical areas worldwide. Of the remaining four species, three can be found in Madagascar and only one, *Basella paniculata* Volkens, is endemic to eastern and southern Africa. *Basella paniculata*, also a perennial, succulent climber have been reported from the Limpopo Province as well as KwaZulu-Natal at altitudes ranging from 30-100m (Jordaan 2000; 2003).

Within the family Basellaceae, three species are cultivated as vegetables. Madeira vine yields the “basell potato”, while the leaves of Madeira vine, *Basella alba* L. (Malabar spinach) and *Ullucus tuberosus* Caldas can be eaten as spinach. *Ullucus tuberosus* has a tuber similar to potato and is native to the Andes Mountains, ranging from Colombia to northern Argentina (Innvista; Jordaan 2003; USDA)

1.4.3 Dispersal

Madeira vine rarely sets seed with the major means of dispersal assumed to be by transportation of underground- and aerially-borne tubers, stem fragments and rooting leaves (Vivian-Smith & Panetta 2002). In New Zealand the plant is regarded as a non-fruiting weed. It is easily spread through garden waste, contaminated and eroding soil, machinery, road clearing and water (tubers are buoyant in both fresh and salt water). Buoyancy studies conducted by Vivian-Smith & Panetta (2002), showed that Madeira vine tubers has a very poor floating ability with the majority of tubers sinking in less than a day. The tubers that did remain buoyant and viable throughout the experiment suggested that occasional long distance dispersal by water might be possible, even though 66% of tubers placed in water decayed over a period of 30 days.

1.4.4 Invasiveness

Anredera cordifolia has a history of weediness in warm, moist climates, aggressive vegetative growth that competes with and replaces other vegetation, and difficulty of control once established. Growth rates of stems in

warmer, moister regions can exceed 1m per week and up to 6-10m in a growing season (LCC 2001). Dry conditions as well as snow and frost can be tolerated and aerial tubers may remain viable on cut branches and in the soil for at least 5 years before growing to form new plants (Blood 2002; Starr *et al*. 2003).

1.4.5 Noxious Weed Act

According to "The Conservation of Agricultural Resources Act" (Act 43 of 1983), *Anredera cordifolia* has been classified as a category 1 plant. This regulation states that *A. cordifolia* is prohibited on any land in South Africa and it must be controlled, or eradicated where possible, except in biological control reserves (areas designated by the executive officer in terms of the regulations for the breeding of biological control agents) (Henderson 2001). *Anredera cordifolia* is described as a transformer elsewhere in the world and is showing definite signs of this ability in southern Africa where locally very dense growth has caused concern (WESSA 2002). Weedy vines are particularly problematic and many are labelled as "transformer species" because they act as agents of ecosystem change (Vivian-Smith & Panetta 2002). This is particularly true for *A. cordifolia*, described as one of the heaviest creepers. The sheer weight is capable of breaking branches off trees, thereby reducing them to poles, potentially causing the collapse of a forest canopy (PIER 2000; WESSA 2002; Starr *et al*. 2003). Madeira vine not only competes for space, light and water, but also smothers and replaces indigenous vegetation (WESSA 2002).

Transformer species pose a huge threat to riparian ecosystems, which are important for maintaining biodiversity and ecosystem function within landscapes. Riparian ecosystems are however prone to exotic invasion and when the functionality of riparian systems is compromised, downstream ecosystems such as wetlands, lakes, estuaries and other coastal habitats may be negatively affected. This may occur via reduced water quality and increased nutrient sediment flows. Once invaded, riparian systems can act as a source of weed propagules for downstream habitats (Vivian-Smith & Panetta 2002).

1.4.6 Management

Eradicating or containing Madeira vine takes considerable effort and patience over a long period. Infestation size should determine the mode of attack, and the depletion of the below- and above ground tuber store should be the main aim for control.

1.4.6.1 Mechanical control

Physical control of this species is very difficult because of above- and underground tubers that need to be destroyed or removed. A plastic sheet can be placed below the plant before any manual control is done so that all parts of the plant, especially aerial tubers, can be bagged and removed. In suitable areas, covering the soil with a weighed down, opaque plastic sheet until tubers have sprouted and decayed, may work to prevent re-growth.

1.4.6.2 Chemical control

The numerous tubers, roots and slightly succulent, waxy leaves make chemical control almost as difficult. Riparian weeds present a special challenge, as both the range of herbicides, and the methods of their application are limited by the proximity to water (Vivian-Smith & Panetta 2002). In South Africa Garlon 480 (triclopyr) is recommended for cut stump application (WESSA 2002). Starane 200 (fluroxypyr) on the other hand can either be used for cut stump application (when used with diesel), or as an overall spray when used with water. It has a slower action than glyphosate but translocates much better into the tubers resulting in less re-growth and no grass damage. One of the biggest problems with herbicide application is the non-target effects, especially when one takes into account that *A. cordifolia* is sometimes embedded in the forest canopy. Follow-up spraying, and timing, is of the utmost importance. If left too long, enough foliage will recover to support the development of new underground tubers, delaying successful control (Blood 2002).

For vines in the canopy, hand removal or cutting of the vine stems, without the use of herbicides, should be avoided, as this will allow fertile aerial tubers to drop to the ground. Stems should rather be scraped and treated with herbicides at 30cm intervals. Vines will die back over a period of several weeks leaving aerial tubers and vines to decompose in the canopy (Wilsons Creek Landcare Project 2003)

1.4.6.3 Biological control

No biological control programme has been considered for *A. cordifolia* anywhere in the world, but the need for possible biocontrol has been recognized in many countries. Opportunistic surveys for promising natural enemies made in South America by PPRI over some years yielded at least three species of destructive leaf-feeding chrysomelids, and an unidentified leaf-mining moth that was also collected on *Talinum* sp.. The chrysomelids include *Plectonycha correntina* Lacordaire, *Isotes eruptiva* (Bechyné) and *Phenrica* sp. 2 (Olckers & Neser 2003). Lai *et al*. (1996) reported a leaf spot disease of Madeira vine caused by *Alternaria alternata* in Taiwan. Red, pimple like leaf spots initially develop on the leaves, gradually turning into necrotic spots with red margins. Similar lesions were recorded in Argentina, and also a geometrid larva feeding on aerial tubers (Neser pers. comm.).

For vegetative reproductive plants, like Madeira vine, weed populations are expected to be genetically uniform. Genetic diversity on the other hand plays an important role in protecting the plant against herbivore attack. It could therefore be expected that genetically uniform species (monoculture) might be easier to control biologically, and therefore less costly, than species, which have a wider genetic diversity (Burdon *et al.* 1980).

Management of most creeping and climbing invasive plants requires special attention, as mechanical and chemical control are largely unsuccessful and undesirable in most situations. In these situations biological control seems to be the only cost effective, long-term solution. A fair number of biological

control agents have been released on climbing plants with mixed results (Table1.1), (Julien & Griffiths.1998). Although many of the agents released failed to establish, biological control should not be deemed as unsuccessful as numerous factors affect the success of a biological control programme. Cruttwell McFadyen (2003) quoted various authors stating that overall, about 60% of the agents establish, with 33% of these resulting in the control of the weed. The data should be interpreted with care; for example, of the12 agents released on *Opuntia stricta* in Australia only 7 established and only 2 of these were responsible for the successful control. This would imply that 42% of the agents failed and only 17% contributed to success. However, this was one of the programmes that can boast a 100% success rate (Cruttwell McFadyen 2003). A programme can thus only be deemed as a failure if the weed is still not adequately controlled, not when individual agents have failed to achieve the aim for which they had been introduced.

Table 1.1 Biological control agents released against vines and creepers

Plant species and family	Biocontrol agent	Country	Degree of control
Asparagus asparagoides (L.)[1,2,3] (Family Asparagaceae)	*Puccinia myrsiphylli* (Uredinales)	Australia	Apparently successful
	Zygina sp. (Cicadellidae: Typhlocibinae)	Australia	Too early to judge
	Crioceris sp. (Chrysomelidae: Criocerinae)	Australia	Too early to judge
Caesalpinia decapetala (Roth) Alston [4] (Family Fabaceae)	*Sulcobruchus bakeri* (Coleoptera: Bruchidae)	RSA	Established (aim: only limiting regeneration and spread by seed)
Chondrilla juncea Linnaeus [6] (Family Asteraceae)	*Bradyrrhoa gilveolella* (Lepidoptera: Pyralidae)	Argentina	Not established
		Australia	Not established
	Cystiphora schmidti (Diptera: Cecidomyiidae)	Argentina	Not established
		Australia	Damaging but parasitism limits impact

Clematis vitalba Linnaeus[6] (Family Ranunculaceae)	*Phoma clematidina* (Fungus: Coelomycetes)	New Zealand	Establishment not confirmed
	Phytomyza vitalbae (Diptera: Agromyzidae)	New Zealand	Establishment not confirmed
Coccinia grandis (Linnaeus) Voigt[6] (Family Cucurbitaceae)	*Melittia oedipus* (Lepidoptera: Sesiidae)	Hawaii and U.S.A.	Established and under assessment
Convolvulus arvensis Linnaeus [6] (Family Convolvulaceae)	*Aceria malherbae* (Acarina: Eriophyidae)	Canada U.S.A.	Establishment not confirmed Established in Montana, Texas and Washington.
	Tyta luctuosa (Lepidoptera: Noctuidae)	Canada U.S.A.	Not established Released in Maryland ('91) and Washington ('96) but establishment not confirmed
Cryptostegia grandiflora (Roxburg) R. Brown [6] (Family Asclepadaceae)	*Euclasta gigantalis* (Lepidoptera: Pyralidae)	Australia	Recovered 4 years after release. Widespread in N-E Australia. Cause total defoliation during localised outbreaks.
	Maravalia cryptostegiae (Uredinales: Chaconiaceae)	Australia	Established over most of Queensland high rainfall areas. Cause extensive damage and repeated defoliation at some locations.
Delairea odorata [5] Lem. (Family Asteraceae)	*Digitivalva delairea* (Lepidoptera: Acrolepiidae	U.S.A.	Applying for release
	Parafreutreta regalis (Diptera: Tephritidae	U.S.A.	Applying for release
Lygodium microphyllum [7]	*Floracarus perrepae* (Eriophyidae)	U.S.A.	Applying for release in Florida
Macfadyena unguis-cati (L.) A.H. Gentry [8] (Family Bignoniaceae)	*Charidotis auroguttata* (Coleoptera: Cassidinae)	R.S.A.	Established in Gauteng, North West, Mpumalanga. No confirmation for Limpopo.
	Hypocosmia pyrochroma (Lepidoptera: Pyralidae)	R.S.A.	Applied for permission to release
	Carvalhotingis visenda & *C. hollandi* (Hemiptera: Tingidae)	R.S.A.	Applied for permission to release
	Hylaeogena jureceki (Coleoptera: Buprestidae)	R.S.A.	Applied for permission to release
Mikania micrantha Kunth [6] (Family Asteraceae)	*Liothrips mikaniae* (Thysanoptera: Phlaeothripidae)	Malaysia Solomon Islands	Failed to establish as a result of predators Not established

Passiflora tripartita (C. Jussieu) Poir var. *triparita* [6] (Family Passifloraceae)	*Cyanotricha necyria* (Lepidoptera: Dioptidae)	Hawaii and U.S.A.	Not established - predation might be reason for failure
	Pyrausta perelegans (Lepidoptera: (Pyralidae)	Hawaii and U.S.A.	Established on Hawaii and Maui. Not confirmed for Kauai
	Septoria passiflorae (Fungus: Coelomycetes)	Hawaii and U.S.A.	Established on Kauai, Hawaii and Maui. Under assessment
Pereskia aculeata Miller [6] (Family Cactaceae)	*Phenrica guérini* (Coleoptera: Chrysomelidae)	R.S.A.	Survived winter in Eastern Cape but failed to establish in KwaZulu-Natal. Impact minimal

[1] Morin et al.,2002,[2&3] Witt 2000 ; 2002, [4] Coetzer *et al*., 1999, [5] Balciunas *et al*., 2003, [6] Julien & Griffiths, 1998, [7] Goolsby *et al*., 2003, [8] Williams, 2002

1.5 Aims of the present study

The aim of this research project was to study the suitability of *Phenrica* sp. 2 as a biological control agent for *A. cordifolia*, an emerging weed with the potential to invade many coastal and inland subtropical, and temperate regions. For the purpose of this study the theory, specific objectives methods and results of each aspect of the research will be introduced and dealt with separately in the relevant chapters.

Chapter 2 describes the biology of *Phenrica* sp. 2, which forms the core of many aspects that need to be considered in selecting a suitable biological control agent. Knowledge of the insect's biology allows a better understanding of the insect-plant relationship, which in return will provide necessary information on the host specificity (Chapter 3) and possible impact that can be expected on the target weed (Chapter 4). Even if the agent should prove to be suitable for release, and gets established, efforts will be futile unless the insect can be shown to have an acceptable level of impact on

biomass production and/or reproduction and/or competitiveness. The laboratory study of the likely impact of *Phenrica* sp. 2 on the plant may aid in revealing which other candidates(s) should be considered in future. Chapter 5 will discuss the final conclusions and recommendations with regards to the suitability of *Phenrica* sp. 2 for the biological control of *A. cordifolia*.

Chapter 2

The selection of biological control agents and the biology of *Phenrica* sp.2

2.1 Introduction

2.1.1 Agent selection

For most weeds that have been targeted for biocontrol, there is a pool of host-specific insects and diseases present in the country of origin, all potentially available as biocontrol agents (Cruttwell McFadyen, 2003). The deliberate introduction of exotic organisms into a new environment does however make us accountable to future generations and the scientific discipline, to ensure that the selection and release of agents is both a good ecological and financial investment (Briese *et al.* 2003). There is a general consensus amongst biocontrollers that the biggest challenge to increase biological control success is to improve agent selection, and yet, there is still no widely accepted scientific approach available (Sheppard 2003; Dennill & Moran 1989; Hokkanen 1989).

Agent selection is one of the big controversies that exist in the biocontrol fraternity. Areas of conflict include debates surrounding the issue on “new” and “old” associations, the release of a single insect *vs.* a suite of complementary insects, and the role of ecology in the selection of biocontrol agents. In classical biocontrol projects, agents are selected from the target plant species in its area of origin. The basic assumptions of classical biological control are that these specialist insect herbivores are most likely to be host specific and safe for introduction to other countries, and that

irrespective of the evolutionary age of such relationships, it is the release of the agents from their natural enemies in the country of introduction, that is the crucial factor in the achievement of biological control of the weed (Dennill & Moran 1989). Hokkanen & Pimentel (1984) suggested a new approach. They argue that the impact of an insect herbivore species on its host plant, is restricted by the level of homeostasis that exists between the phytophage and its host. Old insect-plant relationships could therefore limit the efficacy of the agent in a biocontrol attempt. They advocate the selection of agents from a close relative of the pest plant, rather than from the target species itself. The release of such an agent on the target weed would constitute a new insect-plant relationship which, because of its lack of co-evolved inherent homeostasis, would result in the agent being more damaging to the weed. In their analysis of 286 cases of successful biocontrol, Hokkanen & Pimentel (1984) claim that new associations are 75% more successful than old associations. Goeden & Kok (1986) and Moran *et al.* (1986) have argued that Hokkanen & Pimentel's (1984) data base is confounded by incorporating both insect and weed biocontrol programmes, and by being too selective and limited in respect of the latter. On the other hand, Dennill & Moran (1989) analysed herbivore-crop associations in South Africa which they considered to be similar to insect-weed associations. These associations indicate that new herbivore-plant associations can be as damaging to host plants as old associations. They also recommend the routine incorporation of new associations in the search for effective biocontrol agents, but they stressed that it should be used along with, and not in preference to the classical procedure. Dennill and Hokkanen (1990) concluded the heated debate that

started in 1984, by emphasizing that the achievement of success in biocontrol is dependent on both the release of the agent from its natural enemies, and the degree of homeostasis between the host and the agent.

Ehler (1995) is of the opinion that the conventional division of new *vs.* old is insufficient to characterize the nature of pest-enemy associations that arise in classical biological control, and suggested two further categories. In addition to “old” and “new” associations he suggested (a) “recent associations” where the agent and the weed have co-existed in the region of origin for a relatively long period of time, and have undergone some level of evolutionary adjustment, and (b) “quasi-old associations” where previously co-evolved agent and weed associations is reunited in a new locality, but only after the weed has had time to have undergone some evolutionary adaptation (Ehler 1995).

Myers (1985) first introduced the term “lottery model” to describe where weed biological control is usually achieved by only one of the suite of introduced agents, implying that finding this agent is a lottery. This contrasted with the cumulative stress model where a suite of agents is selected to attack the target in a number of different ways leading to overall suppression (Sheppard 2003).

Two schools of thought developed. One maintains that agent efficacy on the target weed following release; results from complex interactions between the agent, the target weed, and the new environment (Myers 1985). Therefore,

predictions of efficacy of biological control agents following the release into the new environment has been branded by some critics as not being very useful (Cruttwell McFadyen 2003). The alternative school argues that the application of ecological studies of both the agent and the weed enables prioritisation of agents based on likely efficacy. A scientific process is thus applied in agent selection, which allows for the adoption of the "holistic approach" where the best long-term suppression may come from a community of natural enemies (Sheppard 2003). Most biological control practitioners' views lie along a continuum between these two schools of thought, and the practitioner's level of confidence in the value of ecological theory and practice for agent selection will be the determining factor.

Retrospective assessment of agent release strategies can improve the science of agent selection. These strategies should state which agents will be released and in what order, as well as why agents are to be released in that order. This should allow strategies across targets of similar and contrasting life history to be compared and improved (Sheppard 2003). Unfortunately available resources will largely determine the level to which ecological studies can be carried out. The importance of the weed and the complexity of the agent selection process will determine whether each activity is worth the resource cost (Sheppard 2003). When one is faced with a complex weed with a high number of possible agents to choose from, the investment in the decision-making process should be greater. When a high number of potential agents are available in the country of origin, Sheppard (2003) even suggested that it might be of value to retrospectively analyse decisions taken in a

biological control programme against *Lantana camara* L.. As lantana is considered to be the largest and longest running biological control programme in Australia and probably the world, and investment wise, the least successful to date. However, *L. camara* is not a single, homogenous species, and should be seen as a large complex of hybrids with parents of diverse origins, probably in South, Central and North America, including the Caribbean region.

Certain groups e.g. weevils, leaf beetles, gallflies or feeding guilds, such as sap suckers, gall formers, seed feeders etc. have been identified as being more effective biological control agents (Sheppard 2003). Selecting effective biocontrol agents is however hindered by the fact that there are many exceptions to the generalities (Sheppard 2003). Crawley (1989) maintains that biological attributes associated with success have yet to be understood, but this did not prevent intuitive lists of characteristics of agents that should be given high priority being published. Harris (1991) for example, considered high priority agents as being those that: a) have been successful elsewhere, b) have wide geographic ranges, c) have high native parasitism rates, d) attack early in the weed's lifecycle and/or e) are endophagous (to avoid predation). In addition he argued in favour of root feeders for competitive and clonal plants, defoliators for stress-tolerant plants and any agent that reduces fecundity for annual weeds. Harris (1991) also maintains that plants with an intermediary life strategy may require complementary agents. Cullen (1995) argued, that despite the existence of many exceptions, each project could adopt " a questioning approach" that revolves around understanding three factors that will dictate the ultimate success of an agent. These factors are a)

the per capita destructive capacity of the agent, b) ecological and environmental factors likely to determine final agent densities and c) the levels of damage required to suppress the population of the weed.

According to the abovementioned, experience-based value judgements on agent prioritisation/selection seem to be something of the past, as sufficient ecological tools are available. Research activities such as clearly assessed and defined release strategies will enable researchers to review all projects, and the most costly decision made by biocontrolers need not be left to chance (Sheppard 2003).

Cruttwell McFadyen (2003) extracted the number of agents introduced for all successful programmes from Julien & Griffiths (1998). The aim was to determine how many agents were introduced to achieve success; therefore unsuccessful programmes were left out because we are unable to predict whether these programmes will achieve success. For 75% of programme, success was achieved with three or fewer established species, and Cruttwell McFadyen (2003) questions the use of any feasible ecologically based method to improve on the results that is being obtained by the traditional subjective method. Developing predictive models for each weed to determine the critical stage of the weed's life cycle, as well as separate predictive models that will determine if an agent has the potential to inflict sufficient and timely damage on the weed, need to be developed. These models can be validated against past programmes where the results are known. Models should also be developed for weeds that are currently targeted for biological

control so that the selection of new agents can be tested against the model predictions. Cruttwell MacFadyen (2003) concludes by saying that the challenge is to demonstrate that useful predictive models can be developed without the expenditure of huge amounts of time and money, which could have been used for testing and importation of new agents.

2.1.2 Importance of insect biology and behaviour in biological control

Understanding the biology as well as the role of insect demography and behaviour in weed biological control is essential (Gassmann 1996). Unfortunately a literature survey may provide little or no information on a potential agent. Many potential agents have not been studied because they occur in low numbers on unimportant plants that are part of a complex, species-rich community in which the plant is not particularly conspicuous and may even be rare (De Clerck-Floate 1996). They might also be part of the estimated 10 million insect species that have not been described (Harley & Forno 1992). Basic biology (traits such as where the eggs are laid, the number of larval instars, female fecundity, adult longevity etc.) is valuable information that is needed for further stages in the evaluation of a potential biological control agent, whilst also documenting much needed information on an undescribed insect. Comparative studies of the reproductive biology of closely related beneficial insect species are rare, but one that has been documented is the study on the intrinsic rates of increase of *Cyrtobagous singularis* and *C. salviniae* on *Salvinia molesta* Mitchell. At all temperatures tested, *C. salviniae* laid seven times more eggs than *C. singularis*. Differences in the population levels of the two species and their different

feeding behaviours have contributed towards the different impacts on the weed by the two species (Sands *et al.* 1986).

Insect behaviour is almost certain to change when caged under laboratory conditions and therefore it is important to establish the “normal behaviour” of an insect before one is to venture on to further evaluation such as host-specificity. Some insect species have immature stages that are sedentary, while other species have very mobile larvae or nymphs that can move onto other plant species. Larvae of the tortoise beetle, *Charidotis augroguttata* (Boheman) on cat’s claw creeper, are sedentary and will stay on the plant where they have hatched (Williams pers. comm.). On the other hand, the highly mobile nymphs of *Cornops aquaticum* Brüner (grasshopper) on water hyacinth are able to move to other plants and other hosts despite the fact that the female makes a decision on where to oviposit (Hill & Cilliers 1999). Knowing where the female is likely to oviposit, as well as which plant parts will be attacked by which stage of the insect can also be determined when studying insect biology. Another advantage of knowing the insect biology is the ability to understand how the insect will fit into the biology or phenology of the host plant. In some instances a tight phenology matching is required for establishment and control. The Acacia seed weevils (different *Melanterius* spp.) have only one generation per year, which ties in with annual seed development. Adults feed on the developing seeds and eggs are laid in small holes that are chewed into the walls of developing wattle seed pods. When fully developed, larvae chew their way out of the pods and drop to the ground where they pupate in the soil (Impson 2001). Other examples include

Trichilogaster acaciaelongifoliae (Froggatt) on *Acacia longifolia* (Andr.) Willd., where adult females live for only 3-4 days during which they insert eggs into immature flower buds (Hoffmann 2001a) and, *Rhyssomatus marginatus* (Fåhraeus) on *Sesbania punicea* (Cav.) Benth. (Hoffmann 2001b).

When an agent is considered safe for release, attention should be given to various factors such as mass rearing, release strategies and the possibility of integrated control. Mass rearing will require a thorough knowledge of the insect's biology in order to meet the demands, and researchers should be well aware of special conditions required by all the developmental stages. When agents have high reproductive rates and are highly dispersive, this might not pose a problem; however, many promising weed bioglogical control agents are univoltine and have relatively low fecundities. This inherently limits their build-up at some of the release sights (Briese *et al.* 1996). One such agent is the stem-boring weevil, *Lixus cardui* Ol., that was introduced for the control of *Onopordum acanthium* L. and *O. illyricum* L. thistles. In order to increase insect numbers, large field rearing cages were used. This ensured good agent establishment over a relatively short period of time (Briese *et al.*1996). Another example of the importance of biology when releasing an agent, is the European root weevil, *Mogulones cruciger* Herbst., that was released on hound's tongue (*Cynoglossum officinale* L.) in Canada. Variation among host plant populations in the field where the agents were to be released, suggested the implementation of different release strategies to enhance the establishment and increase weevil numbers. Females indicated a strong ovipositional preference for large and flowering plants and therefore a release

was firstly made on large plants and secondly on a section of the plant population that had a high percentage of plants in the reproductive stage (DeClerck-Floate 1996). Harris (1991) also suggested that competitively inferior agents should be released first to avoid highly competitive agents preventing their establishment or spread.

Biological control is often used as part of an integrated management plan and insect biology and behaviour are once again key factors that need to be considered during the decision making and planning phase. Ueckermann & Hill (2001) found that the choice of herbicide formulation and the species of insect in the system are important factors to consider in the integrated control of water hyacinth, *Eichhornia crassipes* (Mart.). The weevil *Neochetina eichhorniae* Warner is nocturnal, sheltering at the base of the petioles during the day and feeding on leaves during the night. Therefore it is very unlikely that they would come into direct contact with the wet, newly applied herbicide. The mirid *Eiccritotarsus catarinensis* (Carvalho) on the other hand, feeds externally during the day and will be more likely to come into contact with the herbicide. This study showed that the herbicides used were, at least to some extent, toxic to the mirid whereas the adult weevils seemed to be unaffected by most of the herbicide formulations.

2.1.3 Chrysomelids as biological control agents

Successful biological control agents appear to have correlated taxonomic and biological attributes such as high rates of population increase and multiple generations per year (Sheppard 2003). The family Chrysomelidae includes a large number of important insect pests of crop plants, e.g. the adult crucifer

flea beetle, *Phyllotreta cruciferae* (Goeze) on canola (*Brassica napus* L.), and it is likely that characteristics that make insects pests may also make members of a group attractive as potential biological control agents (Aslan *et al*. 2003; Yang *et al*. 2003; Syrett 1996). Julien & Griffiths (1998) catalogues the biological control agents, and their target weeds and provides the most up-to-date published information on insects introduced for biological control of weeds worldwide. Table 2.1 gives a summary of all the Alticinae that have been released as biological control agents throughout the world.

This chapter will focus on studies conducted on the biology of *Phenrica* sp. 2 (Fig. 2.1; 2.2; 2.3 & 2.4) as a thorough knowledge and understanding of the biology, as well as the life strategies of control agents and their interaction with host-plant population dynamics, are essential (Briese 1991).

Table 2.1 The release and establishment of Alticinae (Coleoptera: Chrysomelidae) biological control agents (summary of Julien & Griffiths 1998)

Weed	**Biocontrol agent**	**Released**	**Established**
Alternanthera philoxeroides (Martius) Grisebach (Alligator weed) Family: Amaranthaceae	*Agasicles hygrophila* Selman & Vogt	Australia, New Zealand, People's Republic of China, Thailand and the United States of America	Australia, New Zealand, People's Republic of China, Thailand and the United States of America
	Disonycha argentinensis Jacoby	Australia and New Zealand	Not established
Amaranthus retroflexus Linnaeus (pig weed) Family: Amaranthaceae	*Disonycha glabrata* (Fabricius)	United States of America	United States of America
Cirsium arvense (Linnaeus) Scopoli (Canada thistle) Family: Asteraceae	*Altica carduorum* Guérin-Méneville	Canada, Great Britain, New Zealand and the United States of America	Not established
Senecio jacobaeae Linnaeus (ragwort) Family: Asteraceae	*Longitarsus flavicornis* (Stephens)	Australia and Canada	Australia and Canada
	Longitarsus jacobaeae	Australia, Canada, New Zealand and	Australia, Canada, New Zealand and

	(Waterhouse)	the United States of America	the United States of America
Echium plantagineum Linnaeus (Paterson's curse) Family: Boraginaceae	*Longitarsus aeneus* Kutschera	Australia	Note established, released out of season
	Longitarsus echii Koch	Australia	Establishment not confimed
Heliotropium europaeum Linnaeus (common helitrope) Family: Boraginaceae	*Longitarsus albineus* (Froudras)	Australia	Not established
Pereskia aculeata Miller (leaf cactus) Family: Cactaceae	*Phenrica guérini* Bechyné	Republic of South Africa	Republic of South Africa
Euphorbia cyparissias Linnaeus (cypress spurge) Family: Euphorbiaceae	*Aphthona cyparissiae* (Koch)	Canada	Canada
	Aphthona czwalinai (Weise)	Canada	Canada
	Aphthona flava Guillebeau	Canada	Canada
	Aphthona nigriscutis Foudras	Canada	Canada
Euphorbia esula Linnaeus (leafy spurge) Family: Euphoribaceae	*Aphthona abdominalis* Duftschmidt	United States of America	Establishment not confirmed
	Aphthona cyparissiae (Koch)	Canada and the United States of America	Canada and the United States of America
	Aphthona czwalinai (Weise)	Canada and the United States of America	Canada and the United States of America
	Aphthona flava Guillebeau	Canada and the United States of America	Canada and the United States of America
	Aphthona lacertosa (Rosh)	Canada and the United States of America	Canada and the United States of America
	Aphthona nigriscutis Foudras	Canada and the United States of America	Canada and the United States of America
Myriophyllum aquaticum (Vellozo Conceiçao) *Verdcourt* (parrot's feather) Family: Haloragaceae	*Lysathia* sp.	Republic of South Africa	Republic of South Africa
Acacia nilotica ssp. *Indica* (Bentham) Brenan (prickly acacia) Family: Mimosaceae	*Homicloda barkeri* (Jacoby)	Australia	Establishment not confirmed
Ludwigia adscendens (Linnaeus) Hara (water primrose) Family: Onagraceae	*Altica foveicollis* Jacoby	Thailand	Thailand
Lantana camara Linnaeus (lantana) Family: Verbenaceae	*Alagoasa parana* Samuelson	Australia and the Republic of South Africa	Not established

Figure 2.1 *Phenrica* sp. 2 adult

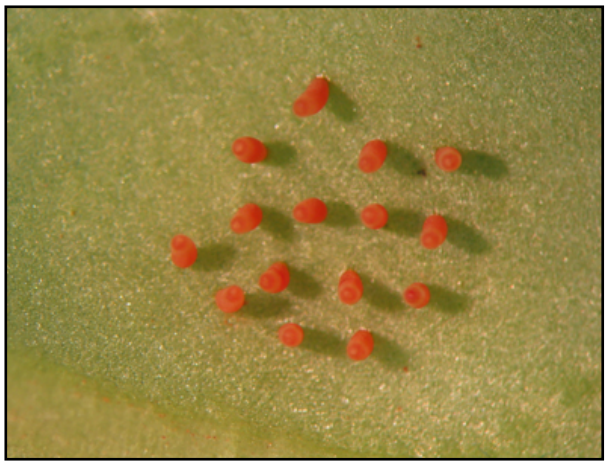

Figure 2.2 Egg group on underside of leaf

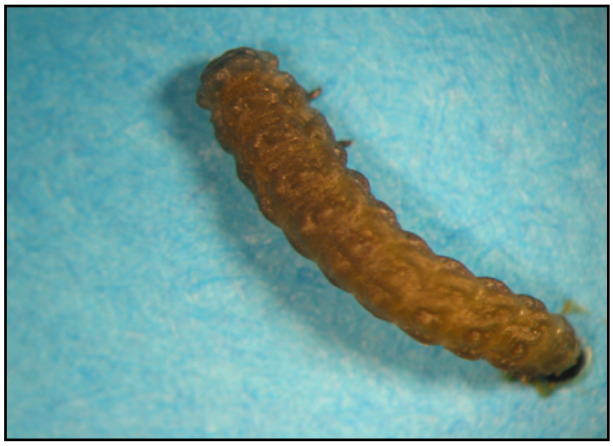

Figure 2.3 Final larval instar of *Phenrica* sp. 2

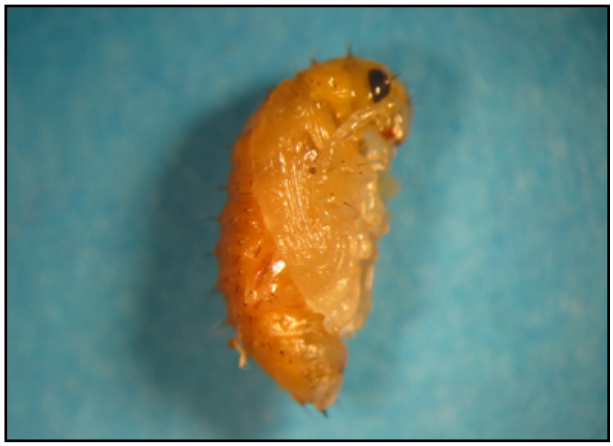

Figure 2.4 *Phenrica* sp. 2 pupa

2.2 Materials and methods

Adults, larvae and eggs of a chrysomelid beetle, belonging to the subfamily Alticinae, were field-collected from *Anredera cordifolia* SSW of Cascavel (Paraná Province, Brazil) during a survey in November 2003. Voucher specimens (AcSN2578) have been lodged with Dr. N.C. Cabrera (Dipartamento Scientifico de Entomologia, Argentina) and Dr. C.N Duckett (Smithsonian Institution, Washington, D.C., United States of America).

Phenrica sp. 2 was cultured in the quarantine laboratories and glasshouses of the Plant Protection Research Institute (Pretoria, South Africa), for life history studies. Adults were initially reared on potted plants in sleeve cages but as insect numbers increased, 55cmx55cmx75cm cages were used instead. The glasshouse cultures were exposed to temperatures of 22ºC (night) and 27ºC (day), with 24% - 65% relative humidity and a prevailing natural photoperiod of about 14h. Biological studies were conducted in a controlled environment room in quarantine where cultures were maintained at 25ºC (night) and 27ºC (day), with 45-77% RH and a 12H photoperiod supplied by overhead fluorescent light –banks.

A homogenous stand of *A. cordifolia* on the PPRI grounds was used as a source for underground tubers, collected and transplanted into pots within a standard soil mixture of equal parts of coarse river sand, loam and a milled pine bark based compost. Plants were kept in a nursery area under 50% shade net with overhead irrigation. Fertiliser was applied when necessary and plants were pruned and kept free of pests and diseases.

The biology studies of the immature stages of *Phenrica* sp.2 included the biology and duration of the eggs, larval instars and pupae, while studies on the pre-oviposition period, female fecundity and female longevity concluded the biology studies on the adult stage. Newly deposited egg groups were tagged on the plant and the time to hatching recorded. Neonate larvae were transferred into ventilated containers and fed on cut leaves (which did not deteriorate visibly) that were replaced every second day until they were ready to pupate. The number of larval instars was determined, and the duration of each was recorded. Fully-grown larvae were allowed to pupate in a moist mixture of soil and vermiculite. The duration of the pupal stage was taken as the time from when the fully-grown larvae burrowed into the soil to adult emergence from the soil. Newly emerged adults were paired and placed onto potted plants that were covered with an upside down, ventilated honey jar. The pre-oviposition period was recorded. Thereafter plants were checked on a weekly basis to determine female fecundity. Males were replaced as necessary and female longevity was noted.

2.3 Results

2.3.1 Collection localities and identification

Figure 2.5 indicates the locality where *Phenrica* sp. 2 was collected in November 2003.

Figure 2.5 Locality where *Phenrica* sp. 2 was collected in November 2003 (the yellow square indicates the collection site)

Correctness of identifications and descriptions are vitally important to biological control. The name is essential for finding possible available information on a recorded host and distribution of a specific organism, and classifications developed by taxonomists reflect probable evolutionary relationships. Misidentification can be costly and time consuming, and it can mean the difference between establishment and failure for a natural enemy. Examples where initial misidentification affected success, are *Pectinophora*

gossypiella (Saunders) in Australia, *Aonidiella aurantii* (Maskell) in California, and *Planococcus kenyae* (LePelley) in Kenya (Gordh & Beardsley 1999). Biological control can also supplement information by supplying taxonomists with zoogeographical-, biological-, behavioural- and ecological data as well as hybridisation studies thereby maximising the usefulness of taxonomy as an accessory to biological control.

The Alticinae (flea beetles) with its over 7000 described species is one of the largest chrysomelid subfamilies (Begossi 1988; Jolivet 1988; Scherer 1988), which are characterized by a peculiar spring-like structure contained in the enlarged metafemora that permits most species to jump with considerable force (Begossi 1988).

Bechyné described several new species and under the generic name *Phenrica* made several new combinations of species formerly described in the genus *Disonycha*. Bechyné however only formally described the genus in 1959 (Duckett 1999). A more recent study by Duckett (1999) suggests that *Phenrica* may be a junior synonym of *Disonycha*. However, as the genus *Disonycha* is so large and morphologically diverse, it is impossible to form a definitive conclusion based on the one species that was analysed. A revision of the two genera is in progress and some species that were previously ascribed to *Phenrica* are being placed in *Disonycha* though this may not be the case with all species classified in *Phenrica* (Duckett pers. comm.).

2.3.2 Life stages

Elongated, orangey-red eggs with pale tips are laid in groups of 16 (Mean ± SE =15.9 ± 0.6; n=17), usually on the underside of leaves. The eggs become progressively lighter in colour from the base to the tip as they mature, prior to hatching (Mean ± SE =6.24 ± 0.14; n=17) (Table 2.2).

There are three larval instars. They live and feed singly on leaves and young growth, with a duration of 4.3 (Mean ± SE =4.3 ± 0.16; n=67); 4.0 (Mean ± SE =3.98 ± 0.15; n=67) and 5.6 (Mean ± SE =5.6 ± 0.4; n=67) days respectively. They pupate just below ground level in flimsy “cocoons” after cessation of feeding. The pupal stage lasts for 17.4 days (Mean ± SE =17.4 ± 0.25; n=67).

When the adults first emerge, the head, pronotum and abdomen have a pale yellow colour and the elytra are black with yellowish-white markings. After a period of 8-12 days, the pale colouration changed to an orangey-red in the males, and a red in the females. Adults jump readily if disturbed and feed on leaves and young growth. Females had a pre-oviposition period of 14 days (Mean ± SE =14.04 ± 1.1; n=23) and laid an average of 6 eggs per day (Mean ± SE =5.96 ± 0.41; n=24) with an 89% fertility rate (n=55). The average reproductive lifetime of a female was about 187 days although it varied considerably in the laboratory (Mean ± SE =187.75 ± 19.12; n=24). The egg-to-egg period under laboratory conditions used was 7-8 weeks.

Table 2.2 *Phenrica* sp. 2: summary of biological information and duration of stages in the laboratory (as described in the text).

	Mean ± SE	n	Range
Number of eggs/group	15.94 ± 0.59	17	10-19
Egg stage (days)	6.24 ± 0.14	17	5-7
Percentage of eggs hatching	89%	55	
Reproductive lifetime of females (days)	187.75 ± 19.12	24	57-413
No eggs/day during reproductive lifetime	5.96 ± 0.41	24	1.9-10.5
Pre-oviposition period (days)	14.04 ± 1.1	23	7-30
First instar	4.3 ± 0.16	67	2-8
Second instar	3.98 ± 0.15	67	2-7
Third instar	5.62 ± 0.4	67	2-22
Pupal stage	17.37 ± 0.25	67	12-25
Generation from egg-egg 7-8 weeks			

2.4 Discussion

Feeding damage of adults and larvae of many chrysomelids cause complete defoliation of their host plants (e.g. *Chrysolina quadrigemina* (Suffrian) on *Hypericum perforatum* L.). They can also be highly fecund and frequently experience high levels of attack by predators, parasitoids and diseases. These factors can be identified with a potentially effective weed biological control agent (Harris 1973). Other practical considerations when working with biological control agents include ease of handling. Chrysomelids are robust and they characteristically may be reared in large numbers in a confined space, and they can usually be transported easily. This undoubtedly leads to a higher probability of establishment once released (Memmott *et al* 1996). It

is also evident that most of the Alticinae are able to fly high and well, and can easily colonize the forest canopy as do the Galerucinae (Jolivet 1995).

Biological traits of *Phenrica* sp. 2. studied during the study period seem to favour the flea beetle as a possible biological control candidate for *A. cordifolia*. Both adults and larvae feed extensively on leaves and stems (Chapter 4). Adults are long-lived and at least 3 generations can be expected during the summer period. Although it is expected that developmental rates will slow down during the winter period, no indication of a definite diapause was found under the prevailing laboratory conditions. This is consistent with observations made by Selman (1994), that seems to indicate that the general tendency for spring breeding chrysomelid species is to over winter as adults, whereas those that breed in late summer and autumn often over winter as diapausing eggs.

Usually large numbers of eggs that are laid in a group contain initially poorly developed embryos, which develop to maturity over a week, as with *Phenrica* sp. 2, or several weeks (Selman 1994). The females prefer laying their eggs on older leaves, possibly to prevent them from being consumed by other adults or larvae, which tend to feed on the younger growth, which might lead to ant predation.

The natural enemies of insects are believed to be a major reason for failure in the biological control of weeds, but their real importance is not known although rates of predation or parasitism are often given to support the

assumption (Grassmann 1996). These given rates can be misleading because of compensatory mechanisms (e.g. fecundity and fertility) that are not taken into account. For example, egg predation of *Cactoblastis cactorum* (Bergroth) by ants, as well as the effects of climate, considerably reduced the effectiveness of this biological control agent in South Africa (Robertson & Hoffmann 1989). In Australia where the moth is very successful, there is no clear indication as to what extent egg mortality induced by predators is compensated for by density-dependant larval mortality (Grassmann 1996).

Hill & Hulley (1995) analysed by order and family the biological control agents that were parasitised after they established on weeds in South Africa and found that 35% (29 of the 62 spp.) of all Coleoptera agents released were parasitised, and 66.67% of all Chrysomelidae (2 of the 3 spp.). *Phenrica* sp. 2 females lay their eggs on the underside of leaves, and both adults and larvae feed externally on foliage, and are therefore exposed to parasitism and predation. At the same time external feeding allows herbivores to move away from telltale signs of their presence (Gross 1991). Freedom of movement also permits various evasive manoeuvres if the herbivore is found, such as dropping off the leaf or jumping. Most alticines are strong jumpers and may be difficult for predators to catch, and difficult-to-catch ('dysleptic') prey or unpalatable prey can negatively reinforce predators. This should enable *Phenrica* sp. 2 adults to escape predation to some extent. Hawkins and Gross (1992) also stated that species that are either more mobile or better concealed support fewer, and more specialised parasitoids species.

Adults display bright, contrasting colour patterns that could signify unpalatibility or possibly even mimicry. Williams (2003) distinguished three colour morphs displayed by the adults of *Alagoasa extrema* Jacoby, and reasoned that the colour morphs might either be the unpalatable models in a Batesian mimicry, or be part of a Müllerian mimicry cycle. One colour morph strongly resembles the coloration found on *Phenrica* sp. 2 and *P. guérini* adults (red abdomen and pronotum; black elytra with yellowish-white spots). Unfortunately not enough information is available with regards to the distribution, genetics and physiology or the mimetic species present in the distribution range of both the *Phenrica* spp. to draw any firm conclusions as to the significance of their particular colour morph. Unlike in *A. extrema* and many other related spp., only a single colour morph has been encountered for each of the 2 *Phenrica* spp. referred to here.

As *Phenrica* sp. 2 is still unidentified, and the possibility exists that it may be ascribed to the genus *Disonycha*, this can very well be an indication that predation and parasitism will be from similar sources as recorded for *Disonycha* spp.(Table 2.3). The distributions of both genera (*Phenrica* and *Disonycha*) are “however” restricted to South and Central America and the new world respectively. Should *Phenrica* sp. 2 be released they will possibly be attacked by a high percentage of generalist parasitoids that can extend their host range more easily from native hosts. Specialist parasitoids might then evolve appropriate traits to exploit the new resource (Cornell & Hawkins 1993).

Table 2.3 *Hymenopterous* and *dipterous* parasitoids listed for the genus *Disonycha* (Cox 1994)

Family and genus	Host subfamily and genus	Stage attacked
Encyrtidae: *Homalolytus*	Alticinae: *Disonycha*	Eggs
Euphorinae: *Microctonus*	Alticinae: *Disonycha*	Adults
Exoristinae: Blondeliini: *Medina* *Myiopharus*	Alticinae: *Disonycha* Alticinae: *Disonycha*	Larvae Larvae
Phasiinae: Strongygastrini: *Strongygaster*	Alticinae: *Disonycha*	Adults

Under laboratory conditions a fungus, later identified as *Laboulbenia dorstii* Balazuc (by Dr. E.J. van der Linde, Biosystematics Division: Mycology, ARC, PPRI), infected a number of adults. As a direct consequence of their obligate relationship with their hosts, Laboulbeniales fungi exhibit an often-high degree of host specificity (Weir 1993). The host range for any given parasitic fungus appears, with very few exceptions to be restricted taxonomically, and generally encompasses only species that belong to the same genus or group of closely related genera (Majewski 1994). *Laboulbenia dorstii* was also reported on *Phenrica aemula* Weise, from Brazil (Balazuc 1988)

2.5 Conclusion

The biological traits discussed in this chapter seem to indicate that *Phenrica* sp. 2 is an agent well worth considering in the biological control of *A. cordifolia*. *Anredera cordifolia* loses its leaves during the winter period in most of the provinces in South Africa, but because the adults are long-lived they should be able to survive the leafless periods. The relatively short life cycle, high fecundity and three generations per year should theoretically ensure a

strong population build-up that would improve the chances of establishment in the field.

Chapter 3

Laboratory host range of *Phenrica* sp. 2

3.1 Introduction

Biological control systems have helped transform perceptions in terms of control, from an industrial to an ecological model (McEvoy & Coombs 2001). Resulting in the acceptance of more environmentally acceptable methods of control such as biological control. This change has also brought about a heightened public and scientific awareness and concern, for the environmental impact of introduced organisms (Ehler 1990; McEvoy & Coombs 2001). Classical biological control programmes, which centre on the introduction of foreign organisms, are generally irreversible as they cannot be withdrawn and discontinued if they have proven to be ineffective or harmful to non-target plants (McEvoy & Coombs 2001). Furthermore, biological control agents cannot be restricted to a specific area as pesticides can, and thus, local governments need to sanction any prospective biological control programme as a matter of public interest (Harris 1989; Briese 2005; Sheppard *et al.* 2005). Host specificity testing plays a pivotal role in the decision making process, as it assures decision makers that the release of a biological control agent would not result in unacceptable non-target impacts (McEvoy 1996; van Klinken 2000).

Host specificity is a term used to rank insect species within a continuum, from specialists to so-called generalists (van Klinken 2000). The objective of host specificity testing in weed biological control is to determine whether candidate biological control agents will attack non-target plant species once released.

These tests aim to determine either the tendency of adults and immatures to feed and oviposit on test plants, or the degree to which a test plant species can support pre-reproductive and reproductive development (Marohasy 1998).

Host specificity test results are often ambiguous, and over-estimating an insect's host range may lead to the rejection of candidate agents that would be adequately specific under field conditions, this is a phenomenon referred to as false positives (Wapshere 1989; Cullen 1998). False negatives could lead to the release of potentially unsafe agents. It is therefore very important that where possible, all examples of false negative results under cage conditions that were not followed by attack on non-target plants in the field, are identified and published (Cruttwell McFadyen *et al.* 2002). Cages in particular, place restrictions on an insect's natural host-finding behaviour, allowing only a small part of the normal sequence of host selection used by the insect to be expressed (Wapshere 1989), which often lead to its selection of unnatural hosts (Marohasy 1998).

Van Klinken (2000) described host specificity testing as a three step process, where 1) the aspects of an insect's life history that need to be specific should be identified; 2) the fundamental host range is estimated and 3) the information gathered is extrapolated to the field.

3.2 Host range, host finding and acceptance and host shifts

The host range of an insect is the sum of plant phenotypes that are hosts (van Klinken 2000), i.e. plants on which an insect can complete its normal development in nature (Hanson 1989). Describing the host range can be complicated by the fact that the host range observed in experiments is frequently broader than what occurs in the field. This problem can be overcome by differentiating between fundamental and realised host ranges (van Klinken 2000).

The fundamental host range is the most inclusive because it includes all the plant species that an insect is capable of accepting and/or utilising, and is constrained by factors such as its metabolic and sensory capabilities, physical limitations and behavioural programming. The realised host range on the other hand is how the fundamental host range is actually expressed under particular conditions. In biological control we are concerned with predicting how the fundamental host range will be realised if the agent were to be released, i.e. the field host range. In the field insects only accept or use a portion of those that they are capable of using in the laboratory (van Klinken 2000). One such an example is that of *Teleonemia scrupulosa* (Stål), where the preliminary field observations indicated that the extension in the accepted host-range during no-choice laboratory trials, were not realised under field conditions in South Africa (Baars 1999).

Singer (2003) describes host range as an aspect of preference, where preference can be defined as an insect trait that would normally be measured

as the set of likelihoods of accepting resources that are encountered. In order to predict evolution in plant-insect systems, Singer (2003) emphasises the need to define potentially heritable traits of plants that describe how they interact with insects, and potentially heritable traits of insects that describe how they interact with plants.

Sensory input, processed by the central nervous system, determines which host plants phytophagous insects find, examine, consume and oviposit on (Marohasy 1996). Host finding and acceptance is a chain sequence with each step operating on a threshold system. A positive stimulus will lead to the acceptance at each step and progression to the next (Wapshere 1989; Marohasy 1996). Insects are also capable of modifying their behaviour as a result of numerous factors such as physiological state and previous experience, nervous system changes with experience, external and internal conditions etc. (Bernays 1999). Plasticity within phytophagous insects also appear to have adaptive value which allows for compensatory feeding to supply sufficient nutrients, avoidance of bad experiences and the maximization of the numbers of eggs laid (Bernays 1999). Amongst the different feeding guilds, generalist herbivores also tend to have a bigger range of potential hosts to choose from, and therefore also a bigger gradation of host suitabilities to make use of should the need arise (Bernays 1999). The categorisation of potential biological control agents is very often done without truly understanding their association with their host plant. Insects tend to have two main evolutionary pulls namely to become a specialist by continuously adapting to the changes posed by one specific host plant (plant

deterrents) or, to adapt to changes in more than one host plant, which results in the insect becoming a generalist (Neser 2005). Being a very slow process, biological controllers have often misidentified a potential agent on the continuum ranging from generalists to specialists. *Melanterius servulus* Pascoe was at first categorised as an oligophage, then as a monophage and finally as being specific. The initial impression of an insect's specialisation will change as more information becomes available and researchers should therefore refrain from branding an insect at an early stage (Neser 2005). For biological control the risk posed by specialist insects to non-target plants are very low compared to that of generalist. A specialist biological control agent is however not always the best option, as they tend to restrict the damage to their host plant in order to ensure food security (Neser pers. comm.).

When a biological control agent is introduced into a new environment, it will inevitably encounter novel plant species. Coincidentally, one or more of these species may possess visual and/or chemical attributes, which result in the biocontrol agent recognising and exploiting these species (Marohasy 1996). The only reason for this phenomonen is that these plants possess all the attributes necessary for acceptance at every step in the biological control agent's host finding and acceptance process. The insect species is therefore pre-adapted to utilise these novel hosts. The terms "host shift", "host switch" and "host range expansion" imply that the insect has somehow changed, and should therefore not be used (Marohasy 1996; Stiling & Cornelissen 2005). Most alien invasive plants have been in South Africa for 150-350 years, some even 600 years. However, of all the alien invasive plants studied to date none

has been reported as being affected by local “monophagous” insects or mites, ruling out the ever present fear of “immediate evolution” (Neser 2005). Cases used to support the “host shift” or “host range extension” theories are often poorly scrutinised and often readily explainable. Most perceived “host shifts” are also often expected and predictable based on field and laboratory studies (e.g. *Trichilogaster acaciaelongifoliae* Froggatt used to control *Acacia longifolia* (Andr.) Willd.) (Neser 2005).

At high densities of biological control agents, females can often not find suitable oviposition sites, forcing them to lower their acceptance threshold and resulting in a situation of “host substitution” or “threshold change” (Harris 1990; Neser 2005). This is however a rare phenomenon and will occur only in some situations when biological control agents become very abundant following a sudden reduction of the weed population caused by the biological control agent (Marohasy 1996). The chrysomelid beetle *Chrysolina quadrigemina* (Suffrain), is a documented example in weed biocontrol where sustained attack on a previously unexploited host occurred. The chrysomelid was introduced into North America in the 1950s to control *Hypericum perforatum* L. (Guttiferae), and twenty years after the introduction it was reported utilising the ornamental, *Hypericum calycinum* L. (Andres), which is closely related to *H. perforatum* (Marohasy 1996). *Rhinocyllus conicus* Frölich, a flower head weevil released in North America in 1968 to control *Carduus nutans* L is now also reducing seed production by multiple native North American thistle species, and local population densities of *Cirsium canescens* Nutt. The inclusion of a native thistle in the host range of *R.*

conicus was known, and to be expected and should therefore not be seen as a “host shift”. Another well-known example is the overspill of *T. scrupilosa* onto *Sesamum indicum* Linn. in Uganda after it’s release on *L. camara* (Davies & Greathead 1967). Again showing that if the preferred weedy species becomes less available, the acceptable secondary native species may become more vulnerable (Arnett & Louda 2002). Louda *et al*. (2005), argues that host range and preference from host specificity test are not sufficient to predict ecological impact if the introduced natural enemy is not strictly monophagous. The environment can alter host use and population growth, leading to higher than expected direct impact on the less preferred native host species. Therefore we should take great care when host specificity data is used in risk assessments as the assumption is made that population impacts are proportional to the relative preference and performance of the insect (Louda *et al.* 2005).

3.3 Selecting test plants for host specificity testing

Initially the primary aim of host specificity testing was to determine whether particular crop plants were safe from attack. More systematic testing protocols were developed where congeneric and related plants form broader taxa were included, until Wapshere (1974) formulated the centrifugal phylogenetic method (CPM) that has been used worldwide for the past three decades (Briese 2002).

The CPM method is based on two components. Firstly, the way in which insects choose their hosts determine the sequence of testing from more

closely related organisms to that of less closely related organisms, with the safe guard criteria, based on the known gaps in this knowledge, as the second component (Wapshere 1974). Even though this method has been serving biological control well, Harris & McEvoy (1995), have called for testing procedures to be expanded to include phylogenetic, bioclimatic and biological constraints, and Briese (2002) sees it as worth while to close the gaps that exists within the CPM model by incorporating the existing knowledge of plant phylogenetic relationships.

As researchers may never be able to claim with absolute certainty that a biological control agent is safe to release, the ultimate goal should be to ensure that decisions to release an agent or not are knowledge-driven and not based on unspecified or nonspecific fears (Briese 2005).

3.4 Host specificity testing techniques

The results of current host specificity procedures have been sufficient to largely prevent unexplained or unpredictable and damaging host shifts in the history of the discipline (Marohasy 1996). The accuracy of host specificity testing is however constrained by the environmental conditions of the tests, which in return hamper or influence the behavioural response of the agent (Sheppard *et al*. 2005). Three basic test designs can be distinguished namely no-choice tests, choice tests, and field tests.

3.4.1 No-choice tests

No-choice tests or starvation tests require the agents to be confined on the test plant until death, or at least for sufficient time to reach a highly deprived state. It is frequently used to determine the fundamental host range but it can also provide information on the relative suitability of hosts for development (e.g. survival, development rate and size of life stages, fecundity etc.) and adult feeding (e.g. frequency and duration of feeding).

3.4.2 Choice tests

Choice tests are normally used to rank hosts for preference after host suitability has been determined. They are invaluable when dealing with highly mobile and discriminatory life stages or where plants are small and test plant phenology can be synchronised (Sheppard *et al*. 2005). However, when used alone they may inaccurately predict field host range. Choice tests can be used as a choice-with-target test which assesses the basic preferred rank of a test plant relative to the target or, a choice-minus-host tests which allows for a number of test plants to be used in different designs and test plants can be offered at once or sequentially (Sheppard *et al*. 2005).

3.4.3 Field tests

These test are conducted in the native range with the use of choice-tests. It can be used for screening multiple potential agents or to clarify results found form other types of tests. Insects that are hard to rear, highly mobile or highly sensitive to artificial experimental conditions make ideal candidates. As agent deprivation is required, the tests consist of two phases whereby the host is

included in the first phase but removed during the second phase thereby forcing the insects to either use the available plants or to move out of the system (Sheppard *et al*. 2005).

3.4.4 Cut foliage *vs.* whole plants

There are practical (e.g. limited space in laboratory and the number of test plants to be tested) and scientific advantages (e.g. pilot studies on key plants during collection trips and permitting more detailed measurements) in using cut foliage for feeding tests when determining the initial host range of a potential biological control agent (Palmer 1999). This method can be used for all types of tests and insects but it is most commonly used for testing the growth, development and survival of leaf feeding insects. When opting to make use of these tests the researcher should be aware of the possible effects it would have on plant chemistry, the possibility of laboratory artefacts and the problems that may arise when choosing representative samples from the whole plant. A comparative study done by Palmer (1999) concluded that the data collected from cut foliage tests would have produced the same final conclusion on host range than that generated by whole plant testing. Cut foliage tests can therefore be considered an appropriate method for determining host range but it would be wise to include comparative data in order to confirm the results obtained from such trials.

It would seem that choosing the appropriate testing procedures are closely related to the characteristics of both the insect and test plant involved. The inability of having a standard test that can be applied to all potential agents

does not add any inherent risk to weed biological control (Sheppard 1999), as the results of current host specificity procedures have been sufficient to largely prevent unexplained or unpredictable and damage to non-target plants (Marohasy 1996).

In this chapter the aim was to establish the host range of *Phenrica* sp. 2 under quarantine laboratory conditions.

3.5 Materials and methods

Studies to determine the host range of *Phenrica* sp. 2 included larval no-choice, and adult choice trials. In order to determine if non-target species can support larval development, no-choice trials were conducted in a controlled environment room within quarantine. Temperatures were maintained at 25ºC (night) and 27ºC (day), with 45-77% RH and a 12H photoperiod supplied by overhead fluorescent light-banks. Adult choice trials give an indication of the acceptability of non-target species and its suitability for feeding and oviposition. These trials were conducted in a quarantine glasshouse where the adults were subject to temperatures of 22ºC (night) and 27ºC (day), with 24% - 65% relative humidity and a prevailing natural photoperiod of about 14h.

3.5.1 Test plant species

Test plant species were selected using Wapshere's (1974) centrifugal phylogenetic testing method, and included species on which *Disonycha*

(Chapter 2) and *Phenrica* species were recorded (Clark *et al.* 2004), as well as some commercially important plant species (Table 3.1).

Culture and test plants were planted into pots within a standard soil mixture of equal parts of coarse river sand, loam and compost. Plants were kept in a nursery area under 50% shade net with overhead irrigation and allowed to grow to a suitable size of about 20cm.

3.5.2 Larval no-choice trials

The larval no-choice trials included twenty-seven plant species from 14 plant families. Five newly emerged and unfed larvae were placed onto the foliage of potted plants of the test plant species and the control, *A. cordifolia*. Ventilated honey jars were used to cage the larvae. The number of larvae surviving to pupation and the duration were recorded. A minimum of three replicates was conducted for each test plant species. Data were analysed using the statistical program GenStat (2003). Differences between larval survival and development were tested for in an analysis of variance (ANOVA). Means were separated using Fishers' protected t-test least significant difference (LSD) at the 5% level of significance (Snedecor & Cochran 1980), if the F-probability from the ANOVA was significant at 5%.

3.5.3 Adult choice trials

The adult choice trial was conducted in 55cmx55cmx75cm cages within a quarantine glasshouse and replicated three times. Potted plants of test plants species that could sustain larval development were used during these trials (Table 3.1). *Anredera cordifolia* was included as a control plant. An experienced female and two males were released in the centre of each cage and removed after 14 days. After an exposure period feeding damage was rated visually (as normal, minor and exploratory) and the number of eggs laid on the test plants was recorded.

Table 3.1 Test plant species used to determine the host specificity of *Phenrica* sp. 2

Plant species	**Common names**	**Trials conducted**
Basellaceae		
Anredera cordifolia (Ten.) Steenis	Madeira vine	LNC, AC
Basella alba L.	Malabar spinach	LNC, AC
Ullucus turverosus Caldas	ulluco	LNC, AC
Amaranthaceae		
Alternanthera ficoidea cv.	Joseph's coat	LNC
Ameranthus deflexus L.	pigweed	LNC
Asphodelaceae		
Bulbine frutescens (L.) Willd *	Snake flower	LNC
*Bulbine sp. (*identity to be confirmed)		LNC
Asteraceae		
Aster sp. (to be identified)		LNC
Brassicaceae		
Brassica oleracea L.	cabbage	LNC
Cactaceae		
Opuntia		LNC
Pereskia acculeata Mill	Barbados gooseberry	LNC
Rhipsalis baccifera (J.S.Mill) Stearn subsp. *Mauritiana* (DC.) Barthlott *	mistletoe cactus	LNC
Caryophyllaceae		
Dianthus chinensis cv.	carnation	LNC
Stellaria media (L.)Vill	chickweed	LNC
Silene acaulis (L.) Jacq. cv.	Moss campion	LNC
Chenopodiaceae		
Beta vulgaris subsp. *cicla* (L.)Koch	chard	LNC
Malvaceae		
Gossipium herbaceum L. subsp. *africanum* (Watt) Vollesen *	wild cotton	LNC
Mesembryanthemoideae		
Aptenia cordifolia (L.f.) Schwantes *	heartleaf ice plant	LNC
Nycaginaceae		
Mirabilis jalapa L.	marvel of Peru	LNC
Phytolacaceae		
Rivinia humilis L.	blood berry	LNC
Plumbaginaceae		
Plumbago auriculata (L.) *	Cape lead worth	LNC
Portulacaceae		
Portulacaria afra (L.) Jacq. *	elephant bush	LNC
Portulaca oleracea L.	purslane	LNC
Portulaca kermesina N.E.Br. *		LNC
Talinum caffrum (Thunb.) Eckl. & Zeyh *	flame flower	LNC
Talinum paniculatum (Jacq.) Guertn.	jewels-of-opar	LNC, AC

LNC – larval no-choice trials and AC - adult choice trials

***** Indigenous plants

3.6 Results

3.6.1 Larval no-choice tests

Larval development was supported by all 3 Bacellaceae species (including the control *A. cordifolia*) and one Portulacaceae species (Table 3.2). None of the remaining 13 plant families tested, including some commercially important species, could sustain larval development. *Anredera cordifolia* proved to be the most preferred host (78% pupation) followed by *B.alba* (70%), *T. paniculatum* (56%) and *U. tuberosus* (55%). First instar mortality was 100% for all the other test plant species.

Table 3.2 Mean number of *Phenrica* sp. 2 larvae developing to pupation on different test plant species during larval no-choice trials.

Plant species	*n*	Number of pupating larvae (Mean ± SEM) (% survival)	Duration of development (Mean ± SEM)(days)
Basellaceae			
Anredera cordifolia (Ten.) Steenis	15	3.9 ± 0.24 (78)[a]	20 ± 1.39[b]
Basella alba L.	5	3.5 ± 0.29 (70)[a]	19 ± 1.11[b]
Ullucus turberosus Caldas	3	2.75 ± 0.75 (55)[a]	18 ± 0.83[b]
Amaranthaceae			
Alternanthera ficoidea cv.	5	0	0
Ameranthus deflexus L.	4	0	0
Asphodelaceae			
Bulbine frutescens (L.) Willd	5	0	0
Bulbine sp. (identity to be confirmed)	5	0	0
Asteraceae			
Aster unidentified	5	0	0
Brassicaceae *Brassica oleracea* L.	5	0	0
Cactaceae			
Opuntia	5	0	0
Pereskia acculeata Mill	5	0	0
Rhipsalis baccifera (J.S.Mill) Stearn subsp. *Mauritiana* (DC.) Barthlott	5	0	0
Caryophyllaceae			
Dianthus chinensis cv.	5	0	0
Stellaria media (L.)Vill	3	0	0
Silene acaulis (L.) Jacq. cv.	3	0	0
Chenopodiaceae			
Beta vulgaris subsp. *cicla* (L.)Koch	5	0	0
Malvaceae			
Gossipium herbaceum L. subsp. *africanum* (Watt) Vollesen	5	0	0
Mesembryanthemoideae			
Aptenia cordifolia (L.f.) Schwantes	5	0	0
Nycaginaceae			
Mirabilis jalapa L.	5	0	0
Phytolacaceae			
Rivinia humilis L.	4	0	0
Plumbaginaceae			
Plumbago auriculata (L.)	4	0	0
Portulacaceae			
Portulacaria afra (L.) Jacq.	5	0	0
Portulaca oleracea L.	5	0	0
Portulaca kermesina N.E. Br.	5	0	0
Talinum caffrum (Thunb.) Eckl. & Zeyh	5	0	0
Talinum paniculatum (Jacq.) Guertn.	5	2.8 ± 0.75 (56)[a]	18 ± 0.41[b]

[a] – means do not differ significantly at the 5% level

[b] – means do not differ significantly at the 5% level

SEM – is the standard error of the mean

3.6.2 Adult choice tests

During adult choice tests, *Phenrica sp*. 2 adults displayed clear feeding preferences for *A. cordifolia* and *B. alba*, with minor feeding on *T. paniculatum*. Eggs were only laid on *A. cordifolia* (Table 3.3).

Table 3.3 The feeding damage and number of eggs laid by *Phenrica* sp. 2 adults during adult choice tests

Plant family	Plant species	Feeing damage	No. reps.	Mean no. of eggs laid (SD)
Bacellaceae	*Anredera cordifolia*	+++	3	14.2 (1.7)
	Basella alba	+++	3	0
	Ullucus turberosus	+	3	0
Portulaccaceae	*Talinum paniculatum*	++	3	0

"+++" normal feeding; "++" minor feeding; "+" exploratory feeding

3.7 Discussion

3.7.1 Larval no-choice

The larval stage has been indicated as the most damaging stage (Chapter 4), and therefore it was decided to firstly initiate larval no-choice trials. Larval development depends on the insect having inherent behavioural responses to initiate and continue feeding, having nutritional requirements that can be met by the plant, having the physical ability to consume sufficient plant material to obtain necessary nutrients, and having the metabolic, behavioural and other capabilities to overcome any toxic properties. (van Klinken 2000).

The host specificity trials clearly indicate that the Basellaceae and Portulacaceae families include plant species that are able to fulfil all the

developmental requirements of the *Phenrica* sp. 2 larvae. Very similar results were obtained from trials done with *Plectonycha corretina* Lac. (Chrysomelidae), another possible biological control agent for *A. cordifolia*. In a no-choice trial the larvae of *P. corretina* only developed on *A. cordifolia*, *B.alba* and *U. tuberosus*, all members of the Basellaceae family (Gandolfo *et al*. unpbulished). Although the larvae of *Phenrica* sp. 2 are mobile, it is more likely that the females would select the most preferred host.

3.7.2 Adult choice-trials

The first signs of adult feeding were always observed on *B. alba*, and the adults seem to prefer sitting/resting on this particular test plant. Only towards the latter part of the trial did the adults feed on *A. cordifolia,* with minor feeding occurring on one *T. paniculatum* test plant. Oviposition preference was restricted to *A. cordifolia*.

Anredera cordifolia is more than capable of sustaining a healthy population of *Phenrica* sp. 2, and it is most certainly a host plant for *Phenrica* sp. 2, although it may not be the primary host in the country of origin. Why *Phenrica* sp. 2 prefers feeding on *B. alba* even though it doesn't co-occur with *A. cordifolia* in the country of origin, has not been resolved. *Basella alba* clearly falls within the physiological host range of *Phenrica* sp.2 and is therefore recognised as a potential host. The possibility also exists that *B. alba* is a "neutral plant" that does not repel the adults. By placing both these plants in close proximity of each other, a situation might have been created whereby the adults were satisfied that they were close enough to the more preferred

host, *A. cordifolia*. It would therefore be well worth repeating the trial in a walk-in cage where a more natural situation can be simulated.

Basella alba, *T. paniculatum* and *U. tuberosus* are all introduced species. *Basella alba*, the second most acceptable host, has a wide distribution throughout Africa and Asia, but is only occasionally grown as an oddity. The likilihood of it being utilised as a host in South Africa is remote, as there is no records of *B. alba* occurring anywhere in South Africa except for the few specimens that were used during the host specificity trials. *Basella alba* is however, being utilised in other parts of Africa and one would have to assess the risk of this plant species being used as an alternative host once the insects are released.

Basella paniculata Volkens, a succulent twining perennial herb is the only indigenous *Basella* species in South Africa (Chapter 1). However, because of its peripheral distribution and its inconspicuous nature, it is seldom recognised, and even less often collected, and therefore it could not be included in the present trial.

3.8 Conclusion

Phenrica sp. 2 has been shown to be adequately host specific, as no commercially important – or native plant species could sustain larval development, and ovipositing was restricted to *A. cordifolia*.

In the country of origin, *A. cordifolia* and *P. aculeata* have been found growing at the same locality (Neser pers. comm.). *Pereskia aculeata* could however not sustain larval development under laboratory conditions, even though it is host to another flea beetle, *Phenrica guérini* Bechyné. Adults of *P. guérini* on the other hand also rejected *A. cordifolia* as a possible host, as no adult feeding was observed on *A. cordifolia*, even though their own host, *P. acculeata* was almost depleted. It would therefore seem that both *Phenrica* sp. 2 and *P. guérini* have become specialised in utilising their different hosts.

Chapter 4

The impact of *Phenrica* sp.2 on *Anredera cordifolia* under laboratory conditions

4.1 Introduction

Much research has been conducted into the impact of defoliating insects on the growth and development on their host plants. Not only in terms of the consequences of the herbivory for the plants, but also for the insects, as the resources provided by the plant changes in response to damage (Foggo 1996).

The enemy release hypothesis assumes that the primary regulation of plant populations in the native range is by specialist herbivores, and that the escape from these specialist herbivores into a new geographic region results in them becoming invasive, and this forms one of the pillars of classical biological control (Raghu & Dhileepan 2005). Another important pillar is that the reunion of natural enemies with their coevolved hosts will suppress plant performance, and result in the replacement of the invasive species with more desirable vegetation (Pratt *et al.* 2005). Few studies however actually test for the role of specialist herbivores in the native range of the invasive plant to determine whether re-establishment of the missing trophic links between the specialist herbivore and the plant in the invaded range, is likely to result in biological control (Raghu & Dhileepan 2005).

Even though most weed agents that are released on weeds do become established, only a portion contribute to the successful control of the target

weed (Julien & Griffiths 1998). The assumption that any agent that becomes sufficiently abundant must have some sort of an impact on the population of its target, is not necessarily true (Meyers 2000), as a substantial portion of weed biological control agents, even some that have become very abundant after their release, fail to control their target (Cruttwell McFadyen 2003). Possible causes of ineffectiveness of abundant agents include: the use of seed feeders against target weeds whose populations are not seed-limited; feeding on non-essential tissue such as parenchyma or fruit pulp; the ability of target weeds to tolerate or compensate for defoliation or other kinds of injury; damage that comes too late in the phenology of the weed to affect its reproduction or growth; agents that trigger a strong defense response in the target weed (McClay & Balciunas 2005); biological control selection protocols that favour obtaining safe, rather than highly damaging candidates (Harris 1973) and the influence of the environment on both the herbivore and the nutritional quality of the weed (Pratt *et al.* 2005).

4.2 Compensation, re-growth and defence

Although insect herbivory is generally considered to be detrimental to plant fitness, some authors have advocated the contrary. Plant traits that reduce damage (genetic variation for resistance to herbivory) are nearly ever-present in natural plant populations (Pilson 2000). However, most plants do not show maximal resistance, and much theoretical and empirical work has been devoted to explaining the rarity of this phenomenon. Pilson (2000) several models by various authors and propose that an intermediate level of resistance is maintained by a trade-off between the benefits of reduced

herbivory and the cost of allocating resources to resistance. Functions such as growth, maintenance and reproduction cannot be stopped completely, and therefore it can be assumed that plants have a limited budget against herbivores (van der Meijden 1989). Van der Meijden (1989) therefore postulates that the trade-off between defence and re-growth will provide two predictions: (a) re-growth and defence should be negatively correlated and (b) defence implies investment of energy and nutrients in secondary compounds or morphological structures in those plant parts that are subject to attack, whereas re-growth capacity requires saving and storing of energy and nutrients in plant parts that are relatively free from attack. Plants do however have different ways of responding to herbivory and this also applies to the response of weeds to introduced herbivores in biological control programmes.

Re-growth leaves produced by many species following defoliation may be distinctly different in shape, colour, toughness, hairiness or chemical composition from the tissues that were destroyed. The chemistry of re-growth leaves in several species has been found to be richer in resins, phenolics or alkaloids. These attributes of re-growth foliage are thought by some workers to represent facultative defences on the part of the plant. Herbivore fitness should then theoretically be lower on a diet of re-growth foliage and insect behaviour is expected to change in such a way that ovipositing adults avoid re-growth tissues. Crawley & Nachapong (1984) in their studies with *Tyria jacobaeae* (L.) on *Senecio jacobaea* L. found that a facultative response, which consisted of an increase in qualitative defences, is unlikely to be effective against adapted herbivores. Furthermore we must resist the

temptation of assuming that changes in plant chemistry, which follow herbivory attack, are induced defences, rather than the consequences of wound repair or altered hormone balance (Crawley & Nachapong 1984). Re-growth does not necessarily lead to complete compensation and other factors such as unfavourable weather conditions can have a more pronounced effect on the impact of herbivores (Prins & Nell 1990).

An insight into the direct impact of herbivores on plant dynamics can be gained from biological control studies (Prins & Nell 1990), and almost all of the long-term observations on the effects of insect herbivores on plant population dynamics stem from programmes on the biological control of weeds (Hoffmann & Moran 1998). Translation of such data to the study of plant-herbivore dynamics can however lead to problems as the situation before and after introduction of the biological control agent is seldom well described, replication does not occur, and plant and insects are mostly alien (Prins & Nell 1990). Furthermore, there are fundamental differences between insect herbivore/host-plant interactions in natural communities and in biological control situations, and conclusions about these interactions cannot easily be extrapolated from one situation to the other (Moran & Hoffmann 1989).

Biological control practitioners often regard it as self-evident that insect herbivores, released from the constraints of their natural enemies, and despite the compensatory powers of many plant species, have the potential to devastate their hosts. The numerous cases of successful biological control of

weeds in which one or more insect herbivore species have drastically reduced the density, spread and distribution of the target plant substantiates this view (Moran & Hoffmann 1989).

Laboratory proof of an agent's host specificity is not enough to warrant the release of a biological control agent: some critics maintain that the release of herbivores poses an ecological risk to the community into which it is introduced, albeit narrow in host range (Louda & Stiling 2004; McEvoy & Coombs 2000). Furthermore, evidence has shown that a relatively small proportion of agents released against any given target weed actually contributes towards control (Chapter 2). The impact trials will therefore aim to quantify the impact of *Phenrica* sp. 2 on plant biomass, and to assess the incidence and intensity of foliar damage under laboratory conditions.

4.3 Materials and methods

In order to assess the potential impact of *Phenrica* sp 2. larvae on *A. cordifolia* the mass increment of tubers were used. *Anredera cordifolia* tubers were field collected and all excess rootlets, stems and leaves removed before tubers were washed and allowed to dry. After being weighed, all tubers were arranged from the highest to the lowest mass and numbered accordingly. This would help to minimise block variation due to the difference in starting mass of the tubers. Tubers were planted into pots within a standard soil mixture of equal parts of coarse river sand, loam and a milled pined bark based compost. Plants were kept in a nursery area under 50% shade net

with overhead irrigation and allowed to grow to a suitable size, i.e. about 15 cm in height.

When of suitable size, plants were arranged in blocks of 6 (6 treatments) starting with the highest mass. Each treatment was replicated 20 times (20 blocks) and plants within each block were randomly assigned to a treatment (Table 4.1).

Table 4.1 Descriptions of impact trial treatments

Treatment	Description	No. of adults	No. of larvae
T1	Control at time zero: destructively sampled at the start of the trial	0	0
T2	Trial control: destructively sampled at the end of the trial	0	0
T3	Adult feeding: destructively sampled at the end of the trial	2	0
T4	Adult and larval feeding: destructively sampled at the end of the trial	2	5
T5	Adult and larval feeding: destructively sampled at the end of the trial	2	10
T6	Adult and larval feeding: destructively sampled at the end of the trial	2	15

In all treatments, except for T1, the number of leaves and stems were recorded three times: at the start, after the first generation and at the end of the trial. Adults and neonate larvae were transferred to potted plants, which were caged to prevent larvae and adults from escaping. The first generation of larvae were allowed to develop and were removed after 12 days of exposure. During this time the number of adults and larvae were kept constant by replacing missing individuals with ones of similar age. At the end

of the 12-day period, both adults and larvae were removed and counted. The same procedure was followed for the second generation of larvae. After removing the second generation of larvae, plants were cut down and the plant material dried at 70 ºC for 24 hours before the above- and below ground dry mass was recorded.

Data were analysed using the statistical program GenStat (2003). The impact experiment was designed as a randomised block design with 20 blocks. For the above– and belowground dry mass and the total plant dry mass, differences between treatments were tested for in an analysis of variance (ANOVA). While an analysis of covariance was performed to test for differences between treatments on the number of leaves recorded at the end of the trial, adjusting for the number of leaves at the start of the trial. Treatment means were separated using Fishers' protected t-test least significant difference (LSD) at the 5% level of significance (Snedecor & Cochran 1980), if the F-probability from the ANOVA was significant at 5%.

4.4 Results

Results for two of the replicates/blocks, were discarded because of thrips damage that was encountered. Statistical analysis was therefore done on 18, instead of 20 replicates.

The number of *A. cordifolia* leaves did not significantly differ among the larval densities when compared to the initial plant size (T1), indicating that no significant leaf production occurred over the trial period (Fig. 4.1). When

compared to the control, T2, the number of leaves were reduced by 34-55% by the respective larval densities. Except for T3 (adult feeding) all other larval densities reduced the number of leaves to below that of the benchmark figure at the start of the trial (T1). This is a clear indication of the impact of *Phenrica* sp. 2 on leaf production. The aboveground dry mass (including the stems) differed significantly at T4 and T6 but not for T3 and T5 (Fig. 4.2).

Larval and adult feeding had no statistically significant impact on the belowground dry mass at any of the larval densities (Fig. 4.3). Despite the lack of statistical difference, a steady increase in root mass was found at larval densities of 10 and 15 larvae per plant when compared to T2 which could be of biological importance (see discussion).

c c
b a

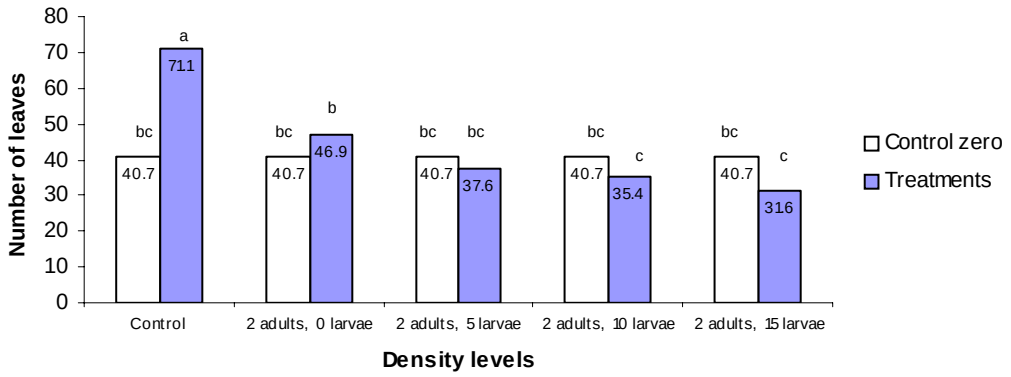

Fig. 4.1 Impact of *Phenrica* sp. 2 adult and larval feeding on the number of leaves produced by *A. cordifolia* plants under laboratory conditions. (Mean no. of leaves followed by the same letter do not differ significantly ($p < 0.05$; ANOVA; F prob. =0.001)

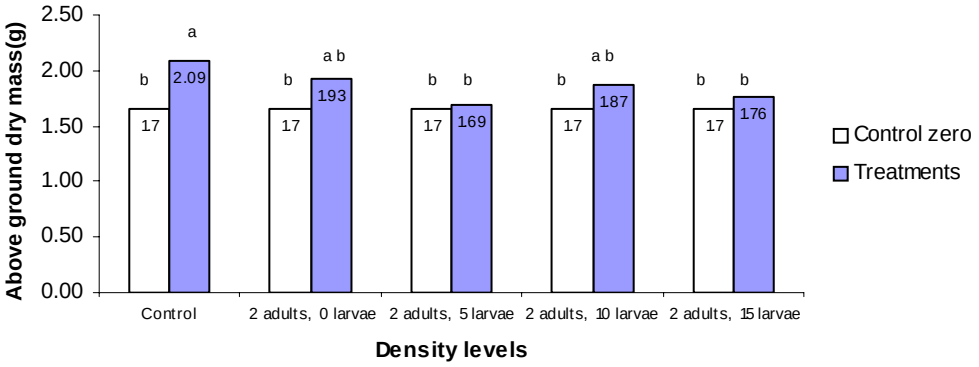

Fig. 4.2 Impact of *Phenrica* sp. 2 adult and larval feeding on the above ground dry mass produced by *A. cordifolia* plants under laboratory conditions. (Mean dry mass followed by the same letter do not differ significantly ($p < 0.05$; ANOVA; F prob. =0.049)

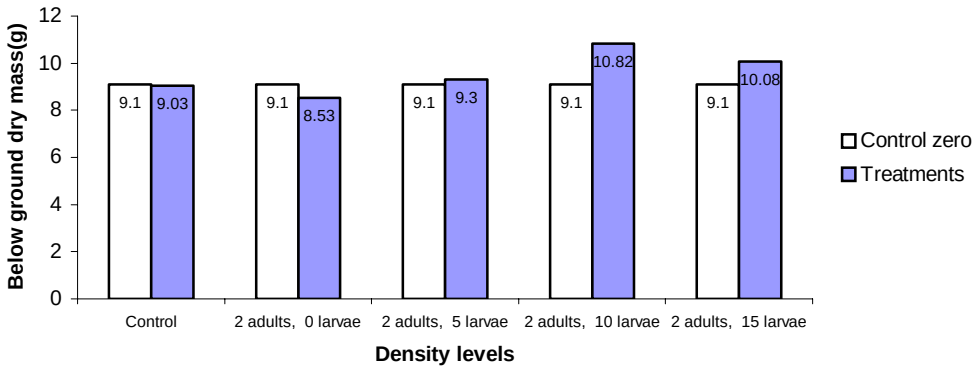

Fig. 4.3 Impact of *Phenrica* sp. 2 adult and larval feeding on the below ground dry mass produced by *A. cordifolia* plants under laboratory conditions. (Mean dry mass did not differ significantly ($p < 0.05$; ANOVA; F prob. =0.343)

4.5 Discussion

Adult feeding alone did not significantly impact on the number of leaves produced by *A. cordifolia* plants. Leaf losses of up to 55% were suffered by the host plant due to both larval and adult feeding, representing a direct reduction in the photosynthetic capacity (Harris 1971). Insect herbivores can

significantly reduce potential fitness of their host plants by reducing current sexual reproduction, as well as investment in asexual reproduction (Wise & Sacchi 1996). *Anredera cordiflia* rarely sets seed, and the major means of dispersal is assumed to be by transportation of underground- and aerially-borne tubers, stem fragments and rooting leaves (Vivian-Smith & Panetta 2002), so that any reduction in vegetative parts should reduce vigour, as well as reproduction and spread.

Although the below ground dry mass showed an initial decrease in treatments with no larvae, a steady increase was observed with treatments at all three larval densities. The temptation is to extrapolate from these results and to speculate about the possible strategies that *A. cordifolia* may have adapted to cope with herbivory at observed levels at least. It is known that long-lived perennials such as *Melaleuca quinquenervia* (Cav.) S.T. Blake, can accumulate root-bound starch reserves that may be mobilised to replace damaged tissue, buffering them from herbivores (Pratt *et al.* 2005).

The present study (as so many others) was concerned with responses over a short period of time. The relative value of different plant structures to the plant at different times of the vegetative cycle, and at different ages, is likely to vary according to their relative contribution to long- and short-term plant fitness. Resource allocation to sink and/or source structures affected by such factors will therefore have an influence on growth and changes associated with growth, and should be taken into account when studying plant responses to herbivory (Foggo 1996).

4.6 Conclusion

A thorough understanding of the ability of plants to tolerate or compensate for the loss in biomass can improve the predictive accuracy of herbivore-plant interactions (Pratt *et al.* 2005). The present study was conducted over a relatively short period of time (24 days), thus not allowing for the study of the impact to the plants of long term exposure to *Phenrica* sp. 2. It may be argued that the number of larvae used for the different larval densities may have been too conservative, and that one may expect higher larval densities when the populations build up under natural conditions.

Other aspects worth investigating include the impact of *Phenrica* sp.2 on the architecture of the plant, the effect of early leaf abscission due to herbivory over a long period of time, and the impact of herbivory on the formation of aerial tubers. Moran and Hoffmann (1989) found that *Trichapion lativentre* (Bèguin-Bellecocq) on *Sesbania punicea* (Cav.) Benth. plants reduced leaf-set and caused extensive damage and resultant premature abscission of the leaflets. A significant reduction in branch development was the direct cause of premature loss of leaflets induced by the feeding activities of the weevils.

The shoot-root mass ratio of *A. cordifolia* was low (0.18), indicating that a comparatively large amount of reserves are stored (van der Meijden 1989). This is supported by the fact that *A. cordifolia* was still capable of enlarging the root mass despite the fact that it had lost 55% of its foliage in the experiments In theory, this would imply that *A. cordifolia* has an effective re-growth capacity and it will only be vulnerable to attack of the storage organs

that enable re-growth, or to repeated attack of other plant parts through which reserves are exhausted, using arguments of van der Meijden (1989). Continued impact trials with *Phenrica* sp. 2 should therefore ideally include studies of re-growth after herbivore damage.

Obvious stress caused to the host plant by biological control agents are often not visible; nonetheless, if they are capable of reducing the biomass and/or alter the pattern of resource allocation to lower the reproductive potential of the plant, they could very well contribute to a reduction in the competitiveness of the target weed and influence its population dynamics (Briese 1996).

Kleinjan *et al*. (2004) demonstrated that sustained attack by *Zygina* sp. (Homoptera: Cicadellidae), a mesophyll feeding leafhopper, reduced both vegetative and reproductive output of *Asparagus asparagoides* (L.) (a plant that also has extensive underground storage organs) when the plants were exposed to sustained, extensive stress. If *Phenrica* sp. 2 can rapidly colonise and sustain severe damage to *A. cordifolia* one could expect similar results. However, it is not possible at present to predict the levels of damage that will be required to impact on stored tuber reserves, nor the long-term outcome of sustained, severe, *Phenrica* sp.2, damage. Should *Phenrica* sp. 2 be unable to impact negatively on existing reserves, and only succeed in hindering reserve accumulation, *A. cordifolia* will persist as a weed, at least in the short term (Kleinjan *et al.* 2004), unless supplemented with additional, possibly complementary agents.

Even though it could not be proven over such a short period that *Phenrica* sp. 2 impacts on the belowground dry mass, it is a very effective defoliating agent capable of defoliating *A. cordifolia* infestations. In a scenario of total defoliation, as witnessed in Argentina (Neser pers. comm.), the plant would be forced to utilise, and perhaps deplete stored resources to compensate for leaf losses. Other complementary agents or unfavourable abiotic conditions can strengthen the effects of defoliation.

Chapter 5

General discussion

5.1 Introduction

Although estimates vary, there is broad agreement that invasive species impose major costs on national economies worldwide. In addition to the direct economic damage, invasive species can also disrupt the provision of non-market environmental goods and services e.g., the adverse effects that it can have on water quality and biodiversity (Anderson 2005).

Unfortunately plant characteristics such as ease of establishment, rapid growth, and high competitiveness are often factors highly desirable in the forestry-, pasture- and nursery industries, and these introduced species are more likely to become invasive weeds (Cruttwell McFadyen 1998). With no single strategy capable of completely solving the invasive species problem, early detection and rapid response to new invaders is seen as the most cost effective and environmentally sound approach (Chapter 1).

The programme launched against *A. cordifolia* bears testimony to the growing awareness of the threat of emerging weeds. Although *A. cordifolia* is still a long way from reaching its predicted full invasive potential, eradication has been shown to be the most cost effective and environmentally sound approach when aiming to restore an invaded habitat to its natural balance (Westbrooks 2004). As chemical and mechanical control have been shown to be ineffective (Chapter 1), biological control will be implemented to limit the

spread, establishment and relative impact of *A. cordifolia*. Although early detection and eradication play an important role in the conservation of biodiversity, it is also invaluable in reducing national expenditure with regards to the control of invasive plants (Chapter 1).

Despite all efforts to increase pre-screening of imported plants and to improve long-term management programmes to provide protection against biological invasions, it is still critically important to create public awareness and to involve the public with any such actions taken. New infestations reported by the public (passive weed detection), should not be underestimated as it can provide meaningful information, even though it is unlikely that all infestations will ever be found in time to be cleared (Dewey & Andersen 2004). For active weed detection, new technology such, as remote sensing offers additional options, which previously relied mainly on extensive, systematic field surveys.

5.2 Invasive creepers and vines

It would seem that invasive creepers and vines have not been targeted at the forefront of biological control. A literature survey conducted for this particular study could only reveal 13 examples of vines or creepers that have been subjected to biological control attempts, with varying results (Chapter 1, table 1.1). No definite reasons could be found to explain this phenomenon, therefore only allowing speculation.

In general, some plant families produce proportionately more invasive aliens than others e.g. Lamiaceae, Brassicaceae and Caryophyllaceae, and some

larger plant families are significantly underrepresented as aliens, e.g. Cyperaceae, Ericaceae, Orchidaceae, Juncaceae, and Polypodiaceae (Crawley 1989). Creepers on the other hand seem to be represented mainly by the Asteraceae - (23% of 13) and Convolvulaceae family (15%). In order to gain some perspective on the percentage of biological control programmes launched against creepers vs. those against other terrestrial plants, one would first have to calculate the ratio of terrestrial plants to creepers on a world wide scale before one can claim one of the two groups to be under- or over represented.

Biological control has historically started off on agriculturally important weeds and not much attention has been given to environmental weeds until fairly recently. Most problematic creepers can be considered environmental weeds and therefore the direct impact on humans is negligible when one compares it to water weeds that directly impact on humans through the loss in water quality, recreational activities and income. In a developing country where food security plays a much bigger role than the conservation of biodiversity, most of the funding will be channelled towards the former, which will ultimately also determine the research objectives.

Most problematic creepers seem to occur associated with forest and grassland, causing them to be fairly inconspicuous, and difficult to detect. Their climbing and scrambling nature also allow them to grow in difficult to reach places such as forest canopies rendering mechanical removal almost useless, especially if aerial parts have reproductive potential over extended

periods (e.g. *P. aculeata, A, cordifolia* and *Delairea odorata* Lem.). Even chemical control does not pose an option as many of the chemicals used can result in drift or over spray that may have a negative impact on the surrounding or supporting vegetation (Chapter 1). On the other hand, biological control presents a distinct, sustainable and environmentally sound method to control the spread of invasive creepers such as *A. cordifolia*.

Biological control is without a doubt one of the best defence mechanism we have for the control of invasive creepers such as *A. cordifolia*, *Macfadyena unguis-cati* (L.) A.H. Gentry and *Cardiospermum grandifolorum* Sw. and should be included in the management plans for these weeds.

5.3 Biological control efficacy

Classical biological control of alien invasive plants has a history of dramatic successes that have earned it a place as one of the primary tools in the effort to lessen the impacts of invasive species with regards to both the agricultural and environmental sectors (Cruttwell McFadyen 1998). It also remains the most cost effective and ecologically friendly method to control invasive plants (Chapter 1). However, for both economic and ecological reasons the release of possibly ineffective agents should be avoided where possible, as it represents a waste of resources, contributes to the perception of biological control as a hit-or-miss strategy, and carry risks of ecological side effects, such as non-target effects (McClay & Balciunas 2005).

Why then does the “lottery model” (Chapter 4) remain so alluring? McEvoy and Coombs (2000), are of the opinion that some scientists and organisations have strong vested interests in making introductions, and that their emphasis is on advertising the release and redistribution of new organisms rather than objectively evaluating impacts on target and non-target organisms. By releasing more and more control organisms, we are challenging the monitoring efforts and the likelihood increases for target and especially non-target effects to go undetected.

If we accept the fact that it takes 10-20 years for the impact of a biological control agent to emerge (Cruttwell McFadyen 1998), and that most releases have been made during the last two decades (McEvoy and Coombs 1999), it could be that a number of released agents have not had enough time to reveal their full impact. We should therefore aim to start monitoring and evaluating the agent soon after release in order to establish its success or failure throughout the phases of establishment, increase, dispersal, suppression of the target and plant succession (McEvoy and Coombs 1999). It may very well be that we are introducing new agents at such a fast rate that we do not allow previously released agents to reach their full potential.

Briese (2005) summarised the modern day dilemma facing biological control by stating that the discipline of biological control can be perceived as being subject to tensions due to differences in philosophical, regulatory or political, and empirical practices. Biological control in itself is also caught up between advocating the view that any organism alien to a particular habitat should be

considered an undesirable invasive and yet, on the other hand we are releasing “alien organisms” and claiming that they are environmentally friendly and desirable. By setting up an appropriate regulatory framework for the introduction of these organisms, a new tension arises between the need to protect non-target species and the need to introduce the most effective biological control agents (Briese 2005).

Pressure is therefore mounting from regulatory authorities as well as the general public to ensure the ever-increasing safety of biological control. One of the biggest challenges facing the science of biological control is therefore to incorporate new or improved theories that will be accepted, and ensure continuous support from all concerned parties.

5.3.1 The target weed

By focusing biological control programmes on the most persistent and environmentally damaging species, especially those where no other feasible alternative control method exists, effective weed control will increase (Louda 2000).

Not including the *Opuntia* cacti and the *Lantana camara* hybrid complex (accounting for over 40% of all weed biocontrol releases), most biological control projects have been targeting weeds from the Asteraceae for example *Centaurea, Carduus, Senecio, Eupatorium* and *Chromolaena* species (Crawley 1989), as this is also numerically one of the largest plant families. A number of traits have been listed that can be associated with plants that

appear to be particularly difficult to control by using insect herbivores. Some of the traits include a long growing period especially a prolonged growing period after a univoltine insect has entered its dormant stage, inaccessible reserves of carbohydrates and proteins (underground rhizomes), growth that is not meristem limited i.e. quick re-growth after defoliation, a large seed bank etc. (Crawley 1989).

Weeds of arid lands are known to be long-lived, reaching a relatively large adult size; they spread mainly by vegetative means, possess high powers of re-growth and represent low-quality food for insects. Although these invasive species have frequently been controlled successfully, the insects tended to spread slowly following release. In contrast successful control of smaller, short-lived perennial weeds of temperate habitats tended to occur more rapidly, with the insects spreading quickly following release. This group of plants tend to reproduce by seed; they have a slower re-growth capacity and offer food of higher quality to the insects (Crawley 1989).

We are currently lacking sound, ecological investigations and therefore we do not have a solid basis from which to predict the numerical consequences of the deliberate introduction of a non-indigenous species on our indigenous biodiversity (Louda 2000).

5.3.2 The biological control agents

Although an expanded external review is often viewed as an “obstruction to progress,” it is often the case that more perspectives are useful when faced

with the decision-making process where possible benefits are weighed up against potential risk (Louda 2000). Practitioners of classical biological control are increasingly conscious of the dual expectation placed on them to achieve successful control of invasive plants, and to avoid damage to non-target plants and adverse indirect effects. The procedures and strategies used to select biological control agents play a central role in meeting these expectations (McClay & Balciunas 2005)(Chapter 2). Factors that contribute to agent success or failure are however only useful in agent selection if they can be assessed or predicted prior to release, something that might be possible when doing pre-release studies (Louda 2003; McClay & Balciunas 2005; Sheppard 2003).

Project efficiency can be improved by quantitatively evaluating factors that may contribute towards the likely success or failure of a specific biological control agent (Louda 2000). McEvoy and Coombs (1999) suggested that the most rational biological control strategy would entail the introduction of the fewest, most effective agents that have the lowest probability of non-target effects. The evidence that a relatively small proportion of agents released against any given target weed actually contributes towards control strengthens the need for a more scientific approach to agent selection (Denoth *et al*. 2002; Myers 2000). The successful prediction of efficacy has been quoted as being the “holy grail” of biological control (Cruttwell McFadyen 2003), as many biological control workers believe that efficacy is affected by many complex, interacting and unforeseeable factors rendering useful predictions impossible (McClay & Balciunas 2005). McClay and Balciunas

(2005) continues their argument by acknowledging that the failure of a biological control programme may be due to various of factors, and that some of the possible causes, such as predation are difficult to predict. However, other causes such as the lack of climatic adaptation, or biotype incompatibility can be foreseen.

The predictive approach emphasises the value of understanding the population dynamics of the weed in the invaded range in relation to likely effects of herbivory, to assess whether specialist herbivores might regulate weed populations (Denoth *et al.* 2002). The selected herbivore/herbivores are then imported for evaluation of the ecological risk they pose to non-target plants.

Although most biological control researchers are aware of the value of ecological data on the target weed and biological control agents, logistics and funding issues often act as obstacles for the collection of such information (Cruttwell McFadyen 2003; Sheppard 2003). Scientists involved in exploration therefore often restrict their research to brief visits to the native range where they meticulously catalogue the herbivores on the plant and select the most apparently damaging species for host specificity testing. Therefore the pre-release data should demonstrate a high probability of control to justify the release of a biological control agent (Louda 2000).

Once the vulnerable life stages of the host plant has been identified, the type of herbivory needed to have the desired effect on the plant must be

determined. In the invaded range, it is however only possible to conduct detailed experiments using the insect herbivores under restrictive quarantine conditions (Raghu & Dhileepan 2005). An alternative approach is to simulate herbivory and use the subsequent plant response as a guide in identifying the insect guilds that will most likely significantly impact on the host weed, thereby narrowing the list of possible agents while also increasing the chances of achieving biological control sooner (Raghu & Dhileepan 2005). Agents can also be prioritised and the results could provide guidance to exploration efforts by allowing the limited time available for exploration in the native range to be targeted at the guilds most likely to yield effective agents. Although simulated herbivory is unlikely to be able to predict the outcome of complex interactions between the agents and the weed that will ultimately influence the efficacy of the biological control agents, it can help to make the rationale for agent selection more quantitative and explicit. Results from simulated herbivory studies need to be verified by comparing it to results gained from impact trials done under laboratory conditions. Data gained through the extrapolation from the simulated herbivory results should be restricted to the individual plant and not extended to more complex ecological interactions such as population level effects or tropic levels (Raghu & Dhileepan 2005).

There are probably two components to this reluctance to rely on pre-release efficacy assessment: one is that such studies will consume scarce resources that could better be spent on the mandatory host-specificity testing required for all agents (Cruttwell McFadyen 2003) (Chapter 2), and the other is the concern that incorrect predictions may result in the rejection of agents that

would, in fact, have been successful if released (the false negative phenomenon) (McClay & Balciunas 2005).

In practice the costs for conducting pre-release evaluations will vary depending on the target weed and the agent being tested. For easy-to-culture, multivoltine agents on target weeds that are easy to grow, costs may be lower than for lengthy host-specificity trials. If the agent quickly kills the target, or entirely prevents seed production this will also reduce the time scale. It may however take many generations of attack before quantifiable impacts are observed on the target weed populatons. For univoltine agents the cost will most probably be too high to be justified (McClay & Balciunas 2005).

It would therefore seem that the main reason to release ineffective agents is as a consequence of our inability to predict agent efficacy (McClay & Balciunas 2005).

5.3.3 Modernising the CPM method

Until recently, it was believed that because the world was green, insect herbivores could not be food limited. The world is however not always green, all that is green is not edible and even if it is edible, it might not be able to sustain the insect population because of insufficient quality (Crawley 1989). Therefore it is crucial for biological control researchers to try and establish the physiological host range of the insect and then narrow it down to the realised or field host range (Chapter 3).

Given the state of knowledge of plant-insect relationships the centrifugal phylogenetic method (CPM) served the biocontrol community well for almost three decades (Chapter 3). An advance in the knowledge surrounding plant-insect relationships and phylogeny does however bring about the ability to modernise the way in which test plants are selected (Briese *et al.* 2003).

Modern day regulatory authorities revolve most of their decision-making in terms of risk. The measurement of such potential risk can be facilitated by breaking it down into three contributory elements, namely, phylogenetic relatedness, biogeographic overlap and ecological similarity, which define the criteria used to select plants for assessing the risk of non-target impact (Briese *et al.* 2003; Sheppard *et al.* 2005). Arguments put forward by Wapshere (1974) are still valid, and the phylogenetic relationships of plants still provides the strongest indicator of the complex behavioural and physiological responses shown by specialist phytophages in choosing a host plant. Phylogeny should therefore remain the key criterion used to choose test plants. Other factors, such as morphological features, potential distributions of agents (determined by habitat and geographical and/or climatic factors) and potential “new hosts” should also be considered.

Briese (2005) summarises the new perspective on test plant selection by saying that the critical feature for determining relatedness to the target weed is the degree of phylogenetic separation rather than applying taxonomic circumscriptions. Therefore relationships are emphasised rather than categories. This will enable researchers to determine what an agent will

attack rather than what will not be attacked, i.e., the host range will be described rather than determining whether or not individual plants are safe. If phylogenetic information was available on the Cardueae thistles twenty years ago, *Rhinocyllus conicus* Fröl., might never have been cleared for release. As all North American *Cirsium* species have the same degree of separation from their Palearctic congeners, including *C. vulgare* (Savi) Ten. The method proposed by Briese (2005) would have focused attention on this group because of their phylogenetic closeness to the target weed *Carduus nutans* L. and, because of their equal phylogenetic distance from *C. nutans* would have required test species to be chosen on the basis of the ecological similarity and/or biogeographic overlap with the target weed.

The CPM should however be placed in context as test plant selection is only the first step in the host specificity testing process. We also need to determine what life-stages should be tested, what types of test to use for the different life stages and finally we need to interpret the data that arises from these tests (Chapter 3). The way in which test plants are selected will loose its relevance if there are flaws in any of the other stages (Briese *et al.* 2003).

5.4 The *Anredera cordifolia* programme in perspective

Although *Anredera cordifolia* is still considered an “emerging weed” in South Africa, it has already become a major problem in Queensland, Australia where chemical and mechanical control failed to impact on the spread of this invasive vine. Prior knowledge of Madeira vine’s invasive nature and difficulty to control with conventional methods prompted the timely initiation of a

biological control programme. The release of a biological control agent will impact on the spread of *A. cordifolia* and it may even result in total suppression if a well designed management plan can be put in place.

Because of limited funding, no studies have so far been undertaken in the country of origin to assess the population dynamics of the weed in relation to the likely effects that herbivory will have in regulating the weed population. Furthermore, no trials were initiated to determine the vulnerable life stages of the weed before the exploration phase was initiated. For the *A. cordifolia* project it was not that crucial as it was known beforehand that seed production was low and that the seeds are typically not viable, which eliminated the need to import any seed- or flower feeders. Foliar- and/or root feeders and possibly stem borers were therefore the only guilds of insects that required investigation.

The insects that were collected in only hours, rather than days of searching, yielded only one species of a possible root feeding chrysomelid beetle, *Isotes eruptiva* (Bechyné), a geometrid larva that fed destructively on the aerial tubers, three species of leaf feeding chrysomelids and one species of a leaf-mining moth. Only one specimen of the tuber-feeding moth was reared to the adult stage, and submitted for identification. Adults of the possibly root-feeding chrysomelid, of which the adults were once seen to entirely defoliate plants (San Ignacio, Misiones, Argentina) were brought back to the ARC-PPRI quarantine laboratories but were diseased and were not reared. The leaf-mining moth appeared to belong to the same species as those also collected

from *Talinum paniculatum* (Jacq.) Guertn, and while being reared in the laboratory it was found that the larvae developing on *A. cordifolia* could also develop on *T. paniculatum*. This species was regarded as having a relatively high risk of having an insufficiently narrow host range and was provisionally rejected. Of the leaf-feeding chrysomelids *cf. Phenrica* sp. 2 was brought back for further evaluation at the PPRI quarantine facility in South Africa. *Plectonycha correntina* was simultaneously studied and evaluated at the USDA-ARS Laboratory in Argentina and shown to have an initial host range very similar to that reported here for *Phenrica* sp. 2. A back-up culture of *P. correntina* has been maintained in quarantine in Pretoria since December 2004.

In the search for biological control candidates the correct insect guilds were investigated but insufficient time and funding prevented collectors from making an in-depth study of all possible leaf -and root feeders, and over a more extensive part of the plants natural occurrence. Searches were done almost exclusively in the Misiones Province of Argentina, and adjacent parts of southern Brazil. It is however useful to have had two chrysomelid beetles for candidate control agents, as the successes obtain with chrysomelids have been well documented (Chapter 2).

Shortcomings in the evaluation of *Phenrica* sp. 2 host specificity and impact have been discussed in detail in chapter 3 and 4 respectively, highlighting a few key risk-related aspects that need to be addressed. These include the need to conduct adult multi-choice trials in a walk-in cage to try and explain

the feeding preference shown by the adults for *B. alba* in captivity, even though *A. cordifolia* was the only host that was found to be selected for oviposition (Chapter 3). Further trials should also be initiated to provide confirmation of the field observations that long term; sustained damage would have a negative impact on plant growth and spread. Some of the options include the use of simulated herbivory that can be ground truthed by laboratory trials that will expose the host weed to higher larval and adult densities over a longer period of time. As the shoot-root ratio was proven to be low (0.18) it is also very important to establish the re-growth capacity of *A. cordifolia*, whilst also estimating the impact *Phenrica* sp.2 will on existing reserves *vs*. accumulated reserves (Chapter 4).

Test plant species were selected using Wapshere's (1974) centrifugal phylogenetic testing method, and included species on which *Disonycha* (Chapter 2) and *Phenrica* species were recorded (Clark *et al.* 2004), as well as some commercially important plant species (Table 3.1). Of the 14 plant families (Chapter 3, Table 3.1) represented in the host specificity trials, ten were in the order Caryophyllales, and the other four from diverse orders. Plant families within the Caryophyllales are presently divided into four nodes according to their phylogeny (Fig 5.1).

Caryophyllales

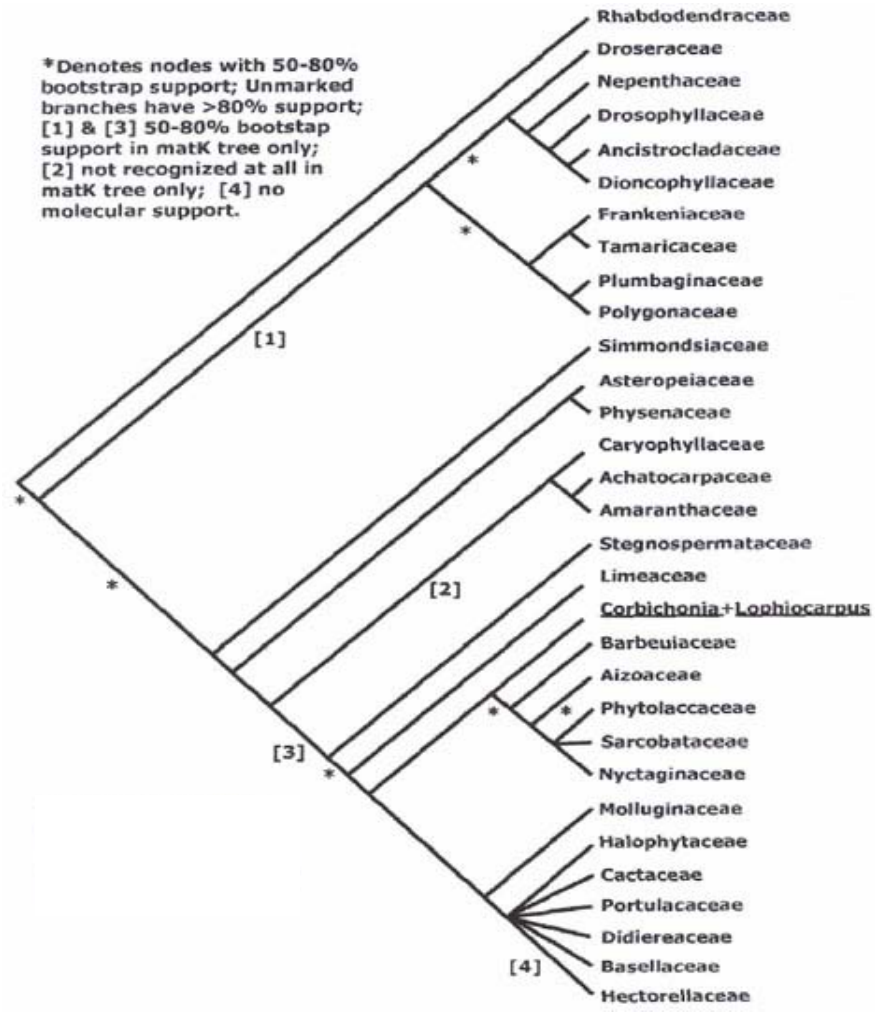

Figure 5.1 The Caryophyllales order (Stevens 2001)

The families that are in node [4], including Basellaceae, do however not have any *molecular* support for their phylogenetic division (Stevens 2001). Other observations made by a number of authors also seem to indicate a fairly close relationship amongst the families in node [4]. Hectorellaceae for instance has a very similar anatomy to that of Portulacaceae and does not seem to be worth recognising as a clade on its own (Stevens 2001), and it is known that a *Didiera* species (family Didiereaceae) may be successfully grafted onto a rootstock of *Pereskia aculeata* (family Cactaceae) (Neser pers. com.). If one

accepts these indications that the Cactaceae, Portulacaceae, Didiereaceae, Basellaceae and Hectorellaceae are all very closely related, it would explain why test plants within the Portulacaceae and Basellaceae were found to be suitable for adult feeding and larval development during the present study. Larvae of *Phenrica* sp. 2 could however not develop on *Pereskia aculeata*, or the species of *Rhipsalis* (Cactaceae) tested, which may also be an indication that the Portulacaceae and Basellaceae are physiologically more closely related to one another than to the Cactaceae. There is but a single species of cactaceous plant still believed to be indigenous in Africa – *Rhipsalis baccifera* (J.S. Mueller) Stearn but this plant, which also occurs in South America, as an indigenous plant may be an example of a species that may have dispersed after the separation of the subcontinents of Africa and South America. More representative indigenous Portulacaceae in the southern African region, and elsewhere where the candidates for biocontrol are considered for release, should also be considered for further testing.

5.5 Conclusion

The *A. cordifolia* programme can be deemed fairly successful thus far, despite a few shortcomings (as discussed in 5.4). The candidate agent that was collected, *Phenrica* sp.2, has proven to have a sufficiently narrow initial host range and a significant impact on the number of leaves. Although no definite proof could be given for the impact on the below ground storage organs, proof thereoff has been found in the country of origin and similar results can be expected once the agent has been cleared for release.

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