Biological Control of *Pereskia aculeata* Miller (Cactaceae)

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Abstract

Pereskia aculeata Miller (Cactaceae) is an environmental weed that is damaging to natural ecosystems in South Africa. The plant is native to Central and South America and was first recorded in South Africa in a botanical garden in 1858. In this thesis, research into the biological control of *P. aculeata* was conducted with the intention of improving the control of the weed. A pre-release study of the relationship between *P. aculeata* density and native plant biodiversity indicated that *P. aculeata* has a negative impact on native biodiversity. The native plant biodiversity associated with different *P. aculeata* densities was used to determine threshold values and goals for the control of the weed. A threshold value of 50% *P. aculeata* density was calculated, indicating that *P. aculeata* density must be maintained below 50% in order to conserve native plant biodiversity. The ultimate goal of the control programme should be to maintain *P. aculeata* densities below 30%. At these densities there was no significant difference in native plant biodiversity from if the weed were absent from the ecosystem.

The biological control agent, *Phenrica guérini* Bechyné (Chrysomelidae), has been released in South Africa but the potential of the agent to impact *P. aculeata* is not known and no post release evaluation has been conducted. Impact assessment studies indicate that *P. guérini* does not impact *P. aculeata*, even at high densities, but the results of greenhouse experiments should be interpreted with caution because of problems with extrapolation into the field. Although observations in the field suggest that *P. guérini* has reduced *P. aculeata* densities at one site, it is clear that new biological control agents are needed to reduce the weed to acceptable levels.

Identifying the origin of the South African *P. aculeata* population was believed to be important to the biological control programme due to the disjunct native distribution and intraspecific variation of the species. Natural enemies associated with plant genotypes in different parts of the native distribution may have developed specialised relationships with certain intraspecific variants of the plant, resulting in differences in agent efficacy on certain host plant genotypes. A molecular study indicated that the

closest relatives to the South African weed population found in the native distribution were in Rio de Janeiro Province, Brazil.

A bioassay experiment in which fitness related traits of the biological control agent, *P. guérini*, were measured on various *P. aculeata* genotypes was conducted to determine the importance of host plant intraspecific variation. There was little variation in fitness traits between genotypes and no evidence of intraspecific host plant specialization. Although intraspecific variation had no effect on agent efficacy in the case of *P. guérini*, it is possible that other natural enemies may be more specialized. Genotype matching is expected to be more important when natural enemies likely to be specialised to individual genotypes are considered for biological control.

Potential biological control agents were prioritized from data collected on surveys in the native distribution. The most promising of these, based on the presence of feeding, incidence, predicted host range, climatic matching, genotype matching and mode of damage, are two species of Curculionidae, the current biological control agent *P. guérini* and the stem boring moth, *Maracayia chlorisalis* Walker (Crambidae). The two curculionid species and *M. chlorisalis* should be considered priorities for host specificity studies.

Releases of *P. guérini* and any new biological control agents should be made at sites where the pre-release study was conducted so that post-release evaluation data can be compared with the pre-release data and the impact of biological control can be evaluated.

Retrospective analyses of biological control programmes provide important ways of improving aspects of biological control programmes, such as methods of agent selection. The evaluation of success in biological control programmes is essential for retrospective analyses because factors that have lead to successes or failures can be analysed. Retrospective analyses of biological control programmes, such as this thesis, may improve weed management, thereby contributing to the conservation of natural resources.

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Publications Arising from this Thesis

Paterson ID, Downie DA, Hill MP (2009) Using molecular techniques to determine the origin of weed populations of *Pereskia aculeata* in South Africa and its relevance to biological control. Biological Control. 48: 84-91

Paterson ID, Coetzee JA, Hill MP, Downie DA. A pre-release assessment of the relationship between the invasive alien plant, *Pereskia aculeata* Miller (Cactaceae), and native plant biodiversity in South Africa. Biological Control. In press.

Paterson ID, Hoffmann JH, Klein H, Mathenge CW, Neser S, Zimmermann HG. Biological Control of Cactaceae in South Africa. African Entomology. In review.

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THIS THESIS IS DEDICATED TO MY FATHER
ANGUS PATERSON
WHO TAUGHT ME TO LOVE NATURE

Chapter 1

Introduction

Invasive alien plants are a threat to biological diversity and have negative economic and environmental impacts (Vitousek 1992, Le Maitre *et al.* 1996, Van Wilgen *et al.* 2001) but these are seldom quantified. Biological control is an environmentally friendly, economically viable and sustainable method of reducing the impacts of invasive alien plants (Andres 1977, McFadyen 1998, McConnachie *et al.* 2003, Müller-Schärer & Schaffner 2008). In this study, research to improve the biological control programme against the invasive alien plant, *Pereskia aculeata* Miller (Cactaceae), was conducted.

This chapter introduces the discipline of weed biological control and the focal organism of the study, *P. aculeata*. The chapter concludes by outlining the rationale and aims of this study.

1.1 Biological control of invasive alien plants

Human society benefits from services that are provided by healthily functioning ecosystems, but the ability of ecosystems to provide these services is threatened by a number of anthropogenic factors, including biological invasions (Palmer *et al.* 2009). Biological invasions are often associated with a loss in native biodiversity (Olden & Poff 2003), and reduced biodiversity has been shown to impair ecosystem functioning and the ability of the ecosystem to provide essential services (Hector *et al.* 1999, Tilman *et al.* 2001, Worm *et al.* 2006).

Invasive alien plant species are considered the most economically and environmentally damaging taxa of invasive species (McFadyen 1998). Various invasive alien plant species have been shown to impact negatively on natural ecosystems as well as human health and wealth by reducing native biodiversity (Weiss & Noble 1984, Braithwaitte *et al.* 1989, Richardson *et al.* 1989, Tyser 1992, Fensham *et al.* 1994, Adair & Groves 1998, Schooler *et al.* 2006, Coetzee *et al.* 2007a, Gooden *et al.* 2009), disrupting natural fire regimes (Van Wilgen & Richardson 1985, Brooks *et al.* 2004),

reducing water quality and availability (Hill 2003, Görgens & Van Wilgen 2004), reducing the value of agricultural land (Moran & Zimmermann 1991a, DiTomaso 2000, Harminder *et al.* 2005) and in some cases impacting directly on human health (Rao *et al.* 1985, McFadyen 1992).

Methods of reducing the impacts of invasive alien plants include land or water use management, herbicidal control, mechanical control and biological control. More recently, integrated control strategies involving different combinations of control methods have been favoured (Zimmermann & Neser 1999, Greathead 2003, Zimmermann & Olckers 2003). The most appropriate control method or combination of control methods depends on the biology of the target weed and the extent and nature of the infestations.

Land or water use management is an appropriate tool when poor agricultural or industrial practices have resulted in degraded and thus easily invaded environments, but cannot be used to reduce weed impacts in undisturbed and well managed ecosystems. Herbicide applications are historically the most widely used method of controlling weed species, but the negative impacts of the chemicals on ecosystems and human health (Pimental & Edwards 1982, Richards *et al.* 1987) as well as non-target effects on other plant species makes this method undesirable in many cases (Moran & Zimmermann 1991a, Klein 1999, Hill 1999, Sparks 1999). For effective herbicidal control many weed species require regular follow ups which may continue indefinitely, making this method expensive and in some cases unsustainable (Hoffmann *et al.* 1999, Olckers *et al.* 1999, Hill 1999, Coetzer & Neser 1999, Cronk & Fuller 2001). Mechanical control involves physically removing weeds using manual labour or machinery. This too requires regular follow ups, is labour intensive and is therefore often expensive and unsustainable on a large temporal or spatial scale (Ikuenobe & Ayeni 1998, Olckers *et al.* 1999, Hill 1999, Cronk & Fuller 2001, Caffrey *et al.* 2010).

Biological control is an environmentally friendly, sustainable and safe method of controlling invasive alien plants (McFadyen 1998). The major advantages of biological control are that introduced natural enemies are host specific and will have no non-target effect on other plant species, that the environment will not be exposed to potentially

harmful chemicals and that control is sustainable and does not require continuous follow-up operations.

Biological control differs fundamentally from conventional control methods in that the control method is permanently incorporated into the ecosystem. The ecosystem is altered by the introduction of the biological control agent in a way that will result in a permanent reduction in the density of the target weed. Biological control can therefore be seen as a form of ecosystem engineering and requires an understanding of both the invaded ecosystem and the ecosystem in the target weed's native distribution. Since the agent is intended to be incorporated into the new ecosystem, indirect effects on aspects of the ecosystem besides the target weed are expected. The majority of indirect effects are desired results of biological control, such as an increase in native biodiversity, and are viewed as positive (Barton *et al.* 2007, Van Driesche *et al.* 2010) while others, such as the utilization of native plant species by biological control agents and population increases of organisms which utilize biological control agents as food resources, are viewed as negative (Turner *et al.* 1987, Simberloff & Stiling 1996, Louda *et al.* 1997, Louda *et al.* 2003, Pearson & Callaway 2006). A full evaluation of all the potential indirect effects of biological control is impractical due to the complexity of natural systems but the negative impacts of biological control are usually minimal when compared with the negative impacts of the weed itself or the negative impacts of conventional control methods (Howarth 1991). A risk assessment approach, in which the benefits of biological control are weighed against the potential risks, indicates that the use of biological control is generally justified (Sheppard *et al.* 2003).

The most recent world catalogue of biological control agents and their target weeds lists over 350 natural enemies that have been released on 133 target weeds (Julien & Griffiths 1998). In South Africa 109 biological control agents have been released for the control of 49 weed species (Klein. in press). Biological control contributes towards control in the majority of South African control programmes (Klein. in press) and in many cases the negative impact of the weed has been dramatically reduced (Annecke & Moran 1978, Moran & Annecke 1979, Moran & Zimmermann 1991a, Zimmermann & Moran 1991, Hoffmann *et al.* 1999, Hoffmann & Moran 1999, McConnachie *et al.* 2004).

1.2 *Pereskia aculeata* Miller (Cactaceae)

Pereskia aculeata is a primitive creeping cactus differing from typical cacti in that it has well developed leaves and woody stems and branches (Fig. 1.1) (Moran and Zimmermann 1991b, Leuenberger 1986). The plant is armed with paired hooked thorns on the young green shoots and clusters of long spines which develop progressively on the older woody stems. Flowers are creamy-white with creamy white or purple stamen filaments. The fruits are yellow and spiny when immature but as the fruit ripens the spines are lost and the fruit changes to an orange colour (Leuenberger 1986).

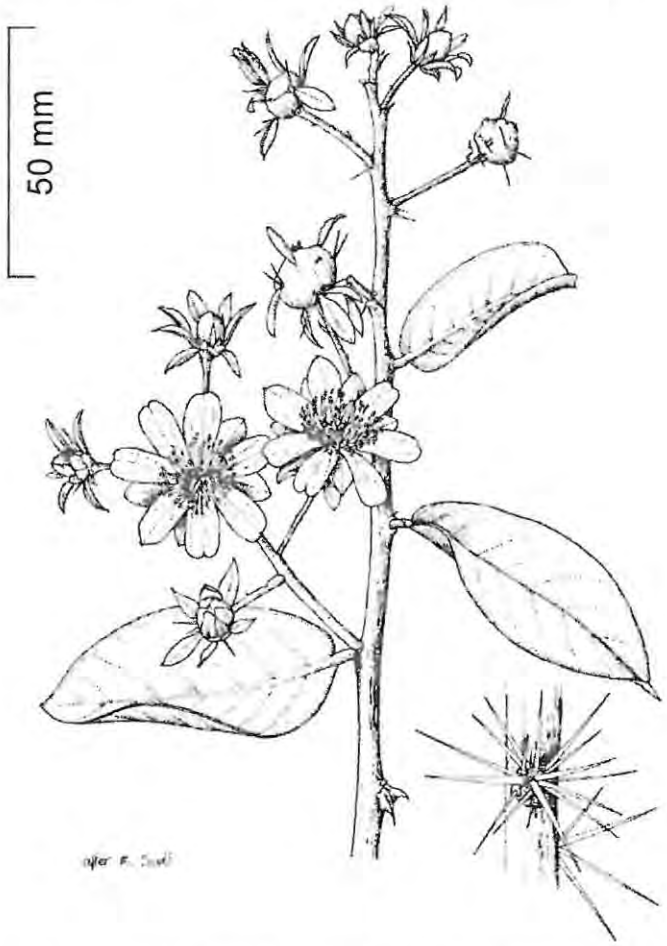

Figure 1.1. *Pereskia aculeata*. (Drawn by Gill Condy, first published in Henderson 1995, ARC-Plant Protection Research Institute, Pretoria.) (Henderson 1995).

The plant was first recorded in South Africa in McGibbon's (1858) catalogue of plants in the Cape Town Botanical Gardens. Records of the species as a horticultural plant go back as far as 1696 (Britton & Rose 1919). In Brazil the leaves are eaten as a pot herb or vegetable (Leuenberger 1986) and the fruits are eaten in the Caribbean (Britton and Rose 1919) and were used to make preserves in the introduced range in South Africa as early as 1881 (De Beer 1988). The plant is often used as a hedge plant because the large thorns make it difficult for people and livestock to move through the barrier and because it is easily propagated from cuttings (Bruton 1981). *Pereskia aculeata* was declared a weed in 1979 (Proclamation R35 of 1979) and is now a declared weed in South Africa under the Conservation of Agricultural Resources Act (1983). It is also listed as an invasive alien plant in the draft regulations in terms of the National Environmental Management: Biodiversity Act (2004). *Pereskia aculeata* is considered a major threat to the native biodiversity of South Africa (De Beer 1988, Bruton 1981, Moran and Zimmermann 1991b). The impact of *P. aculeata* on native plant biodiversity is investigated in Chapter 2.

1.2.1. Taxonomy

Pereskia Miller is a genus in the family Cactaceae, exhibiting plesiomorphic characteristics such as broad non-succulent to slightly succulent leaves and a shrubby or tree-like habit (Butterworth & Wallace 2005). The genus is comprised of 17 species (Edwards *et al.* 2005) and is distributed from southern Mexico southwards through Central America and the eastern side of the Andes and northern Argentina, eastern Brazil, Venezuela, northern Uruguay and the Caribbean (Leuenberger 1986). There are high levels of endemism in the Caribbean islands. Three species are endemic to the island of Hispaniola where two species, *P. quisquenyana* Liogier (Leuenberger 1986) and *P. marcanoi* Areces (Areces-Mallea 1992) are known to survive naturally only at their type localities. *Pereskia zinniiflora* De Candolle is another Caribbean endemic found only in southern and south-western Cuba (Leuenberger 1986). Genetic analyses indicate that *P. aculeata* is most closely related to an Andean clade of the genus (Edwards *et al.* 2005, Butterworth & Wallace 2005). Other *Pereskia* species in the Andean clade do not occur

naturally in the same regions as *P. aculeata*, so no naturally occurring *Pereskia* species sympatric with *P. aculeata* is part of the same clade.

Pereskia aculeata is considered a polymorphic species comprising of varieties, forms and clones including a number of garden variety plants (Leuenberger 1986, Britton & Rose 1919). The intraspecific relationships between the various forms and races of the plant are not distinguishable because of a lack of robust morphological characteristics (Leuenberger 1986). The polymorphic nature of the species may have consequences for the selection of potential biological control agents (Chapter 4, Chapter 6) but the only biological control agent that has already been released, *Phenrica guérini* Bechyné (Chrysomelidae), does not distinguish between different plant races and varieties (Chapter 5).

1.2.2. Biology and Distribution

In its native distribution *P. aculeata* grows on secondary dunes and in forest vegetation. On the secondary dunes the plant usually grows as a shrub and can form dense thickets or creep along the ground. In forest vegetation the plant grows up into the canopy supported by forest trees. In South Africa, *P. aculeata* grows in forest and coastal vegetation where it out-competes native vegetation by covering small plants and shrubs and even large forest trees (Moran & Zimmermann 1991b, Chapter 2).

Although *P. aculeata* is primarily a C3 plant it shifts to an internal recycling of CO_2 that is similar to Crassulacean Acid Metabolism (CAM) when water stressed (Rayder & Ting 1981). CAM photosynthesis is usually associated with drought resistant plants that grow in dry regions as it is possible to conserve more water using this photosynthetic pathway than the more common C3 pathway. This characteristic may contribute to the success of *P. aculeata* in areas of the introduced distribution that may be periodically affected by drought. Frost is believed to be an important factor in restricting the distribution of the weed in South Africa, confining the majority of infestations to the milder eastern coast of the country (Fig. 1.2) (Campbell 1988).

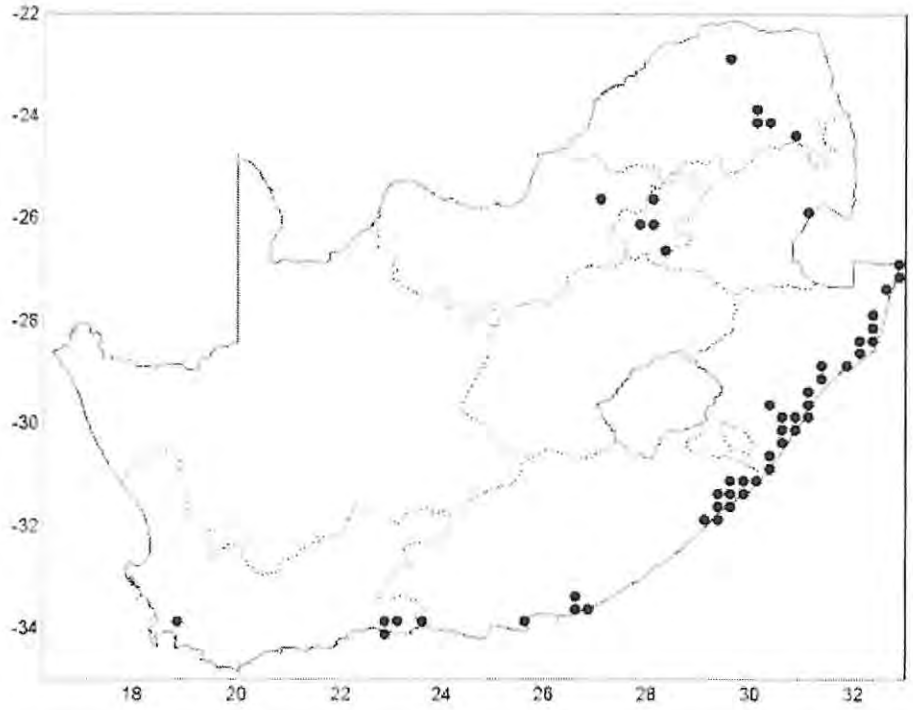

Figure 1.2. Distribution of *Pereskia aculeata* in South Africa. (Drawn by L. Henderson; data source: SAPIA database, ARC-Plant Protection Research Institute, Pretoria.) (Henderson 1995).

Pereskia aculeata reproduces both vegetatively and sexually. Although only about 30% of seeds collected from a South African site were viable (Campbell 1988) the spread of the plant by birds that consume the fruit may be important in starting new nuclear infestations that will then spread vegetatively. Vegetative reproduction is extensive, as any small part of the plant that breaks off has the potential to grow into a new plant. The process of root formation from stems and petioles has been described for the closely related *Pereskia grandifolia* Haw. (Cactaceae) (Carvalho *et al.* 1989).

The native distribution of *P. aculeata* is widespread and divided into two distinct regions. The southern region includes southern and south-eastern Brazil, southern Paraguay and northern Argentina. The northern region includes southern Central America, northern Venezuela and the Caribbean (Fig. 1.3) (Leuenberger 1986). The

disjunct distribution of the plant was considered a possible constraint to biological control (Chapter 4).

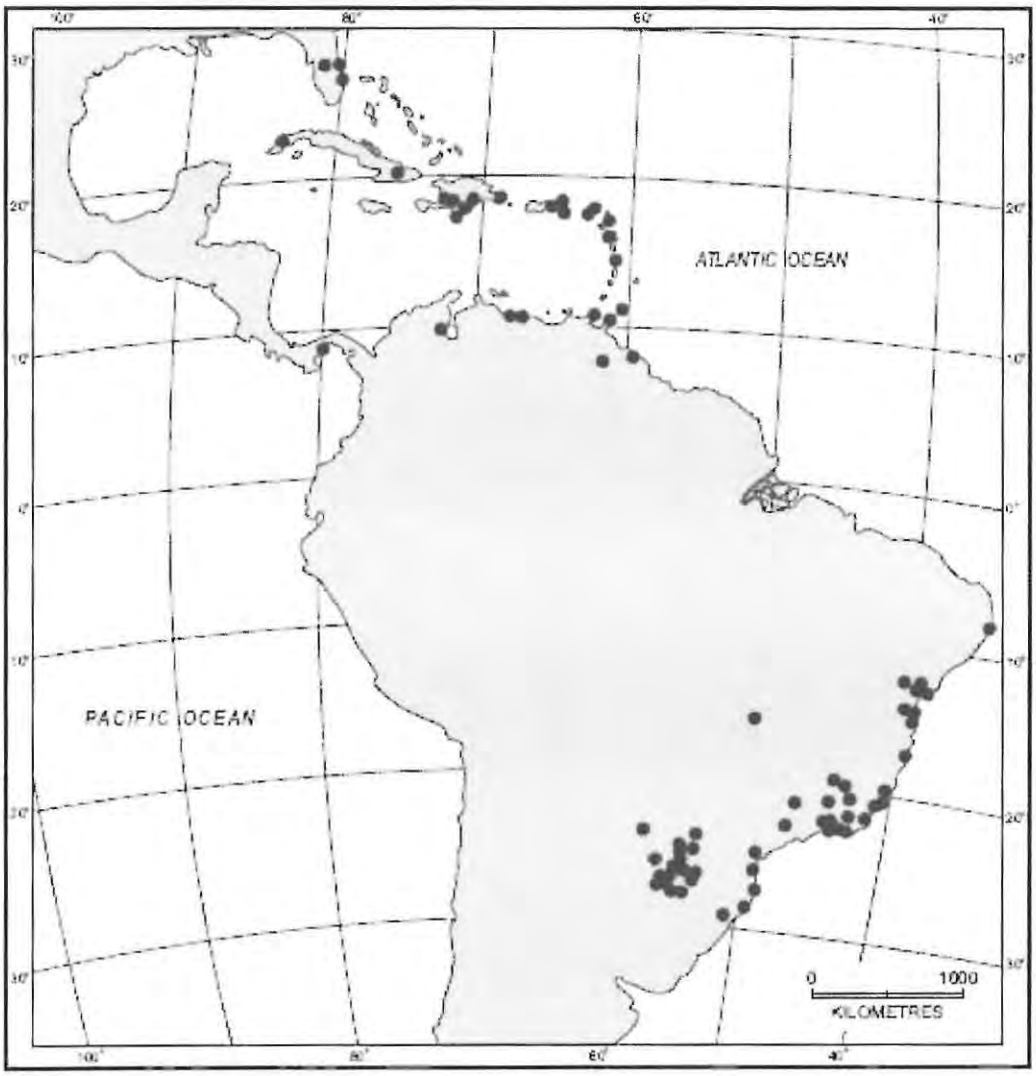

Figure 1.3. The native distribution of *Pereskia aculeata* (After Leuenberger 1986).

The introduced distribution includes areas near Cape Town, greater Johannesburg and in Limpopo Province, but the majority of the infestations are along the east coast of South Africa (Fig. 1.2). *Pereskia aculeata* has also naturalized in riparian vegetation in New South Wales and Queensland, Australia, where it is considered an emerging weed with

the potential to become extremely problematic (Anon 2003, Glanznig 2004). It is also known from at least one location in Hawaii, U.S.A. (Starr *et al.* 2005) but further surveys in Hawaii are likely to result in additional records.

1.2.3. Biological control of Pereskia aculeata *in South Africa*

Pereskia aculeata is not effectively controlled using conventional methods, thus biological control appears to be the only viable and sustainable option against the weed (Moran & Zimmermann 1991b). Although disturbed habitats are invaded by *P. aculeata*, the major concern is the invasion of pristine forest habitats where the plant appears to be driving the loss in biodiversity rather than taking advantage of disturbed areas (MacDougall & Turkington 2005). Land management is not a control option for weed species invading pristine habitats because the infestations cannot be attributed to poor land use.

Physical removal is labour intensive, expensive and ineffective. All parts of the plant, including the roots and the branches in the canopy, must be removed from the infested area because any small piece that is left behind may grow into a new infestation due to the plant's ability to produce adventitious roots from all plant organs.

Triclopyr (butoxy ethyl ester, 480 g/l a.i.) is registered as a herbicidal treatment for *P. aculeata*, but the herbicide is not translocated within the tissue of the plant (Klein 1999). All parts of the plant, including the roots, must come into contact with the herbicide in order to destroy an infestation. Unlike biological control agents, herbicides are not specific and will affect all the native plants with which *P. aculeata* is intertwined, leading to non-target effects. Limited effectiveness and the large non-target effect of conventional methods have been reported in other species of invasive vine due to the tendency of vines to grow intertwined with native vegetation (Sparks 1999, Causton *et al.* 2000, Goolsby 2003). Invasive vines are ideal candidates for biological control because conventional control methods are not viable.

The taxonomic isolation of *P. aculeata* in Southern Africa is promising for biological control. The only species of Cactaceae that is considered native in South Africa is *Rhipsalis baccifera* (J. Müller) Stern (Dyer 1975). Whether this species is in fact

truly native or an early introduction is debatable. *Rhipsalis* species are the only Cactaceae found outside of the new world with eight species in tropical Africa and one in Sri Lanka (Britton & Rose 1919). It is possible that the *Rhipsalis* species in Africa are new world species transported by migratory birds, raising the question of whether the species should be considered native or introduced (Britton & Rose 1919). The lack of cactaceous species makes it possible to introduce oligophagous cactophages without threatening native biodiversity. Cactaceous crops, such as *Opuntia ficus-indica* (L.) Miller, *O. robusta* Wendel and *Hylocereus* spp., are becoming increasingly popular in South Africa, leading to conflicts of interest between biological control practitioners and cactus crop farmers. This is not a new problem in South Africa and is a stumbling block to approving releases of new cactophagous agents (Zimmermann & Moran 1991b, Beinart 2003). The release of *Cactoblastis cactorum* Berg. (Lepidoptera: Pyralidae) in 1933 was a contentious issue due to a conflict of interest with people who utilised the plant for fruits and fodder, but the biological control agent was released because the damage due to *O. ficus indica* was believed to outweigh the benefits of the plant (Beinart 2003). Conflicts of interest of this type are likely when releasing any new cactaceous biological control agent and should be settled by comparing the benefits and costs of the weed species and closely related species that could be affected. In the case of *Hylocereus* spp. crops which are likely to be suitable hosts for the potential biological control agent of *P. aculeata*, *Maracayia chlorisalis* Walker (Crambidae), it is the negative effect of both *P. aculeata* and naturalised *Hylocereus* spp. that should be compared with the benefits of farming *Hylocereus* sp. for fruits (Chapter 6). *Hylocereus* spp. have become naturalised in South Africa and, although not yet registered as invasives, have the potential to be damaging environmental weeds.

The only biological control agent that has been introduced for the control of *P. aculeata* is *Phenrica guérini* Bechnyé (Chrysomelidae). This species was collected in Rio de Janeiro Province in Brazil, was found to be monophagous in host specificity studies and was released in South Africa in 1991 (Klein 1999). Populations established at only two of the 12 sites at which the insect was released (Klein 1999) but more recent releases have led to establishment at a third site. The impact of *P. guérini* on *P. aculeata* was measured in a laboratory experiment (Chapter 3), but no studies have been conducted to

measure changes in the density of *P. aculeata* at sites where *P. guérini* has been released, so the impact of the agent in the field is not known.

1.3. Aims and rationale

The broad aim of this study is to contribute to reducing the impact of *P. aculeata* on native ecosystems in South Africa by improving the biological control programme against the weed.

Observations of *P. aculeata* infestations suggest that it is an extremely damaging environmental weed which covers native vegetation and sometimes causes even large forest trees to collapse under the weight of the vine (De Beer 1988, Bruton 1981, Moran & Zimmermann 1991b). The apparent negative impacts of the weed have resulted in the adoption of drastic control methods in parts of the introduced distribution such as the total destruction of small areas of *P. aculeata* infested natural vegetation (Cronk & Fuller 2001). Although the impacts of *P. aculeata* were widely recognised and used to justify these destructive control methods, the impact of the weed had not been quantified. The impact of *P. aculeata* on native plant biodiversity has been measured, and threshold values which can be seen as goals for the biological control programme are calculated in Chapter 2. Understanding the impact of the weed may be useful in justifying intervention and developing appropriate management strategies. The data in this chapter were intended to be a pre-release measure of the impact of *P. aculeata* that may be used in post- release evaluations of new biological control agents (Chapter 2).

The potential impact of the biological control agent, *P. guérini*, was not estimated prior to its release in South Africa and no post-release evaluation has been conducted (Klein 1999). Before new biological control agents are considered the potential impact of *P. guérini* should be evaluated. Releases of unnecessary biological control agents should be avoided (Louda 2000, Denoth *et al.* 2002, Sheppard *et al.* 2003, McClay & Balciunas 2005) so new agents should be considered only if *P. guérini* is unlikely to reduce *P. aculeata* densities to acceptable levels. The impact of *P. guérini* on *P. aculeata* has been studied in an attempt to determine whether *P. guérini* is a damaging agent and whether new biological control agents are required to control the weed (Chapter 3). Although

further post-release evaluation is required for *P. guérini*, new biological control agents are considered necessary to reduce the weed to acceptable levels (Chapter 3).

The origin of the South African *P. aculeata* populations has been identified to determine the most appropriate region in which to collect new potential biological control agents (Chapter 4). To test whether biological control agents collected at the origin of the weed population are the most suitable potential agent genotypes, a bioassay experiment was conducted in which fitness related traits of *P. guérini* reared on various *P. aculeata* genotypes were measured (Chapter 5). The aims of these two chapters are to ensure that the most effective biological control agent genotypes are used for the control of *P. aculeata* and, more generally, to study the importance of genotype matching in biological control (Chapter 4 & 5).

The most promising potential biological control agents, which should be collected and imported into quarantine for further testing, have been identified from survey data collected during eight field trips conducted between 1988 and 2007 (Chapter 6). A number of criteria are proposed which could be used to identify the most promising potential biological control agents while surveying for natural enemies of weed species (Chapter 6).

Suggestions of how to improve the control of *P. aculeata* in South Africa and the direction of future research, as well as the implications of this study for other biological control programmes, are included in a general discussion chapter (Chapter 7).

Chapter 2

A pre-release assessment of the relationship between *Pereskia aculeata* and native plant biodiversity in South Africa

Abstract

The relationship between invasive alien plant densities and native plant biodiversity is poorly understood for most invasive plant species. Understanding this relationship can be useful in the development of management strategies and goals for biological control. In this study, the relationship between weed percentage cover and native plant biodiversity measured as species richness, Shannon H and Simpson's D diversity were calculated for the invasive alien plant, *Pereskia aculeata*, in South Africa. There was no significant difference in native plant biodiversity between *P. aculeata* cover of 50% and 100% but significantly higher native plant biodiversity was recorded at sites with *P. aculeata* cover of less than 50%. Maintaining *P. aculeata* cover at 40% and below is therefore an appropriate goal for biological control in terms of protection of native plant biodiversity. The ultimate goal for the biological control programme should be to maintain *P. aculeata* at 30% cover or lower because at this percentage cover there was no significant difference in native plant biodiversity from if the weed were absent from the ecosystem.

2.1. Introduction

It is widely accepted that alien invasive plant species have negative effects on native plant biodiversity but those effects have seldom been quantified (Vitousek 1992, Grice 2004, Shabbir & Bajwa 2006). Knowledge of which invasive species are most damaging is important when allocating resources to the control of different weed species, so quantifying pre-determined success threshold values is valuable in that they can define the required levels of control for each weed species, making them useful in selection of appropriate management interventions (Müller-Schärer and Schaffner 2008) and selection of potential biological control agents (Morin *et al.* 2009). Having pre-determined threshold values is advantageous when evaluating success of any weed control programme, but is especially important when biological control has been implemented. Biological control is not expected to eradicate the weed, so even after a weed has been successfully controlled, it will still be present in the ecosystem. For this reason biological control success cannot be defined as the eradication of the weed species. The expectations of biological control are also often unrealistically high due to well known examples of extraordinarily successful programmes in the past (Hoffmann 1995). These problems can be overcome by having measurable levels of success that can be defined prior to the initiation of control (Müller-Schärer and Schaffner 2008, Morin *et al.* 2009). Quantifying the impact of the weed on native biodiversity and the changes in native biodiversity associated with different percentage covers of the weed is a way in which levels of success can be determined.

Methods for determining the impact of alien invasive plant species on native biodiversity can be divided into three different techniques: multi-site comparisons, weed removals or additions, and time sequence studies (Adair & Groves 1998). Multi-site comparison is the most widely used method, but an underlying assumption of this method is that the pre-invasion state of the invaded and un-invaded sites are comparable (Adair & Groves 1998). The advantage of multi-site comparisons is that data can be collected easily in a short period of time, while weed removals or additions and time sequence studies sometimes require decades of data collection (Adair & Groves 1998). When a successful biological control programme is implemented against a weed, there is an

opportunity to determine the impact of the weed on native biodiversity in the form of a weed removal experiment if the pre-release and post-release evaluation includes measures of weed cover and native plant biodiversity (Barton *et al.* 2007). Weed removal experiments should be interpreted with caution because the return in native biodiversity may be affected by residual effects of the weed on the ecosystem or by invasions by other weed species (Gooden *et al.* 2009).

Post-release evaluations are an important yet often neglected component of biological control programmes which are essential in assessing levels of success of biological control (Morin *et al.* 2009). Measuring rates of establishment and changes in agent populations is a logical first step in a post-release evaluation study because if agent populations do not proliferate, then biological control has not been effective and no further study is necessary until new releases have been made or factors limiting establishment have been determined. The majority of post-release evaluations have focused on agent establishment and agent performance (Morin *et al.* 2009). Although high agent population densities are positive indications of the state of biological control, it cannot be assumed that high agent population densities will lead to a reduction in percentage cover of the weed and an increase in native biodiversity if the effect of herbivory on plant population dynamics is not known (Hoffmann 1990). Measuring agent establishment and agent population densities is an important component of post-release evaluations but should not be used to measure the success of biological control.

Change in weed percentage cover before and after biological control is an appropriate indicator of levels of success. There are studies which quantify a change in weed cover associated with biological control (e.g. Hoffmann & Moran 1998, McConnachie *et al.* 2004, Egan & Irwin 2008, Diop & Hill 2009, Overholt *et al.* 2010), but there are fewer studies that measure the increase in native biodiversity associated with a reduction in the percentage cover of environmental weeds (e.g. Barton 2007). There are no studies that determine levels of success or goals for biological control based on either weed cover or the associated increase in native biodiversity prior to control.

Measuring the success of biological control programmes has been problematic due to the subjective way in which success is often rated (Hoffmann 1995, Thomas & Reid 2007). A synthesis of the entire world's biological control programmes with

measures of success given when possible has been compiled by Julien and Griffiths (1998). Although there are data indicating levels of success for many of the biological control programmes, these data are not comparable between countries or weed species and the majority of the data are descriptive and subjective (Julien and Griffiths 1998). A standardized method of measuring success in weed control allowing for comparisons between species and geographic regions is desirable but may not be feasible due to the wide variety of impacts attributed to weed infestations.

In South Africa, categories of biological control success are determined by the amount of alternative control that is needed in conjunction with biological control to reduce the weed species to an acceptable level (Hoffmann 1995). The three control categories are: (1) complete control, when no other control measures are needed to reduce the weed to an acceptable level, (2) substantial control, when other control methods are needed to reduce the weed to an acceptable level but the amount of the alternate methods needed has been reduced due to biological control and (3) negligible control, when control is completely reliant on alternative methods despite the implementation of a biological control programme (Hoffmann 1995). Although this method is useful in that it is comparable between species, it is essentially a measure of the relative importance of biological control to other control methods. A method that measures success of weed control programmes that utilize other control methods integrated with biological control would be useful. In order to quantitatively measure the success of weed control programmes one must define what constitutes an acceptable level of weed infestation in a way that is measurable and repeatable. For weeds that threaten native biodiversity, an acceptable percentage cover would be one at which native biodiversity is not significantly reduced by the presence of the weed.

When quantifying the impact of a weed species, the effect that should be measured depends on the way in which the species is damaging to the environment. For example, if a weed's primary impact is the alteration of fire regimes, the changes to fire regimes should be measured. The primary impact of many environmental weed species is loss in native biodiversity (Pimental *et al.* 2005). The reduction in biodiversity is therefore the appropriate measurement to calculate when quantifying the impact of the weed, and the increase in native biodiversity is a logical measure of success.

Native biodiversity will not necessarily return after weed densities are reduced, and it is unlikely that an ecosystem that has been heavily infested with a weed species will ever return to the pre-infestation state. In cases where native biodiversity does not return, due to negative impacts of the weed through reduced recruitment and allelopathy, or due to invasions by other weed species, restoration and further weed control may be necessary. By understanding the relationship between weed percentage cover and native biodiversity it is possible to determine weed densities that can be seen as management thresholds (Gooden 2009) or goals for biological control.

The impact of the alien invasive vine species, *Pereskia aculeata*, on native plant biodiversity was investigated in this study. Biodiversity, measured as species richness, Shannon H and Simpson's D diversity, was calculated at various weed percentage covers and the differences in biodiversity between weed covers were compared. Using these data, it is possible to identify weed percentage cover values at which there are significant losses in terms of biodiversity, and those percentage cover values at which there is no significant difference in native biodiversity from if the weed species were absent from the ecosystem. These pre-control data may be useful in developing goals for the biological control programme against *P. aculeata* and in evaluation of the success of future biological control agents against the weed.

2.2. Methods

2.2.1. Site selection

Five sites of about 2500m^2 each were chosen in the coastal area of South Africa from Port St. Johns in the Eastern Cape Province to Kosi Bay in KwaZulu-Natal Province on the border of Mozambique (Fig. 2.1). These sites were chosen to cover the areas in South Africa where *P. aculeata* is invasive within natural ecosystems. No sites were selected west of Port St. Johns or in the Johannesburg area and Limpopo Province because, although *P. aculeata* is present in these areas, it grows primarily in disturbed ecosystems and is thought to have less of an effect on native biodiversity than in the more tropical

areas of the country where it invades pristine ecosystems and appears to grow more vigorously.

All sites were in relatively pristine and undisturbed ecosystems, with four of the five sites occurring in protected areas (nature reserves). Sites were chosen where abundance of exotic plant species, with the exception of *P. aculeata*, was low and human induced disturbance was minimal. *Pereskia aculeata* was first recorded at sites 1 and 2 in 1991, site 3 in 1995 and site 5 in 1987 (ARC – Plant Protection Research Institute, South African Plant Invaders Atlas Database (SAPIA)). There were no records of *P. aculeata* at site 4 prior to this study. *Phenrica guerini* was present at Site 2 in low numbers.

Figure 2.1. The five study sites between Port St. Johns and Kosi Bay on the east coast of South Africa. The shaded area represents the introduced distribution of *Pereskia aculeata* in South Africa (from Henderson 2001). Vegetation at site 1 is defined as Scarp Forest, sites 2, 3 and 5 as Northern Coastal Forest and site 4 as Maputaland Coastal Belt (Mucina & Rutherford 2006).

2.2.2. Data collection

At each site, five 35 m transects were set up, evenly distributed across the site. Random numbers were used to select half-meter squared areas on the side of the transect line that would be sampled. Once the end of each transect was reached, the same process was used in the opposite direction but the quadrat was placed on the opposite side of the transect line. In this way, an average of 120 samples was taken per site (Table 2.1).

A quadrat [0.5m X 0.5m X 1.0m ($0.25m^3$)] was placed in each selected area and the number of plant species and percentage cover of each plant species within the quadrat were recorded. Sampling was confined to the understory vegetation using a three dimensional quadrat to exclude plants in the canopy which were inaccessible and therefore could not be sampled. Percentage cover was estimated visually to the nearest 10% cover, but species present in the quadrat with very low percentage covers were assigned a cover value of 5%. Each species was assigned a life form and number which was used to refer to that species on all subsequent samples. Species identified as invasives were referred to using the species name (Henderson 2001). Life forms used were tree, creeper, shrub, bulb, fern or grass; and shrubs, bulbs, ferns and grasses were combined into one category for the purpose of the functional group analysis because of the relatively low number of bulbs, ferns and grasses that were encountered.

Density of *P. aculeata* was recorded as percentage cover, as opposed to number of individuals, because defining an individual *P. aculeata* plant is difficult due to its creeping habit and its ability to produce roots from stems and lateral branches. These habits make the effect of *P. aculeata* individuals on native biodiversity incomparable to each other as a single individual may cover large areas of forest.

Sampling was not intended to be exhaustive, so species richness values are underestimates of total site species richness according to species accumulation curves, and rare species may not have been sampled. The relationship between native plant biodiversity and *P. aculeata* cover would not be affected significantly if rare species were not sampled because of the relatively insignificant contribution that rare species would make to the measures of mean native biodiversity.

2.2.3. Data analysis

Species richness, Shannon H diversity and Simpson's D diversity were calculated for each quadrat and plotted against *P. aculeata* cover. Species richness was defined as the number of species present in each quadrat. Shannon H and Simpson's D diversities were calculated using the following formulae (from Begon *et al.* (1996)):

$$\text{Shannon H diversity: } H = -\sum_{i=1}^{S} P_i (ln\ P_i)$$

$$\text{Simpson's D diversity: } D = 1/\sum_{i=1}^{S} P_i^2$$

Where P_i is the proportion of species i.

Shannon H and Simpson's D diversities were chosen because Shannon H diversity emphasizes rare species while Simpson's D diversity emphasizes common species (Magurran 2004). Species richness, Shannon H diversity and Simpson's D diversity are standard nonparametric diversity descriptors (Schooler *et al.* 2006).

Data were not normally distributed so the nonparametric regression model, Kendall Robust Line Fit Method (Sokal & Rohlf 1995), was used to determine the relationship among the measures of biodiversity and *P. aculeata* cover at each site and for all sites combined using the free software programme KTRLine ver. 1.0 (Granato 2006). The nonparametric correlation coefficient, Spearman's r_s, was calculated for the relationship between *P. aculeata* cover and the three measures of biodiversity using STATISTICA© ver. 8.0.

Threshold values were identified visually and confirmed by piece-wise linear regressions above and below the relevant *P. aculeata* percentage cover category. Threshold values were evident if significant negative relationships were present on one side of the value and non-significant relationships were present on the other. This method of calculating threshold values followed Gooden *et al.* (2009) and analyses were performed in STATISTICA© ver. 8.0.

To determine whether the effect of *P. aculeata* percentage cover was consistent across sites, an ANCOVA (Homogeneity of Slopes test) was carried out using the average of the measures of biodiversity in each *P. aculeata* percentage cover category from each site, which normalized the data. By using averaged data, the variation in the data was ignored, but it allowed meaningful comparison of the average relationship between biodiversity and *P. aculeata* percentage cover among sites.

A linear regression line for species richness of the three functional groups against *P. aculeata* percentage cover was plotted using averages from each site to determine whether the effect of *P. aculeata* differed among functional groups.

Kruskal-Wallis ANOVAs tested for significant differences among *P. aculeata* percentage cover categories and the different measures of biodiversity. These tests used the full data set, and therefore included the variation in biodiversity at each *P. aculeata* percentage cover category. The Multiple Comparison of Mean Rank Post Hoc test was used to calculate p-values for differences in mean rank of biodiversity between individual *P. aculeata* percentage cover categories.

All ANCOVA, Kruskal-Wallis tests and simple regressions were performed using STATISTICA© Ver. 8.0.

2.3. Results

A total of 588 quadrats were sampled in which 1617 observations of various plant species and their percentage covers were made. Only 29 observations (1.79%) were of recognized alien invasive plant species and densities of alien invasive plants, with the exception of *P. aculeata*, never exceeded 0.05% cover at any sites. The only two alien invasive species, besides *P. aculeata*, that were encountered were *Lantana camara* L. (Verbenaceae) and *Chromolaena odorata* (L.) (Asteraceae). *Pereskia aculeata* was present in 55% of the quadrats. Total native species richness varied among sites (Table 1). The ratio of different life forms varied among sites, but with the exception of Site 5, trees were the most species rich life form (Table 2.1).

Table 2.1. Five sites with *P. aculeata* infestations were surveyed on the eastern coast of South Africa. Total native species richness varied between 30 and 57 species per site.

Site number	Site location	Number of quadrats	Trees	Creepers	Others	Species richness	Mean species richness per quadrat
1	Port St. Johns	119	18	7	14	39	2.08
2	Pennington	118	12	9	9	30	3.15
3	Tugela River Mouth	122	26	14	9	49	3.14
4	Ekanyazi Tribal Land	116	32	11	14	57	2.22
5	Kosi Bay	112	16	17	10	43	3.22

As *P. aculeata* percentage cover increased, biodiversity became less variable and consistently lower than at lower *P. aculeata* cover, resulting in skewed variance in the data. The Kendall-Theil robust fit line for species richness against *P. aculeata* cover had a negative median slope at all sites. The regression between *P. aculeata* cover and species richness was statistically significant at all sites except Site 1 (95% confidence intervals did not include zero). There was a significant negative correlation between plant species richness and *P. aculeata* cover at all sites (Spearman's correlation coefficients: Site 1: r_s = -0.389, Site 2: r_s = -0.437, Site 3: r_s = -0.607, Site 4: r_s = -0.579, Site 5: r_s = -0.475, $p<0.05$ at all sites). The relationship between average species richness and *P. aculeata* cover was not significantly different among sites, indicating that *P. aculeata* had a similar effect on species richness at all the sites (Homogeneity of Slopes test, F = 0.405, p = 0.943).

The Kendall-Theil robust fit lines for Shannon H and Simpson's D diversity against *P. aculeata* cover had negative median slopes at all sites. There was a statistically significant regression between *P. aculeata* cover and Shannon H diversity at three of the five sites (2, 4, and 5). For Simpson's D diversity, the regression was significant at all sites with the exception of Site 1. Shannon H and Simpson's D diversity were negatively correlated with *P. aculeata* cover at all sites (Spearman's correlation coefficients for Shannon H diversity: Site 1: r_s = -0.405, Site 2: r_s = -0.437, Site 3: r_s = -0.033, Site 4: r_s = -0.451, Site 5: r_s = 0.371, $p < 0.05$ at all sites. Simpson's D diversity: Site 1: r_s = - 0.406,

Site 2: r_s = -0.417, Site 3: r_s = -0.497, Site 4: r_s = -0.487, Site 5: r_s = -0.337, $p < 0.05$ at all sites). There was no significant difference in the relationship among sites for either diversity index (Homogeneity of Slopes test, Shannon H: F = 0.567, p = 0.840; Simpson's D: F = 0.777, p = 0.660).

When the data from all five sites were combined, there was a significant negative relationship between *P aculeata* cover and species richness, Shannon H diversity and Simpson's D diversity (Kendall-Theil robust fit lines had negative median slopes and 95% confidence intervals did not include zero). Spearman's correlation coefficients (r_s) were significant for all measures of biodiversity (Species richness: r_s = -0.544, $p < 0.05$; Shannon H diversity: r_s = -0.338, $p < 0.05$; Simpson's D: r_s = -0.476, $p < 0.05$). The relationship between *P. aculeata* and all three functional groups was significant (Trees: F = 12.460, $p < 0.001$; Creepers: F = 5.184, p = 0.027; Others: F = 28.477, $p < 0.001$) but the slopes of the regression lines differed between functional groups (Fig. 2.2). 'Creepers' species richness declined to a lesser extent than other functional groups as *P. aculeata* cover increased (Fig. 2.2).

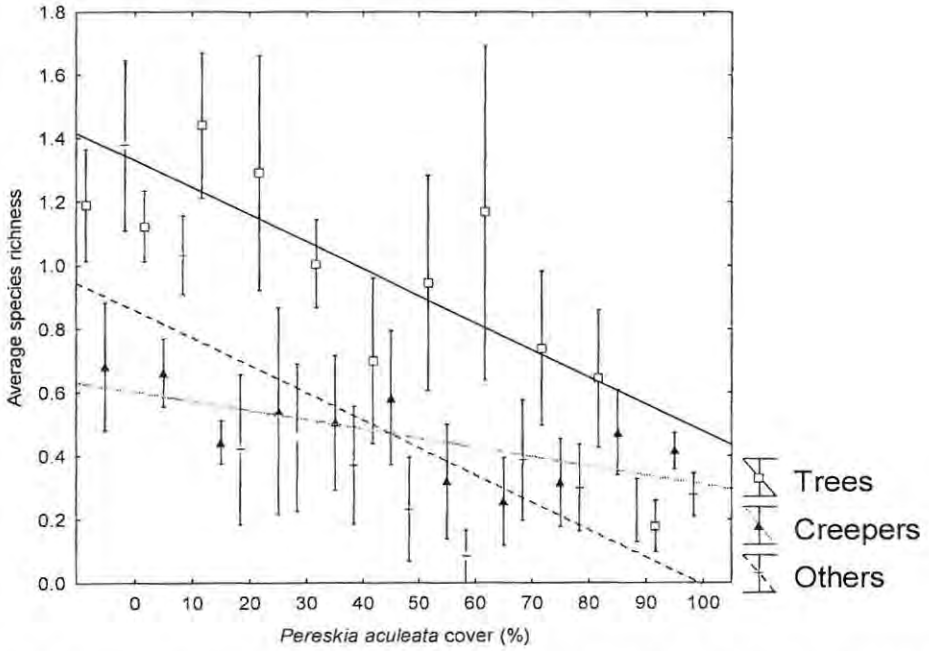

Figure 2.2. Species richness of all functional groups declines as *Pereskia aculeata* cover increases but 'creepers' are not affected to the same extent as 'trees' and 'others'. Error bars represent S.E of the mean.

Species richness and biodiversity declined with increased *P. aculeata* cover but the changes in species richness and biodiversity between each *P. aculeata* percentage cover category were not equal (Fig. 2.3). Significant negative linear regressions were calculated using cover categories between 0% and 50% (species richness: $F = 45.143$, $p < 0.001$; Shannon H: $F = 42.763$, $p < 0.001$; Simpson's D: $F = 28.780$, $p < 0.001$) and non-significant linear relationships were found between 50% and 90% cover categories (species richness: $F = 1.468$, $p = 0.228$; Shannon H: $F = 1.632$, $p = 0.204$; Simpson's D: $F = 0.380$, $p = 0.539$) indicating that a threshold value was present at 50% *P. aculeata* density for all biodiversity indices (Fig. 2.3). There was no significant difference in species richness between 100% *P. aculeata* cover and all cover categories above 50% (Table 2). All cover categories of 30% and less were significantly different from 100%, and categories of 10% and below were all significantly different from categories of 80% and above. Categories of 40% and above were all significantly different from 0% (Table 2).

Differences between Shannon H diversity at different *P. aculeata* cover categories were similar to those of species richness. There was no significant difference between percentage covers of 100% and cover categories above 40%; all categories of 10% and below were significantly different from categories 80% and above; and categories of 40% and above were all significantly different from 0% (Table 3).

Differences in Simpson's D diversity between *P. aculeata* cover categories differed from those between species richness and Shannon H diversity in that there was a significant difference between 40% density and 100%; there was no significant difference between 10% and 80% or 5% and 80%; and only categories of 60% and above were significantly different from 0% (Table 4).

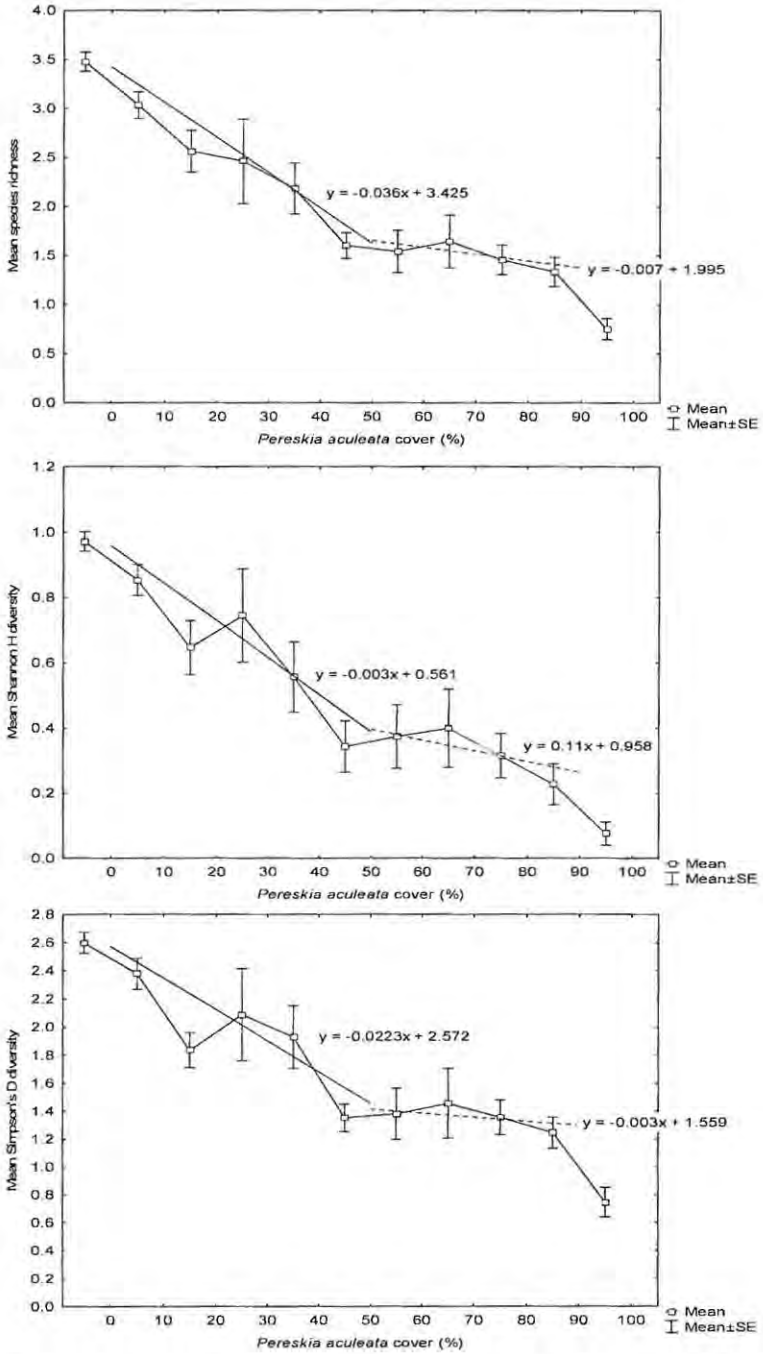

Figure 2.3. Mean species richness, Shannon H diversity and Simpson's D diversity with simple regression on either side of the 50% *P. aculeata* cover threshold values. Error bars represent S.E. of the mean.

Table 2.2. Matrix of p-values from the Kruskal-Wallis Post Hoc test, multiple comparison of mean rank, showing differences in species richness between *Pereskia aculeata* cover categories. Statistically significant values are bold and italicized.

Pereskia aculeata cover (%)	0	5	10	20	30	40	50	60	70	80	90	100
0												
5	1.000											
10	1.000	1.000										
20	0.075	1.000	1.000									
30	1.000	1.000	1.000	1.000								
40	***0.016***	1.000	1.000	1.000	1.000							
50	***0.000***	***0.046***	0.094	1.000	1.000	1.000						
60	***0.001***	0.066	0.127	1.000	1.000	1.000	1.000					
70	***0.004***	0.181	0.311	1.000	1.000	1.000	1.000	1.000				
80	***0.000***	***0.000***	***0.000***	0.499	1.000	1.000	1.000	1.000	1.000			
90	***0.000***	***0.000***	***0.000***	0.244	0.869	1.000	1.000	1.000	1.000	1.000		
100	***0.000***	***0.000***	***0.000***	***0.000***	***0.021***	0.053	1.000	1.000	1.000	1.000	1.000	

Table 2.3. Matrix of p-values from the Kruskal-Wallis Post Hoc test, multiple comparison of mean rank, showing differences in Shannon H diversity between *Pereskia aculeata* cover categories. Statistically significant values are bold and italicized.

Pereskia aculeata cover (%)	0	5	10	20	30	40	50	60	70	80	90	100
0												
5	1.000											
10	1.000	1.000										
20	0.067	1.000	1.000									
30	1.000	1.000	1.000	1.000								
40	***0.029***	1.000	1.000	1.000	1.000							
50	***0.000***	0.060	***0.042***	1.000	1.000	1.000						
60	***0.005***	0.268	0.182	1.000	1.000	1.000	1.000					
70	***0.011***	0.520	0.352	1.000	1.000	1.000	1.000	1.000				
80	***0.000***	***0.001***	***0.001***	1.000	1.000	1.000	1.000	1.000	1.000			
90	***0.000***	***0.000***	***0.000***	0.490	0.402	1.000	1.000	1.000	1.000	1.000		
100	***0.000***	***0.000***	***0.000***	***0.004***	***0.017***	0.185	1.000	1.000	1.000	1.000	1.000	

Table 2.4. Matrix of p-values from the Kruskal-Wallis Post Hoc test, multiple comparison of mean rank, showing differences in Simpson's D diversity between *Pereskia aculeata* cover categories. Statistically significant values are bold and italicized.

Pereskia aculeata cover (%)	0	5	10	20	30	40	50	60	70	80	90	100
0												
5	1.000											
10	1.000	1.000										
20	1.000	1.000	1.000									
30	0.186	1.000	1.000	1.000								
40	1.000	1.000	1.000	1.000	1.000							
50	0.139	1.000	1.000	1.000	1.000	1.000						
60	***0.003***	0.192	0.191	1.000	1.000	1.000	1.000					
70	***0.017***	0.492	0.463	1.000	1.000	1.000	1.000	1.000				
80	***0.032***	0.857	0.799	1.000	1.000	1.000	1.000	1.000	1.000			
90	***0.000***	***0.000***	***0.000***	0.732	1.000	1.000	1.000	1.000	1.000	1.000		
100	***0.000***	***0.000***	***0.000***	***0.000***	***0.025***	***0.035***	1.000	1.000	1.000	1.000	1.000	

2.4. Discussion

There is strong evidence that *P. aculeata* has a negative impact on biodiversity in South Africa, as indicated by the decreases in plant species richness, Shannon H diversity and Simpson's D diversity that were associated with increases in *P. aculeata* cover. Although the relationship between biodiversity and *P. aculeata* cover has been determined using correlative methods, the fact that the relationship is similar at all five sites suggests a causal relationship because other factors that may lead to this correlation, such as the habit of *P. aculeata* to take advantage of vacant plots, are unlikely to be similar at all five sites due to ecological characteristics, such as vegetative composition, which are expected to differ among sites. Disturbance and invasion by other weed species was unlikely to be a causal factor in the loss of native biodiversity because sites were selected in undisturbed, relatively pristine areas where the presence of weed species, with the exception of *P. aculeata*, was negligible.

In order to prove a causal relationship between a weed and reduced native biodiversity, a long-term weed addition experiment (Adair & Groves 1999) could be conducted, but management strategies and goals for biological control should be determined prior to the initiation of control making the time scale for a weed addition experiment impractical. For the purpose of this study, it must be assumed that the relationship between *P. aculeata* cover and biodiversity is causal, taking into account evidence of causality from comparisons in this relationship among sites. This study was intended as a pre-control study of the relationship between the weed species and native biodiversity, which should be followed by a post-release evaluation.

Diversity within plant functional groups was reduced with an increase in *P. aculeata* cover, but the reduction in diversity was not equal between groups. This may alter the functional group composition of the ecosystems by increasing the relative number and abundance of 'creeper' species to 'tree' and 'other' species. Changes in functional group composition and a reduction in functional group diversity may reduce the ability of the ecosystem to provide ecosystem services, because functional group diversity is believed to be the mechanism whereby a reduction in biodiversity leads to loss in ecosystem functioning (Loreau *et al.* 2001).

There were threshold values for species richness, Shannon H diversity and Simpson's D diversity at 50% *P. aculeata* cover, indicating that little biodiversity would be protected by control measures that do not maintain percentage cover of the weed at lower than 50%. Forty percent *P. aculeata* cover should therefore be seen as a goal for biological control. This contrasts strongly with the threshold value calculated for *L. camara* in southeastern Australia, which indicated that the weed had little effect on native plant biodiversity at percentage cover of less than 75% (Gooden *et al.* 2009). The relatively high impact of low *P. aculeata* cover when compared with those calculated for *L. camara* by Gooden *et al.* (2009) could be explained by the different growth habits of the two species. Vines may have a greater impact on native plant biodiversity because of their ability to climb and smother other plants.

Further indications that 40% *P. aculeata* cover is an appropriate goal for biological control come from the fact that the lowest percentage cover with significantly higher Simpson's D diversity than 100% *P. aculeata* cover was 40%. Forty percent is

also an appropriate goal in terms of species richness because species richness was very close to significantly higher than 100% *P. aculeata* cover at 40% cover. The lowest *P. aculeata* cover with significantly higher Shannon H diversity than 100% was 30% cover but higher Shannon H diversity was expected at 40% despite this value not being statistically significant.

Pereskia aculeata had a significant impact on native plant species richness and Shannon H diversity at 40% cover and on Simpson's D diversity at 60% cover. At lower percentage cover than these values, there was no significant difference in native biodiversity from if the weed was not present in the ecosystem. Thirty percent *P. aculeata* cover can therefore be seen as a goal for biological control in terms of species richness and Shannon H diversity and 50% cover can be seen as a goal in terms of Simpson's D diversity. These values are not thresholds (see Panetta & James 1999) but are appropriate goals for biological control of *P. aculeata* because at these percentage covers the negative impact of the weed was no longer significant. In the case of an emerging infestation, biological control should aim to maintain weed percentage cover below these values. In areas of infestations with weed percentage cover higher than these values, biological control should aim to reduce *P. aculeata* to these levels so that native biodiversity has an opportunity to return. If biodiversity does not return, restoration may be necessary to increase biodiversity to levels similar to the pre-infestation state.

The most appropriate management scheme to control alien invasive plant species may differ for the same weed species among sites. This is because biotic and abiotic factors, including weed percentage cover, vary among sites. Knowledge of the effect of weed percentage cover on biodiversity is therefore important in choosing appropriate management techniques for each site (Müller-Schärer and Schaffner 2008). Mechanical control and chemical control are completely ineffective against *P. aculeata*, so the only viable control option for this weed species is biological control (Moran & Zimmermann 1991b). The fact that *P. aculeata* does not need to be eradicated for the weed to have no significant effect on native plant biodiversity indicates that biological control is a viable option.

The methods used in this study were developed for *P. aculeata* specifically and might not be appropriate for other weed species. However, measuring native plant

biodiversity associated with various weed percentage cover values or densities can be used to set management thresholds and quantify the impact of other environmentally damaging weed species (Gooden *et al.* 2009).

Even at relatively low percentage cover, *P. aculeata* has a negative impact on native plant biodiversity on the eastern coast of South Africa. The greatest threat is in the forest biome vegetation types in South Africa because these vegetation types are rare and have high levels of endemism (Mucina & Rutherford 2006). Biological control practitioners should aim to maintain *P. aculeata* cover below the goals and threshold values determined in this study. The success of any future biological control agents released for the control of *P. aculeata* can be evaluated by comparing post-release data with data from this pre-release study and determining whether the percentage covers that are suggested as goals for biological control have been reached or maintained.

Chapter 3

The impact of *Phenrica guérini* on *Pereskia aculeata*

Abstract

Phenrica guérini has established at three sites in South Africa but no quantitative measure of this chrysomelid beetle's impact on *Pereskia aculeata* densities has been made. An estimation of the potential impact of *P. guérini* would be useful for the biological control programme against *P. aculeata* because only agents that have the potential to damage the target weed should be mass reared and distributed. Releases of ineffective agents should be avoided due to the possibility of negative interactions with other biological control agents that may be released in future and because of the intrinsic risks associated with the release of any exotic organism. The impacts of various *P. guérini* densities on *P. aculeata* were assessed in a greenhouse experiment. The impact of *P. guérini* was negligible even at densities much higher than those observed in the field. The results suggest that *P. guérini* is an ineffective agent that cannot impact *P. aculeata* growth parameters, but these results should be interpreted with caution because of the constraints of laboratory or greenhouse impact assessments of this nature. Observations of the impact of *P. guérini* at one field site contradict the results of the greenhouse experiment and suggest that the insect can have an impact on *P. aculeata* under certain conditions. Mass rearing and distribution of *P. guérini* should continue unless a more damaging agent that could be negatively affected by the presence of *P. guérini* becomes available. Releases of *P. guérini* should be made at sites where pre-release records of *P. aculeata* density and native biodiversity are available and should be followed by post-release evaluations.

3.1. Introduction

The risks associated with releasing biological control agents are minimal but there is an intrinsic risk, however small, when releasing any exotic species into a new environment and for this reason the release of ineffective biological control agents should be avoided (Louda 2000, Sheppard *et al.* 2003, McClay & Balciunas 2005). The risks of indirect negative effects increases if agent populations build up to high numbers with no associated decrease in the weed population, because large numbers of the biological control agent will be maintained, increasing the chance of the agent population being utilized by other organisms and therefore disrupting the ecosystem (Balciunas & Smith 2006). Ineffective biological control agents could also have negative interactions with other biological control agents that have been released or may be released in future and this may result in reduced levels of control of the target weed (Denoth *et al.* 2002). Despite the potential negative effects of releasing ineffective agents, a large number of biological control agents that have established and persist on their target weed populations are considered to make little or no contribution towards control (Denoth *et al.* 2002, McClay & Balciunas 2005).

Pre-release assessments of the impact of biological control agents on their target weeds could significantly reduce the chances of releasing ineffective agents if impact studies are conducted as a pre-requisite to host specificity studies (McClay & Balciunas 2005). Impact studies can been conducted in the region of origin of the target weed but under most circumstances biological control researchers are restricted to quarantine when evaluating the impact of new potential biological control agents and very few studies have been conducted under these conditions (Kleinjan *et al.* 2004, Coetzee *et al.* 2005, Balciunas & Smith 2006, Conrad & Dhileepan 2007, Williams *et al.* 2008, Goolsby *et al.* 2009).

McClay and Balciunas (2005) propose the use a conceptual model, developed to calculate the ecological impact of invasive species by Parker *et al.* (1999), to determine the potential impact of biological control agents. In this model, the range, abundance and per-capita effect of the biological control agent are multiplied to give an indication of the agent's potential impact. Although it is difficult to predict the potential abundance and range of a

species prior to release it is possible to assess the per-capita effect of a biological control agent in laboratory studies (McClay & Balciunas 2005). By determining the impact of various biological control agent densities on the target weed it is possible to determine what levels of impact should be expected under different agent densities and whether agent densities comparable with those expected in the field are likely to damage the weed. Extrapolation of these data into the field is difficult because the experiments are generally done under ideal conditions for plant and insect growth and development and in the absence of interspecific plant competition (McClay & Balciunas 2005). A biological control agent that is shown to reduce individual plant survival or growth rate of the target weed will not necessarily have any effect on the weed at a population level, but because agents cannot have a population level effect without being damaging to individual plants, only biological control agents that are damaging on an individual plant level should be considered for control (McClay & Balciunas 2005).

The concept of a "damage curve" was developed in the context of reductions in crop yield as a function of insect damage (Peterson & Higley 2001). The same concept is applicable to the impact of different densities of biological control agents on weed species (McClay & Balciunas 2005). This model distinguishes between the amount of plant tissue removed or injured by the pest (referred to as "damage" in this study) and the resulting effect on plant fitness or vegetative growth (referred to as "impact") (Peterson & Higley 2001, McClay & Balciunas 2005). If damage is plotted against impact (Fig. 3.1) it is possible to predict under what levels of damage a reduction in fitness or vegetative growth (i.e. impact) would be expected in the field. Plants used in the laboratory experiments are grown under ideal growing conditions and are usually not exposed to competition, so if the average levels of damage found in the field, where conditions for plant growth are less favourable, are higher than that required to impact the plants in the laboratory, one can assume that the biological control agent is having an impact in the field. Levels of damage lower than that required to impact the plant in the laboratory could not, however, be used to indicate that there is no effect in the field.

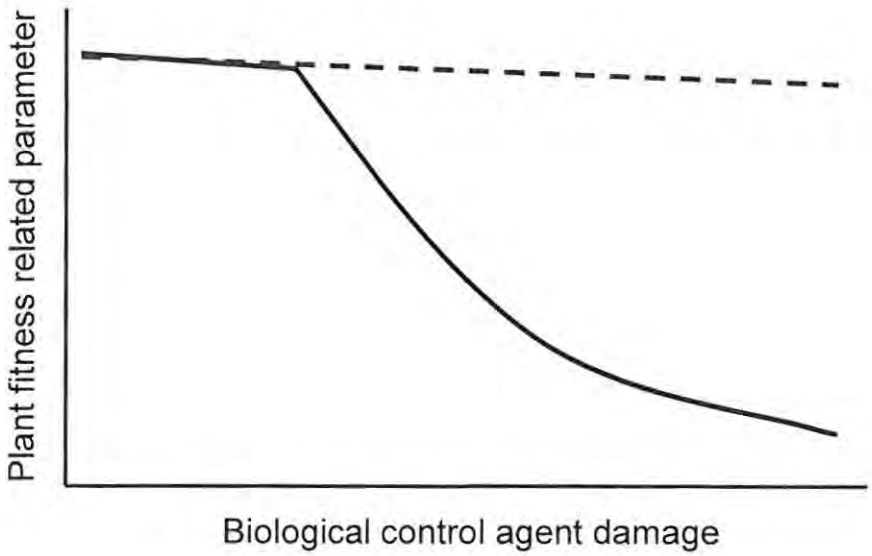

Figure 3.1. An example of two theoretical damage curves for biological control agents. The dotted line represents a biological control agent that damages the target weed but does not reduce weed fitness or vegetative growth significantly. The solid line represents a damaging biological control agent (From McClay & Balciunas 2005, Peterson & Higley 2001).

Phenrica guérini Bechyné (Chrysomelidae) has established at three of the 16 sites at which it was released in South Africa (Klein 1999, Dr. Des Conlong, South African Sugar Research Institute (SASRI), *Pers. comm.*). The impact of *P. guérini* has not been measured at any of the three sites and no pre-release measures of weed density were taken that could be compared with post-release weed densities (Chapter 2). Observations made by biological control practitioners suggest that at two of the sites (Sezela. 30° 23' 33.2" S 30° 40' 34.8" E; Port Alfred. 33° 35' 47.6" S 26° 53' 18.1" E) there was often substantial damage but there was no visible reduction in weed density (personal observations) and that at the third site (Wewe Dam. 29°32'27.7"S 31°08'06.4"E) there was abundant damage and an associated reduction in *P. aculeata* density (Dr. Des Conlong, SASRI. *pers. comm.*). These subjective observations highlight the need for a quantitative post-release evaluation to determine whether *P. guérini* is an effective biological control agent. If *P. guérini* is capable of impacting *P. aculeata* in South Africa, mass rearing and releases should be supported. If no

impact is expected, resources should not be wasted on an ineffective agent because of the intrinsic risks of releasing an exotic organism and because *P. guérini* may have negative interaction with biological control agents that may be released in future.

The life-cycle and development of *P. guérini* was studied by H. E. Sparks (ARC-Plant Protection Research Institute) in the late 1990s with the intention of developing a mass rearing programme (H.E. Sparks, unpublished report). The average duration of development to pupation was 23 days. Eggs were oviposited in batches of between 23 and 33 on the leaf blades of *P. aculeata*. Eggs are pink to red and are usually evenly spaced from one another within in each egg batch. Eggs that are oviposited in a haphazard fashion are often infertile (personal observation). The larvae are pink in the first instar and yellow in the second and the beginning of the third instar. Larvae turn to a dark grey-black towards the end of the third instar when they enter a pre-pupal phase in which feeding no longer takes place. Pupation takes place in the soil and lasted an average of 12 days (H.E. Sparks, unpublished report).

In this chapter, damage curves were constructed for *P. guérini* on *P. aculeata* with the intention of determining what levels of damage in the field would indicate an impact on plant fitness or vegetative growth. If damage levels comparable to those observed in the field, or that could realistically be reached in the field, resulted in decreased plant fitness or reproductive growth this would indicate that *P. guérini* is a potentially effective biological control agent requiring greater mass rearing and release efforts. If damage levels higher than any realistic level of damage that could be reached in the field were required to decrease plant fitness or vegetative growth this would suggest that new biological control agents were required. The implications of the findings for the biological control programme against *P. aculeata* and the limitations of laboratory based impact studies are then discussed.

3.2. Methods

Pereskia aculeata plants were grown from cuttings in pots in a green house with identical fertilizer and watering regimes. All plants were clones of a single genotype originally collected at Port Alfred, Eastern Cape Province, South Africa. This genotype, referred to as

SA3 (Chapters 4 & 5), was chosen because *P. guérini* established on it at the Port Alfred site (Klein 1999) and had persisted for 15 years, indicating that this genotype was a suitable host for *P. guérini* (Chapter 5).

Twenty-three plants of comparable size were selected for the experiment. Plants were cut back so that only a single stem of 15cm protruded from the soil surface. The plants were then uprooted and the roots were trimmed back to 10cm in length and thinned out so that root density appeared similar for all plants. The mass of each trimmed plant was then recorded.

Plants were replanted in pots (25cm diameter, 20cm depth) filled with potting soil and placed on a plant tray in fine mesh insect cages (0.5mX0.5mX1.0m). Plants were watered every second week and fertilized with the organic fertilizer Seagrow® (53:7:17), monthly. Plants were allowed to grow for 36 days after which the number of shoots and length of each shoot was recorded and the plants were inoculated with *P. guerini.*

Phenrica guérini was mass reared at the South African Sugar Research Institute (SASRI) in Mount Edgecomb, KwaZulu-Natal, South Africa. Egg batches were delivered to Rhodes University on *P. aculeata* leaves in petri-dishes. On hatching, the larvae were transferred to the *P. aculeata* plants using a fine paint brush.

Control plants (six replicates) were not inoculated with *P. guérini.* Three density treatments of five larvae per plant (six replicates), ten larvae per plant (five replicates) and 20 larvae per plant (six replicates) were used. Every 20 days the plants were inoculated with new larvae and this process was repeated for 60 days. A 2cm layer of pupation medium (7:7:6 parts loam:sand:vermiculite) covered the cage floor and soil surface so that late third instar larvae could pupate. Twenty days was considered an appropriate length of time between inoculations because most larvae from the previous inoculation had entered the pre-pupal phase or pupated by this stage. Although temperatures were not recorded in the greenhouse, higher temperatures could explain the slightly faster larval development in this study when compared with the study by H.E. Sparks (unpublished report). Adult *P. guérini* were removed from cages soon after eclosion to exclude the effect of adult feeding from the experiment.

Prior to each inoculation event, the number of shoots and length of each shoot was recorded. After the 60 day period all plants were uprooted and the number of shoots, shoot length, root length, leaf tissue mass, below ground mass and above ground mass were recorded. The leaf area consumed (insect damage) was recorded by tracing the damaged and consumed areas of the leaves and measuring the area using WinDias© ver. 2.0 (Delta-T Devices, Cambridge, U.K.). Adult eclosion was intended to be used to measure *P. guérini* mortality but very low survival to the adult stage was recorded. This is believed to be primarily due to mortality during the pupation stage. High mortality during pupation may have occurred due to high temperatures in the pupation medium. The thin layer of pupation medium rested on the metal base of the cages which often became very hot. Pupal mortality would not affect levels of damage in this experiment because only larval feeding damage was measured, but no reliable measure of *P. guérini* larval mortality can be calculated due to the high pupal mortality.

Analyses of variance (ANOVA) were conducted to test for significant differences in levels of insect damage, number of shoots, shoot length, root length, below ground mass and above ground mass among *P. guérini* density treatments. Damage curves were constructed by plotting insect damage against the various plant parameters. Simple regressions were performed to test for any relationships between insect damage and the plant parameters. All statistical analyses were performed in STATISTICA© ver. 8.0.

3.3. Results

All data were normally distributed and met the assumptions of ANOVA.

There were significant differences in the leaf area consumed among *P. guérini* density treatments (ANOVA: $F_{(2,14)} = 4.906$, $p = 0.024$) (Fig. 3.2). Leaf area consumed in five *P. guérini* per plant treatments was significantly lower than in 10 and 20 *P. guérini* per plant treatments but there was no significant difference in leaf area consumed between 10 *P. guérini* per plant treatments and 20 *P. guérini* per plant treatments (Fisher's LSD Post Hoc test) (Fig. 3.2).

There were no significant differences among *P. guérini* density treatments for any of the plant length parameters measured (Fig. 3.3) (ANOVA: Change in number of shoots: $F_{(3,19)} = 2.179$, $p = 0.124$. Change in shoot length: $F_{(3,19)} = 1.072$, $p = 0.385$. Change in root length: $F_{(3,19)} = 1.778$, $p = 0.186$. Below ground mass: $F_{(3,19)} = 1.180$, $p = 0.344$. Above ground mass: $F_{(3,19)} = 1.974$, $p = 0.152$. Change in total mass: $F_{(3,19)} = 2.489$, $p = 0.091$).

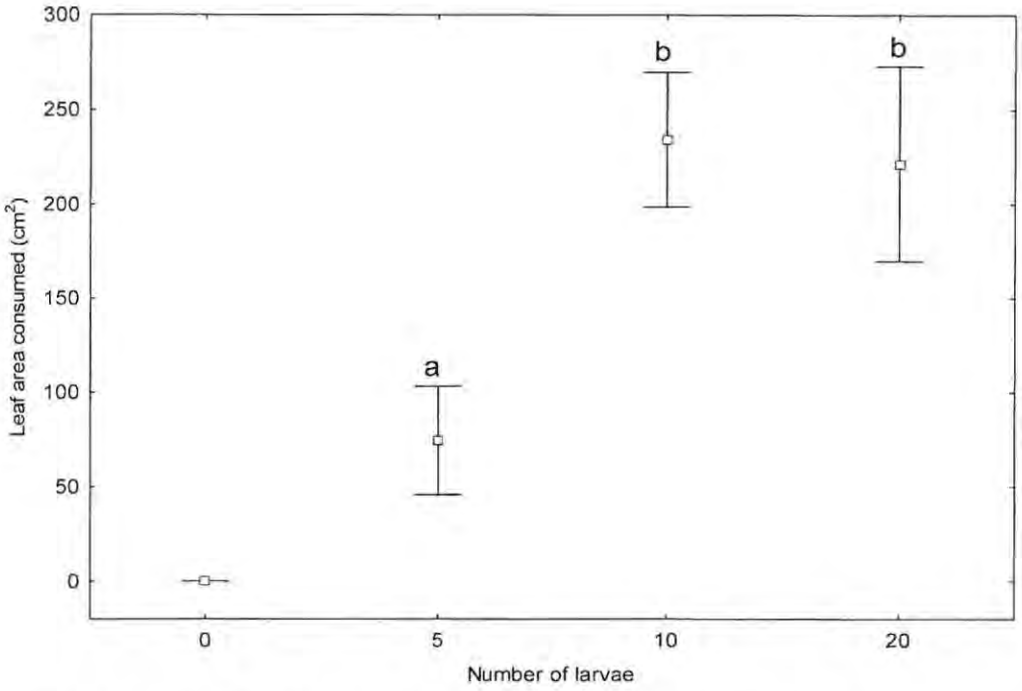

Figure 3.2. *Phenrica guérini* densities of ten and 20 larvae per plant resulted in significantly higher leaf area consumption than five larvae per plant treatments but there was no significant difference in area consumed between densities of ten and 20 larvae per plant. This suggests that higher *P. guérini* densities are unlikely to significantly increase levels of damage to *P. aculeata*. Vertical bars delineate standard errors and letters indicate statistically significant differences ($p<0.05$) in leaf area consumed according to Fisher's LSD Post Hoc test.

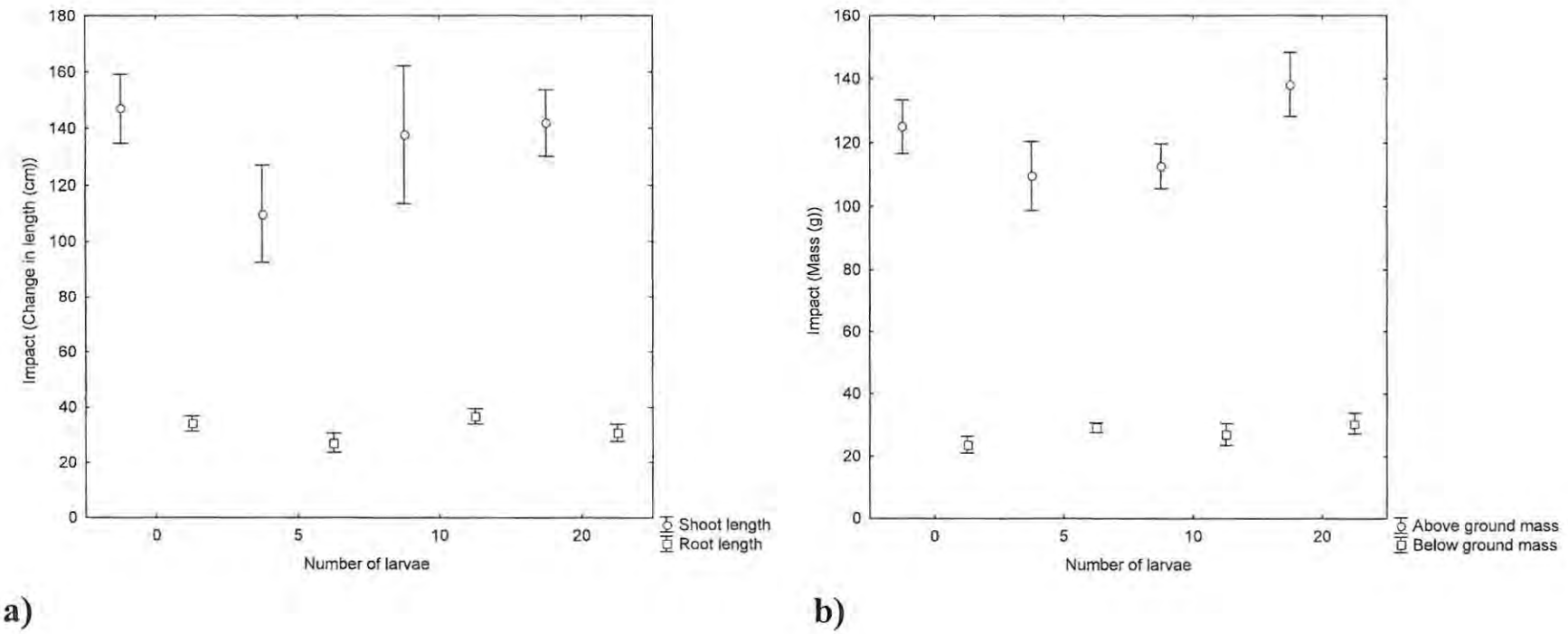

Figure 3.3. The impact of different *P. guérini* density treatments on *P. aculeata* measured as **a)** change in length of shoots and roots, and **b)** above and below ground mass. No significant differences between density treatments were measured for any plant parameters. Vertical bars delineate standard errors.

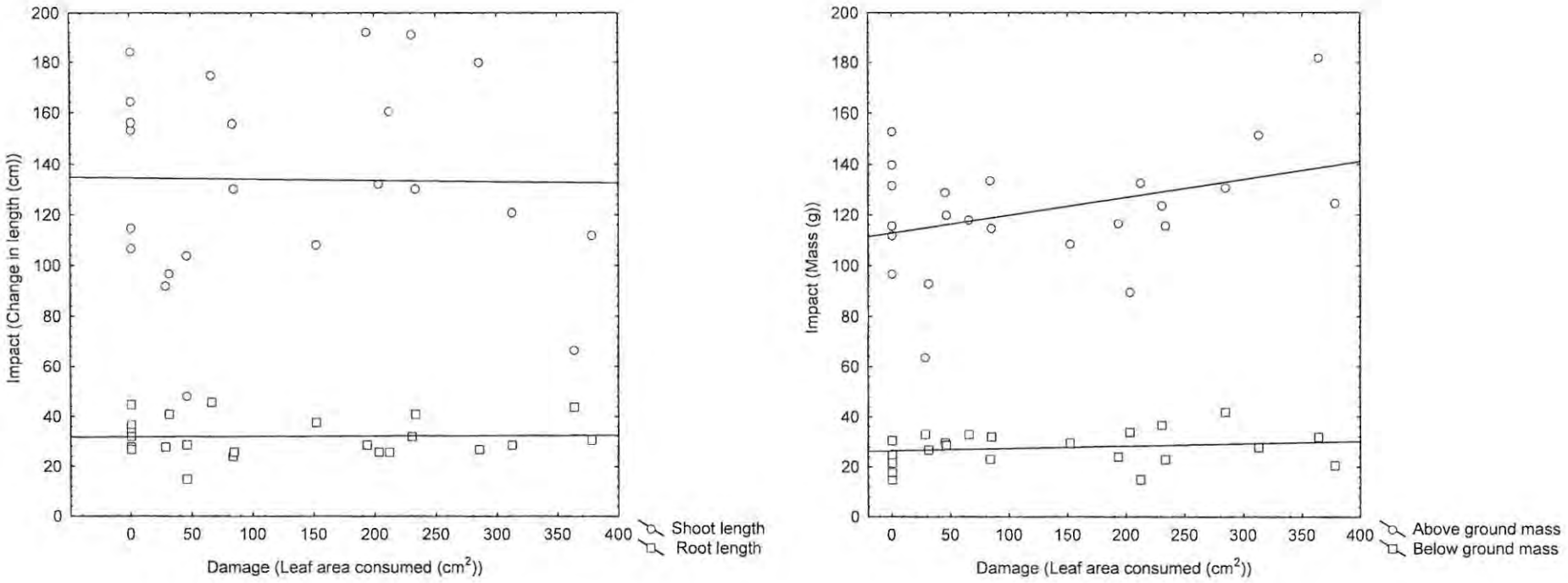

Figure 3.4. Damage curves for *P. guérini* constructed by plotting the impact of *P. guérini* measured as **a)** change in shoot and root length, and **b)** above and below ground mass, against damage measured as leaf area consumed. None of the impact parameters had a significant relationship with damage. The relationship between above ground mass and damage (b) was the relationship that was relatively close to significant (p=0.076) but *P. guérini* damage is unlikely to be causal in the relationship.

Insect damage, measured as leaf area consumed, had no significant relationship with any plant parameters (Fig. 3.4) (Simple regression: Change in number of shoots: $F = 1.587$, $p = 0.222$. Change in shoot length: $F = 0.005$, $p = 0.943$. Change in root length: $F = 0.014$, $p = 0.908$. Below ground mass: $F = 0.672$, $p = 0.422$. Above ground mass: $F = 3.482$, $p = 0.076$. Change in total mass: $F = 0.644$, $p = 0.431$).

The relationship between above ground mass and damage was positive, suggesting that damage may result in increased above ground mass (Fig. 3.4b). This relationship was not statistically significant ($p=0.076$) and could therefore be due to random variation in plant health unrelated to insect damage. By removing two plants, one with low levels of damage and low above ground mass and one with high levels of damage and high above ground mass, the relationship becomes non-significant ($F=0.325$, $p=0.575$).

3.4. Discussion

The relationship between the number of *P. guérini* larvae and leaf area consumed suggests that the optimal density of *P. guérini* was 10 individuals per plant. Densities of 20 larvae per plant did not result in more leaf area being consumed than did ten larvae per plant. At densities of 20 larvae per plant the quality and quantity of the host plant could either not sustain the larval densities which the plants were exposed to, or resulted in decreased per capita larval feeding. The results suggest that larval densities of above ten larvae per plant will not consume significantly more leaf area or that the larval densities are unobtainable due to density dependent larval mortality.

Defining a single *P. aculeata* plant in the field is difficult (Chapter 2) and plants growing in the field are not comparable to those used in this experiment, so the number of *P. guérini* larvae per unit mass of *P. aculeata* leaf tissue is a more appropriate measure to extrapolate into the field. An optimal density of 14.38 *P. guérini* larvae per 100g *P. aculeata* leaf tissue was calculated using the mean of the control plants as an average leaf tissue mass for plants in the experiment. This is the lowest *P. guérini* density at which the maximum damage is done to the plant. At higher densities, *P. guérini* is no more damaging and is likely

to be affected by intraspecific competition. Plants were grown under optimal conditions so it may not be possible to sustain these densities in the field due to lower plant health. Densities observed at field sites where *P. guérini* has established were much lower than this figure (personal observations). The assumption that the *P. guérini* densities used in the experiment were equal to or higher than reasonable maximum densities expected in the field is therefore valid.

Adult *P. guérini* were not included in this impact assessment because adult damage is thought to have relatively little impact on plant growth compared with larval damage. Larvae tend to feed on vigorously growing tissue and disrupt plant growth, while adults tend to feed on older leaves which are likely to be of less importance to overall plant health. It is unlikely that the exclusion of adult damage had a significant effect on the impact levels measured in this experiment.

Damage done by *P. guérini* did not result in reduced plant growth parameters, even at high levels of damage. The impact of *P. guérini* on leaf tissue and young shoots was not concealed by including the stem in the above ground biomass parameter, so the reported parameters in this chapter are appropriate. No reduction in any plant parameters, including leaf mass, were associated with increased *P. guérini* density or area damaged. The damage curve for *P. guérini* resembles that of a biological control candidate that has little effect on plant performance, suggesting that *P. guérini* is an ineffective agent (Fig. 3.1) (McClay & Balciunas 2005). Although *P. guérini* may indeed be an ineffective agent the assumptions made in this experiment that conditions in the greenhouse are comparable to the field and that the experiment was conducted over a long enough time period should be discussed.

The period of time over which the experiment was conducted may have been too short to result in significant differences between treatments. The experiment was terminated after three generations of larval feeding because plants in the higher density treatments appeared too damaged to maintain insect populations. Long-term exposure to *P. guérini* herbivory may have resulted in depletion of resources which could affect the growth parameters measured in this study but *P. aculeata* plants would have needed to recover before *P. guérini* populations could survive on the plants again. It is equally possible that

under the optimal conditions which the plants were grown the damage of *P. guérini* has no effect on plant growth or fitness. *Pereskia aculeata* may be capable of mitigating the damage done by *P. guérini* under these high nutrient and low stress conditions. High nutrients have been shown to reduce the impact of biological control agents for the aquatic weed *Eichhornia crassipes* (Mart.) Solms (Pontederiaceae) so that plant fitness can be reduced by biological control agents at low nutrient levels but not at high nutrients levels (Coetzee *et al.* 2007b).

Phenrica guérini would be an ineffective agent if field conditions were similar to those that the plants were exposed to during this experiment and if interspecific plant competition were absent, but plant health is likely to be lower in the field and competition with native plants may be an important source of stress. The data from this experiment may be useful if used to compare the relative impacts of different potential biological control agents, or combinations of control agents (McClay & Balciunas 2005), but should be interpreted with caution when used to predict whether an agent will have an impact in the field or not. If an impact is recorded at realistic biological control agent densities under ideal growing conditions one can conclude that the agent is likely to have an impact in the field, but if no impact is recorded it cannot be concluded that the agent will be ineffective because the agent's impact on plants growth may increase under suboptimal conditions and under stress of interspecific plant competition.

Laboratory based impact studies of this kind could be improved by including measurements of plant competition and plant health. Plant health can be indirectly manipulated by varying watering and fertilization regimes between treatments. Plant competition and the effect of nutrients was included in impact assessments of the biological control agent *Eccritotarsus catarinensis* (Carvalho) (Heteroptera: Miridae), on water hyacinth, *E. crassipes* (Coetzee *et al.* 2005, Coetzee *et al.* 2007b). The fact that the results of these studies were informative and relevant to water hyacinth management strategies should be seen as evidence for the usefulness of employing these techniques in impact assessment studies for other weed species.

It is evident, from both the greenhouse based impact study and observations in the field, that new biological control agents for the control of *P. aculeata* are needed (Chapter 6).

The impact of new potential biological control agents could be compared with that of *P. guérini* so that the relative impacts of the potential agents are known. Only biological control agents that are likely to have greater impacts than *P. guérini* should be considered for release.

The interactions between new biological control agents and *P. guérini* should also be taken into account because potential negative interactions can lead to reduced biological control efficacy (Ehler & Hall 1982, Crowe & Bourchier 2006). If a potential biological control agent has a greater impact on *P. aculeata* than *P. guérini* and there is a possibility that negative interactions between the two agents could reduce the efficacy of the new agent, then mass rearing and releases of *P. guérini* should be terminated. New potential agents may also interact positively, resulting in cumulative stress that could increase biological control efficacy (Hoffmann & Moran 1999, Seastedt *et al.* 2007, Turner *et al.* 2010). By conducting impact studies using different combinations of potential biological control agents and *P. guérini* the most effective combinations could be determined.

The results presented in this chapter suggest that *P. guérini* is an ineffective agent that has little impact on the growth of *P. aculeata*, but observations at one site (Wewe Dam. 29°32'27.7"S 31°08'06.4"E) contradict this finding. Considering the constraints of this type of impact study, the results should be interpreted with caution and all relevant field observations should be taken into account. The impact of *P. guérini* observed at Wewe Dam indicates that the insect is capable of impacting the plant under certain conditions and should therefore not be discarded as a biological control agent. Mass rearing and releases of *P. guérini* should continue unless a significantly more effective agent becomes available in the future. Release effort should focus at sites where pre-release studies to determine the impact of *P. aculeata* on native plant biodiversity have been conducted and should be followed by post-release evaluations that include measures of insect parameters, *P. aculeata* density and native plant biodiversity (Chapter 2).

Chapter 4

Using molecular methods to determine the origin of weed populations of *Pereskia aculeata* in South Africa and its relevance to biological control

Abstract

New biological control agents are required in order to reach and sustain an adequate level of control of the declared environmental weed *Pereskia aculeata* in South Africa. Identifying the origin of weed genotypes has been important in a number of biological control programmes and is likely to be of importance for the control of *P. aculeata* due to its disjunct native distribution and morphological polymorphisms between plants from different regions of the native and introduced distribution. DNA sequencing of the *trnL* chloroplastic intron and the *phyC* nuclear gene indicate that the South African weed population's origin was in the southern region of native distribution. Inter-Simple Sequence Repeats (ISSRs) confirmed this result and add resolution to the analysis, indicating that the native plants with the closest genetic distance to the South African weed population were found in Rio de Janeiro Province, Brazil. The relationship between the South African weed population and garden variety plants, as well as the large genetic distance between the South African plants and the native plants suggests that the South African population may be the progeny of escaped garden variety plants that have been cultivated and possibly hybridized with other genotypes. The low levels of genetic variation within the South African population and the monophyly of the South African plants indicates that these plants are the progeny of a single introduction or multiple introductions from the same source. Rio de Janeiro Province in Brazil is the most appropriate region in which to survey for new biological control agents.

4.1. Introduction

Determining the origin of weed populations has been important in a number of weed biological control programmes due to polymorphisms within species complexes (Spies & Stirton 1982, Cilliers & Nesser 1991, Madeira *et al.* 1997, Von Senger *et al.* 2000, Strathie and Zachariades 2000, Barker *et al.* 2005, Ye *et al.* 2004, Gaskin *et al.* 2005, Goolsby *et al.* 2006, Madeira *et al.* 2007, Hufbauer & Sforza 2008). Plant genotypes may have varying levels of resistance to herbivorous insects (Höglund *et al.* 2005) making some plant genotypes more susceptible than others to certain biological control agents.

The selection of biological control agents for the programme against *P. aculeata* is likely to be influenced by the origin of the weed which has a disjunct native distribution (Fig. 1.3). Local adaptations can lead to insects being more damaging on plants from populations with which they co-evolved (Kniskern & Rausher 2001, Goolsby *et al.* 2006). This is important because plants from different populations may differ in susceptibility to biological control agents as well as herbicides (Wain *et al.* 1985, Nissen *et al.* 1995). Determining the origin of the South African *P. aculeata* population is therefore an important step towards successful biological control.

Pereskia aculeata is a polymorphic species that includes a number of different varieties, including a number of variegated garden cultivars (Leuenberger 1986). Plants from the northern region of the native distribution have spineless fruits, weakly spiny receptacles and white stamen filaments. The leaves of the plant in the northern region vary from narrow to broad but are generally broader than those in the southern region (Leuenberger 1986). This variation in leaf morphology may partially be due to introductions of plants from other regions of the native distribution (Cicero 1978). Plants in the southern region generally have narrow leaves, spiny receptacles and fruits and carmine-red stamen filaments (Leuenberger 1986). Plants in South Africa have characters from both areas of native distribution, including narrow leaves, spiny fruit and white stamen filaments. There are many garden varieties of *P. aculeata* that have yellow-red upper leaf surfaces with red undersides or variegated pink and yellow leaves (Britton & Rose 1919, Leuenberger 1986). The

morphological characteristics of *P. aculeata* in South Africa cannot be used to determine the region of origin of the South African weed population because the plants share characters from both regions of native distribution.

Levels of genetic diversity within weed populations usually represent only a fraction of the overall genetic diversity of the native plant population due to the limited number of introductions (Burdon & Marshall 1981). The primary mode of reproduction will affect the levels of genetic diversity within these already genetically depauperate populations and this will have an effect on the success of biological control. Predominantly asexually reproducing weeds have a significantly greater chance of being controlled than weeds with higher levels of sexual reproduction (Burdon and Marshall 1981). *Pereskia aculeata* reproduces both sexually and vegetatively and although the relative importance of each mode in the South African population is unknown the levels of seed viability in South African plants indicates that sexual reproduction could be important (Campbell 1988). The effect of sexual reproduction and potential multiple introductions from different source populations could lead to relatively high levels of genetic diversity within a weed population. Knowledge about the genetic diversity of weed populations is important because susceptibility to control may differ between genotypes of the weed in populations with high levels of genetic diversity (Wain *et al.* 1985, Nissen *et al.* 1995).

In this chapter, DNA sequencing and Inter-Simple Sequence Repeats (ISSRs) were used to determine the origin of the South African *P. aculeata* population, with the purpose of identifying locations within the native distribution where potential biological control agents with the highest efficacy on the South African plant genotypes will be present.

4.2. Materials and Methods

4.2.1 Sampling

Stem cuttings of *P. aculeata* were taken from 13 plants in Argentina, 10 in Brazil, two in Venezuela, five in Dominican Republic and 10 in South Africa (Fig. 4.1, Fig. 4.2).

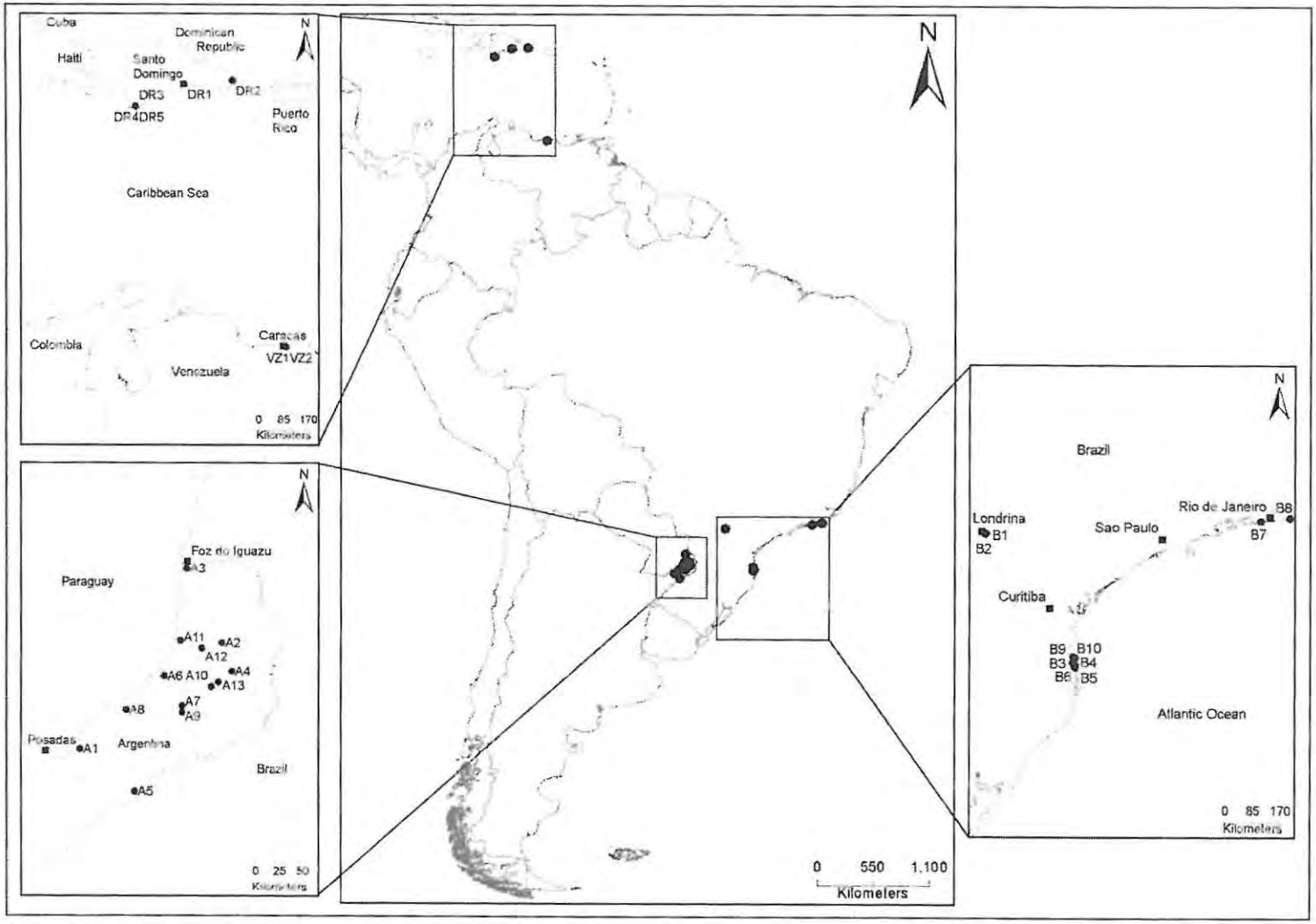

Figure 4.1. Sampling sites in southern and northern regions of native distribution.

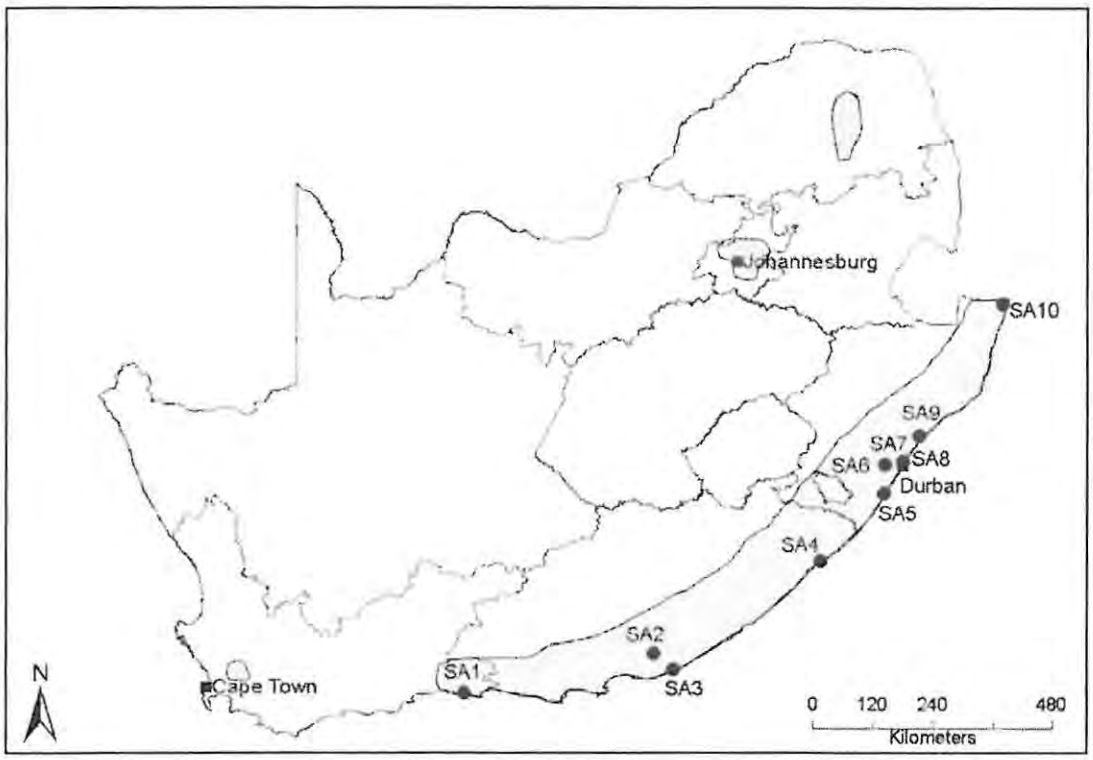

Figure 4.2. Sample sites in the introduced region of distribution in South Africa. The shaded area indicates the introduced distribution (After Henderson 2001).

The cuttings were planted in pots in the quarantine facility at Rhodes University, Grahamstown, South Africa, and kept as a supply of fresh material. The growing tips of new shoots were used for DNA extraction. *Pereskia grandifolia* Haworth (Cactaceae) collected at Durban Botanical Gardens, South Africa, was used as an outgroup for the phylogenetic analyses.

Samples collected from sites covering the widest geographic distribution of both the native and introduced range were used in order to maximize the number of populations sampled and sample the largest genetic diversity (Pons & Petit 1995, Downie *et al.* 2001, Gaskin *et al.* 2005). Although there is a risk that important genotypes within populations were not sampled (Muirhead *et al.* 2008) it is argued that, given the nature of the study, sampling a wider area was more important than sampling within populations.

A small piece (100mg wet weight) of a growing leaf tip was sliced finely using a sterile razor blade and then crushed in the AP1 buffer of the Qiagen DNeasy® Plant Mini Kit (Qiagen Inc., Valencia, CA) using a micro-pestle. DNA was then extracted according to the manufacturer's protocol.

4.2.2 *DNA Sequencing and analysis*

The *trnL* (UAA) cpDNA intron was amplified using universal primers B49317 and A49855 of Taberlet *et al.* (1991). The PCR cycling profile had an initial denaturing step of 5 min at 95°C followed by 30 cycles of 95°C for 1 min, 50°C for 1 min and 72°C for 2 min and a 5 min extension at 72°C.

The nuclear gene phytochrome C (*phyC*) was amplified using primers phyC-F and phyC-R from Mathews and Donoghue (1999). The optimum magnesium chloride concentration for amplification of this gene was found to be 3,5M. The touchdown PCR protocol for amplification of this region was taken from Edwards *et al.* (2005). The PCR products were then re-amplified due to the extremely low yield from the touchdown PCR protocol. A 5 min denaturing step at 95°C was followed by 40 cycles of 95°C for 1 min, 46°C for 1 min and 72°C for 2 min and a final extension of 5 min at 72°C. The re-amplification of the PCR products produced bright bands adequate for cycle sequencing.

PCR products were cleaned up using the MinElute™ PCR Purification Kit (Qiagen Inc., Valencia, CA) or using the Centri-Sep™ protocol (Princeton Separations, USA) at Stellenbosch University, South Africa. Cycle sequencing reactions were done using BigDye Terminator ver. 3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) with the same primers used as in the PCR reactions with the addition of two internal primers for the *phyC* region (phyC454-R and phyC701-F) from Edwards *et al.* (2005). Cycle sequencing products were purified using ethanol-sodium acetate precipitation. Capillary electrophoresis was done at Rhodes University using a ABI 3100® genetic analyser and at Stellenbosch University using a ABI 3130® genetic analyser.

After careful examination of chromatograms contiguous sequences were assembled and manually edited in GeneStudio™ ver. 1.03.72 (GeneStudio, Inc.). Alignment of sequences was done in MEGA ver. 3.1 (Kumar *et al.* 2004) using ClustalW set to default parameters. A statistical parsimony haplotype network of the combined data set from the *trnL* intron and *phyC* was produced using TCS ver. 1.21 (Clement *et al.* 2000) with gaps in the alignment set as a 5th character.

4.2.3 *Inter-Simple Sequence Repeats (ISSR) PCR protocol and analysis*

Fifteen universal ISSR primers were tested of which all but HB14 produced replicable bands (Table 4.1). Primers were designed by Andrea Wolfe and Harvey Ballard (Wolfe *et al.* 1998, McCauley & Ballard 2002, Yockteng *et al.* 2003). All reactions were done at a 2,5mM magnesium chloride concentration and the PCR protocol had an initial denaturing step of 2 min at 94°C followed by 35 cycles of 94°C for 30 sec, 44°C for 45 sec and 72°C for 90 sec and a final extension of 20 min at 72°C. PCR products were run at 80 V in 0.5 X Tris-boric acid-EDTA (TBE) buffer on 1,5% agarose gels for 5 h 30 min. Gels were stained in ethidium bromide and images were acquired with a Bio-Rad ChemiDoc system (Bio-Rad, USA). Reactions were replicated to verify reproducibility and only bands that were consistently observed in all PCR reactions were scored. Replicable bands were scored for presence or absence and the binary matrix was converted to a distance matrix in the software programme FreeTree (Hampl *et al.* 2001) using the Jaccard's coefficient. Although the use of the Jaccard's coefficient has been criticised (Lamboy 2008) it is considered an appropriate coefficient for this study as negative matches are not incorporated into the coefficient (Sokal & Sneath 1963). The absence of a band does not necessarily imply homology and the Jaccard coefficient excludes shared absences as a character. Using Nei and Lei's co-efficient did not have a significant effect on the analysis. A neighbour-joining (NJ) tree with bootstrap support (1000 replications) was constructed from the distance matrix using FreeTree (Hampl *et al.* 2001).

Table 4.1. ISSR primer sequences tested for *P. aculeata*. All primers produced replicable bands with the exception of HB14.

Primer Name	Primer Sequence (5' to 3')	Number of Replicable Bands
814	CTCTCTCTCTCTCTCTTG	6
17898A	CACACACACACAAC	9
17898B	CACACACACACACACAGT	4
17899A	CACACACACACAAG	13
17899B	CACACACACACAGG	9
844A	CTCTCTCTCTCTCTCTAC	4
844B	CTCTCTCTCTCTCTCTGC	6
HB8	GAGAGAGAGAGAGG	5
HB9	GTGTGTGTGTGTGG	10
HB10	GAGAGAGAGAGACC	10
HB11	GTGTGTGTGTGTCC	6
HB12	CACCACCACGC	5
HB13	GAGGAGGAGGC	8
HB14	CTCCTCCTCGC	0
HB15	GTGGTGGTGGC	5

The binary matrix was analysed by maximum parsimony (MP) in PAUP* ver. 4.0 beta 10 (Swofford 2003) by heuristic search with 500 replications of random sequence addition and no limit on maxtrees. Bootstrap support for nodes used 500 bootstrap replications with 10 replications of random sequence addition for each bootstrap replication and maxtrees = 100 000 due to computing constraints. Bayesian analysis was also performed using MrBayes ver. 3.1 (Huelsenbeck & Ronquist 2001) with four Monte Carlo Markov Chain runs with 5×10^6 generations per run using the default settings for standard data. Trees were sampled every 100 generations. Stationarity was reached before 10^6 generations and the first 10 000 trees were excluded from the analysis before generating a consensus tree.

Principle Component Analysis (PCA) of the binary presence/absence data was used to create a covariance matrix and to construct a scatterplot, using PAST: Paleontological statistics package ver. 1.81 (Hammer & Harper 2001). Genetic distances were partitioned

into populations identified by the above analyses using Mega ver. 3.1, and a t-test and ANOVA tested for significant differences between populations (native vs. introduced, and native north vs. native south vs. introduced).

4.3 Results

Alignments of both the *trnL* intron (Genbank accessions EU926671 – EU926704) and the *phyC* gene (Genbank accessions EU926705 – EU926736) showed minimal variation. The *trnL* intron sequences were trimmed to 631 bp and the *phyC* gene sequences were trimmed to 1112 bp. Out of the total of 1743 bp there were only 12 variable sites. Six variable sites were in the *trnL* intron and six were in the *phyC* gene.

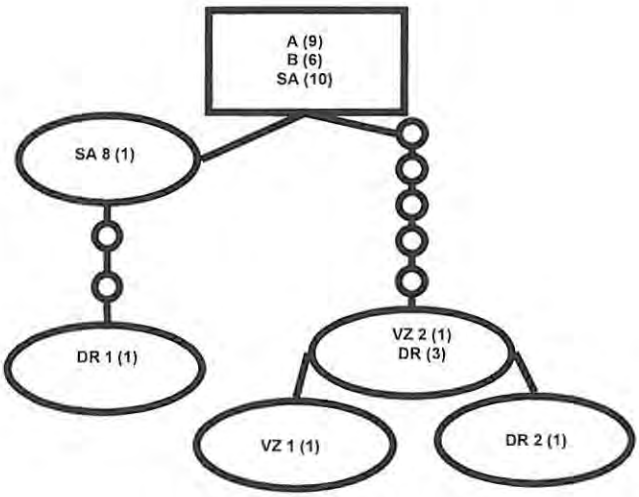

Figure 4.3. Statistical parsimony haplotype network constructed in TCS 1.21 (Clement *et al.* 2000). Numbers in parenthesis represent the number of samples from each country in that haplotype. The open circles correspond to additional mutational steps between the haplotypes detected.

The sample contained six haplotypes. All the South African plants, with the exception of SA8 which is a variegated garden variety plant, were the same haplotype as all the plants

from the southern region of native distribution (Fig. 4.3). Plants from the northern region of the native distribution all form a group six to seven mutational steps from the South African and southern native haplotype. Three plants from the Dominican Republic (DR2, DR4, DR5) and one plant from Venezuela (VZ2) are part of a single haplotype within the northern native distribution group. Sample DR3 and VZ1, from the Dominican Republic and Venezuela respectively, have a single mutational difference to the other northern haplotype but the mutations are not at a shared site (Fig. 4.3). The South African variegated garden variety plant (SA8) shares a mutation in the *Trnl* intron with the garden variety plant from Santo Domingo Botanical Gardens in the Dominican Republic (DR1), but DR1 also has three novel mutations, two of which are in the *Trnl* intron and a single mutation is in the *PhyC* gene (Fig. 4.3).

The 14 ISSR primers produced 100 replicable bands (Table 4.1). In order to match native population haplotypes to the South African population haplotypes a NJ tree was constructed from the ISSR data. The South African plants are part of a southern group, confirming the results from the sequencing data (Fig. 4.4). Plants in group 1 are plants from the northern region of the native range. This group was strongly supported in the NJ analysis but was not as strongly supported in the MP and Bayesian analyses. Within the northern native group plants from Dominican Republic form a strongly supported group and the plants from Venezuela form a separate strongly supported group in all three analyses. The South African plants form a strongly supported distinct group within the southern group (Group 2). The South African plants are included in a poorly supported group with the two garden variety plants (DR1 and SA8). Two Brazilian plants from Rio de Janeiro Province (B7 and B8) fall in as a sister group (Group 3) to this South African and garden variety group with no statistical support. Group 4 is a poorly supported group containing all plants from Brazil and Argentina with the exception of the plants from Rio de Janeiro Province that form group 3. There is little support for any groupings within this southern native group but four plants from Curatiba Province, Brazil form a group (B3, B4, B5 and B6) and the two plants from Londrina region (B1 and B2) fall into a group with a plant from Misiones, Argentina (A9) and, with less support, with two additional plants from Misiones, Argentina (A10 and A11).

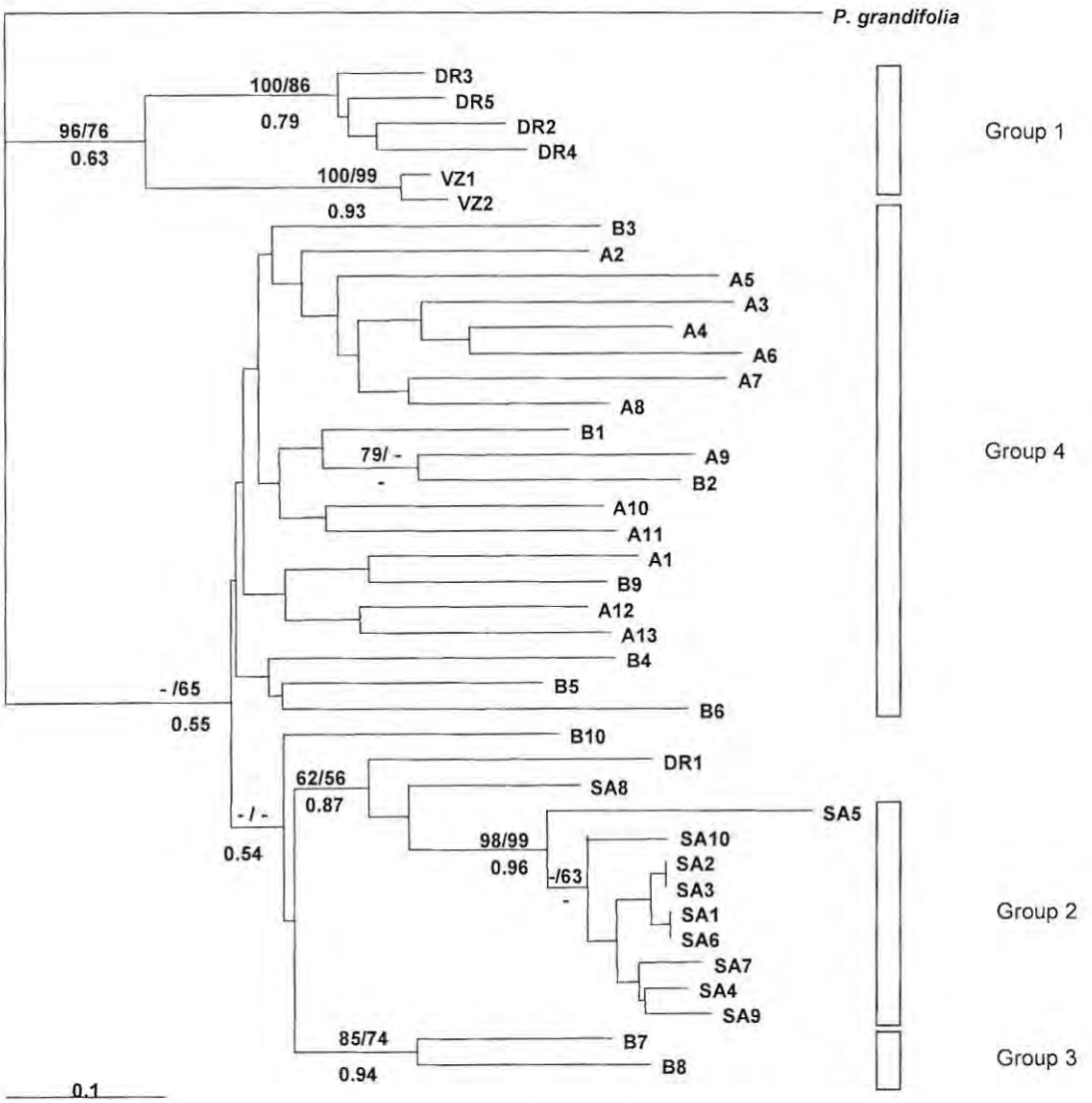

Figure 4.4. Neighbour-joining tree constructed from ISSR data. Bootstrap values and posterior probabilities lower than 0.5 are excluded. NJ bootstrap values/parsimony bootstrap values are given above each node and Bayesian posterior probabilities are given below each node. Removing the garden variety plants from the analysis does not significantly affect the relationship between the plants from Rio de Janeiro Province and plants from South Africa.

The PCA distinguished three major groupings. Group 1 consists of all the plants from the northern region of native distribution, group 2 consists of the South African plants and group 3 consists of the plants from the southern region of native distribution as well as the two garden variety plants (DR1 and SA8). The most closely related plants to the South African population are the two garden variety plants followed by the two plants from Rio de Janeiro Province (B8 and B7) and a plant from the Londrina region, Brazil (B2) (Fig. 4.5).

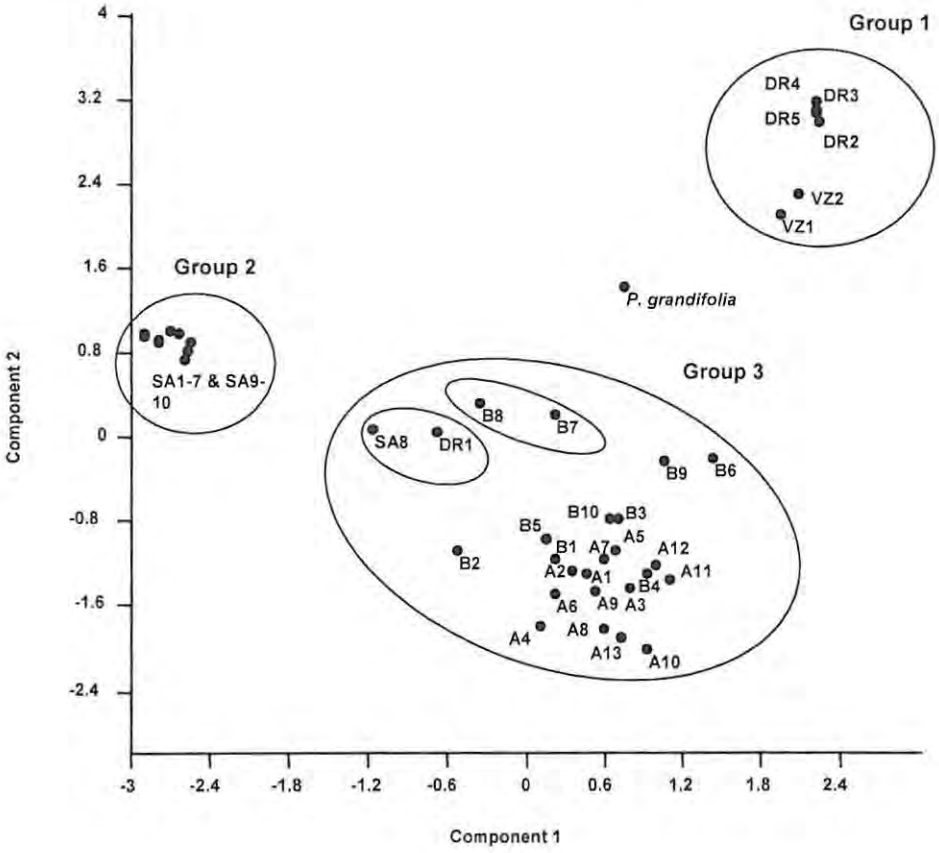

Figure 4.5. Principle components plot from ISSR binary data. 21.9% of the variation is explained by component 1, 19.1% by component 2 and 6.1% by component 3. Component 3 and all other components excluding 1 and 2 are not included as they explain insufficient variation and therefore have little influence on the interpretation of the figure.

Although the sampling methods used in this study did not sample within populations, comparisons of genetic distances within different regions were informative indicating low levels of genetic diversity in the introduced population. The average genetic distance within the South African population was 0.109 while the average genetic distance within the native region was 0.516. The average genetic distance between the introduced and native range was 0.548. Average genetic distance within the southern native region was 0.480 and the average within the northern range was 0.280. The average genetic distance between the southern and northern native region was 0.602. An ANOVA showed significant differences of genetic diversity ($F_{2,301} = 393.709$, $p < 0.001$) and a LSD post hoc test showed significant differences between all three populations ($p < 0.001$). A t-test between the native and introduced plants showed significant differences in genetic diversity ($t = 22.9884$, $df = 440$, $p < 0.001$).

Plants from native populations with the closest average genetic distances to the plants in the South African population include the two plants from Rio de Janeiro Province (B7 and B8, genetic distances 0.435 and 0.429) and the two plants from the Londrina region, Brazil (B1 and B2, genetic distances 0.476 and 0.479).

4.4 Discussion

Based on the results presented here, the origin of the South African weed population of *P. aculeata* is in the southern region of the plant's native distribution. Although the levels of variation in both the *trnL* intron and the *phyC* gene were extremely low these sequencing data provide important support for the ISSR data, which provide much higher resolution. The ISSR data indicate that the most similar native plants to the South African weed population are those in the north of the southern region of native distribution in Rio de Janeiro Province, Brazil (Samples B7 & B8). Phytophagous insects and pathogens collected in this region are most likely to have local adaptations that will suit the South African weed genotype and are therefore expected to have the highest efficacy as biological control agents (Kniskern & Rausher 2001, Goolsby 2006). The coastal region north of Rio de Janeiro should be the starting point for surveys for biological control agents. Surveys should continue further north

along the coast because plants here will not be genetically isolated from those sampled in this study and will therefore be genetically most similar to the South African plants.

However the large genetic distance between the South African weed population and the native populations is an unexpected result and has important implications for biological control. The average genetic distance between plants in the two regions of native distribution (0.608) is very similar to the average distance between the South African and northern region of native distribution (0.602) and the South African and southern region of native distribution (0.533). The South African plants are therefore genetically distinct and have similar distances from the native plants as the two native regions plants have to each other. This is unexpected as the first plants of the South African weed population were probably introduced within the last 160 years which is a fraction of the time that would be expected for such distinct genotypes to arise. One probable explanation for the large genetic distance between introduced and native plants is that the true source population of the South African plants was not sampled. Although a wide range of the native distribution was sampled the results indicate that plants further north from the plants in Rio de Janeiro Province may be closer matches to the South African genotypes. It is also possible that the South African plants are the progeny of a rare haplotype found within the regions sampled.

The South African weed population may also be the progeny of escaped garden variety plants. *Pereskia aculeata* has been a garden plant in England since 1696 (Briton & Rose 1919), so it is possible that cultivation and hybridization has lead to garden variety plants becoming genetically distinct from the original native populations. This is supported by the fact that the two garden variety plants are the closest relatives to the South African population, and by the fact that the first *P. aculeata* recorded in South Africa was a plant at a botanical garden that was most likely taken from the collection of another botanical garden outside South Africa (McGibbon 1858). This has important implications for biological control because insects with local adaptations to any one native plant genotype will have low efficacy on the South African genotype but may be the most effective agents for *P. aculeata* that can be obtained because the introduced population is so distinct.

The low levels of genetic diversity and the monophyly of the South African plants in the NJ, MP and Bayesian analyses indicate that the weed population is the progeny of a single introduction. Biological control has been more successful when used against weed species with low levels of genetic diversity and this has been suggested as a criterion for choosing which weed species should be targeted for biological control (Burdon & Marshall 1981, Nissen *et al.* 1995).

Low levels of genetic diversity within the weed population are promising for biological control, while the large genetic distance between native source populations and the South African weed population may be problematic. Although genetic techniques indicate the region of origin of weed genotypes, and therefore help identify insects and pathogens that have local adaptations to that genotype, there is no indication of the efficacy of biological control agents collected off plants of a different genotype. Insects and pathogens collected on different plant genotypes may have the same levels of efficacy on all plant genotypes due to a lack of local adaptations at this level. For this reason potential biological control agents should not be rejected on the bases that they are not found at the source population. This is especially relevant in the case of *P. aculeata* due to the great genetic distance between the source population and the South African weed population. Potential biological control agents should, however, be collected in the region of origin of the weed population whenever possible and surveys to identify new potential agents should be focused on this region.

Chapter 5

The effect of *Pereskia aculeata* intraspecific genetic variation on the fitness of *Phenrica guérini*

Abstract

Variation in insect fitness related traits when developing on different host plant genotypes with known genetic relationships was tested using the monophagous biological control agent *Phenrica guérini* and its host plant, *Pereskia aculeata*. Differences in insect fitness among tested host plant genotypes were expected because of the geographic isolation and genetic distances between plant genotypes, and because *P. guérini* has been associated with some genotypes while other genotypes have never been exposed to herbivory by the insect. There was little variation in insect fitness related traits among plant genotypes and no differences in the insect's ability to utilize different plant genotypes. The host range of *P. guérini* included all the plant genotypes used in the study and no specialization to any one genotype was detected. The results of the study suggest that, in some cases, biological control agents collected from the wild genotype most closely related to the target weed population will not always be more effective than those collected off more distantly related genotypes.

5.1 Introduction

The majority of phytophagous insects are relatively host specific, with food plants in one or a few genera or a single family (Bernays & Graham 1988). Specialization of phytophagous insects is usually attributed to coevolution or reciprocal adaptations between herbivorous insects and their host plants driven by plant chemistry (Ehrlich & Raven 1964, Futuyma 2000), but other mechanisms by which specialization may be selected for have been proposed (Bernays & Graham 1988, Jermy 1993). Levels of host specialization in phytophagous insects are not limited to preferences for certain plant genera or even species. Many insect species show variation in biological traits between plant varieties within a single species (Karban 1992), and some insect species show variation in performance between individual plant genotypes (Edmunds & Alstad 1978, Karban 1989, Mopper & Strauss 1998, Egan & Ott 2007).

Phytophagous insects often have much shorter generation times than their host plants leading to local specialization of insect populations to certain plant genotypes (Edmunds & Alstad 1978, Karban 1992, Alstad 1998). This can result in variance between insect populations in their ability to utilize host plants from different geographical regions (Fox & Morrow 1981, Goolsby *et al.* 2006). The levels of variation in the ability of the insect herbivores to utilize certain host plant genotypes are likely to depend on the variation in levels of plant defenses between plant genotypes. Defense mechanisms against specific herbivores may evolve in plants if the herbivore has a negative effect on plant fitness. Many herbivorous species have negative effects that are considered strong enough to select for plant defenses, but in some cases herbivores are so rare that the selection pressure to acquire defense mechanisms will be outweighed by the cost of acquiring the defense characteristic (Jermy 1993, Futuyma 2000). Variation in insect fitness between host plant genotypes is therefore expected to differ between insect species.

Sedentary insect species which are more likely to have multiple generations on a single individual host plant are likely to develop specializations to individual genotypes due to reduced gene flow between insect populations on individual plants (Karban 1989, Karban

1992, Hanks & Denno 1994). Asexually reproducing plant species are more likely to have insect populations specialized to individual genotypes because a single genotype can become abundant and can persist for long periods, allowing multiple insect generations on a single genotype.

Variation in the ability of insect populations to utilize certain host plant varieties or genotypes has important implications for biological control of weed species (Spies & Stirton 1982, Cilliers & Nesser 1991, Madeira *et al.* 1997, Von Senger *et al.* 2000, Strathie & Zachariades, 2000; Barker *et al.* 2005, Ye *et al.* 2004, Gaskin *et al.* 2005, Goolsby *et al.* 2006, Madeira *et al.* 2007, Hufbauer & Sforza 2008, Chapter 4). Some weed species are genetically diverse or have large and sometimes disjunct, native distributions (Chapter 4), increasing the probability of insect populations becoming locally adapted to specific plant varieties, populations or genotypes. Biological control agents that have developed local adaptations to certain plant genotypes in the plants' native distribution may have reduced fitness on different genotypes, or may not accept plant genotypes in the introduced distribution as hosts. It is also possible that weed populations may not be suitable or optimum host plants due to genetic changes which occurred in the early phases of introduction or due to artificial selection as a horticultural plant prior to introduction. Conversely, defenses against insect herbivory may be lost due to horticultural selection, making the weed species more susceptible to insect herbivores (Rosenthal & Dirzo 1997).

Whether phytophagous insects or pathogens which have co-evolved with the weed species are the most appropriate agents for biological control is a debated topic (Hokkanen & Pimental 1984, Goeden & Kok 1986, Dennill & Moran 1989, Dennill & Hokkanen 1990, Ehler 1995). There are two opposing schools of thought: one is that herbivores that have evolved specialized relationships with their host plant will be the most appropriate biological control agents due to adaptations of the herbivore to the host plant. This approach is referred to as the use of 'old associations' (Hokkanen & Pimental 1984). Another school of thought is that the most appropriate biological control agents will be those that accept the host plant but have not co-evolved with it. This approach is known as the use of 'new associations' and is based on the assumption that there is a level of homeostasis between insects and plants that

have co-evolved (Hokkanen & Pimental 1984). Insects that have not co-evolved with the host plant are more effective agents because a level of homeostasis between the herbivore and the plant has not been reached (Dennill & Hokkanen 1990). Although the debate of 'new' vs. 'old associations' has focused on different insect species that are considered either 'new' or 'old associations', the opposing approaches can be tested using a single insect species that is differentially adapted to two different host plant populations.

In this chapter various life history traits of the monophagous biological control agent, *Phenrica guérini*, were measured on different genotypes of the insect's host plant, *Pereskia aculeata*, in order to determine host suitability of the different genotypes. The genetic relationship between the plant genotypes and the original host plant genotype of the insect population are known (Chapter 4). Differences in host plant suitability were expected because of the large genetic divergence and geographic distance between populations in the southern and northern regions of the native distribution (Fig. 1.3), as well as the genetic divergence between native plants and plants in the introduced distribution in South Africa (Chapter 4). Genetic differentiation of the introduced *P. aculeata* population in South Africa from the native populations due to artificial selection as a horticultural plant was also expected to have an effect on insect fitness (Chapter 4).

The aim of this chapter was to determine the effect of plant genotypic variation on phytophagous insect fitness, with the intention of testing the relevance of intraspecific plant variation and plant origin in this plant-herbivore system.

5.2 Methods

5.2.1 Host plant genotype selection

The population of *P. guérini* in South Africa was introduced in 1994 for the purpose of biological control of *P. aculeata*. The population is the progeny of insects collected at coastal sites in Rio de Janeiro Province, Brazil (Klein 1999). The original host plant genotype of the South Africa *P. guérini* population is expected to be similar to plant genotypes collected in the same area based on the relationship between genetic distance and geographic distance in the native distribution of the plant (Chapter 4). Plant genotype B7 (Table 5.1) is therefore considered a surrogate original host plant genotype for the *P. guérini* population in South Africa. The population of *P. guérini* used in this study was mass reared for multiple generations on South African plant genotype SA5. This genotype is considered the host genotype of the *P. guérini* population (Table 5.1). Genotype DR2 was collected from the northern region of the native distribution of *P. aculeata* and is genetically distant from both host plant genotypes (Table 5.1). Genotype SA3, collected from the introduced distribution of *P. aculeata*, is the closest genotype to the host genotype in terms of genetic distance and genotypes B9 and B1, which were collected from the southern region of the native distribution of *P. aculeata*, are closest to the original host genotype (Table 5.1). Three of the samples are from within the native range of *P. guérini*, two are from the introduced range where there has been only a short association between the insect and the plant, and one genotype has not been exposed to *P. guérini* at any stage in the past (Table 5.1). The distribution of *P. guérini* is presumed to be limited to southern Brazil based on localities of specimens present in the Bechyné Alticinae Collection, which is an extensive collection of neotropical Alticinae housed at the Museo del Instituto de Zoologia Agricola (Universidad Central Venezuela, Maracay, Venezuala) (H.E. Erb, unpublished report). Codes used to refer to plant genotypes match the codes used in Paterson *et al.* (2009) and Chapter 4.

Table 5.1: The genetic distances from each genotype to the host and original host genotype were calculated using Inter-Simple Sequence Repeat data (Chapter 4). Codes used to refer to plant genotypes are the same as those used in Chapter 4.

Genotype	Province and country of origin	Region of origin	Replicates	Genetic distance to host genotype (SA5)	Genetic distance to original host genotype (B7)	*Phenrica guérini* population
DR2	Dominican Republic	Northern, Native	9	0.68	0.59	None
B9	Rio de Janeiro, Brazil	Southern, Native	7	0.61	0.38	Native
B1	Paraná, Brazil	Southern, Native	6	0.56	0.41	Native
B7	Santa Catarina, Brazil	Southern, Native	3	0.57	0	Native
SA3	Eastern Cape, South Africa	Introduced	8	0.23	0.41	Introduced
SA5	KwaZulu-Natal, South Africa	Introduced	8	0	0.57	Introduced

5.2.2 *Bioassay between genotypes*

Clones of each genotype were grown from cuttings to produce replicates for each plant genotype. The number of replicates for each genotype was limited by the amount of material available on the original plant from which cuttings were taken (Table 5.1).

Plants were grown in randomly distributed pots in a green house with identical fertilizing and watering regimes until they were of a suitable size for the experiment. The plants were then moved to an indoor growth room as a precaution to reduce predation of larvae by arthropod predators (Growth room average temperatures: Min:25°C, Max:30 °C; 8hrs:16hrs (dark:light)).

Phenrica guérini eggs were collected from a culture established on genotype SA5. On hatching, 20 larvae were transferred onto each replicate of each genotype using a small paintbrush. A mesh sleeve was placed over each plant to insure that larvae were restricted to the correct plant. After 22 days, larvae were removed and placed in ventilated tubs with pupation medium (7:7:6 parts loam:sand:vermiculite). Some *P. aculeata* leaves of the

appropriate genotype were placed in each pupation tub so that the larva could feed until pupation. Larvae were moved into the pupation tubs at 22 days because in trial experiments no larvae pupated before 22 days but most had entered the pre-pupal phase when they no longer fed and had changed in colour from yellow to dark grey-black.

The total leaf area and the area of leaf consumed by *P. guerini* was calculated for each plant by photocopying the leaves and measuring leaf area using WinDias© ver. 2.0 (Delta-T Devices, Cambridge, U.K.). Percentage survival and duration of development to the pre-pupal stage, pupation and eclosion were recorded. The masses of pre-pupal larvae and adults were recorded. Adults were sexed because *P. guérini* is a sexually dimorphic species with higher average female mass compared with males.

Six pairs of adults were released back onto plants of the appropriate genotype, with the exception of DR2 which had three females and six males. The numbers of eggs and of hatched larvae were recorded for each genotype. Genotype B7 was not included in this part of the bioassay due to a lack of adult insects reared on the genotype and lack of plant material.

5.2.3 Statistical analysis

Normality of the distribution of variance in all data sets was tested using the Kolmogorov-Smirnov test and Levene's test for homogeneity of variance. Data that were not normally distributed were log or arcsine transformed. If transformation did not result in normalization of the data, non-parametric methods were used. Analyses of co-variance were performed for mass of males and females in the pre-pupal stage; masses of males and females as adults; percentage survival to pupation; leaf area consumed per unit of pre-pupal insect mass; and Maw's Host Suitability Index (Maw 1976, Czypionka & Hill 2007). Total leaf area was used as a co-variant in all analyses in order to control for plant size. It was appropriate to use total leaf area as a co-variant because a large amount of variation in total plant leaf area was found within individual clones indicating that total leaf area was not genetically predetermined.

A Kruskal-Wallis test was performed to test for significant differences in percentage survival to eclosion.

All statistical analyses were performed using STATISTICA© ver. 8.0.

Maw's Host Suitability Index was calculated using the following equation (from Maw 1976):

Host Suitability Index = (pre-pupal mass)(% pupation) / duration of development to pupation

Maw (1976) used the host suitability index to determine which plant species were superior hosts for *Cassida hemisphaerica* Hbst. (Coleoptera: Chrysomelidae). *Cassida hemisphaerica* was considered a potential biological control agent for bladder campion, *Silene cucubalus* Wibel (Caryophyllaceae), but larvae fed and survived well on some plant species within the genus *Silene*. No single insect parameter provided a reliable measure of host suitability for test plant species that were closely related to *S. cucubalus* but when combined in the index the relative suitability of each plant species was apparent and *S. cucubalus* was clearly identified as the superior host plant (Maw 1976). The assumptions of the index are that insects feeding on more suitable host plants are likely to have greater pupal mass, higher survival rates and shorter developmental times than those feeding on inferior host plants (Maw 1976, Czypionka & Hill 2007).

Leaf area consumed per unit of pre-pupal insect mass was considered an informative parameter because pupal mass is a reliable estimate of fitness for another species of chrysomelid, *Grantiana spadicea* (Klug) (Coleoptera: Chrysomelidae) (Czypionka & Hill 2007) and it is expected that insects feeding on less suitable host plants will require more leaf tissue to reach levels of fitness comparable to those that have developed on more suitable host plants.

Pre-pupal mass was used instead of pupal mass because *P. guérini* forms a pupation chamber out of the soil substrate that would need to be removed in order to measure pupal mass reliably. Removing the pupae from the pupal chamber was likely to result in higher

pupal mortality. Pre-pupal mass was comparable to pupal mass because larvae stop feeding in the pre-pupal phase.

5.3 Results

There were significant differences in total plant leaf area among plant genotypes (ANOVA: $F_{(5,35)} = 4.778$, $p = 0.001$) (Fig. 5.1). Genotype SA3 had significantly larger total leaf surface area than other genotypes, and genotype SA5 had significantly smaller leaf surface area than genotype DR2 (Fisher's LSD Post Hoc Test).Total leaf area was used as a covariant in all analyses of co-variance (ANCOVA) but is only reported when a statistically significant effect for total leaf area was found.

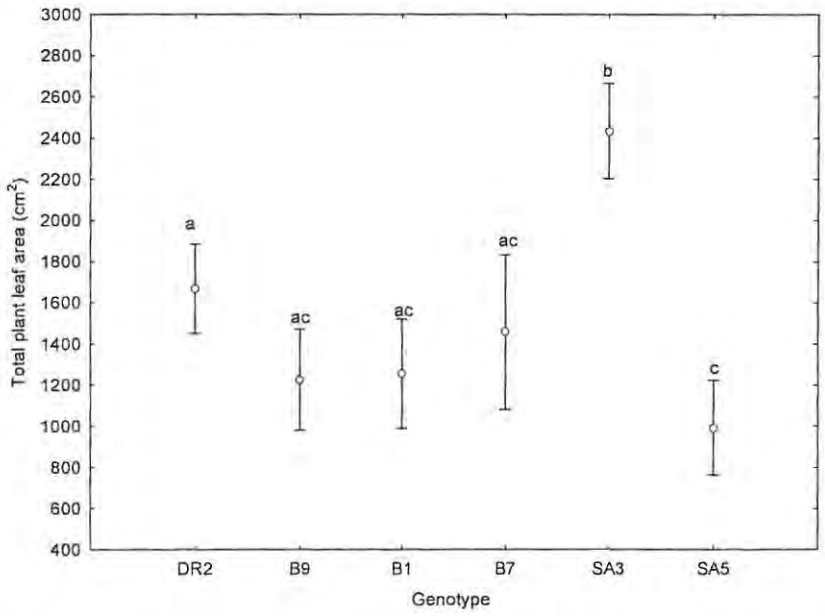

Figure 5.1. Total plant leaf surface area differed significantly among genotypes (ANOVA: $F(5,35)=4.778$, $p=0.002$). Letters indicate statistically significant differences ($p < 0.05$) in total leaf surface area between genotypes according to Fisher's LSD Post Hoc test. Vertical bars delineate standard errors.

There were no significant differences in male pre-pupal larval mass among plant genotypes (ANCOVA: $F_{(5,24)} = 2.085$, $p = 0.102$) but there were significant differences in pre-pupal larval mass of females (ANCOVA: $F_{(5,28)} = 4.309$, $p = 0.005$) (Fig. 5.2). Female *P. guérini* reared on genotypes B9 and B1 were significantly lighter than those reared on genotypes DR2, B7 and SA3 but those reared on genotype SA5 were not significantly different from those reared on any other genotypes (Fisher's LSD Post Hoc test) (Fig. 5.2).

No significant differences in adult female mass were detected among plant genotypes (ANCOVA: $F_{(5,28)} = 0.169$, $p = 0.972$) but there were significant differences in adult male mass (ANCOVA: $F_{(5,23)} = 2.852$, $p = 0.038$) (Fig. 5.3). The mass of adult males reared on genotype SA5 was significantly less than those reared on all other genotypes (Fisher's LSD Post Hoc test) (Fig. 5.3).

Differences in mass among genotypes were not comparable between the pre-pupal stage and the adult stage. This result is unexpected because, with the exception of those that died during pupation, the insects measured in the pre-pupal and adult stage were the same individuals. There were no significant differences in male mass in the pre-pupal stage but significant differences were recorded in the adult stage. The opposite was recorded for female mass where there were significant differences in the pre-pupal stage but not in the adult stage (Fig. 5.2 & Fig. 5.3).

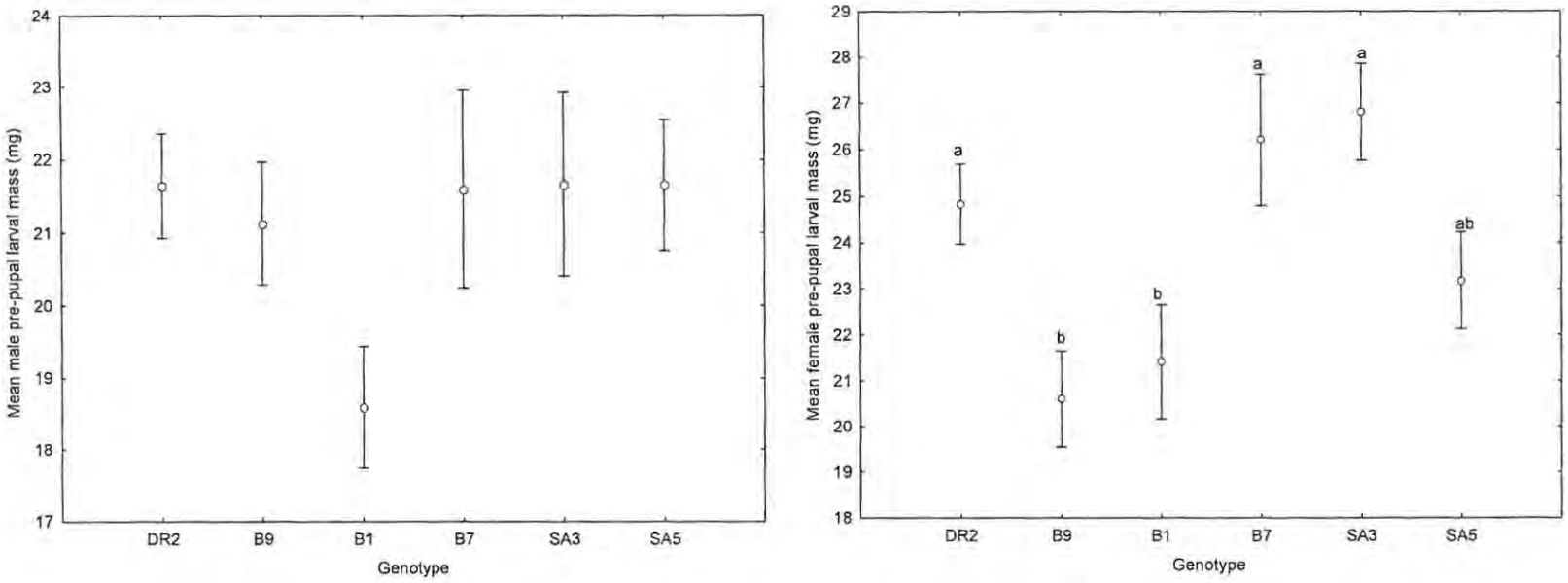

Figure 5.2. Analysis of covariance of pre-pupal larval mass for males and females with total plant leaf area as a covariant. There was no significant difference among genotypes for males ($F_{(5,24)} = 2.0855$, $p = 0.10240$). There was a significant difference between genotypes for females ($F_{(5,28)} = 4.309$, $p = 0.005$). Letters indicate statistically significant differences ($p < 0.05$) in female pre-pupal mass between genotypes according to Fisher's LSD Post Hoc test. Vertical bars delineate standard errors.

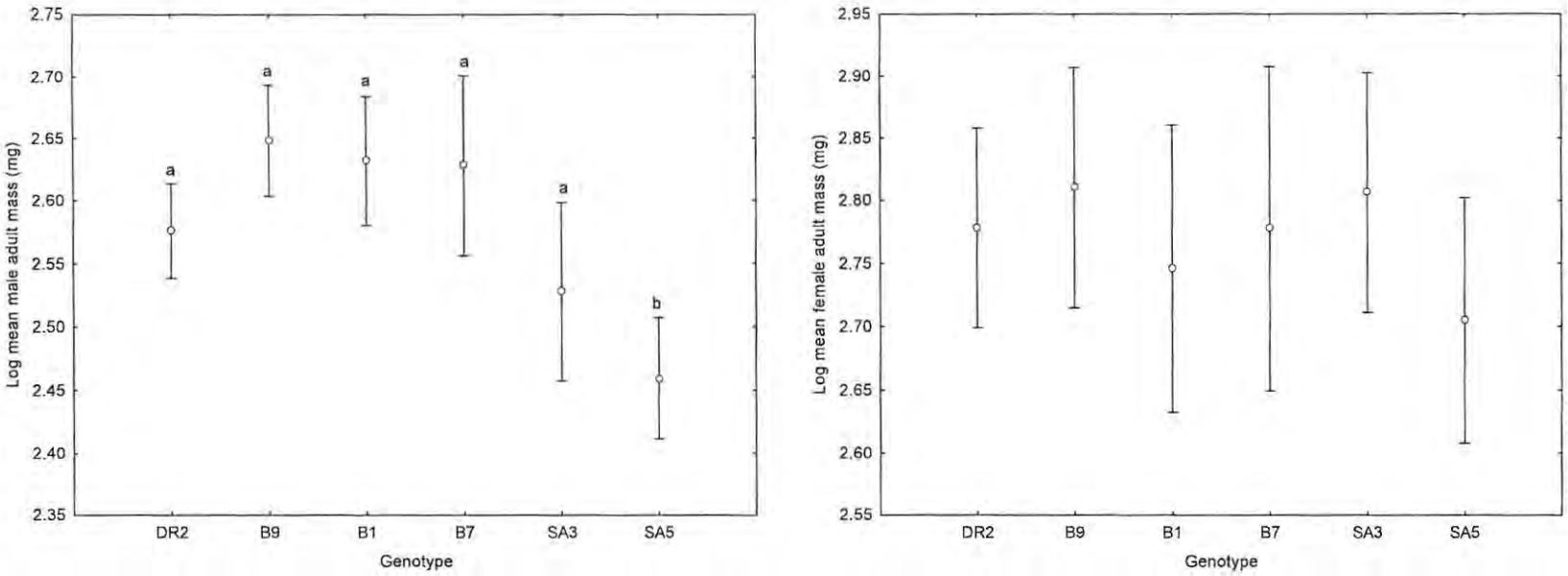

Figure 5.3. Analysis of covariance of adult mass for males and females between genotypes with total plant leaf area as a covariant. There were no significant differences among genotypes in female mass ($F_{(5,28)} = 0.169$, $p = 0.972$) but there was a significant difference for male mass ($F_{(5,23)} = 2.852$, $p = 0.038$). Male *P. guérini* reared on genotype SA5 were significantly lighter than those reared on all other genotypes. Letters indicate statistically significant differences ($p < 0.05$) between genotypes according to Fisher's LSD Post Hoc test. Vertical bars delineate standard errors.

There were no significant differences in percentage survival to pupation (ANCOVA: $F_{(5,34)} = 1.242$, $p = 0.311$) (Fig. 5.4) or percentage survival to eclosion (Kruskal-Wallis test: $H = 1.087$, $p = 0.955$) for *P. guérini* reared on different plant genotypes.

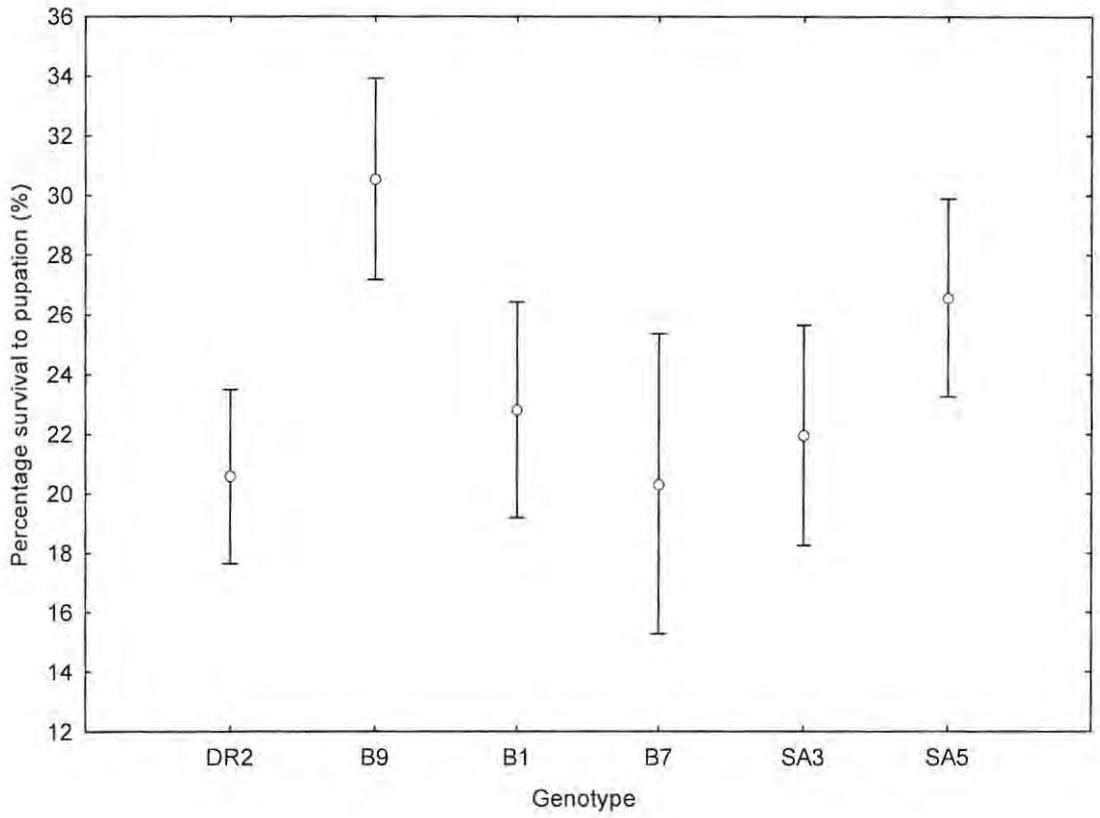

Figure 5.4. Analysis of covariance of percentage survival to pupation between genotypes with total leaf area as a covariant. There are no significant differences in percentage survival to pupation between genotypes ($F_{(5,34)} = 1.242$, $p = 0.311$). Vertical bars delineate standard errors.

Significant differences in duration of development to pupation were detected among genotypes according to a Kruskal-Wallis test (H = 15.077, p = 0.010) but the Kruskal-Wallis Post Hoc test, Multiple Comparisons of Mean Rank, showed no significant differences between any genotypes (Fig. 5.5).

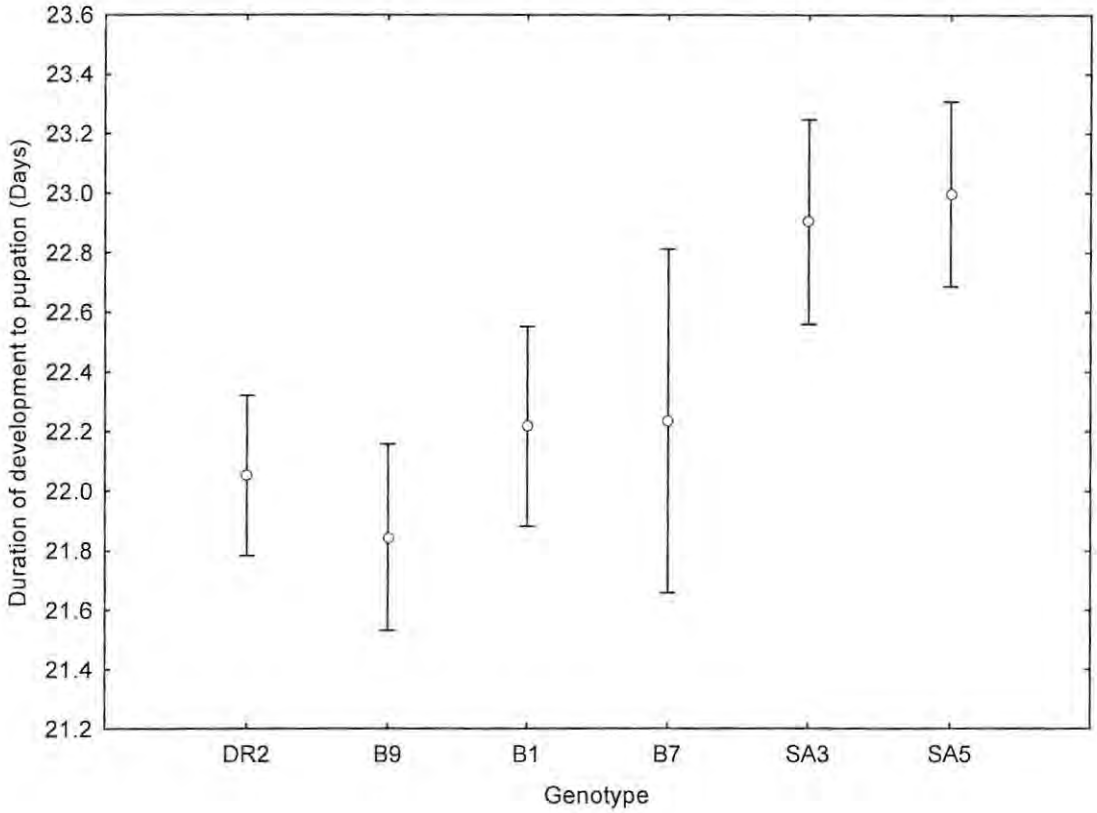

Figure 5.5. Mean duration of development to pupation with total plant leaf area as a covariant. A Kruskal-Wallis test indicated that there were significant differences among genotypes (H = 15.077, p = 0.010) but no significant differences were calculated in the Kruskal-Wallis Post Hoc test, Multiple Comparisons of Mean Rank. Vertical bars delineate standard errors.

No significant differences in the leaf area consumed per mass of pre-pupal larvae were detected among plant genotypes (ANCOVA: $F_{(5,34)} = 0.484$, $p = 0.786$) (Fig. 5.6). The covariant, total plant leaf area, had a significant effect on the analysis ($F = 10.15$, $p = 0.003$).

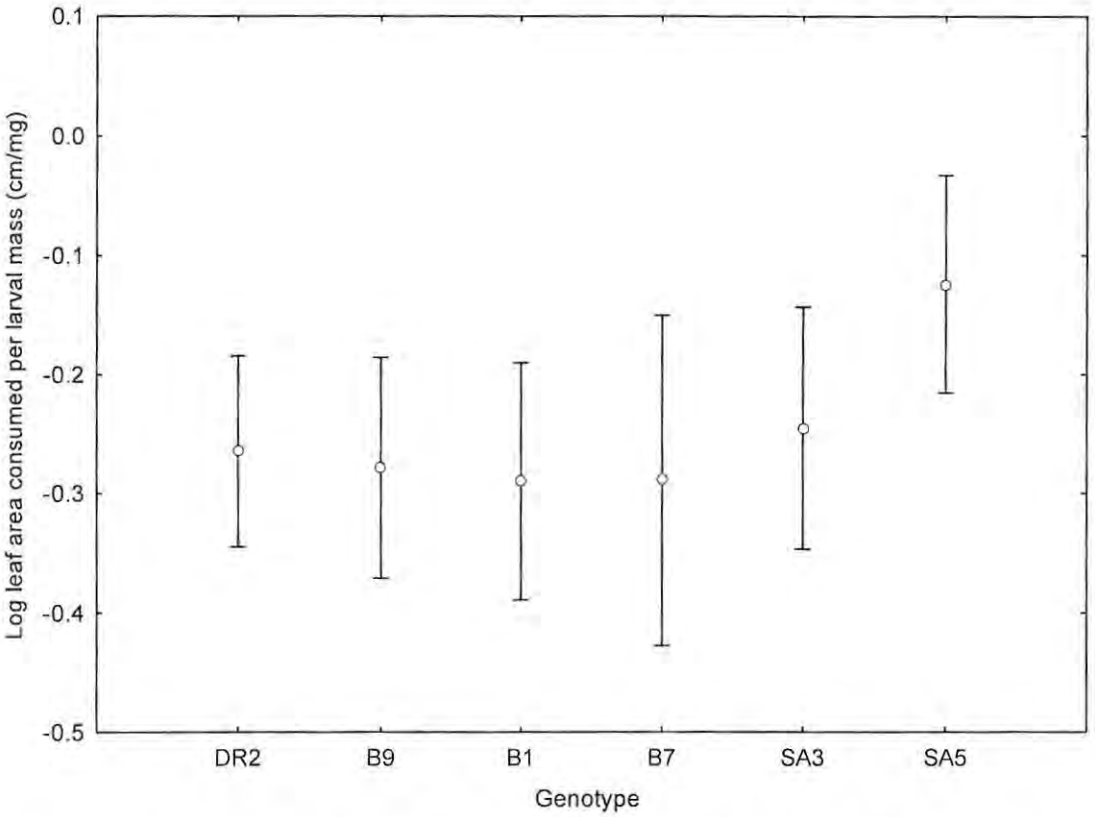

Figure 5.6. Analysis of covariance of leaf area consumed per larval mass between genotypes with total leaf area as a covariant. There were no significant differences among genotypes ($F_{(5,34)} = 0.484$, $p = 0.786$), indicating that there were no differences in the nutritional value of each plant genotype for the purposes of larval mass accumulation. Vertical bars delineate standard errors.

Maw's host suitability index for *P. guérini* did not differ significantly among plant genotypes (ANCOVA: $F_{(5,34)} = 0.440$, $p = 0.818$) (Fig 5.7).

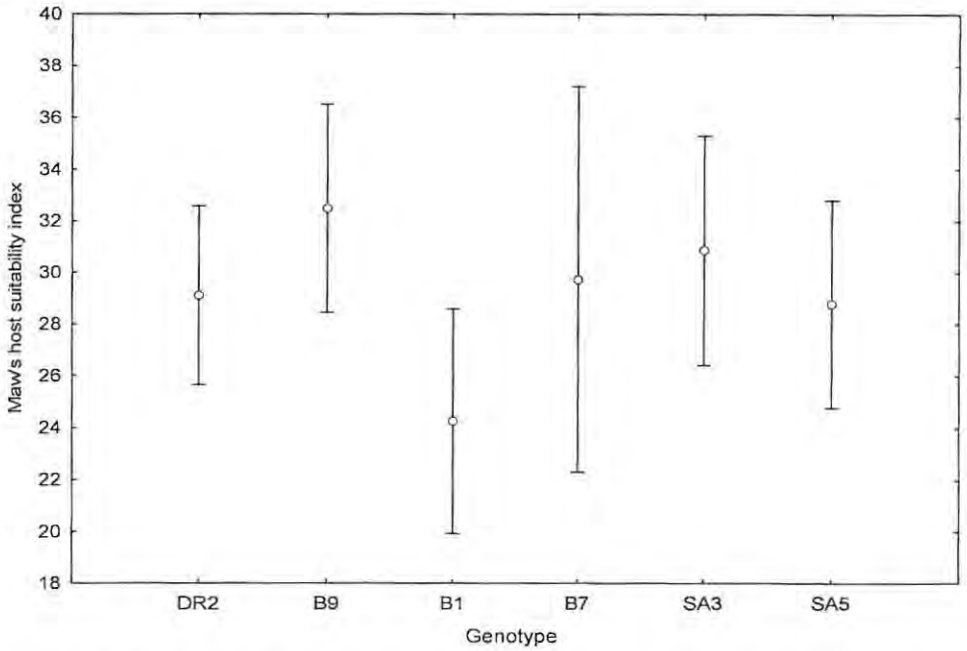

Figure 5.7. Analysis of covariance of Maw's host suitability index with total leaf area as a covariant. There are no significant differences in Maw's index among genotypes ($F_{(5,34)} = 0.440$, $p = 0.818$). Vertical bars delineate standard errors.

Table 5.2. Mean values (±S.E.) for all measured fitness related traits among genotypes.

Fitness parameter	DR2	B9	B1	B7	SA3	SA5
Leaf area (cm^2)	1668 (±183)	1227 (±163)	1256 (±337)	1458 (±568)	2435 (±286)	995 (±159)
Male pre-pupal mass (mg)	21.6 (±0.31)	20.7 (±0.86)	18.7 (±0.74)	21.5 (±0.26)	21.5 (±0.94)	21.7 (±0.93)
Female pre-pupal mass (mg)	24.6 (±0.82)	21.5 (±1.07)	22.4 (±0.65)	26.4 (±1.90)	25.3 (±1.12)	24.1 (±0.18)
Male adult mass (mg)	13.2 (±0.38)	13.5 (±0.67)	13.7 (±0.21)	14.0 (±0.00)	13.2 (±0.98)	11.5 (±0.42)
Female adult mass (mg)	16.3 (±0.59)	16.3 (±0.69)	15.6 (±0.79)	16.2 (±1.77)	18.7 (±3.13)	14.7 (±1.04)
Survival to pupation (%)	17.8 (±2.90)	21.9 (±2.66)	20.0 (±2.18)	18.3 (±6.67)	21.3 (±5.32)	16.9 (±3.89)
Duration of development (days)	22.0 (±0.02)	22.3 (±0.22)	22.0 (±0.00)	22.0 (±0.00)	22.6 (±0.41)	23.3 (±0.49)
Leaf area consumed (cm^2/mg)	373 (±103)	198 (±36)	218 (±42)	249 (±74)	458 (±123)	162 (±33)
Maw's host suitablility index	29.5 (±3.65)	28.9 (±3.28)	22.0 (±3.28)	31.4 (±0.19)	34.0 (±4.79)	26.8 (±3.75)

A summary of mean values for all fitness related parameters is given in Table 5.2.

The highest number of fertile eggs was produced by *P. guérini* reared on plant genotype SA3. No fertile eggs were produced by *P. guérini* reared on plant genotypes DR2 or B9 (Table 2). There were large differences in plant leaf area among plant genotypes, but the number of eggs and fertile eggs produced by *P. guérini* reared on the different genotypes did not increase consistently with total leaf area (Table 5.3).

Table 5.3: The majority of fertile eggs were produced by *Phenrica guérini* reared on genotype SA3 which is closely related to the host genotype. The number of eggs and fertile eggs do not increase consistently with leaf area, suggesting that oviposition sites were not a limiting factor.

Genotype	Egg batches	Eggs	Fertile batches	Fertile eggs	Leaf area (cm^2)
DR2	7	160	0	0	131
B9	13	295	0	0	562
B1	14	402	2	36	441
SA3	23	579	8	135	1694
SA5	9	215	1	13	2354

5.4 Discussion

The *P. aculeata* genotype on which *P. guérini* developed had no effect on the majority of the fitness related traits measured for the insect in this study. The only insect traits that showed statistically significant differences among genotypes were female pre-pupal larval mass and male adult mass.

Differences in female pre-pupal larval mass of *P. guérini* do not indicate a relationship between female pre-pupal larval mass and genetic distance to a preferred host genotype. Fitness parameters of *P. guérini* reared on the host genotype were not significantly different from those reared on any other genotype, and *P. guérini* reared on genotypes B1 and SA3, which have equal genetic distances to the original host genotype, were significantly

different from each other in terms of female pre-pupal larval mass. The genetic distance to a host genotype is therefore not the cause of variation in female pre-pupal larval mass. The fact that differences in female mass were not recorded in the adult stage supports the hypothesis that host plant genotype was not the cause of variation in female pre-pupal mass.

There were no significant differences in masses of adults, with the exception of males reared on the host genotype, which were significantly lighter than males reared on other genotypes. The host genotype was not the least suitable from any other biological traits. Although the mean duration of development and leaf area consumed per larval mass of *P. guérini* reared on the host genotype are slightly higher than on other genotypes, these differences are not significant and should therefore not be considered biologically important. There were no significant differences in Maw's host suitability index between genotypes, indicating that all the genotypes are equally suitable hosts for *P. guérini* in terms of the fitness related traits measured in this study. The genotype that produced the highest fitness in terms of fertile eggs was the closest relative to the host genotype (SA3), followed by genotype B1 and the host genotype (SA5).

No difference in the ability of *P. guérini* to utilize any one *P. aculeata* genotype could be detected by the methods used in this study suggesting that insect fitness between plant genotypes from different regions of the plant's distribution are similar. The genotype from the northern part of the native distribution of *P. aculeata* (DR2) has been geographically isolated from genotypes in the southern region of native distribution (B9, B1, B7) and this has led to genetic divergence of plants from the different regions. Despite this genetic differentiation, and the fact that only the plants in the southern region of native distribution have been exposed to herbivory by *P. guérini* in the past (see Chapter 6), there were no differences in the ability of *P. guérini* to utilize the different genotypes. Genetic differentiation from native plant genotypes due to isolation and artificial selection as a horticultural plant has also not led to any difference in the ability of *P. guérini* to utilize South African genotypes (SA3, SA5).

The fact that *P. guérini* performs equally when developing on plants from both regions of the native distribution of *P. aculeata* suggests that *P. aculeata* from the southern

region of the native distribution has no defense mechanisms that reduce *P. guérini* fitness that are not present in plants from the northern region of the native distribution. Plants from the southern region of the native distribution were expected to have defense mechanisms against *P. guérini* because they have co-evolved with the insect species, while *P. aculeata* plants in the northern region of the native distribution have not been exposed to *P. guérini* in the past and are not expected to have specialized defenses against the insect. Plant fitness between genotypes was not measured in this study so it is possible that plant genotypes from the native distribution of *P. guérini* may be more capable of mitigating the damage done by the insect.

The number of replicates used for some of the plant genotypes in this study may be too low to give adequate statistical robustness to some results. If more replicates were used it is possible that significant differences could be detected between treatments that were not significantly different in the experiment (e.g. mean male pre-pupal larval mass of B1 (Fig. 2) and percentage survival to pupation of B9 (Fig. 4)). Even if these results are assumed to be statistically significant the interpretation of the data would not be affected. Closely related plant genotypes do not show similar trends for the different plant parameters, suggesting that there is no relationship between the measured fitness parameters and the genetic distance to a preferred host genotype.

Collecting *P. guérini* from plant genotypes closely related to the weed population may make no difference in terms of the efficacy of *P. guérini* as a biological control agent. Variation in the ability to utilize different plant genotypes, and hence determining the origin of the weed population, may be important for other potential biological control agents for *P. aculeata* but this study suggests that insects collected on the host plant genotype most closely related to the weed population are not always more effective biological control agents than those collected off more distantly related genotypes. Natural enemies of *P. aculeata* such as the leaf gall wasp *Bruchophagous* sp., the fruit galling wasp species and the Pseudococcidae species (Chapter 6), which are expected to have multiple generations on the same host plant genotype due to poor dispersal potential, are more likely to have local adaptations to particular *P. aculeata* genotypes.

Similarly, it is neither the 'new' nor 'old association' approach that would be favored in this plant-herbivore system. This study does not give evidence as to which approach would be most suitable for other biological control agents but it does suggest that in some cases whether the insect has an 'old' or 'new' association with the target weed is of little practical importance.

The lack in variation in biological traits of *P. guérini* between the *P. aculeata* genotypes in this study should not be viewed as evidence against the importance of local adaptations of insect herbivores in other plant-herbivore systems or biological control programmes. The importance of local adaptations varies depending on the biology and evolutionary history of both the plant and herbivore, and could be an important factor leading to the success of biological control programmes (e.g. Goolsby *et al.* 2006). The results of this study indicate that not all insect herbivores will have greater fitness or higher levels of host suitability on their original host genotype. The origin of weed populations should be taken into account when collecting biological control agents, especially when insect herbivores are highly sedentary, but it should not be accepted as a rule that the insects from the origin of the weed population will have higher fitness or efficacy as biological control agents.

Chapter 6

Surveys for potential biological control agents for *Pereskia aculeata*: selection of the most promising potential agents

Abstract

New biological control agents are required for the control of *Pereskia aculeata* in order to reduce the weed's density to acceptable levels. Data from eight surveys for natural enemies of *P. aculeata* in the native distribution were used to compile a list of insect species associated with the plant. Sixty-two sites were surveyed resulting in a list of 40 insect species associated with the plant. Six prioritization categories were used to identify the most promising potential biological control agents from the suite of insects associated with the plant. Prioritization categories were i) the presence of feeding damage, ii) insect incidence measured as the number of sites at which the insect species was present divided by the total number of sites, iii) the host range of the insect observed in the field, iv) the similarity of the climate where the insect species was found to the climate at the weed's introduced distribution, v) the similarity of the weed genotype to the genotype on which the insect species developed in the native distribution and vi) the mode and levels of damage in the native distribution. The most promising potential biological control agents for *P. aculeata* identified using the various criteria of the prioritization categories are the released biological control agent, *Phenrica guérini* Bechyné (Chrysomelidae), two species of Curculionidae and *Maracayia chlorisalis* Walker (Crambidae). The method used to prioritize the most promising potential biological control agents for future research may be useful when surveying for natural enemies for use as biological control agents for other weed species.

6.1 Introduction

Impact assessments and field observations of the current biological control agent, *Phenrica guérini*, indicate that new biological control agents are required in order to reduce *Pereskia aculeata* densities to acceptable levels (Chapter 3). *Pereskia aculeata* is considered native in parts of Central America, northern Venezuela and the Caribbean, as well as northern Argentina and south-eastern Brazil (Leuenberger 1986) (Fig. 1.3). Eight surveys for potential biological control agents of *P. aculeata* in the plant's native distribution were carried out between 1988 and 2007 (Table 6.1) resulting in the importation of three insect species into quarantine in South Africa for host specificity testing. *Phenrica guérini* was found to be monophagous and was subsequently released in South Africa (Klein 1999). *Epipagis cambogialis* (Guenée) (Pyralidae) was rejected as a biological control agent due to the broad host range of the species, which included other species of Cactaceae as well as species in the families Portulacaceae and Basellaceae (Klein 1999). Some species within the Fabaceae and Amaranthaceae have also been recorded as food plants for *E. cambogialis* (Silva *et al.* 1968, Da Cruz 1992). *Maracayia chlorisalis* Walker (Crambidae) was imported into quarantine but detailed host specificity studies were not possible due to difficulties rearing the moth (Klein 1999). No host specificity testing has been undertaken on any other species associated with *P. aculeata* and very little is known about the life-cycles of other natural enemies associated with the plant. The most promising of the natural enemies that have been recorded on *P. aculeata* during surveys in the native distribution should be identified as priority insects for further studies, including host specificity testing.

A method for prioritizing the most promising potential biological control agents from data collected during surveys in the weed's region of origin would be valuable to the field of biological control (Van Klinken & Raghu 2006). There are often large numbers of herbivorous arthropod species associated with plants (Kennedy & Southwood 1984, Palmer 1987, Gillett *et al.* 1991, Fontes *et al.* 1994, Palmer & Pullen 1995, Balciunas *et al.* 1995a, Harley *et al.* 1995) making host specificity testing of all the potential biological control agents infeasible. In the case of *Lantana camara* L. (Verbenaceae) for example, 550

phytophagous species were recorded on the plant during surveys (Palmer & Pullen 1995) but only 36 species have been released as biological control agents world wide (Julien & Griffiths 1998). The most promising of the potential biological control agents should therefore be determined prior to host specificity testing, although the predicted host range of the potential biological control agent should be taken into account during the prioritization process. Systems for prioritizing biological control agents have been proposed, but the data required for prioritization includes information that is difficult to deduce without detailed studies of the insect's life cycle, levels of parasitism and host specificity (Harris 1973, Goeden 1983). These systems can be used to prioritize potential biological control agents after laboratory based studies have been concluded, but are not appropriate for prioritizing potential biological control agents for further research based on data from surveys in the weed's region of origin.

Table 6.1. Surveys for natural enemies of *Pereskia aculeata* within the plant's native distribution.

Date	Duration	Country (Province)	Report Authors
18/08/1988	10 days	Brazil (Rio de Janeiro Province)	HE Erb (1988)
13/08/1990	1 month*	Brazil (Rio de Janeiro Province), Argentina (Misiones Province), Costa Rica	HG Zimmermann (1990)
15/02/1991	23 days*	Trinidad, Venezuela, Brazil (Rio de Janeiro Province)	HE Erb (1991)
10/2/2005	1 month*	Argentina (Misiones Province), Brazil (Santa Catarina Province)	A McConnachie, S Neser, D Simelane (2005)
19/08/2005	4 days	Argentina (Misiones Province)	MP Hill, F McKay (2005)
26/01/2006	10 days	Brazil (Paraná Province, Santa Catarina Province) Argentina (Misiones Province)	MP Hill (2006)
20/05/2007	6 days	Dominican Republic	MP Hill, HG Zimmermann
26/10/2007	1 month	Brazil (Rio de Janeiro Province, Santa Catarina Province), Argentina (Misiones Province), Dominican Republic	S Neser, ID Paterson

* Surveys on various South African weeds including *P. aculeata* opportunistically.

In this study the most promising natural enemies in terms of potential as biological control agents are identified for the control of *P. aculeata*. All the data for prioritization were collected during field surveys or prior to field surveys in the introduced distribution of the weed. Prioritization was based on the presence of damage, incidence (percentage of sites at which the species was present), field host range, climatic matching, genotype matching and mode of damage. Genotype matching is of specific importance to the biological control of *P. aculeata* (Chapter 4) and may not be of value in surveys for potential biological control agents of all alien invasive plant species. Other prioritization categories could be used to identify the most promising natural enemies during surveys for biological control agents of all weed species.

6.2 Methods

6.2.1 Surveys

Data presented in this study are a synthesis of all data collected during surveys for natural enemies of *P. aculeata* (Table 6.1). Insects and other natural enemies of *P. aculeata* have been collected at 62 sites in the native distribution of the plant (Fig. 6.1). All phytophagous insects found on *P. aculeata* were collected and immatures were reared to adults for identification. All insects collected on the most recent survey were sent to the South African National Collection of Insects at the Plant Protection Research Institute (PPRI-ARC) for identification and are housed at the South African National Collection (PPRI-ARC), Pretoria and referred to by Rhodes University (RH) accession numbers.

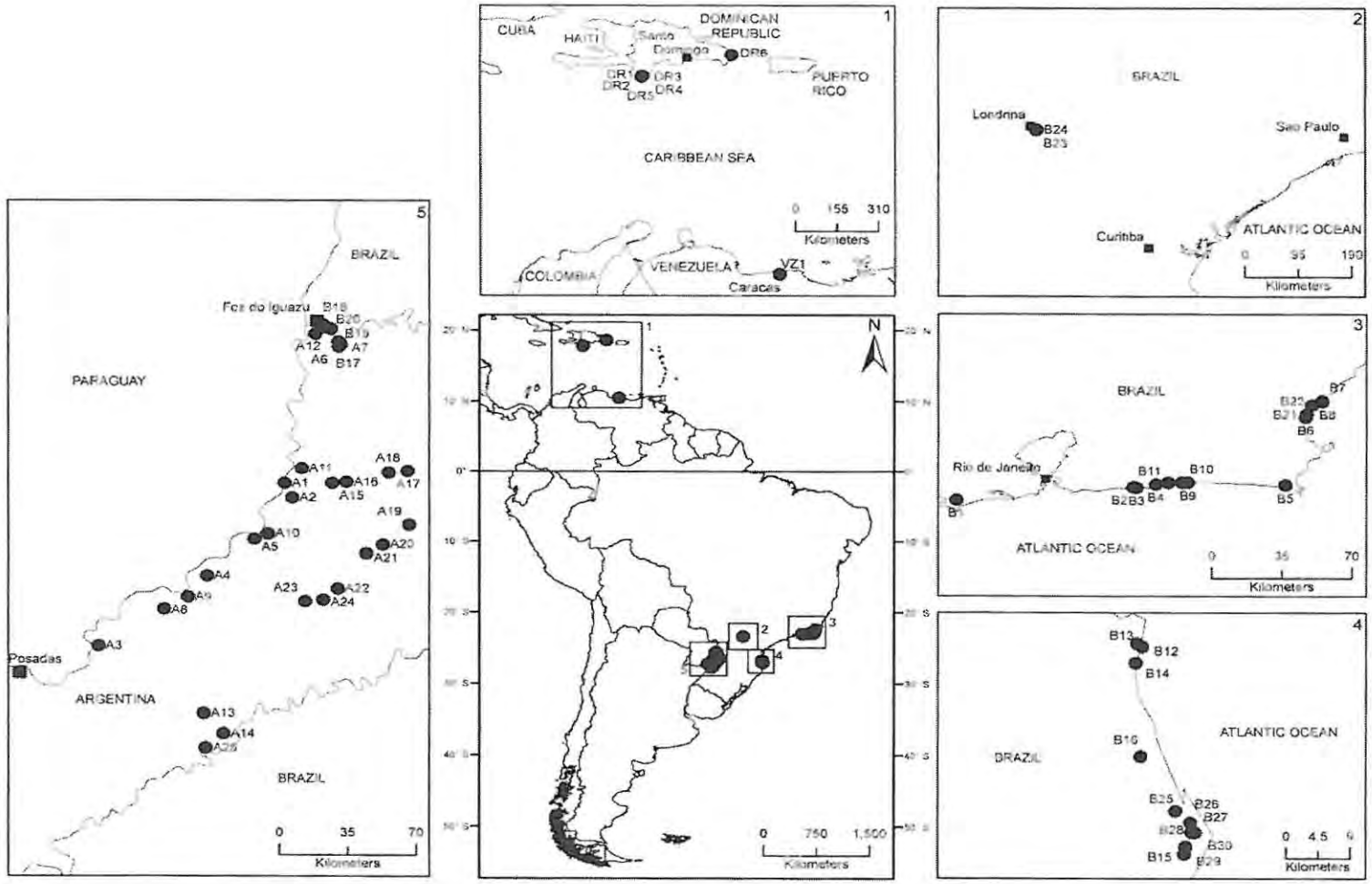

Figure 6.1. Sites surveyed for phytophagous insects associated with *P. aculeata* in the regions of native distribution.

6.2.2 Prioritization

Six criteria were applied sequentially to determine the most promising potential biological control agents in a sequential fashion. At each step the natural enemies considered promising according to the criteria of the prioritization category were separated from those that were not considered promising. Only natural enemies that were considered promising were included in the following step. This system is intended to prioritize the most promising of the potential biological control agents for future research.

6.2.2.1 Feeding damage

Insects, or other arthropods, found on the target plant in the region of origin may be predators that feed on arthropods associated with the plant, detritivores that feed on dead plant material or incidental visitors that have no association with the target plant. Arthropods that are not phytophagous and those that do not feed on the target plant cannot be considered for biological control. Insects found at sites where no damage to the plant could be attributed to the feeding of the insect were excluded from the list of potential biological control agents.

6.2.2.2 Incidence

Incidence was calculated as the percentage of sites at which the species was recorded. Species with very low incidence values were possibly incidental visitors to the plant with no specialized relationship between the plant and the natural enemy. It was therefore unlikely that species with very low incidence values would be suitably host specific for consideration as biological control agents. In this step, incidental visitors were excluded from the list of potential biological control agents. The incidence value below which insects are excluded will differ between biological control programmes depending on the number of sites surveyed. For *P. aculeata* any natural enemy that was found at only one of the 62 sites was

considered an incidental visitor. All natural enemies with incidence values of 0.03 or less were therefore excluded.

6.2.2.3 Field host range

Field host range data can be very useful when determining the levels of host specificity of potential biological control agents (Olckers 2000). In this study, the plants included in the surveys to give an indication of field host range of the natural enemies found on *P. aculeata* were *Talinum paniculatum* Gaertner (Portulacaceae) and *Anredera cordifolia* (Ten.) Steenis (Basellaceae). The two plant species often grow sympatrically with *P. aculeata*, are in closely related families and have similar mucilaginous, succulent leaves. Both plants are also considered weeds in South Africa and have been surveyed for potential biological control agents in the region of origin. Natural enemies of *P. aculeata* that were also associated with *T. paniculatum* or *A. cordifolia* were considered less promising potential biological control agents due to their broad field host ranges.

Pereskia grandifolia Haworth, *Pereskia marconoi* Areces and *Pereskia quisquenyana* Loigier were also surveyed for natural enemies when possible. A natural enemy that is specific to the genus *Pereskia* would be considered safe for release in terms of host specificity due to the lack of native Cactaceae in South Africa (Chapter 1), so natural enemies associated with other *Pereskia* species were not considered less promising. Surveying on other *Pereskia* species could give a good indication of levels of specificity because if a natural enemy does not feed on congeneric species it is unlikely to feed on more distantly related species.

6.2.2.4 Climatic matching

In order to be effective biological control agents, natural enemies must be suited to the climatic conditions of the region where the target plant has become problematic. One way of insuring that potential biological control agents are suited to the climatic conditions expected

after release is to collect natural enemies in areas of the native distribution where climatic conditions are similar to those where the plant is considered a weed (Wapshere 1983, 1985, Robertson *et al.* 2008). Climatic matching was performed using MaxEnt (Phillips *et al.* 2004, Phillips *et al.* 2006) to identify regions in the native distribution of *P. aculeata* that are climatically most similar to those in the introduced distribution in South Africa.

Natural enemies found at sites with similar climatic conditions to the introduced distribution of *P. aculeata* were considered more promising than those found only in areas with different climatic conditions.

6.2.2.5 Genotype matching

Although some natural enemies may have no preference among plant varieties or genotypes (Chapter 3), some may be specialized to intraspecific varieties of the host plant (Edmunds & Alstad 1978, Karban 1989, Mopper & Strauss 1998, Egan & Ott 2007). Selecting potential biological control agents that are most suited to the weed population may be important for biological control success (Goolsby *et al.* 2006). The population of *P. aculeata* in South Africa originated from plants in Rio de Janeiro Province, Brazil (Chapter 4). Natural enemies of *P. aculeata* found in south-eastern Brazil are more likely to be suited to the South African weed population if local adaptations have resulted in specialization of the natural enemy to specific plant genotypes. Insects that are present in south-eastern Brazil are more promising potential biological control agents than those that are only present elsewhere.

6.2.2.6 Mode of damage

Natural enemies that damage the vascular tissue or mechanical support tissue of the plant are more likely to be effective biological control agents than those that damage the leaves, seeds or flowers of the plant (Harris 1973, Goeden 1983). Natural enemies that damage the vascular tissue or mechanical support tissue of the plant are therefore considered more promising potential biological control agents. Natural enemies that appear to transmit

diseases should also be considered more promising potential biological control agents if the disease is host specific.

6.2.3 Sampling effort

When prioritizing the most promising potential biological control agents for a weed species it is important that all the natural enemies of the target weed are considered (Van Klinken & Raghu 2006). Whether all natural enemies associated with the plant have been sampled during surveys can be determined using species accumulations curves. If sampling effort has been adequate to sample all the species associated with the target weed it is unlikely that any new potential biological control agents would be encountered if further surveys were conducted.

Species accumulation curves were constructed using EstimateS Version 7.5 (Colwell 2005). The Chao 2 estimator was used to predict the number of natural enemy species associated with *P. aculeata*. This estimator is appropriate for estimating populations when abundances of species are unknown. Insect species that did not feed on the plant were not included in this analysis because these species were most likely incidental visitors.

6.3 Results

Forty insect species were collected on *P. aculeata* in the plants native distribution (Table 6.2). The order with the highest species richness was Coleoptera, with 23 species, followed by Hemiptera (nine species), Lepidoptera (five species) and Hymenoptera (three species). The families with the highest species richness were Chrysomelidae (14 species) and Curculionidae (six species). Only four species (Cicadelidae sp. 1, Curculionidae sp. 1, *M. chlorisalis* and *E. cambogialis*) were recorded in the northern region of the plants native distribution. Thirty-eight species were recorded in the southern region of the native distribution.

Table 6.2. Insect species found associated with *Pereskia aculeata* during surveys in the native distribution. Accessioned specimens are referred to by Rhodes University (RU) accession numbers. Incidence is calculated as the number of sites at which the insect species was present divided by the total number of sites.

	Life stage and habit	Feeding present	Incidence
Hemiptera			
Coreidae			
sp. 1 (RH 769)	Adult on shoots	Yes	0.016
Catorhintha sp. (RH 780, RH 781)	Adult & Nymphs on shoots	Yes	0.016
Pentatomidae			
sp.1	Adult	No	0.032
cf. Derbidae			
sp. 1 (RH 785)	Adults	Yes	0.048
Tropiduchidae			
sp. 1 (RH 791)	Adult	Yes	0.016
Cercopidae			
sp. 1	Adult	Yes	0.016
Membracidae			
sp. 1 (RH 764, RH 787, RH 798, RH 799)	Adult & Nymphs	Yes	0.113
Cicadelidae			
sp.1	Adult & Nymphs	Yes	0.048
Pseudococcidae			
sp. 1	Adult	Yes	0.016
Coleoptera			
Melyridae			
sp. 1 (RH 768)	Adult	No	0.032
Erotylidae			
sp. 1 (RH 770)	Adult	No	0.016
Cerambycidae			
sp. 1	Larvae in stem	Yes	0.016
Chrysomelidae			
Criocerinae			
Lema sp. 1 (RH 722)	Adult	No	0.016
Lema possibly *extracta* (RH 792)	Adult	No	0.016
Eumolpinae			
Colaspis sp. (RH 790)	Adult	No	0.016
Galerucinae			
Diabrotica sp. (RH 773)	Adult	No	0.016

	Life stage and habit	Feeding present	Incidence
Alticinae			
sp. 1 (RH 765 RH 766 RH 767)	Adult	No	0.016
sp. 2 (RH 786)	Adult	No	0.016
sp. 3 (RH 789)	Adult	No	0.016
Oedionychini sp. (RH 757)	Adult	No	0.032
Omophoita sp. (RH 804)	Adult	No	0.016
Phenrica guérini	Adult	Yes	0.065
Cassidinae			
Possibly *Cistudinella sp.* (RH 793)	Adult	No	0.016
Possibly *Charidotis sp.* (RH 794)	Adult	No	0.016
Charidotis possibly *mansueta* (RH 756)	Adult	No	0.016
Charadotis possibly *auroguttata* (RH 778)	Adult	No	0.016
Curculionidae			
sp. 1 (RH 797)	Adult	No	0.016
sp. 2 (RH 758, RH 759)	Adult	Yes	0.032
sp. 3 (RH 761, RH 762)	Adult	Yes	0.032
sp. 4 (RH 779)	Adult	No	0.016
sp. 5 (RH 763)	Adult	No	0.016
sp. 6 (RH 782)	Adult	No	0.016
Lepidoptera			
Tortricidae			
Anopinella brasiliana (RH 805, RH 806, RH 807)	Larvae in galled fruits	Yes	0.016
Gracillariidae			
Lithocolletinae			
cf. Porphyrosela sp. (RH 747, RH 748)	Adult and larvae	Yes	0.226
Pyralidae			
Epipagis cambogialis	Larvae (Leaf tier)	Yes	0.339
Crambidae			
Maracayia chlorisalis (RH 744, RH 745, RH 746)	Larvae and pupae in shoots and stems	Yes	0.484
Unknown larvae sp. 1			
cf. Notodontoidea	Larvae on leaves	Yes	0.048
Hymenoptera			
Tethredinidae			
sp. 1	Larvae on leaves	Yes	0.048
Eurytomidae			
Bruchophagus sp. (RH 749, RH 750)	In galls on leaves	Yes	0.081
Unknown larvae	In fruit galls	Yes	0.064

There was no evidence of feeding by 20 of the species collected on *P. aculeata* (Table 6.2). All species with no associated damage were collected only as adults. Melyridae sp. 1 is unlikely to be useful as a biological control agent because members of this family are either carnivorous or flower feeders that may be important pollinators (Weiss 1922, Grant *et al.* 1979). At least one species, *Charidotis auroguttata* (Boheman), is known to be host specific to another plant species, *Macfadyena unguis-cati* (L.) Gentry (Bignoniaceae), that was growing in close proximity to the *P. aculeata* site (Sparks 1999). These species are not considered potential biological control agents because they do not appear to feed on the plant.

Seven of the 20 remaining species were not considered promising potential biological control agents due to their low incidence values (Table 6.2). These species were found only at one of the 62 sites surveyed in this study suggesting that they were incidental visitors to the plant.

The remaining 13 species were feeding on *P. aculeata* and were found at more than one site (Table 6.2). With the exception of *cf.* Derbidae sp. 1, all 13 species were associated with *P. aculeata* as adults and immature stages (Table 6.2). These species are unlikely to be incidental visitors because they were found at more than one site.

Epipagis cambogialis, *cf. Porphyrosela* sp. and Tenthredinidae sp. 1 were recorded feeding on host plants from different plant families (Table 6.3). *Epipagis cambogialis* and Tenthredinidae sp. 1 were both found feeding on *T. paniculatum* and *cf. Porphyrosela* sp. was found feeding on *T. paniculatum* and *A. cordifolia*. These species were not considered the most promising of the potential biological control agents due to their broad host ranges.

The majority of the sites sampled in the surveys are within areas with similar climatic conditions to those found in the introduced distribution of *P. aculeata*, with the important exception of sites in Misiones Province, Argentina (Fig. 6.2). Areas with similar climatic conditions to those in the introduced distribution of *P. aculeata* are rare in Venezuela and Dominican Republic (Fig. 6.2) but the plant was not found outside of the climatically similar areas and was rare in both countries (Personal observation, H.E. Erb Unpublished report). Species found only in Misiones Province, such as *cf.* Derbidae sp. 1, unknown Lepidoptera

c.f. Notodontoidea and the unknown hymenopterous fruit galler, may not be suitable biological control agents in South Africa due to climatic incompatibility. The other remaining species were considered more promising potential biological control agents because they were present in areas where the climatic conditions are similar to those found in the introduced distribution of the plant (Table 6.3).

Native genotypes of *P. aculeata* most closely related to the South African weed population are found in Rio de Janeiro Province, Brazil (Chapter 4). All the remaining potential biological control agents, with the exception of Cicadellidae sp. 1, were found associated with genotypes closely related to the weed population genotypes. Cicadellidae sp. 1 was therefore considered less promising than other potential biological control agents due to the possibility of genetic mismatching between the insect and the South African plant genotypes (Table 6.3). *c.f.* Derbidae sp. 1, unknown Lepidoptera *c.f.* Notodontoidea and the unknown hymenopterous fruit galler, which were excluded from the list by the criteria of the climatic matching category, were also considered unpromising by the genetic matching criteria (Table 6.3). Although the possibility of potential biological control agents being incompatible with certain plant genotypes must be considered, not all insect herbivores will have greater fitness, or higher levels of suitability on their original host plant genotype, so potential biological control agents should not be rejected on the criterion of genetic matching alone (Chapter 5).

Membracidae sp.1, Curculionidae sp. 2, Curculionidae sp. 3, *P. guérini*, *M. chlorisalis* and *Bruchophagous* sp. are the only natural enemies that fulfill the criteria of the first five categories (Table 6.3). The most damaging of the remaining potential biological control agents are the Curculionidae species and *M. chlorisalis*, which are both stem borers that destroy structural and vascular tissue. *Phenrica guérini* is also a damaging insect which sometimes destroys shoot tips. Membracidae sp. 1 and *Bruchophagous* sp. attack the leaves of the plant and are not considered promising potential agents in terms of the damage done to the plant (Table 6.3).

Table 6.3. Field host range, climatic matching, genotype matching and mode of damage for prioritization of promising potential biological control agents. The species presented in this table are those which passed the criteria of prioritization categories one and two (see sections 5.2.2.1 and 5.2.2.2).

	Field host range included:		Present in	Present on	Mode of Damage
	Anredera cordifolia	*Talinum paniculatum*	matching climate?	matching genotypes?	
Hemiptera					
cf. Derbidae					
sp. 1 (RH 785)	No	No	No	Yes	Leaf sucker
Membracidae					
sp. 1 (RH 764, RH 787, RH 798, RH 799)	No	No	Yes	Yes	Leaf sucker
Cicadelidae					
sp.1	No	No	Yes	No	Leaf sucker
Coleoptera					
Chrysomelidae					
Alticinae					
Phenrica guérini	No	No	Yes	Yes	External leaf feeder
Curculionidae					
sp. 2 (RH 758, RH 759)	No	No	Yes	Yes	Stem borer
sp. 3 (RH 761, RH 762)	No	No	Yes	Yes	Stem borer
Lepidoptera					
Gracillariidae					
Lithocolletinae					
cf. Porphyrosela sp. (RH 747, RH 748)	Yes	Yes	Yes	Yes	Leaf miner
Pyralidae					
Epipagis cambogialis	Yes	Yes	Yes	Yes	Leaf tier and miner
Crambidae					
Maracayia chlorisalis (RH 744, RH 745, RH 746)	No	No	Yes	Yes	Stem borer
Unknown larvae sp. 1					
cf. Notodontoidea	No	No	No	Yes	External leaf feeder
Hymenoptera					
Tethredinidae					
sp. 1	No	Yes	Yes	Yes	External leaf feeder
Eurytomidae					
Bruchophagus sp. (RH 749, RH 750)	No	No	Yes	Yes	Leaf galler
Unknown larvae	No	No	No	Yes	Fruit galler

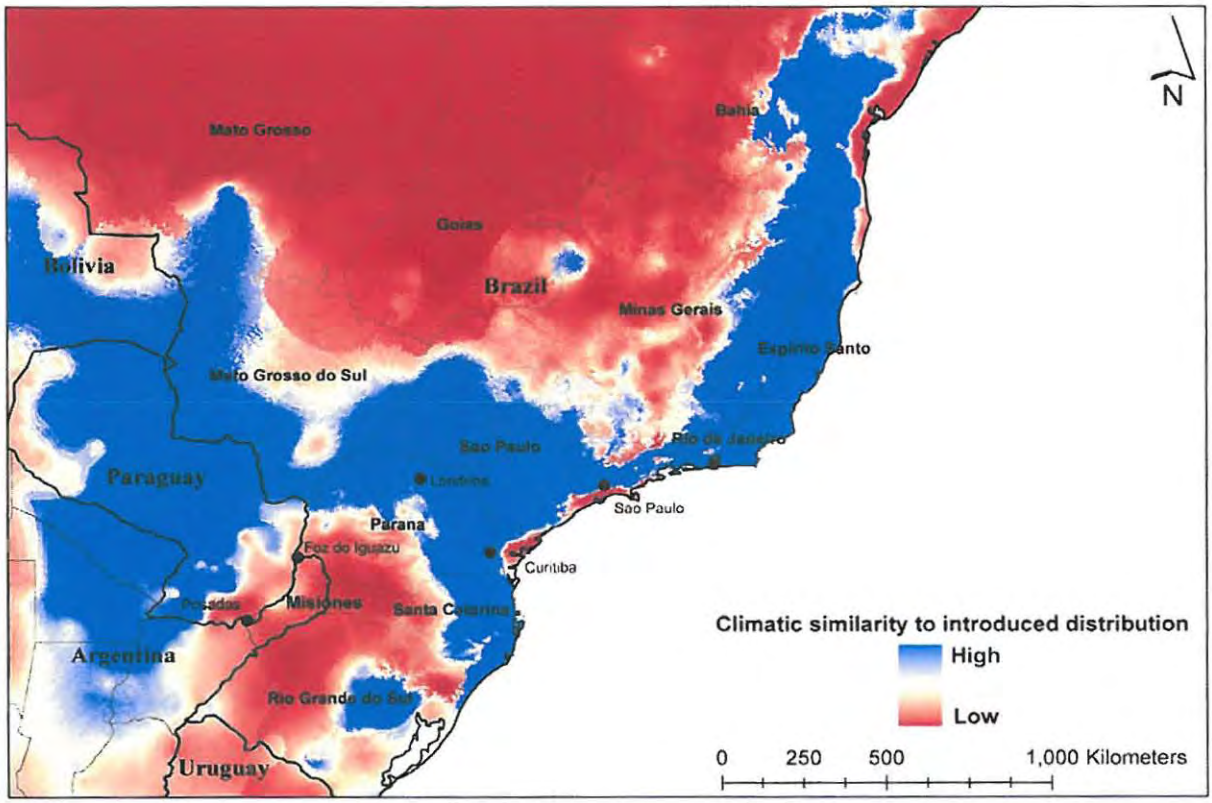

Figure 6.2a. Climatic similarity to the introduced distribution of *P. aculeata* in South Africa in the southern region of the native distribution using Maxent (Phillips *et al.* 2004, Phillips *et al.* 2006).

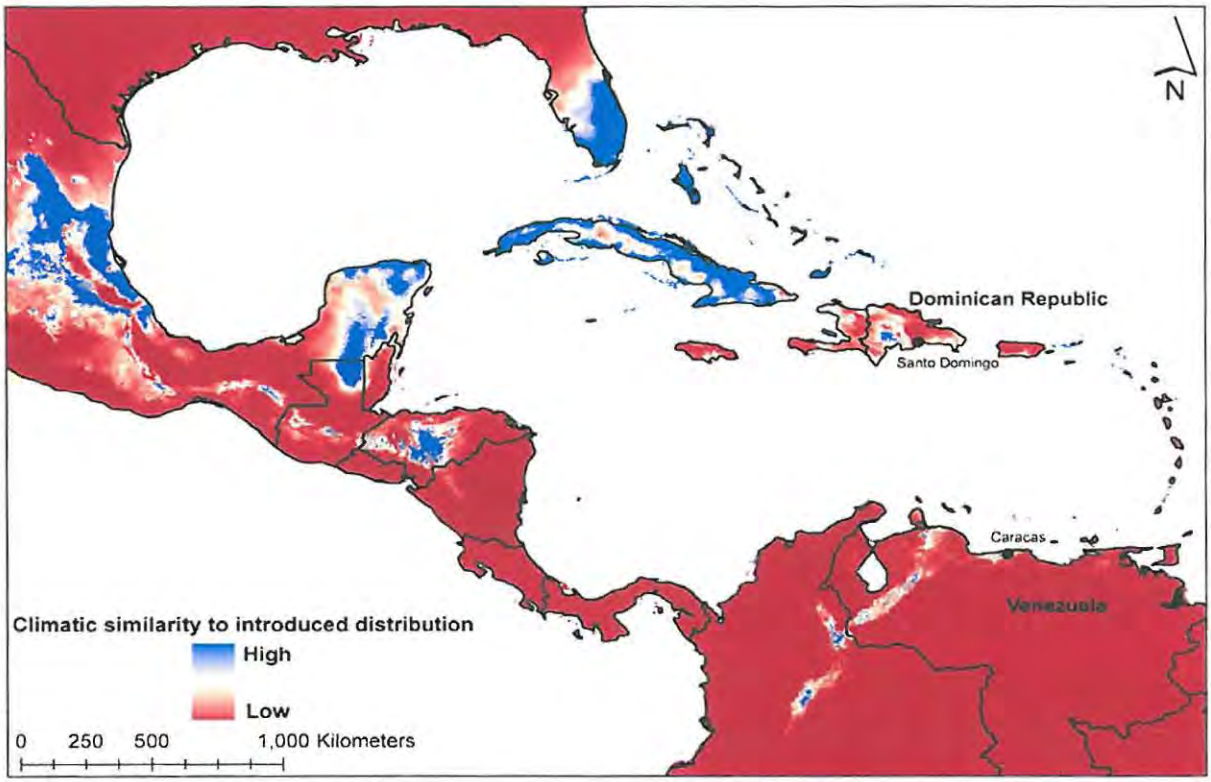

Figure 6.2b. Climatic similarity to the introduced distribution of *P. aculeata* in South Africa in the northern region of native distribution using Maxent (Phillips *et al.* 2004, Phillips *et al.* 2006).

The Chao 2 estimator indicated that, although the majority of the insects associated with *P. aculeata* have been sampled, further surveys are likely to result in three or four additional insect species which feed on *P. aculeata* but the upper 95% confidence interval of the Chao 2 estimator indicates that there may be as many as 20 species associated with the plant in the regions of native distribution (Fig 5.3). Further surveys are warranted and may result in both the addition of new potential biological control agents and an increase in the predictive power of the Chao 2 estimator by narrowing the 95% confidence intervals.

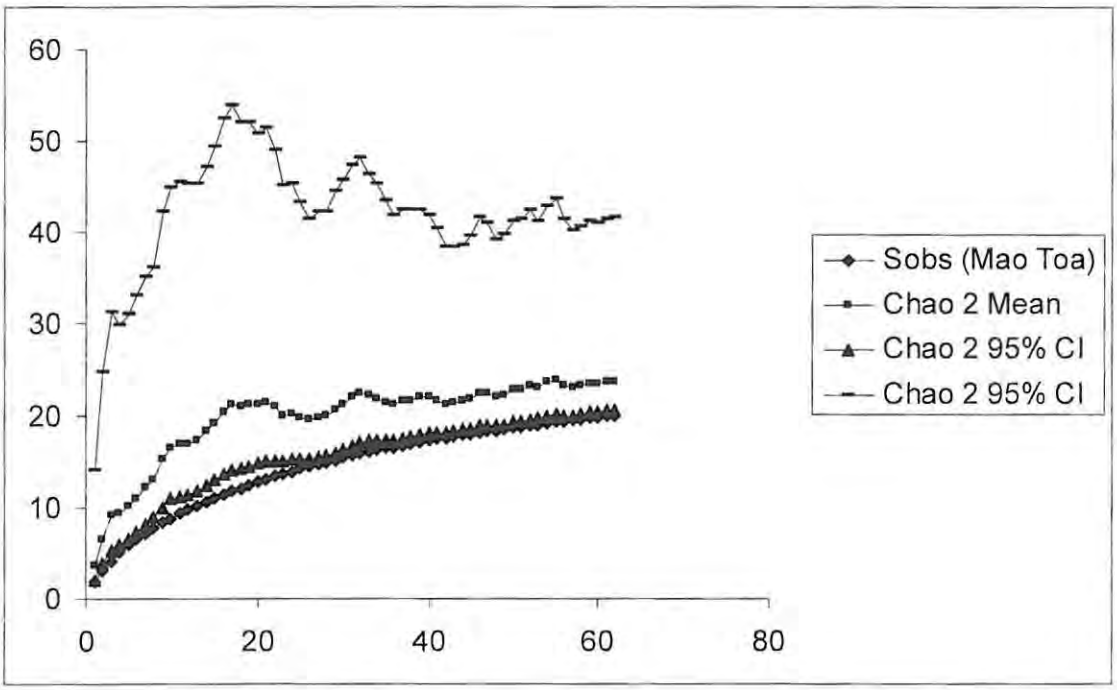

Figure 6.3. The Chao 2 estimator (Colwell 2005) predicts that there are likely to be three or four more insect species associated with *Pereskia aculeata* in the event of further surveys (Chao 2 mean) but there is a possibility that as many as 20 new insect species may be encountered (Chao 2 upper 95% C.I.). Further surveys in the region of origin are necessary to sample all the potential biological control agents for *P. aculeata*.

The most promising potential biological control agents in terms of the criteria of the six prioritization categories presented in this study are *Phenrica guérini*, Curculionidae sp. 2, Curculionidae sp. 3 and *M. chlorisalis*.

6.4 Discussion

The species richness of phytophagous insects associated with *P. aculeata* is low compared with species of *Lantana* (Verbenaceae) (Palmer and Pullen 1995), *Melaleuca quinquenervia* (Cav.) S. T. Blake (Mytaceae) (Balciunas *et al.* 1995a), species of *Solidago* (Asteraceae)

(Fontes *et al.* 1994), *Mimosa pigra* L. (Mimosaceae) (Harley *et al.* 1995) and *Sida acuta* N. L. Burman (Malvaceae) (Gillett *et al.* 1991), but is similar to that found on the aquatic plants *Eichhornia crassipes* (Mart.) Solms-Laubach (Pontederiaceae) (Cordo 1999) and *Hydrilla verticillata* (L.f.) Royle (Hydrocharitaceae) (Balciunas *et al.* 1995b) and to the fern *Lygodium microphyllum* (Cav.) R.Br. (Lygodiaceae) (Goolsby *et al.* 2003). The suite of insects associated with *P. aculeata* is small and unique compared with the 150 – 160 species found associated with over 175 other cactaceous species (Mann 1969, Zimmermann *et al.* 1979). With the exceptions of *E. cambogialis* and *M. chlorisalis*, which are known to feed on other Cactaceae (Mann 1969, Zenner de Polania 1990), insect species associated with *P. aculeata* are apparently not commonly known cactophages. Cochineal insects (*Dactylopius* spp.) are associated with many species of Cactaceae (Mann 1969, Zimmermann *et al.* 1979) and are useful biological control agents on a number of cactaceous weeds (Julien & Griffiths 1998) but no *Dactylopius* species have been found associated with *P. aculeata* or other *Pereskia* species.

The low species richness recorded during surveys in the northern region of the plant's native distribution may be exaggerated due to poor sampling efforts but it is clear that species richness is generally higher in the southern region of native distribution. Future surveys should therefore be conducted in the southern region of the native distribution. This suggests that the species is likely to have originated in the southern region of the native distribution and that plants in the northern region of native distribution may be the result of an early introduction. It is possible that this introduction was induced by human activity but any attempts to identify how *P. aculeata* came to be present in the northern region of its native distribution are based purely on speculation.

The biological control agent, *P. guérini*, is considered one of the four most promising species for biological control based on the prioritization categories. Although it is evident that *P. guérini* alone is unlikely to reduce *P. aculeata* densities to acceptable levels, the insect should not be disregarded as a biological control agent and mass rearing and post-release evaluation of *P. guérini* should continue (Chapter 3).

The two promising Curculionidae species were found at only two sites in Santa Catarina Province, Brazil (Sites B12 and B13). The supposedly limited distribution of the species could be seen as a disadvantage for biological control but climatic matching and genotype matching suggest that these species will be compatible with the South African weed type and climate. Adults of both species were found at both sites, and curculionid larvae were present at both sites, but larvae were not successfully reared to adults so it is impossible to identify which curculionid species is responsible for the larval damage. Further research on these species should focus on identifying the weevil species responsible for the damage found at the sites. If necessary, genetic techniques could be used to match larvae to adults (Ståhls *et al.* 2009).

Maracayia chlorisalis is a promising potential biological control agent and should be considered a priority species for collection and importation into quarantine in South Africa for host specificity studies. The species is often damaging in the native range where the damage done by the insect sometimes kills shoots, resulting in the development of secondary shoots from below the tunnel. Host records and preliminary host specificity testing suggest that *M. chlorisalis* is oligophagous, feeding on a number of cactaceous genera. *Maracayia chlorisalis* is considered a pest of *Hylocereus ocamponis* (Salm-Dyck) Britton & Rose (Cactaceae) crops in Colombia (Zenner de Polania 1990) and *Hylocereus undatus* Britton & Rose (Cactaceae) crops in Mexico (CESAVEP 2006). *Hylocereus* crop species are traditionally cultivated in Asia and Central America and have been cultivated more recently in Israel, Australia and Réunion Island (Van Wyk 2005, Le Bellec *et al.* 2006). The fruit, commonly referred to as pitahaya or dragon fruit, is widely consumed in Asia and is becoming popular in the European market (Nerd *et al.* 2002, Le Bellec *et al.* 2006). A number of small scale pitahaya farms are now present in South Africa (J. Husselman, Institute for Tropical and Subtropical Crops (ARC), *pers. comm.*). There is a potential conflict of interest between biological control practitioners who may support the release of *M. chlorisalis* and *Hylocereus* crop farmers who may object to the release of the insect.

Maracayia chlorisalis is likely to be safe for release in South Africa in terms of the predicted threat to native biodiversity. Only one species of cactus, *Rhipsalis baccifera* (J.

Müller) Stern, is considered native in South Africa (Dyer 1975) and this species is unlikely to be a suitable host plant for *M. chlorisalis* based on differences between *R. baccifera* morphology and the morphology of recorded host plant species.

The most appropriate region to collect *M. chlorisalis* for further research is Rio de Janeiro Province, Brazil, because climatic and genetic matching suggests that insects from this region will be most suited to the weed population and climate in South Africa.

The type of damage expected to impact on weed density at a population level is important in predicting success of potential biological control agents (Van Klinken & Raghu 2006). Stem borers such as the two curculionid species and *M. chlorisalis* are expected to be damaging agents for *P. aculeata* because they destroy structural and vascular tissue and could therefore reduce vegetative growth. *Phenrica guérini* feeds externally but although it is also capable of destroying structural and vascular tissue it was found to have little effect on vegetative growth parameters (Chapter 3). Despite the limited impact of *P. guérini*, insects which destroy structural and vascular tissue, such as stem boring insects, should be the focus of future research because this mode of damage is the most likely to be effective against *P. aculeata*. Root feeding natural enemies have not been encountered on *P. aculeata* but would be considered potentially damaging agents.

Sampling effort has been sufficient to collect the majority of the phytophagous insects associated with *P. aculeata*, but a small number of new species are likely to be encountered during future surveys. More surveys in the region of origin are therefore warranted. Analysing sampling efforts to predict the number of species expected to be associated with weed species could improve efficiency of biological control programmes by indicating whether new natural enemies are likely to be encountered. When the majority of the natural enemies of a weed species have been sampled the focus of surveys should shift from finding new natural enemies to collecting the most promising of the natural enemies already encountered.

The prioritization categories used in this study may be useful for selection of potential biological control agents during surveys on other weed species. With the exception of the genetic matching data, all the information needed for prioritization can be collected in the

introduced distribution of the weed or during surveys in the region of origin. Genetic matching should be included as a prioritization category only when there is evidence to suggest that host plant genetic variation may be an important factor in agent selection. In the case of *P. aculeata* the disjunct and diverse native distribution suggests that some biological control agents may be affected by host plant intraspecific genetic variation (Chapter 4).

The proposed system should not be used to reject potential biological control agents but should rather be used as a method to suggest which potential biological control agents are the most appropriate to target for importation into quarantine for further studies, including host specificity trials, in the introduced distribution of the weed. If all of the most promising natural enemies prove to be unsuitable for biological control, natural enemies from the previous prioritization category should be selected. By selecting the most promising of the potential biological control agents for further study the risk of conducting host specificity studies on unsuitable natural enemies is reduced, resulting in more efficient use of biological control researcher's time and resources.

Chapter 7

General Discussion

The studies conducted for this thesis were intended to improve the biological control programme against *P. aculeata* by conducting a pre-release evaluation of the weed's impact, determining appropriate goals for control, determining whether new biological control agents were required and identifying the most effective potential biological control agent. The results of the studies have implications for the biological control programme against *P. aculeata* and for biological control programmes against environmental weeds in general.

In this chapter, the importance of improving methods of determining the most effective biological control agent and evaluating success in weed biological control is discussed. The response of natural ecosystems to weed management and the potential importance of restoration following weed management is then considered. Finally, the implications of this research to the biological control programme against *P. aculeata* and the direction of future research for the control of *P. aculeata* are discussed.

7.1. Determining the most effective agent

Selection of the most effective biological control agent has long been considered the 'holy grail' of biological control research (McFadyen 1998). There have been a number of studies that propose agent characteristics which may increase the likelihood of natural enemies being successful biological control agents (Hokkanen & Pimental 1984, Wapshere 1983, 1985, Harris & Shorthouse 1996) but only two proposed systems attempt to take more than a single characteristic into account when selecting the most effective agent (Harris 1973, Goeden 1983). Although these systems for agent selection have contributed greatly to debates around agent selection in the biological control research literature, they have generally not been utilised (Van Klinken & Raghu 2006) and some researchers have suggested that predicting agent efficacy prior to release is inaccurate, impractical and unnecessary (Simmonds 1976).

Retrospective analyses of biological control programmes, such as those presented in this thesis (Chapter 3, 4, 5 & 6), are valuable ways to examine biological control methods and identify ways in which methods could be improved. Factors which may have affected success or failure of biological control agents often become apparent only some time after the release of the agent. A retrospective analysis of these factors could result in improved agent selection in future because results are likely to be applicable to other biological control programmes.

There are a large number of factors that could contribute towards agent success, such as the biology of the weed and the agent, mode of damage, climatic matching, genotype matching, release effort, interactions between control agents and integration with other control methods. In this thesis, agent selection can be divided into selection of the most effective agent species or combination of species (Chapter 3 & 6) and the selection of the most effective agent populations or variants from within a single agent species (Chapter 4 & 5). While greenhouse impact assessments (Chapter 3) and genotype matching (Chapter 4 & 5) examine just one aspect of the myriad of possible factors limiting success, the agent prioritization system proposed in Chapter 6 attempts to bring as many of the possible factors that can be examined during surveys in the region of origin together.

The usefulness of greenhouse or laboratory impact assessments as a method of determining agent success is limited due to problems with extrapolation into the field (Chapter 3). Accurately predicting agent densities prior to release is difficult (Balciunas & Smith 2006) but even in a retrospective study, when densities of *P. guérini* in the field were known, no strongly supported conclusions could be made about the agent's potential impact, and the results of the greenhouse study contradicted observations made in the field (Chapter 3). Better simulation of conditions in the field, by including nutrients and plant competition, may improve the predictive power of laboratory or greenhouse impact studies.

Genetic matching was expected to be a possible constraint to biological control of *P. aculeata* (Chapter 4) but the biological control agent, *P. guérini*, accepts *P. aculeata* plants from all regions of the introduced and native distribution as equally suitable hosts (Chapter 5). This result has important implications for other biological control programmes because it

indicates that biological control researchers do not need to identify the native population most closely to the weed population in order to ensure that the most effective agent genotype is collected. Although no differences in fitness were recorded for *P. guérini* reared on different *P. aculeata* genotypes, there may be differences for other natural enemy species (Chapter 5), so determining the origin of the weed population could be very important for some biological control agents but not others. Differences in the ability of a natural enemy to utilise various host plant genotypes are expected in natural enemies that are sedentary and have limited gene flow between insect populations on individual plant genotypes (Karban 1989, Karban 1992, Hanks & Denno 1994). A 'rule of thumb' for biological control researchers that could predict which insect guilds are likely to have intraspecific host plant specialisations would be useful. Genetic studies to identify the origin of a weed population could then be initiated only if it were likely that the biological control agent in question would have greater fitness if collected from the weed population's origin.

A method of ranking potential agents in order of predicted success would be of considerable value to biological control researchers, but only one such system has been proposed (Harris 1973), and very little has been done to improve this system besides the modifications to Harris's scoring system made by Goeden (1983). The complexity of the task and the lack of predictive power in pre-release studies (Simmonds 1976) may explain why so little progress has been made in refining a generalised system for agent selection. The method described in Chapter 6 differs from the methods proposed by Harris (1973) and Goeden (1983) in that laboratory based studies are not needed to prioritise the potential agents (Chapter 6). The proposed method is unlikely to have strong predictive power because many factors that may affect success in the introduced range are not taken into account but the unpredictability of agent success could be refined using this method, resulting in better utilisation of resources (Chapter 6).

Interactions between agents are important factors that may affect biological control success (Denoth *et al.* 2002). In Chapter 3, the impact of *P. guérini* was evaluated with the intention of determining whether new biological control agents were necessary for the control of *P. aculeata*. Impact assessment techniques similar to those used in Chapter 3 could

be used to measure the relative impacts of different combinations of potential biological control agents. Impact assessments of this nature would have the same problems of poor predictive power as those discussed in Chapter 3, but may be useful in identifying the agent or combination of agents that is the most potentially damaging. Impact assessments of all the potential agents or combination of agents on *P. aculeata* would be impractical but impact assessments of the most promising potential agents identified in Chapter 6 may be feasible. If the combination of agents that is potentially the most effective is chosen for release then the use of the 'Lottery model' (Myers 1985) could be avoided. Pre-release studies of this nature could reduce the number of ineffective agents that are released by identifying a combination of agents that damage the target plant in a way that results in cumulative stress (Seastedt 2007).

It can be argued that accurately predicting agent success prior to release is impossible due to the large number of variables and the complex interactions between variables that may determine success. Releasing safe agents without attempting to predict success may therefore be the most appropriate approach (Simmonds 1976). This approach has been taken in many biological control programmes that have unintentionally adopted a 'Lottery model' approach (Denoth *et al.* 2002). If predicting agent success is considered impossible or impractical, agent selection in biological control would remain more of an art than a science and although this would not necessarily detract from the usefulness of biological control in weed management, it would leave little room for improvement. The incremental improvements in biological control agent selection that have been made since Harris highlighted the need for such a system in 1973 have not contributed significantly to weed control as yet but have fuelled further interest and research which will hopefully result in a better understanding of the factors contributing to agent success and ultimately improved biological control of weeds.

The lack of progress in agent selection methods could also be attributed to the way in which biological control researchers' performances are reviewed. Although biological control researchers are sometimes associated with universities, their traditional seat is with government institutions that are mandated to control invasives. The performance of

biological control researchers is therefore generally measured by the number of agents that have been released or number of weeds that have been managed rather than the contribution that the researcher has made to the scientific literature. This encourages researchers to focus on standardised aspects of biological control, such as host specificity, which are essential to the process of biological control but contribute little to improving methods or understanding the reasons for successes and failures in biological control. If the academic output of biological control researchers is compared with a similar field that is traditionally seated at universities, such as invasion biology, it is clear that the academic outputs of biological control are not on a par with similar fields despite the high levels of government funding generally allocated to biological control research. The Web of Science® was used to compare the average h-index (an index of both productivity and impact) of three senior researchers from the field of biological control and compared to three senior researchers in the field of invasion biology who were considered comparable in terms of their position in their respective scientific communities. Only articles relevant to the respective topic were included in calculating the h-indices. Researchers in invasion biology had substantially higher h-indices than biological control researchers (biological control: mean of 18, range 17-19; invasion biology: mean of 30, range 26-38). In order to improve biological control methods, research into complex systems, such as predicting biological control success is essential. This type of research will contribute towards scientists' h-indices but will not directly contribute towards the traditional measures of performance of biological control researchers in the short term. Studies that aim to better understand and improve biological control should be supported. One way to encourage this type of research is to support biological control research at universities or similar academic institutions.

7.2. Evaluation of success in biological control

Evaluations of the success of biological control programmes are valuable because assessments of success can be used to justify continued investment in biological control and because retrospective analyses of success and failures may improve future control

programmes (Morin *et al.* 2009). Integration of biological control with other control methods is now considered the most appropriate management option for many weed species (Zimmermann & Neser 1999, Greathead 2003, Zimmermann & Olckers 2003), so an informative method of evaluation should be of the entire control programme rather than just the biological control aspect (Chapter 2). When evaluating success of weed control programmes it is essential that an appropriate parameter is measured. Ideally a reduction in the negative impact of the weed should be measured but if this is not possible a parameter that is clearly related to the weed's impact should be chosen. Selection of appropriate parameters to evaluate success is challenging for environmental weeds due to the diverse impacts that environmental weed species have on ecological functioning (Chapter 2). In the case of many environmental weed species, the impact is a reduction of native biodiversity. Although conservation of biodiversity is often justified by citing the negative consequences of biodiversity loss to ecosystem services and functioning (Vitousek 1992, Scholes *et al.* 2005), the biodiversity itself is worthy of conservation due to its intrinsic cultural value (McCauley 2006). The protection of native biodiversity is therefore an appropriate measure of success for many environmental weed species.

In order to develop standardised methods for weed control it is necessary to divide weed species into groups based on the impact of each species and develop an appropriate method for each group. Examples of impact based groups are weeds that reduce crop yield, weeds that reduce grazing capacity, weeds that disrupt fire regimes, weeds that reduce water availability and weeds that reduce native biodiversity. Where a weed species fits into more than one group the methods from each respective group could be applied. In this way, a relative measure of success of control of weed species within each group could be developed. This system would be valuable as a tool for retrospective analyses because the characteristics of successful and failed weed control programmes could be analysed.

The benefits of biological control of weeds in natural ecosystems are substantial (Van Driesche *et al.* 2010) and evaluation of success of biological control is essential to justify the continued use of biological control for conservation (Morin *et al.* 2009). Long term post-release evaluations are required in order to evaluate success in most cases but these studies

are often neglected due to lack of resources and funding (Syrett *et al.* 2000, Thomas & Reid 2007, Morin *et al.* 2009). Relatively few resources are required for long term post-release evaluations because expensive equipment is not necessarily required and visits to sites may need to be conducted only every few years. Allocations of resources into long term post-release evaluations are therefore justified.

7.3. Restoration in natural ecosystems

A reduction in weed densities will not necessarily result in an increase in native biodiversity, but because protection of biodiversity is the goal of biological control of environmental weeds, it is native biodiversity that should be measured when evaluating success (Chapter 2). Data from long term post-release evaluations that include measures of weed densities and associated native biodiversity will indicate whether native biodiversity is returning at an acceptable rate. If native biodiversity does not return, restoration of the native communities in the form of stimulating native seed bank germination through controlled fires or adding native seeds or seedlings to the community is required (Reid *et al.* 2009). In the case of *P. aculeata*, additions of seedlings or saplings would be an appropriate restoration method rather than stimulating native seed germination because natural fires are rare in the forest biomes in which the plant primarily occurs. The level of biodiversity and proportions of functional groups measured in plots where *P. aculeata* was absent should be seen as a goal for restoration (Chapter 2).

It is not feasible to add seeds or plant seedlings of all the native species present in sites that have not been infested with *P. aculeata*. A small number of representative species from each functional group should be selected based on ease of propagation, the speed at which the species grow and the time taken for the species to reach maturity. Restoration is intended to counteract any residual negative effects of the *P. aculeata* infestation, such as reduced recruitment, rather than adding plant species that simulate the pre-invaded state. The addition of native species could possibly reduce invasions by other invasive plant species by occupying vacant niches that would be susceptible to invasions (Elton 1958, MacArthur

1970). It is essential that long term post-release evaluations, including measures of weed density and native biodiversity, are continued after restoration efforts have been completed.

Although restoration may be necessary in some cases, the use of weed density related goals, as proposed in Chapter 2, is appropriate because without the initial reduction in weed densities, restoration efforts would be futile.

7.4. Biological control of *Pereskia aculeata*

Future surveys for potential biological control agents for *P. aculeata* are warranted (Chapter 6). Surveys should be conducted in the southern region of the plant's native distribution (Fig. 1.3) because plants in this region are genetically more similar to plants in the introduced distribution (Chapter 4) and because higher species richness was recorded during surveys in the southern region of the native distribution (Chapter 6). Rio de Janeiro Province, Espírito Santo Province, Bahia Province, Santa Catarina Province, Sao Paulo Province, Rio Grande do Sul Province and Paraná Province in south eastern Brazil are the most appropriate parts of the southern region of the native distribution of *P. aculeata* in terms of climatic matching and should therefore be the focus of future surveys (Fig. 6.2). Surveys in other parts of the southern region of native distribution that are climatically similar to the introduced distribution, such as the Brazilian provinces of Mato Grosso, Mato Grosso do Sul and Bolivia, Paraguay and northern Argentina, are unlikely to be productive because *P. aculeata* has not been recorded in these areas (Leuenberger 1986) (Fig. 6.2). If new potential biological control agents are likely to have local adaptations to certain *P. aculeata* genotypes, such as sedentary, short lived insects, these species should be collected from Rio de Janeiro Province, Brazil, where the closest relative of the South African *P. aculeata* population was found (Chapter 4).

Maracayia chlorisalis, Curculionidae sp. 2 and Curculionidae sp. 3 should be considered priority species for host specificity testing but surveys in areas of the southern region of native distribution that have not yet been visited, such as parts of Paraná Province, Bahia Province, Espírito Santo Province and Rio Grande do Sul, may result in new promising

potential agent species (Chapter 6). A survey of species of Cactaceae that are closely related to *P. aculeata*, including wild and cultivated pitahaya, in south-eastern Brazil may be informative in terms of predicting the host range of the *M. chlorisalis* populations present in the region. *Maracayia chlorisalis* populations in Colombia and Mexico are considered pests on cactaceous crop species (Zenner de Polania 1990, CESAVEP 2006) but populations in south eastern Brazil may have different host ranges (Chapter 6). Surveys for *M. chlorisalis* on cultivated pitahaya in Brazil will give a good indication of whether the insect is likely to be problematic to pitahaya farmers in South Africa.

The future success of the biological control programme against *P. aculeata* depends heavily on field trips to collect prioritized potential agents and, possibly, to identify new natural enemies that have not been encountered on previous surveys (Chapter 6). Acquiring permits to collect and export insects from South American countries is often a long and difficult process. A streamlined process to obtain collection and export permits from a single institution in each country would be extremely valuable. Collaboration with South American institutions is therefore essential for biological control of all South American plants that are considered problematic in South Africa. A serious limitation of the requirements of many permits is that insects that are to be exported must be identified prior to the survey. Many insects in South America have not been described and it is impossible to predict what species could be encountered during surveys. More realistic permit requirements which consider the process of surveying for unidentified biological control agents are needed.

The low number of natural enemies found associated with *P. aculeata* compared with other Cactaceae (Mann 1969, Zimmermann *et al.* 1979) leaves limited options for biological control. It is surprising that such low species richness of phytophagous insects would be associated with *P. aculeata* because there are ample resources for phytophagous insects in the form of leaves, fruits and stems and because high numbers of natural enemies have been found on other members of the Cactaceae. One possible reason for the low species richness associated with *P. aculeata* is that the true region of origin within the areas of South and Central America where *P. aculeata* is considered native has not been surveyed. For this reason surveys to areas where *P. aculeata* is native that have not been visited during previous

surveys are warranted. The possibility that *P. aculeata* simply has a depauperate phytophagous insect fauna for unknown reasons should not be ruled out.

Mass rearing and releases of *P. guérini* should continue unless further evidence suggests that it is an ineffective agent, or if *P. guérini* is likely to interact negatively with a new, and more effective, agent (Chapter 3). Post-release evaluations at sites that were used in the pre-release study may be useful in evaluating the potential of *P. guérini* as a biological control agent. Pre-release densities of *P. aculeata* are known at these sites so if densities are measured after the release of *P. guérini* it will be possible to determine whether *P. guérini* has reduced the weed population to the appropriate densities calculated as goals in the pre-release study (Chapter 2). The limited number of sites at which *P. guérini* has established may be due to poor release efforts (Klein 1999). Augmentative releases of large numbers may improve establishment rates and may result in a decrease in *P. aculeata* density in a shorter time.

The current list of phytophagous insects associated with *P. aculeata* includes insects that have good potential as biological control agents (Chapter 6). *Maracayia chlorisalis* is the most promising new agent and future research should focus on conducting host specificity testing for this species. If *M. chlorisalis* is safe for release in terms of its host specificity then studies to determine the interactions between *M. chlorisalis* and *P. guérini* should be conducted.

7.5. Conclusion

Retrospective analyses, such as this thesis, are valuable for the purpose of improving evaluations of success and agent selection in weed biological control programmes. By evaluating success and analysing the possible reasons for successes and failures of other biological control programmes the process of agent selection could be significantly improved. Improvements to methods of evaluating success and selection of biological control agents could lead to more efficient and effective weed biological control programmes and would therefore contribute to the control of environmental weeds.

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Appendix

Table A1. Locality data of *Pereskia aculeata* plants collected for DNA analysis and the two sites where *Phenrica guérini* was collected.

Sample name	Location	Longitude	Lattitude
SA1	Knysna, South Africa	S34°02'0.0"	E23°04'0.0"
SA2	Grahamstown, South Africa	33°18'57.17"S	26°31'50.90"E
SA3	Port Alfred, South Africa	33°35'47.78"S	26°53'17.35"E
SA4	Port St. Johns, South Africa	31°36'56.23"S	29°32'29.90"E
SA5	Umdomi, South Africa	30°23'12.60"S	30°40'15.35"E
SA6	Shongweni, South Africa	29°52'0.0"S	30°42'0.0"E
SA7	Durban, South Africa	29°48'53.4"S	31°01'18.7"E
SA8	Durban, South Africa, Variegated	29°48'53.4"S	31°01'18.7"E
SA9	Stanger, South Africa	29°20'33.9"S	31°18'41.3"E
SA10	Kosi Bay, South Africa	26°57'49.17"S	32°48'40.19"E
VZ1	Caracas, Venezuala	10°27'0.0"N	66°48'21"W
VZ2	Caracas, Venezuala	10°27'0.0"N	66°48'21"W
DR1	Santo Domingo, Dominican Rep., Variegated	18°29'34.63"N	69°57'30.42"W
DR2	Punta Cana, Dominican Rep.	18°35'51.98"N	68°28'02.80"W
DR3	Pendernales, Dominican Rep.	17°47'37.78"N	71°28'06.73"W
DR4	Pendernales, Dominican Rep.	17°47'51.24"N	71°28'03.19"W
DR5	Pendernales, Dominican Rep.	17°47'51.17"N	71°27'38.44"W
B2	Londrina, Brazil	23°22'40.40"S	51°04'26.9"W
B3	Londrina, Brazil	23°22'19.20"N	51°03'54.8"W
B4	Curatiba, Brazil	26°55'08.00"N	48°38'31.5"W
B5	Curatiba, Brazil	26°59'37.00"N	48°35'54.7"W
B6	Curatiba, Brazil	27°00'36.3"S	48°34'46.8"W
B7	Curatiba, Brazil	27°01'23.7"S	48°34'42.9"W
B8	Curatiba, Brazil	27°01'25.1"S	48°34'27.5"W
B9	Curatiba, Brazil	27°03'14.1"S	48°35'15.8"W
B10	Rio de Janeiro, Brazil	23°00'57.37"S	43°25'24.88"W
B11	Rio de Janeiro, Brazil	22°55'59.44"S	42°36'37.46"W
B12	Curatiba, Brazil	26°46'00.32"S	48°38'27.48"W
B13	Curatiba, Brazil	26°47'26.16"S	48°35'16.66"W
A1	Misiones, Argentina	27°21'49.20"S	55°35'11.00"W
A2	Misiones, Argentina	26°21'05.80"S	54°12'54.80"W
A3	Misiones, Argentina	25°38'12.60"S	54°33'10.00"W
A4	Misiones, Argentina	26°37'22.60"S	54°07'19.20"W
A5	Misiones, Argentina	27°45'46.00"S	55°03'31.90"W
A6	Misiones, Argentina	26°40'10.90"S	54°46'15.40"W
A7	Misiones, Argentina	26°57'04.10"S	54°36'00.10"W
A8	Misiones, Argentina	26°59'30.20"S	55°08'24.90"W
A9	Misiones, Argentina	27°00'56.30"S	54°36'00.10"W
A10	Misiones, Argentina	26°46'11.00"S	54°19'05.70"W
A11	Misiones, Argentina	26°19'41.10"S	54°36'54.30"W
A12	Misiones, Argentina	26°24'00.90"S	54°24'34.60"W
A13	Misiones, Argentina	26°43'26.20"S	54°14'56.40"W
Phenrica guerini	Rio de Janeiro, Brazil	22°59'56"S	43°11'27"W
Phenrica guerini	Rio de Janeiro, Brazil	22°17'59"S	41°37'55"W