

**Interactions between two biological
control agents released on
Pereskia aculeata Miller (Cactaceae), in
South Africa**

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Abstract

Pereskia aculeata Miller (Cactaceae) is an alien invasive plant introduced into South Africa from Brazil, which has negative impacts on native biodiversity in South Africa. *Phenrica guerini* Bechyne (Chrysomelidae) and *Catorhintha schaffneri* Brailovsky & Garcia (Coreidae) are two biological control agents released against *P. aculeata* in South Africa. *Phenrica guerini* was first released against *P. aculeata*, in 1991, followed by *C. schaffneri* in 2014. The overall aim of this study was to improve the biological control programme against *P. aculeata* in order to decrease its density to a level where it does not threaten the native biodiversity of South Africa.

The first part of this study evaluated the efficacy of *P. guerini* on *P. aculeata* at Port Alfred (Eastern Cape) to better understand the role of *P. guerini* in the biological control of *P. aculeata*. An insecticide exclusion experiment was conducted over 100 days. Plots with *P. guerini* had a mean of 187 (SE $\pm$ 62) fewer leaves/m² than plots without *P. guerini*. The agent reduced percentage cover in plots with *P. guerini*, with a mean of 19.42% (SE $\pm$ 3.15) lower cover than plots without *P. guerini*. Although *P. guerini* had an impact on *P. aculeata* at Port Alfred previous studies have indicated that a reduction to below 50% cover is required for native biodiversity to recover and the agent only reduced cover to 62% at Port Alfred. *Phenrica guerini* has therefore not reduced percentage cover sufficiently to completely control the weed. The data collected from Port Alfred was compared to the performance of the agent nationwide. Although *P. guerini* was found at far more sites than previously recorded, there were very few sites with comparable levels of damage to Port Alfred. This evidence suggests that *P. guerini* is not sufficiently damaging to reduce *P. aculeata* to acceptable levels and other biological control agents should be considered.

Interactions between two biological control agents can have complex and unexpected impacts for a biological control programme. The second part of this study was to investigate interactions between *C. schaffneri* and *P. guerini* under laboratory conditions to test whether the two agents, individually or jointly, enhanced or reduced their impact on *P. aculeata*. Potted *P. aculeata* plants were exposed to one of four treatments: control (no agents), *P. guerini* only, *C. schaffneri* only and both species in combination. Four stocking densities, ranging from 2 to 12 insects per plant were used. *Catorhintha schaffneri* alone at high densities was more damaging than all other treatments with a significantly greater reduction in the mean number of leaves, 11.7 (SE $\pm$ 1.29), and shoot lengths, 2.17cm (SE $\pm$ 0.75). Even at lower density treatments, the combination of the two agents was not significantly more damaging than *C. schaffneri* alone and *C. schaffneri* was always more damaging than *P. guerini* alone. Mortality of *P. guerini* was significantly higher than *C. schaffneri* at the highest stocking density when in combination.

Phenrica guerini contributes towards the biological control of *P. aculeata* at some sites in South Africa but not enough to completely control the weed. The antagonistic interaction between *P. guerini* and *C. schaffneri* suggests that these agents should not be released together because this would impact negatively on the overall biocontrol programme against *P. aculeata*. *Catorhintha schaffneri* should be released at sites where *P. guerini* is not present and evaluations of the success of this agent in the field should be conducted. Extrapolation of laboratory-based studies into the field is often challenging so mass-rearing of *P. guerini* should continue until there is convincing proof that *C. schaffneri* alone is more effective than *P. guerini* in the field.

Table of Contents

Abstract	ii
List of figures	viii
List of tables	xiii
Acknowledgements.....	xiv
Chapter 1: General Introduction	1
1.1. Plant-insect interactions.....	1
1.2. Insect-insect interactions between phytophagous insect species	4
1.3. Invasive alien plants	4
1.4. Biological control of invasive alien plants.....	8
1.5. Is a biological control program best achieved by single or multiple agents?.....	10
1.6. Importance of post-release evaluation and quantifying success	12
1.7. <i>Pereskia aculeata</i> Miller (Cactaceae).....	13
1.7.1. Description of <i>Pereskia aculeata</i>	13
1.7.2. Biological control of <i>Pereskia aculeata</i> in South Africa	16
1.8. Thesis aims and rationale	19
Chapter 2: Impact and abundance of <i>Phenrica guerini</i> on <i>Pereskia aculeata</i>	21
2.1. Introduction.....	21

2.1.1. Post-release evaluations	21
2.1.2. The importance of post-release evaluations	21
2.1.3. Types of data collected to assess agent effectiveness	23
2.1.4. Methods of post-release evaluations.....	24
2.1.5. Comparing sites (or plots) with and without agents.....	24
2.1.6. Comparing before and after release data	25
2.1.7. Manipulative experiments.....	27
2.1.8. Evaluating success in biological control	28
2.1.9. Post-release evaluation of <i>Phenrica guerini</i>	29
2.2. Materials and Methods	30
2.2.1. The details of the insecticide chosen	30
2.2.2. The impact of insecticide on <i>P. guerini</i> and <i>P. aculeata</i>	30
2.2.3. Effect of <i>Phenrica guerini</i> on growth of <i>P. aculeata</i> under field conditions.....	32
2.2.4. Nationwide survey.....	34
2.3. Results	36
2.3.1. The impact of insecticide on <i>P. guerini</i> and <i>P. aculeata</i>	36
2.3.2. Effect of <i>Phenrica guerini</i> on growth of <i>P. aculeata</i> under field conditions.....	42
2.3.3. Nationwide survey.....	51

2.4. Discussion.....	53
2.5. Conclusion	57
Chapter 3: Interactions between <i>Phenrica guerini</i> and <i>Catorhintha schaffneri</i> , two biological control agents for <i>Pereskia aculeata</i> in South Africa	59
3.1. Introduction.....	59
3.1.1. Insect-insect interactions	59
3.1.2. Multiple versus single biological control agents.....	62
3.1.3. Classification of the effects of interactions between insects on their host plants	64
3.2. Materials and methods	67
3.2.1. <i>Pereskia aculeata</i> potted plants	67
3.2.2. Rearing of <i>Phenrica guerini</i>	67
3.2.3. Rearing of <i>Catorhintha schaffneri</i>	68
3.2.4. Experimental design.....	69
3.3. Data collection	72
3.3.1. Parameters measured	72
3.4. Data analysis	72
3.5. Results	73
3.5.1. Numbers of leaves	73

3.5.2. Shoot length	75
3.5.3. Plant biomass	78
3.5.4. Insect mortality	80
3.6. Discussion.....	83
3.7. Conclusion	88
Chapter 4: General Discussion	89
References	95

List of figures

Chapter 1

Figure 1.1 *Pereskia aculeata* (Drawn by G. Condry; first published in Henderson (1995), ARC-Plant Protection Research Institute, Pretoria.) 14

Figure 1.2 Distribution of *Pereskia aculeata* in South Africa. (Drawn by L. Henderson; data source: SAPIA database, ARC-Plant Protection Research Institute, Pretoria.) 15

Chapter 2

Figure 2.1 Change in the number of leaves for different treatments. The treatments included were control=no herbivory and no insecticide applied, insecticide only=insecticide applied on plants with no *P. guerini*, *P. guerini* only=insects not excluded by insecticide, and *P. guerini* and insecticide=plants inoculated with *P. guerini* and then sprayed with insecticide to exclude the agent. Error bars indicate standard errors around each mean. Different letters indicate significant difference ($p < 0.05$) according to Fisher's LSD test. 37

Figure 2.2 Change in the shoot length for different treatments. The treatments included were control=no herbivory and no insecticide applied, insecticide only=insecticide applied on plants with no *P. guerini*, *P. guerini* only=insects not excluded by insecticide, and *P. guerini* and insecticide=plants inoculated with *P. guerini* and then sprayed with insecticide to exclude the agent. Error bars indicate standard errors around each mean. Different letters indicate significant differences ($p < 0.05$) according to Fisher's LSD test. 38

Figure 2.3 Change in the number of damaged leaves for different treatments. The treatments included were *P. guerini* only=insects not excluded by insecticide, and *P. guerini*

and insecticide=plants inoculated with *P. guerini* and then sprayed with insecticide to exclude the agent. Error bars indicate standard errors around each mean. Different letters indicate significant differences ($p<0.05$) according to Fisher's LSD test. 39

Figure 2.4 Mean ($\pm$ SE) plant biomass (g) for different treatments. The treatments included were control=no herbivory and no insecticide applied, insecticide only=insecticide applied on plants with no *P. guerini*, *P. guerini* only=insects not excluded by insecticide, and *P. guerini* and insecticide=plants inoculated with *P. guerini* and then sprayed with insecticide to exclude the agent. Error bars indicate standard errors around each mean. Different letters indicate significant differences ($p<0.05$) according to Fisher's LSD test. 40

Figure 2.5 Mean ($\pm$ SE) survival of *P. guerini* for different treatments after 7 days. The treatments included were *P. guerini* only=insects not excluded by insecticide, and *P. guerini* and insecticide=plants inoculated with *P. guerini* and then sprayed with insecticide to exclude the agent. Error bars indicate standard errors around each mean. Different letters indicate significant differences ($p<0.05$) according to Fisher's LSD test. 41

Figure 2.6 Mean ($\pm$ SE) survival of *P. guerini* for different treatments after 14 days. The treatments included were *P. guerini* only=insects not excluded by insecticide, and *P. guerini* and insecticide=plants inoculated with *P. guerini* and then sprayed with insecticide to exclude the agent. Error bars indicate standard errors around each mean. Different letters indicate significant differences ($p<0.05$) according to Fisher's LSD test. 42

Figure 2.7 Number of leaves per m^2 on different sampling days for each treatment. The treatments included were *P. guerini* present=plots with insects not excluded by insecticide, and *P. guerini* absent=plots sprayed with insecticide to exclude the agent. Error bars

indicate standard errors around each mean. Different letters indicate significant differences (p<0.05) according to Fisher's LSD test. 44

Figure 2.8 Shoot lengths (cm) per m² on different sampling days for each treatment. The treatments included were *P. guerini* present=plots with insects not excluded by insecticide, and *P. guerini* absent=plots sprayed with insecticide to exclude the agent. Error bars indicate standard errors around each mean. Different letters indicate significant differences (p<0.05) according to Fisher's LSD test. 45

Figure 2.9 Percentage cover per m² on different sampling days for each treatment. The treatments included were *P. guerini* present=plots with insects not excluded by insecticide, and *P. guerini* absent=plots sprayed with insecticide to exclude the agent. Error bars indicate standard errors around each mean. Different letters indicate significant differences (p<0.05) according to Fisher's LSD test. 47

Figure 2.10 Number of shoots per m² on different sampling days for each treatment. The treatments included were *P. guerini* present=plots with insects not excluded by insecticide, and *P. guerini* absent=plots sprayed with insecticide to exclude the agent. The error bars indicate standard errors around each mean. Different letters indicate significant differences (p<0.05) according to Fisher's LSD test. 48

Figure 2.11 Number of damaged leaves per m² on different sampling days for each treatment. The treatments included were *P. guerini* present=plots with insects not excluded by insecticide, and *P. guerini* absent=plots sprayed with insecticide to exclude the agent. The error bars indicate standard errors around each mean. Different letters indicate significant differences (p<0.05) according to Fisher's LSD test. 50

Figure 2.12 Adults of *P. guerini* found per m² on different sampling days for each treatment. The treatments included were *P. guerini* present=plots with insects not excluded by insecticide, and *P. guerini* absent=plots sprayed with insecticide to exclude the agent. Error bars indicate standard errors around each mean. Different letters indicate significant differences ($p<0.05$) according to Fisher's LSD test. 51

Chapter 3

Figure 3.1 The Latin Square Design representing the four treatments by the letters C= Control, S= *Catorhintha schaffneri*, P= *Phenrica guerini* and B= Both insects in combination. Numbers 2, 4, 6 and 12 indicate different stocking densities. 71

Figure 3.2 Mean ($\pm$ SE) changes in the number of leaves of different treatments at different stocking densities. Numbers 2, 4, 6, and 12 indicate different stocking densities. Treatments: S=*Catorhintha schaffneri*; P=*Phenrica guerini*; and B=both insects in combination. Different letters indicate significant differences ($p<0.05$) according to Fisher's LSD test. Error bars indicate the standard error around each mean. 75

Figure 3.3 Mean ($\pm$ SE) changes in shoot lengths (cm) of different treatments at different stocking densities. Numbers 2, 4, 6, and 12 indicate different stocking densities. Treatments: S=*Catorhintha schaffneri*; P=*Phenrica guerini*; and B=both insects in combination. Different letters indicate significant differences ($p<0.05$) according to Fisher's LSD test. Error bars indicate the standard error around each mean. 77

Figure 3.4 Mean ($\pm$ SE) plant biomass (g) after different release treatments at different stocking densities. Numbers 2, 4, 6, and 12 indicate different stocking densities. Treatments: S=*Catorhintha schaffneri*; P=*Phenrica guerini*; and B=both insects in combination. Letters

indicate significant differences ($p < 0.05$) according to Fisher's LSD test. Error bars indicate the standard error around each mean..... 78

Figure 3.5 Mean ($\pm$ SE) mortality of *C. schaffneri* and *P. guerini* when each was released alone over a 10-day period at different stocking densities. Numbers 2, 4, 6, and 12 indicate different stocking densities. Treatments: S=*Catorhintha schaffneri* and P=*Phenrica guerini*. Different letters indicate significant differences ($p < 0.05$) according to Fisher's LSD test. Error bars indicate the standard error around each mean..... 81

Figure 3.6 Mean ($\pm$ SE) mortality of *C. schaffneri* and *P. guerini* when both agents were released over a 10-day period at different stocking densities. Numbers 2, 4, 6, and 12 indicate different stocking densities. Treatments: S=*Catorhintha schaffneri* and P=*Phenrica guerini*. Different letters indicate significant differences ($p < 0.05$) according to Fisher's LSD test. Error bars indicate the standard error around each mean. 82

List of tables

Chapter 2

Table 2.1. Release history of *P. guerini* abundance and level of damage measured at each site of the 23 sites in KwaZulu-Natal and Port Alfred in the Eastern Cape.52

Table 2.2. Comparison of number of damaged leaves per m² between Port Alfred and sites in KwaZulu-Natal. The only site with similar levels of damage to Port Alfred was Paradise Valley, which is highlighted in bold text.53

Chapter 3

Table 3.1. The combinations of species and total number of insects released in each treatment of the experiment.69

Table 3.2. Means ($\pm$ SE) of *Pereskia aculeata* plant growth during a 10 day period when plants were exposed or not exposed to herbivory by a single or both agents in combination. Treatments: S=*Catorhintha schaffneri*, P=*Phenrica guerini*, B= both insects in combination. Letters given after the standard errors indicate differences ($p < 0.05$) according to Fisher's LSD.79

Table 3.3. Comparison of *P.guerini* larval mortality when released in combination with *C. schaffneri* nymphs or alone at various stocking density over a 10-day period. This table shows that *P. guerini* experienced higher mortality when in combination with *C. schaffneri* compared to when it is released alone.83

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Chapter 1: General Introduction

Invasive alien plants, also referred to as invasive alien weeds, are a serious global problem (Vitousek *et al.*, 1997). Biological control, which utilizes host specific natural enemies to reduce invasive alien plant densities, is one of the methods implemented to manage this problem (McFadyen, 1998). One of the essential phases of a biological control programme is post-release evaluation where the success of the agents released is evaluated and the need for additional agents is assessed (Morin *et al.*, 2009). If more agents are required to successfully control the target plant, then the interaction between the two agents needs to be assessed (Myers, 1985).

In this study, a post-release evaluation was conducted for a biological control agent that was released 25 years ago for the control of the invasive alien cactus, *Pereskia aculeata* Miller (Cactaceae), and the interactions between this agent and a new biological control agent were investigated. In this chapter, a general introduction to the topics of plant-insect and insect-insect interactions is provided. This is followed by an introduction to alien invasive plants, biological control of weeds and the plant-insect interaction that is the focus of the thesis, *Pereskia aculeata* and the two biological control agents, *Phenrica guerini* Bechyne (Chrysomelidae) and *Catorhintha schaffneri* Brailovsky & Garcia (Coreidae).

1.1. Plant-insect interactions

It is estimated that 50% of eukaryote biodiversity in terrestrial ecosystems is composed of plants and insects that eat them (Price, 2002). Insect herbivores can be categorised according

to their feeding specialisations; namely specialist insect herbivores and generalist insect herbivores (Crawley, 1989a). Some can be both generalists and specialists at different times because their feeding behaviour changes depending on the availability of a food source. For example, some aphids are specialists on different plants at different times of the year (Crawley, 1989a). The generalist (polyphagous) insects feed on a variety of host plants of different plant families and the specialist (monophagous and oligophagous) insects feed on one or a few host plants which belong to the same family or genus (Howe and Jander, 2008; Lampert, 2012). As examples, the leafhopper, *Nilaparvata lugens* (Stål) (Delphacidae), has a narrow host range and prefers to feed only on rice species, (Tang *et al.*, 2010; Shivshankar *et al.*, 2012; Xu *et al.*, 2014) whereas the autumnal moth, *Epirrita autumnata* (Borkhausen) (Geometridae), has a wide diet breadth and feeds on several deciduous tree species belonging to several families (Ruohomaki *et al.*, 2000).

Herbivorous insects have different feeding guilds that damage different plant parts such as leaf feeders, sap-suckers, stem and shoot borers, fruit and flower feeders, and seed feeders (Crawley, 1983, Strong *et al.*, 1984). The effect of insect herbivores on plant growth is different for each insect and it depends on the type of plant part which is damaged (Begon *et al.*, 2006). Insect herbivory may disturb the plant's developmental stages by feeding on the reproductive structures, for example, flower-bud feeders may stop the plant from producing flowers and will, therefore, reduce seed set (Blossey and Schat, 1997; Barber *et al.*, 2011). The influence of insect herbivory can also sometimes be indirect, for example by causing flowers to have a degraded appearance and resulting in a lower rate of pollination (Halpern *et al.*, 2010). The impact of herbivory on a plant is also influenced by the quantity; location and timing of attack

relative to plant developmental stages (Crawley, 1983). An example of the differential impact of feeding location can be illustrated by a comparison between the damage of leaf feeders, which reduce the photosynthetic area of the leaf, as opposed to stem and shoot borers, which affect the physical structure of the plant and often damage xylem and phloem and so disturb the movement of nutrients and water within the plant (Crawley, 1983). The timing of the feeding can also influence the impact of herbivory. For example, damage to the youngest leaves results in more severe impacts compared to the impact on old leaves, and seedlings are more likely to be killed by herbivory than older plants (Crawley 1983). The feeding methods, quantity, timing, and location of the herbivory can therefore affect host plant fitness, survival, and growth rate (Lehndal and Agren, 2015).

Insect herbivory can often reduce a plant's ability to compete for resources with other plants (Crawley, 1989b). This happens in many different ways, one of which is by changing the plant's relative ability to acquire limited resources (for example, reducing its height if the limited resource is sunlight) (Louda *et al.*, 1990). Such competition, which may be interspecific (occurring between individuals of different species) (Tilman, 1997) or intraspecific (occurring between individuals of the same species), is defined as the response of an organism to acquiring a resource that is limited within an environment (Louda *et al.*, 1990; Kaplan and Denno, 2007). Keddy (1989) referred to competition as the negative response which one organism shows to another by consuming or restricting its access to a resource that is limited. Competition may alter the physiological functioning and structure of individuals which may, in turn, lead to changes in their growth form, reproduction, and fitness (Booth *et al.*, 2010). Competition can be classified as either exploitative or interference (Keddy, 1989). Exploitative

competition occurs indirectly when one individual uses shared resources so depriving others of using the resource (Keddy, 1989). Interference competition is a direct form of competition where individuals harm each other by fighting or producing chemicals (as with allelopathy) which limit access of other individuals to the resource (Schoener, 1983; Keddy, 1989). It is, therefore, important to consider the impacts of competition with other plants when investigating the impact of herbivorous insects on their host plant's populations.

1.2. Insect-insect interactions between phytophagous insect species

Interspecific interactions influence the performance and fitness of phytophagous insects and also play a role in structuring insect communities (Kaplan and Denno, 2007). Insect-insect interactions may be positive or negative (Petersen and Sandström, 2001). Negative interactions are observed as direct competition, which can occur through the exploitation of a common limited food resource (Petersen and Sandström, 2001). Insect herbivores targeting the same plant part are most likely to compete for a resource (Petersen and Sandström, 2001). Competition between these insects can be reduced by resource partitioning, such as feeding and emerging at different times or targeting different parts of the host-plant (Schoener, 1974). From an applied perspective, insect-insect interactions can have a negative impact on biological control programmes by reducing or enhancing the overall efficacy, fitness, and performance of insect biological control agents (Briese, 1997; Crowe and Bouchier, 2006).

1.3. Invasive alien plants

Invasive alien plants grow outside their native distribution where conditions are favourable for their growth. They grow where they are not needed and not wanted (Gullan and Cranston,

2000; Myers and Bazely, 2003; Catford *et al.*, 2009). Invasive alien plants pose a threat to natural ecosystems and often have a negative impact on native biodiversity (Simberloff, 2005; Paterson *et al.*, 2011b; Quentin and Fuller, 2013). The mechanisms that contribute to biological invasion are not well understood and predicting which exotics will become invasive among introduced species is a challenge (Meyers *et al.*, 2005).

Introduction, establishment and spread are the three steps that must take place for a species to become an invasive (Williamson, 1993; Jeschke, 2008). The 'Tens Rule' explains how often invasive species establish themselves within introduced ranges and how likely they are to become pests (Williamson *et al.*, 1986). The 'Tens Rule' proposes that approximately 10% of species introduced in a new range survive about 10% of those introduced species will become established and about 10% of these will spread and become invasive. Thus, of 1000 alien species introduced, the 'Tens Rule' holds that one will become invasive (Williamson and Fitter, 1996).

Invasive alien species are the second leading factor, after habitat destruction, contributing to the loss of biodiversity worldwide (Srivastava *et al.*, 2014). This is caused by their capability to outcompete and replace native species (Byers *et al.*, 2002; Nuñez *et al.*, 2010; Hornoy *et al.*, 2011). It is therefore necessary to respond to the issue of biological invasions urgently and to acquire knowledge of major factors that contribute to an exotic plant species becoming invasive (Shea and Chesson, 2002). A number of hypotheses have been proposed in an attempt to explain and predict the factors that contribute to the establishment and spread of invasive alien species outside of their native distribution (Jeschke *et al.*, 2012).

The Enemy Release Hypothesis (ERH) explains that non-native species lack natural enemies (i.e. predators, herbivores, and pathogens) found in their native communities, which is why plant populations are greater in the introduced range than in the native range (Crawley, 1997; Maron and Vilà, 2001; Keane and Crawley, 2002; Vilà *et al.*, 2004). The absence of natural enemies within the introduced ranges makes it possible for non-native species to outcompete native species by increasing in abundance and distribution (Maron and Vilà, 2001; Keane and Crawley, 2002; Cappuccino and Carpenter, 2005). There are many examples supporting the predictions of this hypothesis in which there are higher levels of damage inflicted by herbivory where the plant is native compared to where the plant is introduced (Wolfe, 2002; DeWalt *et al.*, 2004; Vilà *et al.*, 2004; Liu and Stiling, 2006; Adams *et al.*, 2009). On the other hand, some studies do not associate specific alien invasions with a release from natural enemies (Blaney and Kotanen, 2001; Beckstead and Parker, 2003; Novotny *et al.*, 2003).

The Evolution of Increased Competitive Ability (EICA) hypothesis postulates that the absence of natural enemies in the introduced range of an invasive alien plant could increase competitive ability because the traits that were used to defend the plant against herbivory are re-allocated to improve growth and reproduction processes. This change in resource allocation is possible because defences are no longer needed as the natural enemies of the plant are not present in the introduced range (Blossey and Notzold, 1995; Keane and Crawley, 2002).

The Novel Weapons Hypothesis (NWH) proposes that invasive alien plants have a greater competitive ability because they release allelopathic chemicals that have a negative impact on other plants within the introduced range (Callaway and Ridenour, 2004). These allelopathic

chemicals do not affect plants in the native range because of their co-evolutionary history (Schaffner *et al.*, 2011). This results in higher densities of the exotic plants within their introduced range compared to the native range (Cappuccino and Carpenter, 2005).

The Vacant Niche Hypothesis (VNH) suggests that non-native species use resources such as space, water, and light that are not used by native species (Levine and D'Antonio, 1999; Mack *et al.*, 2000). The invasive alien species, therefore, proliferate and spread more effectively within the introduced range compared to the native range because competition for resources in the introduced range is reduced (Burslem *et al.*, 2005).

There are other hypotheses that have been proposed to explain why alien species become problematic outside of their native distribution. Many of these hypotheses are similar and interrelated with each other (Catford *et al.*, 2009). For example, there are hypotheses that focus on ecosystems into which invaders were introduced (biotic resistance hypothesis (Von Holle, 2005), disturbance hypothesis (Colautti *et al.*, 2006) and some on invader-ecosystem interactions (invasional meltdown) (Jeschke *et al.*, 2012)). Although these hypotheses have been discussed individually, none are mutually exclusive and a combination of different hypotheses will most likely explain why plants become problematic outside of their native distribution with the relative importance of each hypothesis varying depending on the invasive species in question and the nature of the ecosystem that is invaded.

1.4. Biological control of invasive alien plants

Biological control of invasive alien plants involves the use of natural enemies, such as phytophagous insects and pathogens, to reduce densities of a target weed to a level where it is no longer a significant threat to native biodiversity (Myers, 1985; van Wilgen *et al.*, 2013; Seastedt, 2015; Hajek *et al.*, 2016). The theory of biological control is grounded in the Enemy Release Hypothesis (Keane and Crawley, 2002) in which a lack of natural enemies is proposed to results in the invasive alien plant gaining a competitive advantage over indigenous plant species within the introduced range (Maron and Vilà, 2001). Biological control aims to reduce the competitive advantage of the invasive alien plant by introducing natural enemies (biological control agents) from the native range into the introduced range where the plant has become problematic. Biological control of weeds has been practiced for many years in some countries, for example in India (1863), Sri Lanka (1865), Hawaii, U.S.A. (1902), Australia (1912) and in South Africa (1913) (Tryon, 1910; Coulson *et al.*, 2000; Julien *et al.*, 2012). It is a method that has resulted in complete control of some invasive alien plants and is considered a safe and environmentally friendly method of control although some authors have emphasized the non-target effects and unintended consequences of the release of biological control agents (Simberloff and Stiling, 1996; Louda *et al.*, 2005; Simberloff, 2011; Seastedt, 2015).

Suckling and Sforza (2014) conducted a study to review the magnitude of non-target impacts from weed biocontrol agents. They found that more than 99% of the biological control agents that have been introduced had no major non-target effects (Suckling and Sforza, 2014). *Rhinocyllus conicus* Frölich (Curculionidae) and *Cactoblastis cactorum* (Bergroth) (Pyralidae)

were the only two biological control agent species which were considered to have major non-target effects (Suckling and Sforza, 2014) but even the release of these agents is believed to have a positive net benefit for biological diversity because the impacts of the weeds, which were successfully controlled by the biological control agents, were far more damaging than the relatively small non-target effects caused by the agents (Hinz *et al.*, 2014; Downey and Paterson, 2016). It must also be emphasised that the host ranges of both *R. conicus* and *C. cactorum* were accurately determined prior to release, so the non-target effects that were recorded were predictable and expected (Downey and Paterson, 2016).

Biological control is regarded as the only available option when mechanical and chemical methods are ineffective (Moran and Zimmermann, 1991). It is environmentally-sustainable and this sustainability makes it economically viable compared to mechanical and chemical control (McFadyen, 2000; Clewey *et al.*, 2012). It does not eradicate invasive alien weeds but aims to reduce the weed to a level where the weed is no longer a significant threat or problem (Paterson *et al.*, 2011b) and therefore helps to protect the remaining native biodiversity in natural ecosystems (Culliney, 2005; Müller-Schärer and Schaffner, 2008).

South Africa celebrated 100 years of biological control of weeds in 2013. The use of biological control in the country has decreased the negative effects of alien invasive plants on economic productivity and natural ecosystem services (Zimmermann *et al.*, 2004; Moran *et al.*, 2013) and has significantly reduced the costs of using other control methods for a number of invasive alien plant species (van Wilgen *et al.* 2013; van Wilgen and Wannenburgh, 2016). Biological control was initiated in South Africa in 1913 with a highly successful biological control

programme for the drooping prickly pear, *Opuntia monacantha* Haw. (Cactaceae), originally from South America, using the cochineal insect, *Dactylopius ceylonicus* (Green) (Dactylopiidae) (Zimmermann *et al.*, 2004). This early success was followed in 1993 by the introduction of the cochineal insect *Dactylopius opuntiae* (Cockerell) (Dactylopiidae) and the cladode mining moth, *C. cactorum*, which generated high levels of damage and reduced infestation of *Opuntia ficus-indica* (L.) Mill. (Cactaceae) in South Africa by approximately 90% (Zimmermann and Moran, 1991).

Not all biological control agents are successful (Malecki *et al.*, 1993). Some agents that have been released have not established or are not effective (Zimmermann *et al.*, 2004). Biological control agents can fail because they cannot establish or they may establish but not reach high enough population densities to reduce the density of the target weed (Myers, 2000). In some instances, when two or more agents have been released; particular agents fail to establish because their performance and fitness have been affected by the presence of other agents. It is important to review the biological control programmes that have failed and investigate the possible causes of failure in an attempt to improve both present and future biological control programmes (Myers, 2000).

1.5. Is a biological control programme best achieved by single or multiple agents?

There are conflicting views on whether one or several agents are more likely to successfully control a weed (Denoth *et al.*, 2002). In some cases, one agent has been sufficient to achieve complete control, while for others multiple agents have been required (Myers, 1985). Sometimes when multiple agents have been released only one agent has contributed to the

control (Myers, 1985). The prediction of how many and which agents will be effective is a continuing issue of debate (Weyl and Hill, 2012). The release of multiple biological control agents on the same target weed may cause interference which could reduce the efficacy of other agents. Negative interactions between agents will reduce the effectiveness and performance of one or more insects and if the effective biological control agent happens to be an inferior competitor; this will have an impact on the success of the biological control programme (Myers, 1985). Conversely, the release of multiple agents could result in complementary interactions between the agents, improving the level of control.

The effect of several biological control agents introduced on a target weed is often viewed as cumulative (Myers and Bazely, 2003). In such a case, the target weed experiences increased stress from additional biological control agents that work synergistically, targeting different plant parts to increase the stress received by the target weed (Myers and Bazely, 2003). This is the cumulative stress model originally proposed by Harris (1985).

Myers (1985) and McEvoy & Coombs (2000) related the introduction of biological agents to a lottery where the introduction of more agents maximises the chances of finding the 'right' biological control agent. This approach is supported by many weeds that have multiple agents but only one that causes the majority of the damage (Myers, 1985). One agent is sometimes enough to reduce the density of a target weed but it remains difficult to identify which of the potential agents is likely to be the most effective prior to release (Myers, 1985; 2008) because predicting the efficacy of agents before release is extremely difficult (McFadyen, 1998). In a case when one agent works effectively in decreasing the density of the weed and results in a

successful biological control programme, it is recommended that introducing additional biological control agents should be avoided in order to reduce the risk of negative effects (McClay and Balciunas, 2005). Formal post-release evaluations should be done to determine the rates of establishment and damage of an agent before additional agents are considered in order to avoid the release of unnecessary agents. There is a small intrinsic risk to any release, so only biological control agents that are likely to suppress the target weed and are unlikely to have indirect effects should be released (Simberloff and Stiling, 1996; Thomas and Willis, 1998; McClay and Balciunas, 2005; Barratt *et al.*, 2010).

1.6. Importance of post-release evaluation and quantifying success

Post-release evaluation is an important phase of a biological control programme that is often neglected (Morin *et al.*, 2009). It is conducted to evaluate the abundance of biological control agents, the impact of agents on target weeds, the potential for non-target effects, as well as the response of native communities to the reduction of the weed populations (Carson *et al.*, 2008). Post-release evaluations provide data that should be used to indicate whether new biological control agents are desirable for a particular alien invasive plant, to improve release strategies of existing agents and to improve future biological control programmes by learning from past successes and failures (Morin *et al.*, 2009). The data collected during post-release evaluations are also used to assess if the damage inflicted by the agents has achieved control of the target weed and contributed to aesthetic, recreational, human health, biodiversity, and economic benefits. This assessment can be used to justify continued investment in biological control research as well as implementation (Morin *et al.*, 2009).

1.7. *Pereskia aculeata* Miller (Cactaceae)

1.7.1. Description of *Pereskia aculeata*

Pereskia aculeata, commonly known as Pereskia or Barbados gooseberry is a problematic alien vine in South Africa (Moran and Zimmermann, 1991; Klein, 1999). It belongs to the Cactaceae family, but it is different from what most people consider typical cacti species because it has well-developed leaves and lacks the swollen stems or cladodes, that are morphological features of most species of Cactaceae (Leuenberger, 1986; Moran and Zimmermann, 1991). *Pereskia aculeata* is a spiny, shrubby, climbing vine with long, whip-like branches (Fig. 1.1). The young stems of the plant have paired hooked spines and the older, woody stems have clusters of straight spines (Fig. 1.1). The leaves are bright, glossy green; its flowers are white, cream or yellowish in colour and the fruits are yellow and contain spines but change to orange and lose the spines when ripe (Leuenberger, 1986; Henderson, 1995; 2001).

Pereskia aculeata was first recorded in South Africa in 1858 at the Cape Town Botanical Garden (McGibbon, 1858). The plant can be proliferated from cuttings and viable seeds, which makes it easy to grow as a garden plant (Bruton, 1981; Campbell, 1988). It is often used as a barrier hedge because the large thorns restrict the movement of people and livestock (Bruton, 1981). It was first recognised as a weed in 1971 (Pickworth, 1972) and has been declared an environmental weed in South Africa as a Category 1b invasive species (weeds which cannot be grown and must be controlled) by the National Environmental Management: Biodiversity Act 10 of 2004 (NEM:BA) (Department of Environmental Affairs, 2014). The control of species that

belong to this category should be prioritised because of their negative impact on native ecosystems (Henderson, 1999; 2001).



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

The native distribution of *P. aculeata* occurs in two distinct regions in Central and South America (Leuenberger, 1986). The southern region includes south and south-eastern Brazil, northern Argentina and southern Paraguay whereas the northern region includes the Caribbean and northern Venezuela (Leuenberger, 1986). Populations in Florida (USA), Mexico and Central America are considered naturalized populations outside the true native distribution (Leuenberger, 1986). In South Africa, *P. aculeata* now occurs in seven of the nine provinces (Fig.

1.2) where it reduces native biodiversity due to its abundance and ability to outcompete and cover indigenous plants (Paterson *et al.*, 2011b).

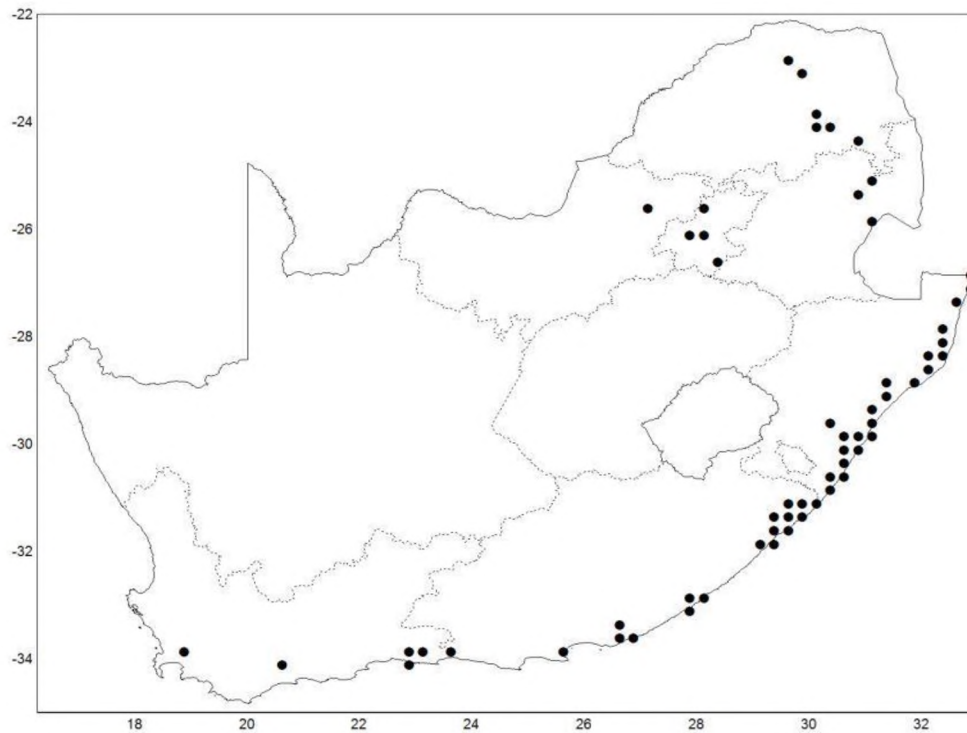


Figure 1.2 Distribution of *Pereskia aculeata* in South Africa. (Drawn by L. Henderson; data source: SAPIA database, ARC-Plant Protection Research Institute, Pretoria.)

Mechanical and chemical control options have been implemented against this species. These two control options are labour-intensive, expensive and generally ineffective (Moran and Zimmermann, 1991). Mechanical and chemical controls against *P. aculeata* have non-target effects because the plant becomes intertwined in native vegetation that must be sprayed or cleared in order to treat the *P. aculeata* (Klein, 1999). Triclopyr (butoxy ethyl ester; 480g/l) is a registered herbicide to control *P. aculeata* in South Africa (Vermeulen *et al.*, 1996) but it has been reported that herbicides do not effectively translocate within the plant, so all parts of the

plant must come into contact with the herbicide in order to kill the whole plant (Klein, 1999). All plant parts must be killed or removed carefully because any fragment left behind can produce roots and is, therefore, capable of growing into a new infestation. Both mechanical and chemical control options are ineffective and restrictively expensive, so biological control is regarded as the only possible option to reduce *P. aculeata* to acceptable densities (Moran and Zimmermann, 1991; Paterson *et al.*, 2011a).

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

Surveys for potential biological control agents of *P. aculeata* were conducted during eight field trips between 1984 and 2007 in southern Brazil, northern Argentina, Venezuela and Dominican Republic (Paterson *et al.*, 2011a; 2014a). Only fifteen insect species were recorded on *P. aculeata* during these field trips (Paterson *et al.*, 2014a). Six of these insect species, which were considered as promising potential control agents because of their mode of damage and impact on the plant in the native range, are discussed below (Paterson *et al.*, 2014a).

The stem borers, *Acanthodoxus machacalis* Martins & Monné (Cerambycidae), *Pereskiophaga brasiliensis* Anderson (Curculionidae), and *Maracayia chlorisalis* (Walker) (Crambidae) and the fruit galling midge, *Asphondylia* sp. (Cecidomyiidae), as well as the leaf-feeding beetle, *P. guerini*, and stem-wilter, *C. schaffneri*, were identified as promising potential biological control agents for *P. aculeata* (Paterson *et al.*, 2014a, Anderson, 2015). *Acanthodoxus machacalis* and *P. brasiliensis* damage the structural tissues of old stems and destroy the vascular system, causing the plant tissue above the damaged area to die (Paterson *et al.*, 2014a). *Maracayia chlorisalis* mines the young shoots which result in wilting and eventually death of the shoot

(Paterson *et al.*, 2014a). *Asphondylia* sp. forms galls in the ovaries of the flowers and hinders seed production (Paterson *et al.*, 2014a). Any reduction of seed dispersal would limit the spread of the weed to new areas as *P. aculeata* seeds are dispersed by birds and bats (Campbell, 1988). All these species have been taken into consideration as biological control agents for *P. aculeata* but only two, *Phenrica guerini* Bechyne (Chrysomelidae) and *Catorhintha schaffneri* Brailovsky & Garcia (Coreidae) have been released to date.

Phenrica guerini

Phenrica guerini adults and larvae are external leaf-feeders (Klein, 1999). The eggs of *P. guerini* are laid in groups of 23-33 eggs per batch on the upper or lower leaf surface. *Phenrica guerini* undergoes three larval instars and pupation takes between seven and nine days before adults emerge (de Beer *et al.*, undated). When the eggs hatch, the larvae are pink to red in colour, the first instar larvae then turn grey; later changing to yellow when approaching the second instar and during the third instar the larvae change back to a dark grey colour. The pre-pupation stage follows, when no feeding takes place, in preparation for pupation in the soil (de Beer *et al.*, undated).

An initial collection of this agent was started from ten adults collected at Barra de São João, Brazil in 1988 (Klein, 1999). Two years later, nine adults were collected from Lagoa de Marapendi and Praia da Barra de Tijuca in Brazil. More than 40 adults were collected at Barra de São João and added to the culture in 1991 and 1994 (Klein, 1999). Host specificity testing showed that *P. guerini* is monophagous and the larvae did not complete their development on any of the non-target plants tested, not even the closely related, *Pereskia grandifolia* Howard

(Cactaceae) (H. der Beer, unpublished; Klein, 1999). Permission to release was granted and *P. guerini* was mass-reared and released at 12 sites in the Eastern Cape and KwaZulu-Natal between 1991 and 1997 (Klein, 1999). A sum of 1319 individual beetles was released with the highest number of 300 beetles released at any single site (Klein, 1999). The beetles established at two sites, one at Sezela Sugar Mill in KwaZulu-Natal and the other one in Port Alfred in the Eastern Cape (Klein, 1999). *Phenrica guerini* was not mass-reared between 1997 and 2009 at any institution and there are no records of any releases of *P. guerini* available for these years. *Phenrica guerini* mass-rearing efforts were re-initiated in 2009 and between 2009 and 2014; the South African Sugar Research Institute (SASRI) released large numbers of the beetle at several sites. There were only three sites known with populations that continued to exist in 2011 (Paterson *et al.*, 2011b) and the impact of *P. guerini* was believed to be negligible (Klein, 2011). *Phenrica guerini* was considered an ineffective agent but no formal post-release evaluation had ever been conducted (Paterson *et al.*, 2011a).

Catorhintha schaffneri

Catorhintha schaffneri is a stem-wilting bug that was introduced from southern Brazil into quarantine in South Africa in May 2012 (Paterson *et al.*, 2014b). The culture of *C. schaffneri* in South Africa was initiated from twenty-three individuals that were collected in Santa Catarina Province, Brazil (Paterson *et al.*, 2014b). Host specificity testing proved that it feeds only on *P. aculeata* and is unable to develop on even close relatives of the target plant (Paterson *et al.*, 2014b). Permission to release *C. schaffneri* was granted and the bug was released in South Africa in October 2014. The nymphs and adults of *C. schaffneri* both play crucial roles in

damaging the plant; they feed on the shoot tips, causing the stem to wilt and eventually die (Paterson *et al.*, 2014b). The damage inflicted by *C. schaffneri* reduces the shoot lengths of plants and could reduce the potential of *P. aculeata* for reaching the canopy in forest biomes and so reduce competition with native plants for light (Paterson *et al.*, 2014b).

The egg batches of *C. schaffneri* are deposited on the leaves and sometimes on the stem or even on another dry surface, like the sides of a cage. Under standard laboratory conditions, the time taken for eggs to hatch from the day they were oviposited averaged 15.4 days (SE $\pm$ 0.82; n=10) (Paterson *et al.*, 2014b). At the same temperature, the first instar nymphs moulted after 3.7 days (SE $\pm$ 0.26), duration to the second ecdysis was 6.6 days (SE $\pm$ 0.34), and then the third ecdysis was reached after 13.5 days (SE $\pm$ 0.65) followed by the final ecdysis to the adult stage after 23.8 days (SE $\pm$ 1.51). Adults survived for an average of 25.8 days (SE $\pm$ 3.74) after the final ecdysis (Paterson *et al.*, 2014b).

Catorhintha schaffneri has been released in KwaZulu-Natal and Eastern Cape provinces where it has established and survived the winter seasons of 2015 and 2016 at some sites. It is too early to determine whether *C. schaffneri* will be an effective biological control agent but laboratory impact trials have indicated that it is suitably damaging to warrant release (Paterson *et al.*, 2014b).

1.8. Thesis aims and rationale

This study aims to quantify the impact of *P. guerini* as a biological control agent in order to determine whether additional agents are required for the control of *P. aculeata*. This was done

through a survey of sites where *P. guerini* has been released and an insecticide exclusion experiment at a site in South Africa where the agent has established (Chapter 2). The interaction between *P. guerini* and the new biological control agent, *C. schaffneri*, was then investigated in order to determine if the relationship with the new agent would be synergistic or antagonistic (Chapter 3). These data are valuable to inform biological control practitioners of the best methods of implementing biological control against *P. aculeata*. The overall aim of the study is to improve the biological control programme against *P. aculeata* in order to reduce its density to a level where it is no longer a significant problem to native biodiversity of South Africa.

Chapter 2: Impact and abundance of *Phenrica guerini* on *Pereskia aculeata*

2.1. Introduction

2.1.1. Post-release evaluations

Post-release evaluations of biological control programmes have received little attention compared to other stages of the process (McEvoy and Coombs, 1999; McClay and Balciunas, 2005; Van Klinken and Raghu, 2006; Morin *et al.*, 2009). The focal point of many post-release studies has been on the establishment, abundance, and damage by biological control agents (Morin *et al.*, 2009). Few studies have focused on measuring the reduction of weed populations due to the damage inflicted by biological control agents and very few have evaluated the change in plant communities in response to weed population decline (e.g. Barton *et al.*, 2007). It is, however, important to assess the degree to which biological control agents contribute to the conservation and restoration of native biodiversity (Morin *et al.*, 2009).

2.1.2. The importance of post-release evaluations

There are several reasons as to why it is important to invest resources in post-release evaluations (Carson *et al.*, 2008). Data collected during post-release evaluation can be used to improve release strategies of biological control agents or justify further efforts and investments in mass-rearing and releases (Morin *et al.*, 2009). Another valuable contribution of post-release evaluations is that the data can indicate whether additional biological control agents are required or if the existing biological control agents are sufficiently effective (Thomas and Willis,

1998). It is also essential to quantify the levels of success of biological control as this will justify previous investments in the science and encourage future investments (Morin *et al.*, 2009).

The establishment and performance of biological control agents are often used to measure success in post-release evaluations (Morin *et al.*, 2009). Although biological control agent establishment is an important parameter to measure, it does not guarantee that any damage is inflicted, or that the damage will result in a reduction of the target weed population (Hoffmann, 1990). The establishment of biological control agents is therefore important but should not be the only parameter used to measure the success of biological control (Syrett *et al.*, 2000; Paterson *et al.*, 2011b). In order to fully assess the success of a biological control programme, the damage done by the agent must be linked with a decline in the target weeds population densities and an associated decrease of the negative impacts of the weed (e.g. reduced biodiversity or agricultural productivity) (Paterson *et al.*, 2011b).

The effectiveness and establishment of biological control agents may differ between geographic areas depending on climatic and environmental conditions (McClay, 1995; Müller-Schärer and Schaffer, 2008). It has been recommended that post-release evaluations should be conducted over a period of years and in different regions to minimise the confounding factors of climatic variability (Denoth and Myers, 2005). It is also important to note that biological control is a long-term process and that significant impact on the target weed populations should not be expected immediately after release. It has been suggested that a period of 10-20 years should be allowed before post-release evaluations are conducted (McFadyen, 2000), but in at least

some cases the impacts of biological control have been much more rapid (e.g. McConnachie *et al.*, 2004).

2.1.3. Types of data collected to assess agent effectiveness

Different types of data can be collected when conducting post-release evaluations including agent-related measurements, weed-related measurements, and ecosystem-related measurements (Morin *et al.*, 2009). The number of adults and each life-stage of the agent can be counted to determine the density of the agent and gain an understanding of agent establishment, population, phenology and dispersal patterns (Morin *et al.*, 2009). To determine the damage inflicted by an agent, plant parameters which may be measured include the number of damaged leaves or the damaged area of leaves, number of damaged shoots or stems; the number of galls formed or the number of damaged flowers or seeds (Dhileepan *et al.*, 2000; Goolsby *et al.*, 2004; Paynter *et al.*, 2006).

Weed-related measurements can be made at the individual and population levels and may include plant parameters such as number, size, and biomass of above and below ground parts as well as reproductive parameters such as a number of flowers, fruits, and seeds. Parameters at the population level include density, cover and spread (Dhileepan *et al.*, 2000; Morin *et al.*, 2009). Age structure is another population level parameter that can provide valuable information about the health of a weed population (Paynter, 2005). This type of data should also be collected from different areas in order to evaluate the response of agents to different weed populations and the impact of the agent in different climatic regions (Shea and Kelly, 1998; Davis *et al.*, 2006).

Ecosystem-related measurements are done to evaluate the response of an ecosystem after the reduction of a weed population (Morin *et al.*, 2009). It is important to measure the changes in the ecosystem that the weed is known to impact. These measurements may include diversity and abundance of native and invasive alien plant species, and productivity in agricultural or forestry systems (Reid *et al.*, 2008). It is also important to consider other characteristics of ecosystems such as water quality, the rate of ecosystem processes, nutrient cycling, decomposition and changes in hydrological cycles (Reid *et al.*, 2008).

Agent, plant and ecosystem parameters should all be measured for a full assessment of the biological control efficacy. It is also important to understand how changes at one level will be translated into changes at other levels. For example, how damage at the individual plant level translates into population-level impacts and what effect this has on the invaded ecosystem.

2.1.4. Methods of post-release evaluations

There are several methods used to assess the changes in the parameters mentioned above (Morin *et al.*, 2009). Some methods are more successful than others, some are required for different circumstances and each method has advantages and disadvantages (Reid *et al.*, 2008). These approaches include 1) comparing sites (or plots) with and without agents, 2) comparing before and after release data, and 3) manipulative experiments (Morin *et al.*, 2009).

2.1.5. Comparing sites (or plots) with and without agents

Comparing sites or plots with and without agents is possible in cases where the biological control agents have not been released yet or have not spread to all the areas where the target

weed is present (Dhileepan, 2003a; Cruz *et al.*, 2006; Reid *et al.*, 2008; Morin *et al.*, 2009). Plots with and without the agent should be paired and replicated (Carson *et al.*, 2008). The advantages of using this approach are 1) it is not a manipulative approach so manipulations (such as the application of insecticides) will not affect the results, and 2) this approach collects data within a short period of time and provides a quick evaluation (Adair and Grove, 1998). This approach has been used to evaluate the efficacy of the biological control agent, *Carmentis mimosae* Eichlin and Passoa (Sesiidae), on the invasive alien plant, *Mimosa pigra* L. (Mimosaceae), in Australia (Paynter, 2005). The results showed that the sites with the highest densities of *C. mimosae* had a decrease of more than 90% of seed rain compared to sites without *C. mimosae* (Paynter, 2005).

2.1.6. Comparing before and after release data

The first step of a biological control programme evaluation should be a pre-release assessment (Paterson *et al.*, 2011b). This provides detailed baseline pre-release data that can be used to evaluate the efficacy of agents after they have been released (Thomas and Reid, 2007; Carson *et al.*, 2008). Comparing before and after release data includes 1) photo points, 2) comparing historical and contemporary data 3) stakeholder surveys and 4) comparing pre- and post-release weed population densities directly (Morin *et al.*, 2009). Photo points are the cheapest and simplest method to evaluate the efficacy of biological control agents (Dhileepan, 2003b). This method involves establishing fixed reference points at the sites before or immediately after the releases of agents and taking, several photos at regular intervals after release (Morin *et al.*, 2009). Photo points alone are not enough to assess the efficacy of biological control

agents on target weeds because the data they provide may not give supporting evidence that the reduction observed is caused by the damage inflicted by biological control agents (Syrett *et al.*, 2000; Wilson *et al.*, 2004). The use of photo points may also provide confusing results if other vegetation displaces the target weed (Syrett *et al.*, 2000; Wilson *et al.*, 2004). In areas where the impact of agents and the reduction in weed density and percentage cover is clearly visible, it is easy to implement this approach. For example, it has been used to measure the infestations of *Azolla filiculoides* before and after the release of its biological control agent, *Stenopelmus rufinasus* in South Africa (McConnachie *et al.*, 2004). Large declines of *A. filiculoides* populations were recorded by comparing photos taken before and after the release of *S. rufinasus* (McConnachie *et al.*, 2004).

Comparing historical and contemporary data uses historical maps of weed distributions before release compared with maps generated over years after agents have been released (Henderson, 1999). Both published and unpublished historical data, such as aerial photos and field ecological data can be compared with the current data taken at the same sites (Morin *et al.*, 2009). This approach is relatively cheap and easy to conduct because it uses previously collected data, therefore, it does not require long-term experiments and is most appropriate when the biological control agent is widely distributed and pre-release data is available (Paynter, 2005; Morin *et al.*, 2009). The comparison of historical and contemporary data cannot be strictly used to conclude that weed decline is due to the damage inflicted by biological control because it is possible that other factors may have caused the decline in the weeds populations (Reid *et al.*, 2008).

Stakeholder surveys involve information received from landowners about the target weed and are conducted before the release of biological control agents and then repeated years after the release (Morin *et al.*, 2009). This approach is relatively easy and cheap to implement but, as with many of the other approaches, the results are correlative and so other factors may have impacted the weed populations besides biological control (Morin *et al.*, 2009). Stakeholder opinions may also change over time, so the perceived negative impact of the weed may change due to changes in opinion, rather than changes due to the action of a biological control agent (Bardsley and Edwards-Jones, 2006; Andreu *et al.*, 2009).

Pre-release data can also be directly measured, by evaluating weed densities prior to release and comparing this data to post-release measurements (Paterson *et al.*, 2011b). If agent population data and damage measurements are included during post-release sampling, then a link between the damage by the agent and the decline in weed densities can be made.

2.1.7. Manipulative experiments

Manipulative experiments consist of inclusion and exclusion experiments. This involves manipulating the agent densities, either by releasing the agent and confining it to certain plots or by excluding the agent from some plots and comparing this to plots with the agent. The changes to the density of the target weed and the changes to other vegetation can then be compared between the plots (Paynter *et al.*, 2006). The drawback is that these experiments are time-consuming and expensive and for this reason, they are commonly performed over small ranges and for short periods of time (Morin *et al.*, 2009). Inclusion experiments are conducted by the release of an agent to a caged area in the field. These experiments are rarely used

because the results they provide may be unrealistic as agent population densities may be inflated in the cages (Morin *et al.*, 2009). It is important to take into consideration these negative effects when analysing and interpreting the results for inclusion experiments (Hunt-Joshi and Blossey, 2004).

Agent exclusion experiments involve the use of cages, insecticides or fungicides to assess the efficacy of the agent on the target weed by excluding the agent from some plots and not from other plots (McClay, 1995; Dhileepan, 2003b; Goolsby *et al.*, 2004; Tipping and Center, 2002). Field exclusion experiments are advantageous because they provide more reliable and realistic information than other experiments (Morin *et al.*, 2009). The chemical composition of the insecticide or fungicide might have phytotoxic effects which could affect the plant's growth and performance so it is important to conduct laboratory trials before applying them in the field (Tipping and Center, 2002; Siemann *et al.*, 2004). Laboratory experiments may help: a) to verify that the insecticide chosen will effectively exclude the biological control agent, b) to check how long the insecticide is effective and when re-application will be required, and c) to find out if the chosen insecticide had any negative or positive effect on the quality of the target weed. The application of insecticide to exclude insects may not be suitable for areas with high rainfall because these areas require frequent re-applications of the insecticide (Reid *et al.*, 2008; Paynter *et al.*, 2006).

2.1.8. Evaluating success in biological control

For many biological control programmes in South Africa, there are no quantitative data on the success of biological control. The lack of data is partially due to a lack of suitable capacity and

funding within the biological control research community to conduct post-release evaluations on all the weeds for which agents have been released, and partially due to the fact that post-release evaluations have been somewhat neglected in the past (Morin *et al.*, 2009). Hoffmann (1995) developed a basic method of evaluating the success of biological control in South Africa that does not require detailed post-release evaluations for each weed species. This system is regarded as the most appropriate method to compare and evaluate the success of biological control programmes and has been adapted and improved over time, first in the appendix of Olckers and Hill (1999) and then by Klein (2011). The method divides biological control projects into the categories of complete, substantial or negligible control based on the need for alternative control methods such as mechanical and chemical control. The damage by each agent is also categorised as extensive, considerable, moderate or trivial. Each category is given a clear and unambiguous definition (Klein 2011).

2.1.9. Post-release evaluation of *Phenrica guerini*

Phenrica guerini was first released in 1991 and has established at some sites in South Africa but no formal post-release evaluation has ever been conducted (Paterson *et al.*, 2011b). Prior to this study, there were three confirmed sites where populations of *P. guerini* had established and persisted (Paterson *et al.*, 2011a). Visual observations have indicated that *P. guerini* had established at Port Alfred and although damage was visible at the site, it is unclear as to whether the damage has resulted in a reduction in *P. aculeata* density. *Phenrica guerini* was considered a poor agent that had a trivial impact and resulted in a negligible degree of control (Paterson *et al.*, 2011b; Klein, 2011).

In this chapter, a post-release evaluation of *P. guerini* was conducted in order to estimate the impact of the biological control agent in South Africa. First, the impact of *P. guerini* was evaluated using an insect exclusion experiment at a single site at Port Alfred in the Eastern Cape Province. This detailed evaluation from a single site was followed by a broad assessment of the impact of the agent in a survey of sites where the agent has been released around South Africa in the past. The aim of this chapter is to quantify the impact of *P. guerini* at Port Alfred and use this to extrapolate and estimate the impact throughout South Africa. The data is useful for the implementation of biological control of *P. aculeata* as it can be used to determine whether further agents are required in the country and whether mass-rearing and releases of *P. guerini* should be continued.

2.2. Materials and Methods

2.2.1. The details of the insecticide chosen

Rosecare 3 (Agro- Serve (Pty) Ltd EFEKTO TM) is a broad acting insecticide and was chosen as a suitable insecticide to exclude *P. guerini* on *P. aculeata* after conducting trial experiments. The insecticide will exclude or kill all insects but *P. guerini* is the only insect that feeds extensively on *P. aculeata* in South Africa so the exclusion of all insects does not confound the experiment. All applications followed the instructions provided by the manufacturer.

2.2.2. The impact of insecticide on *P. guerini* and *P. aculeata*

This experiment was conducted to determine whether Rosecare insecticide was effective at excluding *P. guerini* and to test whether the insecticide had any impact on plant parameters

(such as a phytotoxic effect). A common garden experiment was conducted in a shaded greenhouse at the Waainek Research Facility, Rhodes University in Grahamstown, South Africa. Potted *P. aculeata* plants with two shoots ranging between shoot lengths of 8cm and 13cm were used. Potted plants of a similar size were selected for each control and treatment pair so that starting parameters were similar. Second instar larvae were used for the experiment because first instar larvae are extremely fragile and do not survive being transferred onto new plants. Five day old second instar larvae were used because this is the first day that all larvae would have completed the first ecdysis. Treatments that required the agent were inoculated with five second instar larvae per plant. The larvae were inoculated again to check the efficacy of the insecticide after seven days. The potted plants were arranged in a Latin square design. The four different treatments were as follows: **treatment 1**: no insecticide; no *P. guerini* (control), **treatment 2**: insecticide; five larvae of *P. guerini* (this was done to evaluate the effect of both insecticide and biological control agent on plant growth), **treatment 3**: insecticide; no *P. guerini* (this was done to evaluate the effect of insecticide on plant growth), and **treatment 4**: no insecticide; five larvae of *P. guerini* (this was done to evaluate the effect of the biological control agent on plant growth).

The number of leaves and shoot length were measured before and after the experiment. Insect mortality was recorded daily and insects that had died were replaced after seven days in order to check whether the insecticide was still effective seven days after the treatment. Insect mortality was recorded after seven and 14 days, after which the experiment was terminated. The insecticide was applied as a foliar spray to exclude *P. guerini*. One litre of water was mixed with ten millilitres of the insecticide and applied as a foliar spray as per the manufacturer's

instructions. All the leaves of the potted plants were thoroughly sprayed with the insecticide. The experiment was replicated ten times.

Data analysis

Data analysis was conducted using the statistical programme STATISTICA version 13.0 (©Stat Soft, Inc., USA). The number of leaves, shoot length and plant biomass data were normal distributed and therefore analysed using one-way ANOVA. Insect parameter and damaged leaves data were non-normally distributed and did not fulfil the requirements of analyses of variance (ANOVA) therefore, they were analysed by the generalized linear model (GLZ) with a normal distribution and a log function. Fishers LSD post hoc tests at a confidence of 0.05 were conducted to identify homogenous groups of the plant parameters.

2.2.3. Effect of *Phenrica guerini* on growth of *Pereskia aculeata* under field conditions

Study area

The field study was conducted from December 2015 to May 2016 at Port Alfred (33°36'S 26°53'E) in the Eastern Cape Province, South Africa. Port Alfred is one of the sites where *P. guerini* has been established for many years (Klein, 1999). Five releases were done between 1994 and 1995 with a total of 300 individuals of *P. guerini* released (Klein, 1999). Two years after release, establishment was confirmed (Klein, 1999). *Phenrica guerini* population significantly increased in 1997 and there was a high number of the beetles found in early 1998 (Klein, 1999). The study site is located within the Subtropical Thicket Biome characterised by closed shrubland and low forest of evergreen, succulent trees, shrubs, and vines (Mucina and

Rutherford, 2006). *Lantana camara* L. (Verbenaceae), *Ricinus communis* L. (Euphorbiaceae), *Solanum mauritianum* Scopoli (Solanaceae) and *Canna* spp. are other invasive alien plants found within the study site. The average daily minimum and maximum temperatures are 12.4°C and 22.4°C (South African Weather Service, 2015). The area receives a mean annual rainfall of 750 mm, which falls between October and April (Mucina and Rutherford, 2006).

Experimental design

Five paired 1m² plots were permanently marked within the *P. aculeata* infestations. Five plots were designated as control plots and were left unsprayed to allow the biological control agent to continue feeding on the weed at the natural densities that were present at the site. Five plots were sprayed with the insecticide every 10 days to exclude *P. guerini* for a period of 100 days. All the paired plots were 5m apart from each other and the gap between all the control and insecticide exclusion plots was 1m. The same insecticide mixing instructions and application method followed during the common garden experiment was used for the field study with the same intensity of spraying.

Plant and insect parameters were measured and recorded after every 20 days. Plant parameters that were measured included the number of leaves, the number of shoots, shoot length, the number of leaves damaged by *P. guerini* and the percentage cover, which was visually estimated. Shoot lengths were measured from the shoot axils to the shoot tips. The number of *P. guerini* in each life-stage found within each quadrant was recorded. *Phenrica guerini* adults were counted before plant parameters to prevent disturbing the agent.

Data analysis

Data analysis was conducted using the statistical programme STATISTICA version 13.0 (©Stat Soft, Inc., USA). The number of leaves, shoot lengths, percentage cover, the number of shoots and number of *P. guerini* found per quadrat were compared between the five plots for both control and spray treatment between sampling days. Data collected for the number of leaves, the number of shoots and the number of damaged leaves were normally distributed and analysed using a one-way ANOVA. The shoot lengths, percentage cover and *P. guerini* adult abundance data were non-normally distributed and therefore, were analysed by GLZ model with a normal distribution and a log function.

2.2.4. Nationwide survey

A survey was conducted in November 2014 with the intention of estimating the distribution and abundance of *P. guerini* across South Africa. *Phenrica guerini* is a poor disperser and so only sites where it has been released were included. Out of 54 sites where *P. guerini* has been released, 22 (40.7%) sites were visited (Table 2.1). The historical data records of each release site between 2009 and 2014 were taken from the SASRI Biological Control Agent Mass-Rearing Facility and release records between 1997 and 1999 were from Klein (1999). This covered all the releases ever done in South Africa from 1997 until the time of the survey. During each site visit, the presence or absence of *P. guerini* was evaluated by actively searching for the insect or associated damage on the plant for half an hour by two people (one-person hour). *Phenrica guerini* damage can be easily differentiated from other insect herbivory by the pattern of feeding on the leaves, as well as frass marks left by the insect.

At each site, a 50m tape measure was used to measure the size of the *P. aculeata* infestation around its perimeter. For very large *P. aculeata* infestations, Google Earth was used to measure the area of the site and GPS co-ordinates were taken around the perimeter if necessary. Patches of *P. aculeata* that were less than 50m distance apart from each other were considered a single site. A 50m tape measure was used to run a transect across a section of each infestation to measure the abundance and damage of *P. guerini* on *P. aculeata*. A 1m² quadrat was then placed on the transect and parameters were measured within each quadrat. The position of each quadrat on the transect was determined using randomly generated numbers, for example, if a random number of four was generated then the quadrat would be placed between meters four and five on the transect. Ten quadrats were done in this way at each site. Within each quadrat, parameters measured included the percentage cover of *P. aculeata* which was visually estimated, the number of *P. aculeata* shoots, the number of *P. guerini* in each life stage and the number of leaves damaged by *P. guerini*.

Data analysis

The data collected for the number of damaged leaves and abundance of *P. guerini* from Port Alfred was compared to the nationwide survey sites where *P. guerini* has established. Data was analysed using STATISTICA version 13.0 and T-tests were conducted to compare if there were significant differences in the number of leaves damaged by *P. guerini* between Port Alfred and each of the sites sampled in KwaZulu-Natal individually.

2.3. Results

2.3.1. The impact of insecticide on *P. guerini* and *P. aculeata*

Number of leaves

The mean starting number of leaves for each plant was 28 ($SE \pm 5.39$) and there was no significant difference in the number of leaves between plants selected for different treatments ($F_{39}=39.11$, $p<0.05$; Fig. 2.1). The control treatment, which was not inoculated with *P. guerini* and not sprayed with insecticide, had a mean increase of 11.5 ($SE \pm 0.96$) leaves (Fig. 2.1). This treatment was not significantly different from the treatment where *P. guerini* was inoculated onto the plants and then excluded with the insecticide or the treatment without any *P. guerini* that were sprayed with the insecticide (Fig. 2.1). The treatment that was inoculated with *P. guerini* and not sprayed with the insecticide had a mean decrease of 1.3 ($SE \pm 1.12$) leaves and this was significantly different to all other treatments ($F_{3,36}=12.58$, $p<0.05$; Fig. 2.1).

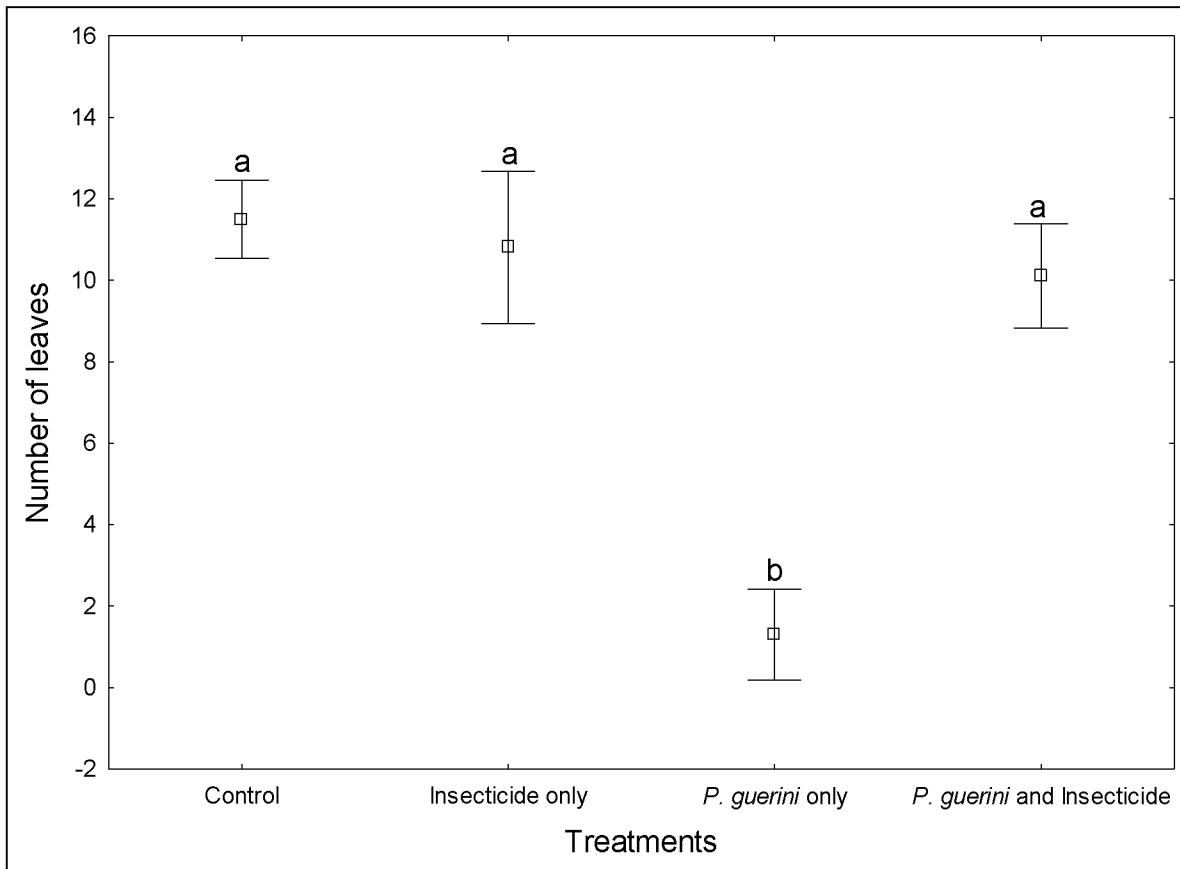


Figure 2.1 Change in the number of leaves for different treatments. The treatments included were control=no herbivory and no insecticide applied, insecticide only=insecticide applied on plants with no *P. guerini*, *P. guerini* only=insects not excluded by insecticide, and *P. guerini* and insecticide=plants inoculated with *P. guerini* and then sprayed with insecticide to exclude the agent. Error bars indicate standard errors around each mean. Different letters indicate significant difference ($p < 0.05$) according to Fisher's LSD test.

Shoot length (cm)

The mean starting number of leaves for each plant was 18.8 ($SE \pm 4.11$) and there was no significant difference in the number of leaves between plants selected for different treatments ($F_{39}=16.5$, $p < 0.05$; Fig. 2.2). There was no significant difference between the control and the treatment with *P. guerini* in terms of shoot length (Fig. 2.2). There was, however, a significantly

greater increase in shoot length in the treatment where *P. guerini* was excluded using insecticide compared to both the control and *P. guerini* treatment but not compared with the insecticide only treatment (Fig. 2.2).

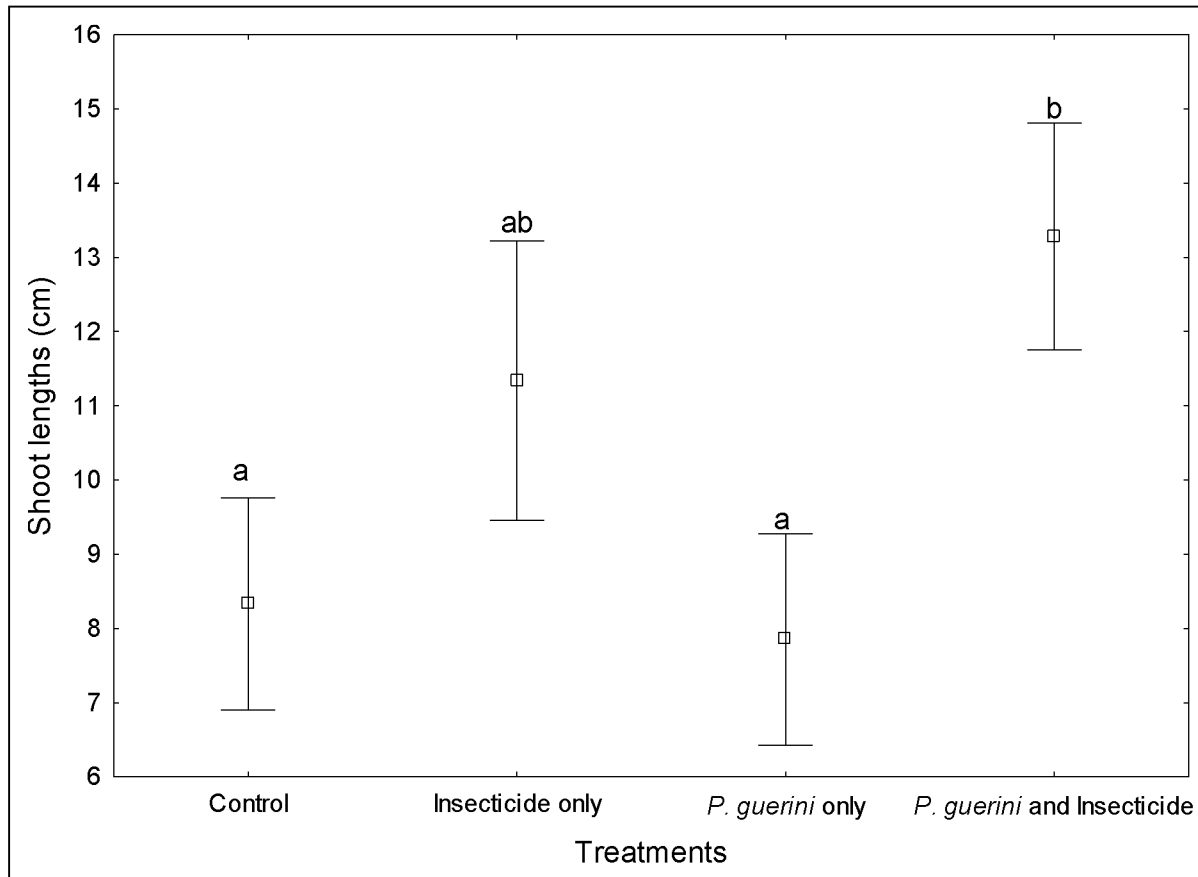


Figure 2.2 Change in the shoot length for different treatments. The treatments included were control=no herbivory and no insecticide applied, insecticide only=insecticide applied on plants with no *P. guerini*, *P. guerini* only=insects not excluded by insecticide, and *P. guerini* and insecticide=plants inoculated with *P. guerini* and then sprayed with insecticide to exclude the agent. Error bars indicate standard errors around each mean. Different letters indicate significant differences ($p < 0.05$) according to Fisher's LSD test.

Number of damaged leaves

Only the treatments that were inoculated with *P. guerini* were included in this analysis as no damage was recorded in the other treatments. The treatment that was inoculated with *P.*

guerini and not sprayed to exclude the agent had a significantly greater mean number of damaged leaves than treatments where the agent was excluded using the insecticide (Wald $X^2=3.16$, $p<0.05$; Fig. 2.3). There was a mean of 19 (SE ± 1.12) damaged leaves in treatments where *P. guerini* was not sprayed with insecticide compared with 2.6 (SE ± 0.75) damaged leaves in treatments that were sprayed.

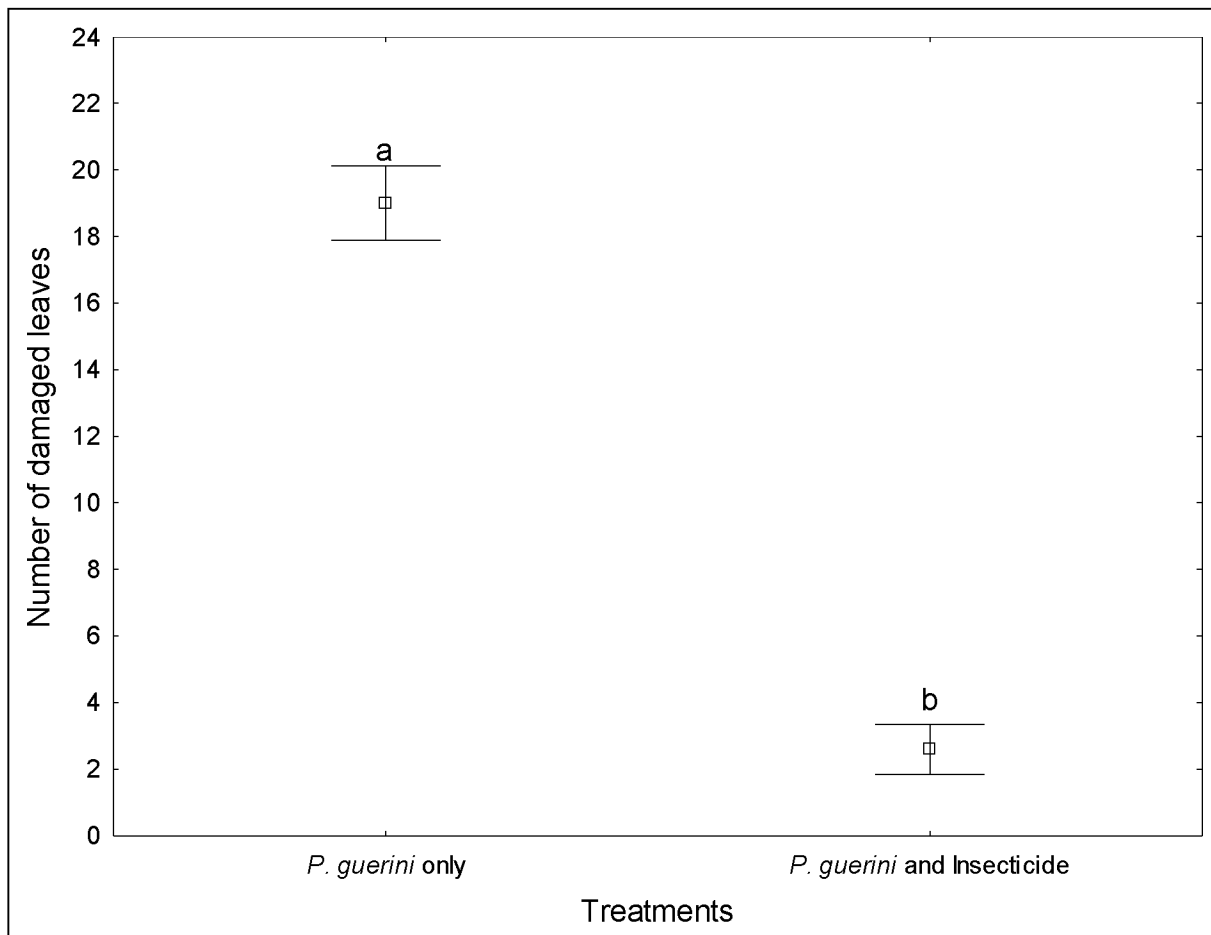


Figure 2.3 Change in the number of damaged leaves for different treatments. The treatments included were *P. guerini* only=insects not excluded by insecticide, and *P. guerini* and insecticide=plants inoculated with *P. guerini* and then sprayed with insecticide to exclude the agent. Error bars indicate standard errors around each mean. Different letters indicate significant differences ($p<0.05$) according to Fisher's LSD test.

Plant biomass

There was no significant difference in plant biomass for all treatments ($F_{3,36} = 0.93$, $p > 0.05$; Fig. 2.4). The control had the greatest plant biomass of 14.2g (SE ± 0.91) but this was not significantly different from other treatments (Fig. 2.4).

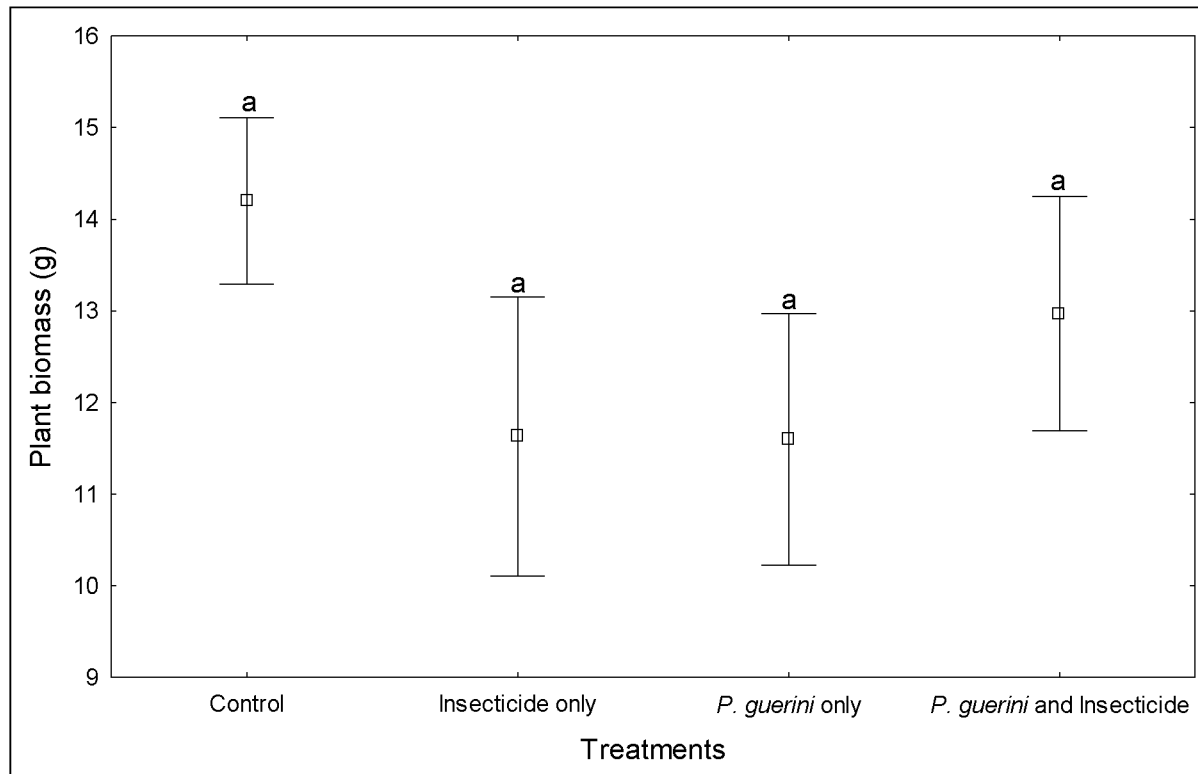


Figure 2.4 Mean ($\pm$ SE) plant biomass (g) for different treatments. The treatments included were control=no herbivory and no insecticide applied, insecticide only=insecticide applied on plants with no *P. guerini*, *P. guerini* only=insects not excluded by insecticide, and *P. guerini* and insecticide=plants inoculated with *P. guerini* and then sprayed with insecticide to exclude the agent. Error bars indicate standard errors around each mean. Different letters indicate significant differences ($p < 0.05$) according to Fisher's LSD test.

Insect parameters

Phenrica guerini survival was significantly greater in the treatment that was not sprayed with insecticide after seven days (Wald $\chi^2 = 4.49$; $p < 0.05$; Fig. 2.5) and 14 days (Wald $\chi^2 = 21.54$, $p < 0.05$; Fig. 2.6). The treatment where *P. guerini* was not sprayed had 60% (SE ± 0.31) larval survival after seven days while there was 100% mortality in the sprayed treatment (Fig. 2.5). Plants were re-inoculated with the agent after seven days and 100% mortality was recorded in the sprayed treatment after 14 days, while only 30% mortality was recorded in unsprayed treatments (Fig. 2.6). After 7 and 14 days, *P. guerini* larvae experienced 100% mortality in the treatment where *P. guerini* was excluded and this occurred two days after they were inoculated (Fig. 2.6).

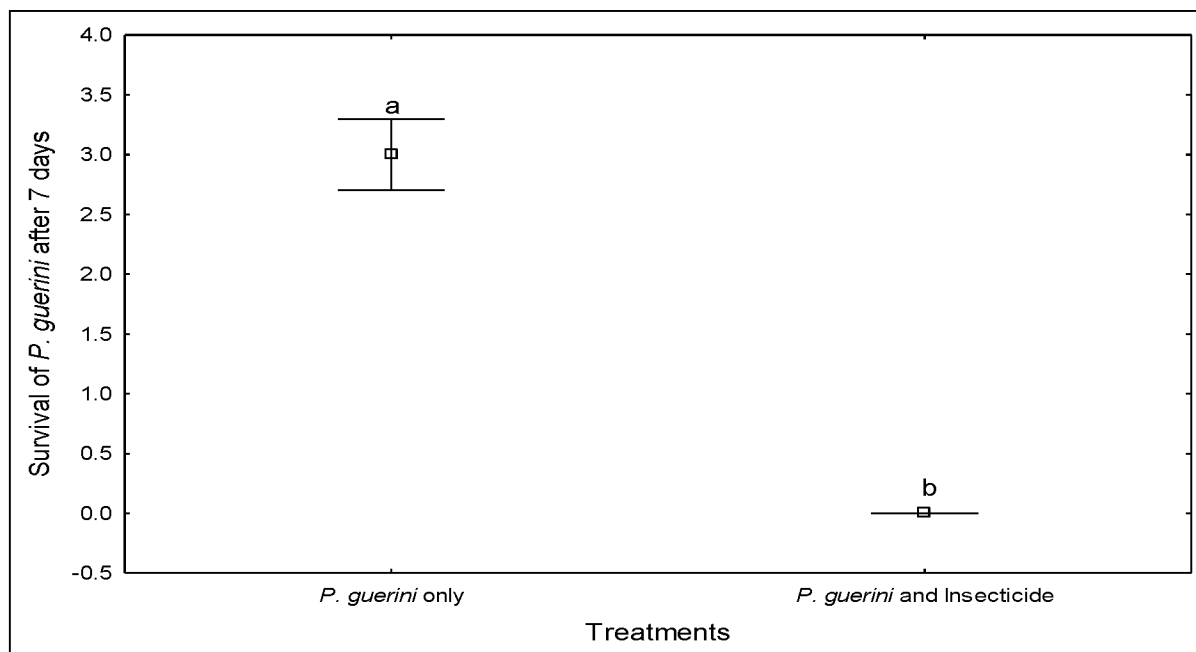


Figure 2.5 Mean ($\pm$ SE) survival of *P. guerini* for different treatments after 7 days. The treatments included were *P. guerini* only=insects not excluded by insecticide, and *P. guerini* and insecticide=plants inoculated with *P. guerini* and then sprayed with insecticide to exclude the agent. Error bars indicate standard errors around each mean. Different letters indicate significant differences ($p < 0.05$) according to Fisher's LSD test.

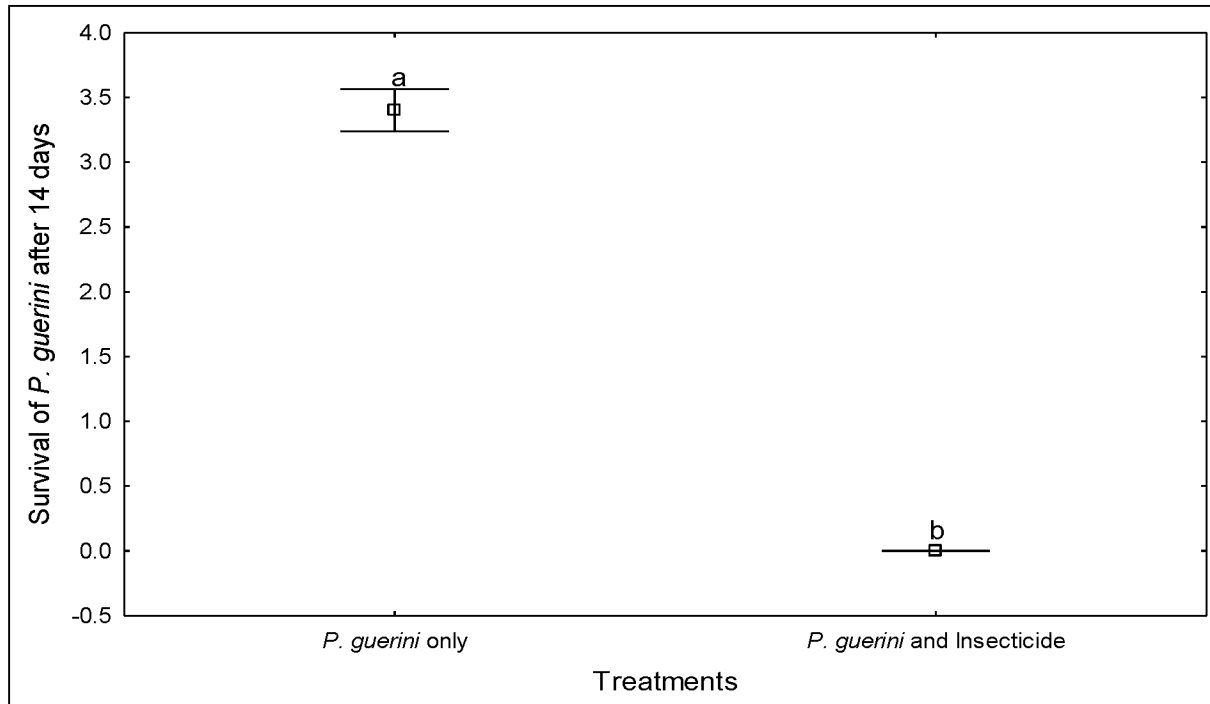


Figure 2.6 Mean ($\pm$ SE) survival of *P. guerini* for different treatments after 14 days. The treatments included were *P. guerini* only=insects not excluded by insecticide, and *P. guerini* and insecticide=plants inoculated with *P. guerini* and then sprayed with insecticide to exclude the agent. Error bars indicate standard errors around each mean. Different letters indicate significant differences ($p < 0.05$) according to Fisher's LSD test.

2.3.2. Effect of *Phenrica guerini* on growth of *Pereskia aculeata* under field conditions

Number of leaves

Before the start of the experiment, there was no significant difference in the number of leaves between plots chosen for *P. guerini* insecticide exclusion and those selected as controls (Fig. 2.7). The number of leaves in plots where *P. guerini* was excluded increased significantly over time, increasing with each sampling event until day 60 and then remaining stable until the experiment was terminated at 100 days (Fig. 2.7). There was a decrease in the number of leaves

in plots with *P. guerini* over the period of the experiment but this was not significant (Fig. 2.7). After 20 and 40 sampling days, the mean number of leaves for plots with *P. guerini* excluded increased but this was not significantly different from plots with *P. guerini* during the same period (Fig. 2.7). At 60, 80 and 100 sampling days, the mean number of leaves for plots without *P. guerini* had increased significantly to a mean of 674 (SE $\pm$ 5.61) leaves per m² whereas plots with *P. guerini* decreased to a mean of 379 (SE $\pm$ 3.49) leaves per m² (Fig. 2.7). After 60 days, the difference between plots with and without the agents was statistically significant and this was maintained for the next 40 days until 100 days and the termination of the experiment. At the end of the experiment, the mean number of leaves for plots with *P. guerini* was 382 (SE $\pm$ 26.91) leaves per m² and was significantly less than the mean number of leaves for plots with *P. guerini* excluded that had 666 (SE $\pm$ 68.33) leaves per m² (Fig. 2.7). This resulted in a mean difference of 284 (SE $\pm$ 142) leaves per m².

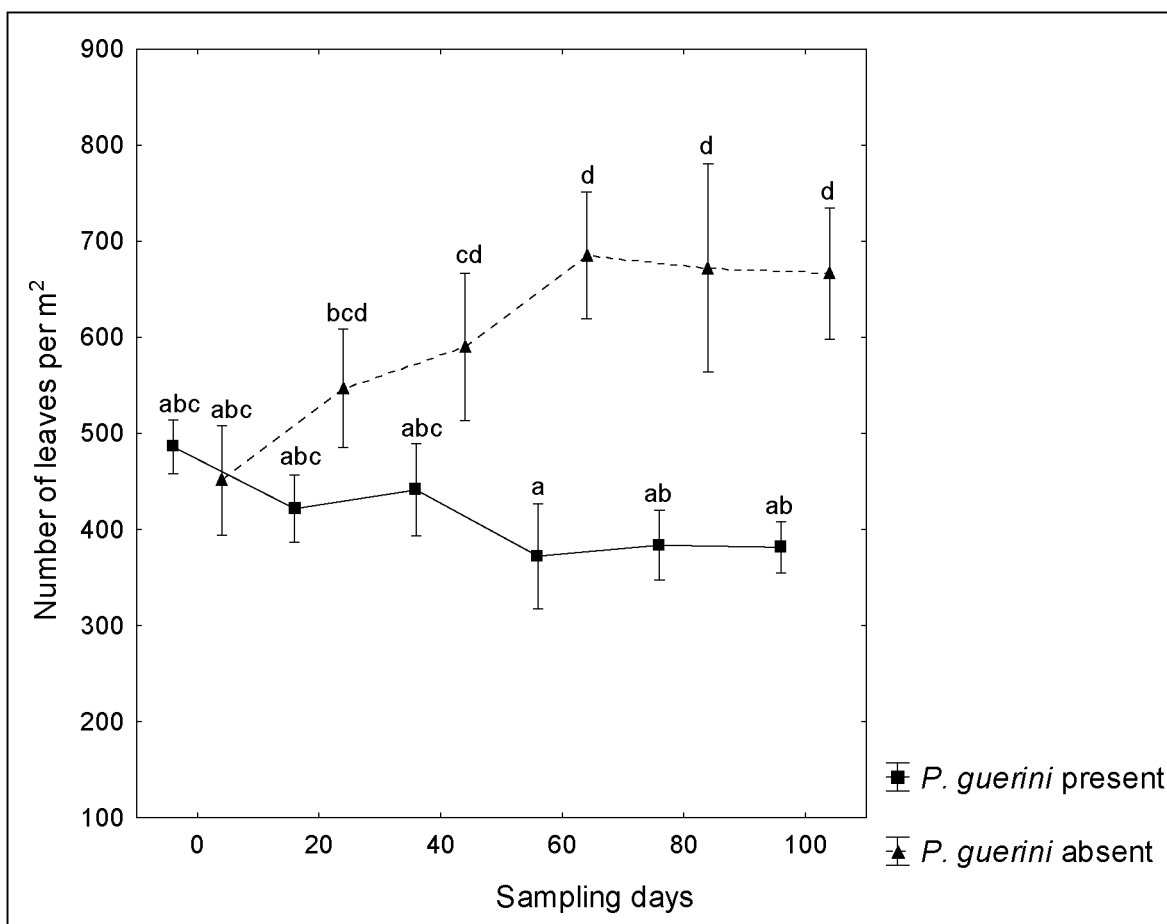


Figure 2.7 Number of leaves per m² on different sampling days for each treatment. The treatments included were *P. guerini* present=plots with insects not excluded by insecticide, and *P. guerini* absent=plots sprayed with insecticide to exclude the agent. Error bars indicate standard errors around each mean. Different letters indicate significant differences ($p < 0.05$) according to Fisher's LSD test.

Shoot length

There were significant differences in shoot length over time in both plots with *P. guerini* and plots without *P. guerini* but there were no significant differences for plots with *P. guerini* and plots without *P. guerini* at any sampling event (Fig. 2.8). Between 40 and 60 days, there was a slight decrease in shoot length for both treatments but after 60 days, the shoot length increased again for both treatments until the end of the experiment (Fig. 2.8). The difference in

mean shoot length from the start to the end of the experiment was 27.6cm (SE $\pm$ 13.8) per m² for plots with *P. guerini* and 35.1cm (SE $\pm$ 17.6) per m² for plots with *P. guerini* excluded (Fig. 2.8). Shoot length was always greater in plots with *P. guerini* excluded but this was not significantly different at any sampling events (Fig. 2.8).

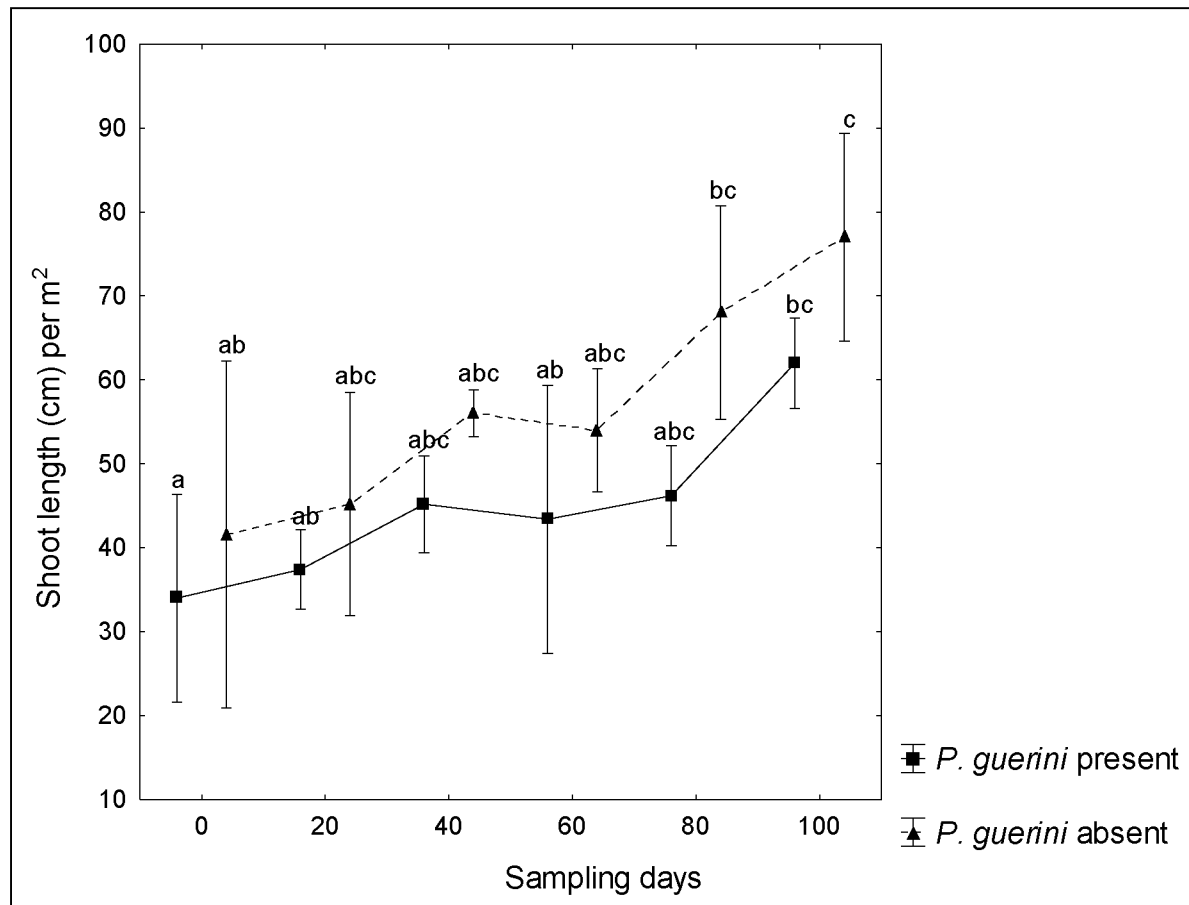


Figure 2.8 Shoot lengths (cm) per m² on different sampling days for each treatment. The treatments included were *P. guerini* present=plots with insects not excluded by insecticide, and *P. guerini* absent=plots sprayed with insecticide to exclude the agent. Error bars indicate standard errors around each mean. Different letters indicate significant differences ($p < 0.05$) according to Fisher's LSD test.

Percentage cover

Percentage cover for plots with *P. guerini* was constant from the start of the experiment until 20 days with 82% (SE $\pm$ 9.2) cover (Fig. 2.9). After 20 days, percentage cover reduced to 70% (SE $\pm$ 7.5) (Fig. 2.9). Between 40 and 60 days, there was a slight increase but it was not statistically significant and after 60 days, percentage cover continued to decrease to 63% (SE $\pm$ 6.4) cover at the end of the experiment (Fig. 2.9). For plots with *P. guerini* excluded, percentage cover increased from a mean of 78% (SE $\pm$ 5.83) at the start of the experiment to a mean of 100% (SE $\pm$ 2.0) after 100 days (Fig. 2.9). After 60 days, there was a slight decrease in percentage cover from 100% to 98% but this was not significant (Fig. 2.9). At the end of the experiment, percentage cover was 98% (SE $\pm$ 2.0) cover and this was statistically different from plots with *P. guerini* which had a mean of 63% (SE $\pm$ 6.44) (Wald $X^2 = 35.35$; $p < 0.05$; Fig. 2.9). Percentage cover was always less in plots with *P. guerini* compared to plots without *P. guerini* at all sampling events except for the percentage cover recorded at the start of the experiment.

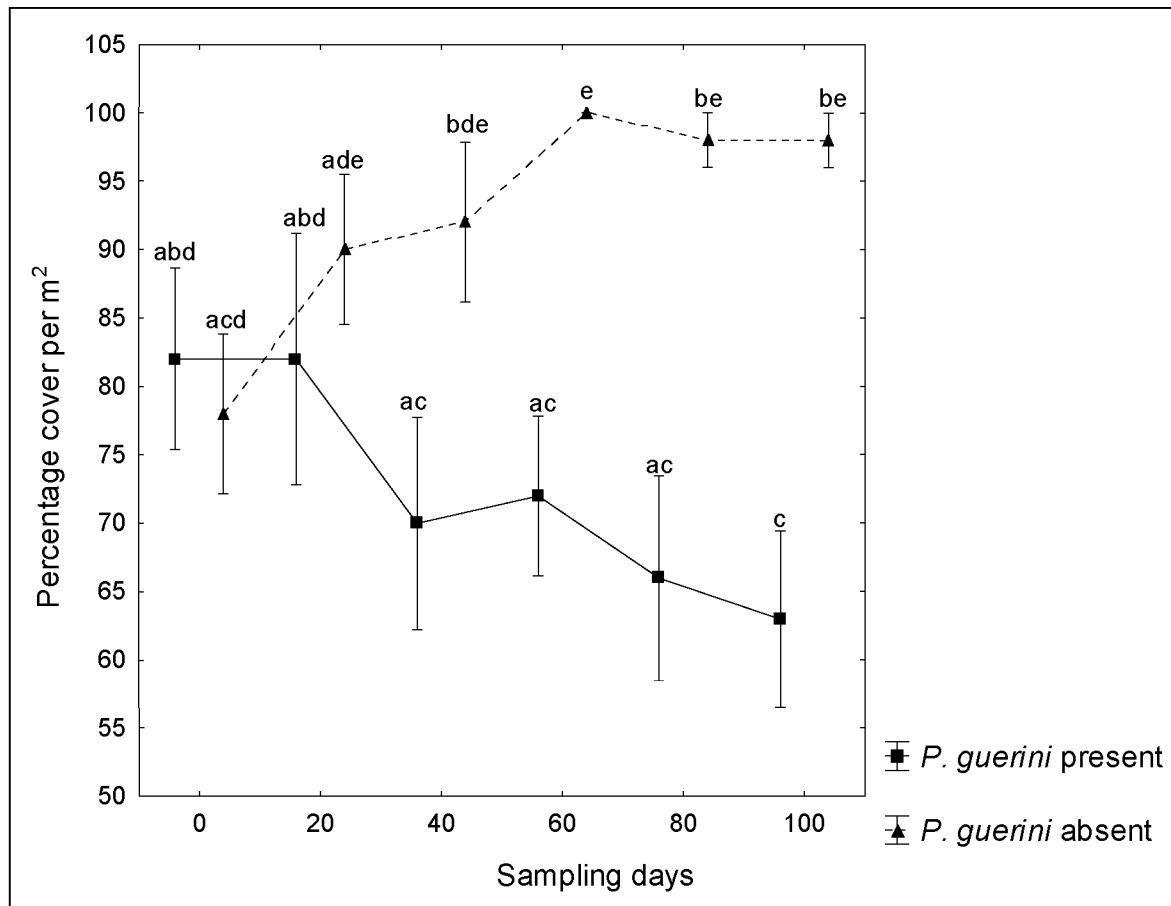


Figure 2.9 Percentage cover per m² on different sampling days for each treatment. The treatments included were *P. guerini* present= plots with insects not excluded by insecticide, and *P. guerini* absent=plots sprayed with insecticide to exclude the agent. Error bars indicate standard errors around each mean. Different letters indicate significant differences ($p < 0.05$) according to Fisher's LSD test.

Number of shoots

Significant differences were observed between plots without *P. guerini* and plots with *P. guerini* for the number of shoots ($F_{1,58} = 8.59$, $p < 0.05$; Fig. 2.10). The number of shoots measured for both plots with *P. guerini* and with *P. guerini* excluded was not significantly different at the start of the experiment (Fig. 2.10). Before the experiment, the mean number of shoots for plots that were not to be treated with insecticide was 26.4 (SE $\pm$ 1.99) shoots per m² whereas plots selected for *P. guerini* exclusion had 25.6 (SE $\pm$ 3.75) shoots per m² (Fig. 2.10). The number of

shoots increased for both treatments over the period of sampling (Fig. 2.10). The number of shoots was always greater in plots with *P. guerini* excluded but this was not significantly different at any sampling event (Fig. 2.10).

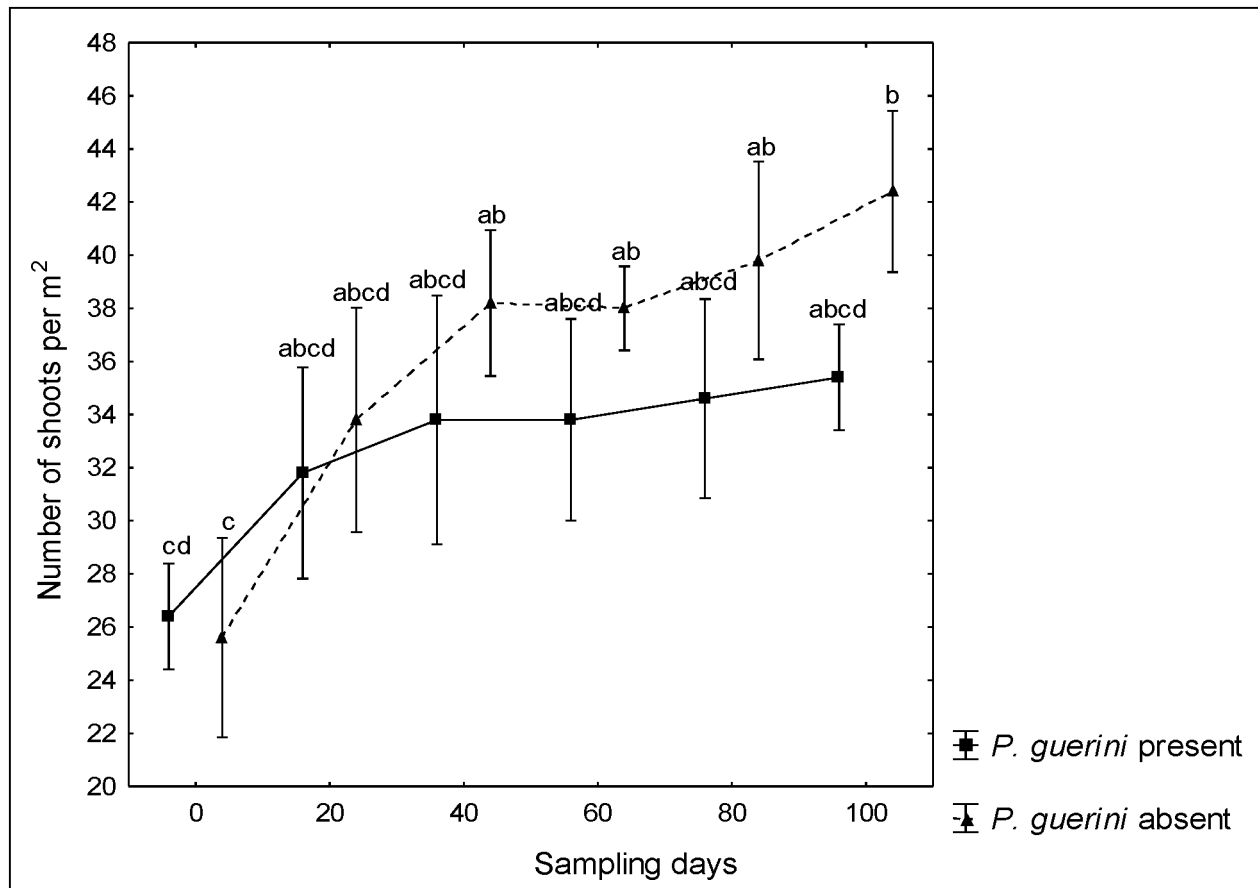


Figure 2.10 Number of shoots per m² on different sampling days for each treatment. The treatments included were *P. guerini* present= plots with insects not excluded by insecticide, and *P. guerini* absent =plots sprayed with insecticide to exclude the agent. The error bars indicate standard errors around each mean. Different letters indicate significant differences ($p < 0.05$) according to Fisher's LSD test.

Number of damaged leaves

The number of damaged leaves per m² for plots with *P. guerini* present remained relatively constant over the 100-day sampling period with a mean of 366 (SE $\pm$ 17.9) damaged leaves per

m² (Fig. 2.11). The number of damaged leaves decreased from 353 (SE ± 62.16) to 158 (SE ± 33.04) leaves per m² after 40 days of the beetles being excluded from the plots (Fig. 2.11). The number of damaged leaves remained constant after 40 days until the end of the experiment with a mean of 120 (SE ± 16.29) damaged leaves per m² (Fig. 2.11). Plots with *P. guerini* had a significantly higher number of damaged leaves than the plots without *P. guerini* at all sampling days except for the first measurement taken at the start of the experiment ($F_{1,58}=34.91$, $p<0.05$; Fig. 2.11).

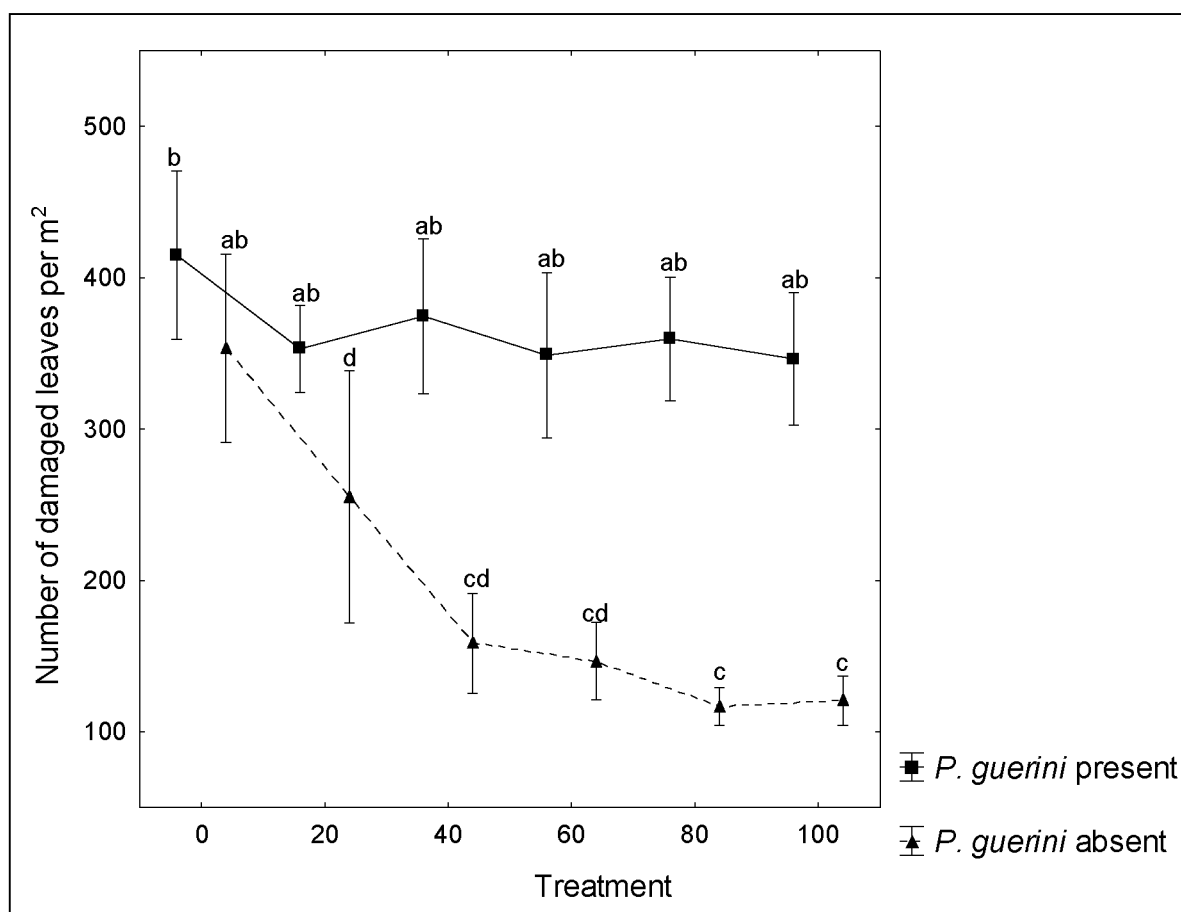


Figure 2.11 Number of damaged leaves per m² on different sampling days for each treatment. The treatments included were *P. guerini* present= plots with insects not excluded by insecticide, and *P. guerini* absent =plots sprayed with insecticide to exclude the agent. The error bars indicate standard errors around each mean. Different letters indicate significant differences ($p < 0.05$) according to Fisher's LSD test.

Phenrica guerini abundance

The overall number of *P. guerini* found per m² was not significantly greater except for the first and last sampling events in plots with *P. guerini* than plots where *P. guerini* was excluded (Wald $\chi^2 = 0.00$, $p < 0.05$; Fig. 2.12). Although there were no significant differences plots that were sprayed with insecticide always had a mean of less than one individual, while plots that were not sprayed had a mean of 5.6 (SE ± 1.04) adults per m² across all sampling events (Fig. 2.12).

The mean of larvae for plots that were not sprayed with the insecticide was $3.13 (SE \pm 2.32)$ and for plots that were treated with the insecticide was $0.15 (SE \pm 0.25)$.

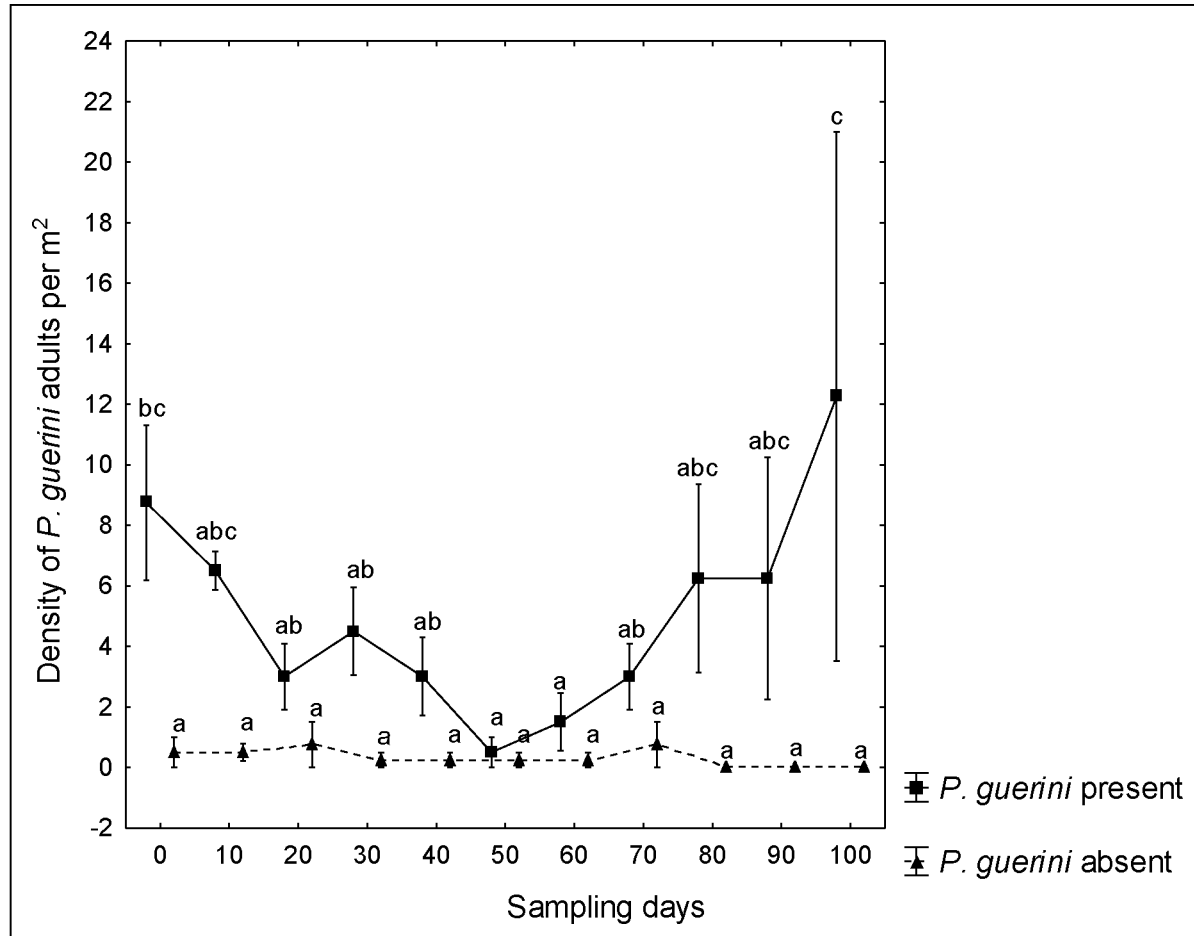


Figure 2.12 Adults of *P. guerini* found per m² on different sampling days for each treatment. The treatments included were *P. guerini* present= plots with insects not excluded by insecticide, and *P. guerini* absent =plots sprayed with insecticide to exclude the agent. Error bars indicate standard errors around each mean. Different letters indicate significant differences ($p < 0.05$) according to Fisher's LSD test.

2.3.3. Nationwide survey

The total area of *P. aculeata* infested sites visited during the survey was 65 254m² with an average *P. aculeata* cover of 66%. This corresponds to an area of 42 408m² or 4.24ha covered

with 100% *P. aculeata*. Populations of *P. guerini* have established at 14 of the 23 sites (60.8%) that were visited during the survey (Table 2.1). The majority of the sites had very low densities of *P. guerini* and very low levels of damage (Table 2.1). Port Alfred, Umtentweni, Paradise Valley, and Amanzimtoti had the highest density of *P. guerini* that resulted in the highest levels of damage. Paradise Valley and Port Alfred had a significantly greater number of damaged leaves per m² and were significantly different from all other sites (Table 2.2).

Table 2.1. Release history of *P. guerini* abundance and level of damage measured at each site of the 23 sites sampled in KwaZulu-Natal and Port Alfred in the Eastern Cape.

SITE	ESTABLISHED	SIZE (m ²)	DENSITY PHENRICA PER M ²	DAMAGED LEAVES PER M ²
PORT ALFRED	YES	5	5.6	366
LEISURE BAY	NO	472	0	0
PALM BEACH	NO	39	0	0
BENDIGO	YES	126	0	21.6
UMTENTWENI	YES	60	5.7	91.4
ZINKWAZI	YES	3000	0	1.6
UMHLALI	YES	6750	2	33.2
WEWE	YES	638	0	2.8
BALLITO	NO	75	0	0
PEACE COTTAGE	YES	4000	1.6	36.4
THORNY PARK	NO	576	0	0
TUGELA RIVER	NO	400	0	0
RICHARDS BAY	YES	240	0.8	32
RICHARDS BAY COMMUNITY	NO	630	0	0
NONOTI	NO	168	0	0
NONOTI COMMUNITY	YES	1950	0.4	7.6
BELLAIR	YES	144	4.4	45.2
BRIDGEVAIL	YES	84	2.4	48
PARADISE VALLEY	YES	1500	36	375.6
AMAMZIMTOTI	YES	27	13.6	163.2
LUTHULI	NO	45	0	0
INYEZINE	YES	208	0	4.8
THE BLUFF	NO	12	0	0

Table 2.2. Comparison of a number of damaged leaves per m² between Port Alfred and sites in KwaZulu-Natal. The only site with similar levels of damage to Port Alfred was Paradise Valley, which is highlighted in bold text.

SITE	DAMAGED LEAVES PER M2	p-value	t-value
Port Alfred	366		
Bendigo	21.6	0.69	0.53
Umtentweni	91.4	0.53	0.92
Zinkwazi	1.6	0.73	0.44
Umhlali	33.2	0.67	0.57
Wewe	2.8	0.74	0.44
Peace Cottage	36.4	0.67	0.58
Richards Bay	32	0.68	0.55
Nonoti Community	7.6	0.73	0.45
Bellair	45.2	0.65	0.61
Bridgevail	48	0.65	0.62
Paradise Valley	375.6	0.00	377.8
Amanzimtoti	163.2	0.38	1.47
Inyezine	4.8	0.75	0.42

2.4. Discussion

Phenrica guerini has established at 14 sites in South Africa than the three sites recorded by Paterson *et al.*, (2011a) in November 2014. At Port Alfred, the damage inflicted by *P. guerini* has resulted in a reduction in the number of leaves as well as the percentage cover and it can therefore be regarded as damaging at this site. The spraying of insecticide does not confound the results of the field experiment at Port Alfred because the number of leaves of *P. aculeata* was not affected by the application of insecticide in the laboratory based common garden experiment. There is, however, some evidence that shoot length may increase due to the application of insecticide because plants with the agent that were treated with insecticide had

greater shoot lengths than controls in the laboratory based common garden experiment. This is not problematic for the interpretation of the results of the field based exclusion experiment because shoot length was never significantly different between plots with and without the agent in the field. While Port Alfred had the highest density of *P. guerini* and the highest level of damage, there are other sites, such as Paradise Valley, that had comparable levels of damage and we can assume that some impact on the number of leaves and percentage cover might be expected at these sites. All of these results highlight that *P. guerini* should not be deemed as an ineffective biological control agent even though it has not resulted in complete control of *P. aculeata* in South Africa.

There are no accurate pre-release data of *P. aculeata* densities from before the release of *P. guerini* so it is not possible to determine what the density of *P. aculeata* would be without the release of the agent. Although the exclusion experiment clearly indicates some impact from the agent on *P. aculeata* density, it was not done on a country-wide scale, does not include the rate of spread of the weed, and was only conducted over a 100-day period. *Phenrica guerini* has been established at sites in South Africa since 1991 and the long-term impact at these sites is difficult to determine from these data. Similarly, the impact of *P. guerini* may have reduced the spread of the weed to other sites and this would not have been evident from this study.

It is important to define what will be perceived as successful biological control of the target weed before the initiation of biological control programmes (Hoffmann and Moran, 2008; Müller-Schärer and Schaffer, 2008). The targets set before the start of a biological control programme are important to assist in determining and evaluating success (Hobbs, 2003; van

Klinken and Raghu, 2006). Biological control programme success should not only be restricted to a drastic reduction in weed density because any reduction in weed fitness could have a positive impact on control (Hoffmann and Moran, 2008). Although there was no pre-release data collected before the release of *P. guerini*, goals for the biological control programme against *P. aculeata* have been set (Paterson *et al.*, 2011b), and it is possible to determine whether *P. guerini* has achieved these goals at Port Alfred. A reduction of *P. aculeata* densities to below 30% has been suggested as the ultimate goal of the biological control programme against *P. aculeata* (Paterson *et al.*, 2011b). The impact to native biodiversity is considered the most important negative impact of *P. aculeata* because it is primarily an environmental weed with minimal impacts to agriculture and other industries. There was a significant increase in native plant biodiversity when *P. aculeata* densities were below 50% compared to when the density of *P. aculeata* was between 50% and 100%, and at 30% or below, native biodiversity was not statistically different to plots without the weed (Paterson *et al.*, 2011b). A percentage cover of 50% can, therefore, be regarded as a threshold of success as there are benefits in terms of an increase in native biodiversity at *P. aculeata* densities below this threshold value (Paterson *et al.*, 2011b). A percentage cover of 30% can be regarded as the threshold of complete control because at densities below this value there is no difference in native biodiversity from plots where *P. aculeata* is absent (Paterson *et al.*, 2011b). At Port Alfred, the percentage cover of *P. aculeata* was reduced from about 98% to about 65%, so although this is a significant reduction in cover, it will not result in any statistically significant increase in native plant biodiversity at the site as the *P. aculeata* has not been reduced below 50% cover. The Paterson *et al.*, (2011b) study was also conducted at sites where few other invasive alien plant

species were present, while the diversity and abundance of invasive alien plant species at Port Alfred were high. This suggests that even if there is an increase in plant diversity due to the action of *P. guerini* it is likely to be due to an increase in other alien species rather than natives.

Although no direct measurements of changes in biodiversity were recorded in this study, it is possible to estimate the benefits of *P. guerini* to biodiversity by using the data from Paterson *et al.*, (2011b). The data collected in this study would, therefore, be considered weed-related measurements according to Morin *et al.*, (2009) but the data can be extrapolated to the level of ecosystem measurements using the threshold values from Paterson *et al.*, (2011b). Biodiversity recovery is a process that is likely to take many years, so in order to directly measure ecosystem recovery, the exclusion experiment would have to have been continued for a much longer period of time.

During the nationwide survey, only 14 sites out of the 23 sites sampled had populations of *P. guerini*. Of the sites that had *P. guerini*, five sites had such low levels of damage and agent abundance that very little impact on weed density was expected. Three of these sites (Umtentweni, Amamzimtoti and Paradise Valley) had high agent abundance and extensive damage. It was not possible to quantify how much the damage inflicted by *P. guerini* contributes towards the biological control programme against *P. aculeata* from the data collected during the nationwide study but the level of damage was comparable with that recorded in the exclusion experiment at Port Alfred and this suggests that some impact should be expected. The high levels of damage at the three sites where *P. guerini* was most abundant are very likely to have been causing a negative impact to plant growth and reproduction of *P.*

aculeata. At these three sites, the number of leaves damaged by *P. guerini* was greater than the number of leaves that was not damaged. The results of the nationwide study highlight that *P. guerini* is clearly a more damaging agent than previously reported. There may also be more sites that were not sampled during the survey where there is a high level of damage inflicted by *P. guerini*.

The level of damage inflicted by *P. guerini* on *P. aculeata* is categorised as trivial by Klein (2011) and the level of control of *P. aculeata*, as estimated using the system first proposed by Hoffmann (1995), is negligible (Klein, 2011). The data from this study suggests that the level of damage inflicted by *P. guerini* should be changed from trivial to moderate for at least some sites where the agent has become abundant. There could also be an argument to change the status of negligible control, to substantial control, but only at a very limited number of sites.

2.5. Conclusion

Post-release evaluations assist in decision-making about whether additional biological control agents are needed in a case where the existing biological control agents are ineffective (Morin *et al.*, 2009). This study has shown that more biological control agents are required to reduce *P. aculeata* densities to appropriate levels. *Phenrica guerini* does contribute to the control of *P. aculeata* in South Africa and is a more effective agent than previously published reports have suggested (Klein, 1999; Paterson *et al.*, 2011a; Paterson *et al.*, 2014a, b). Mass-rearing of *P. guerini* should continue until there are new agents that are proven to be more effective. The level of damage inflicted by *P. guerini*, even at high densities such as at Port Alfred, is however clearly not sufficient to reduce the density of *P. aculeata* below the proposed threshold values

(Paterson *et al.*, 2011b). Further biological control agents must therefore be considered but the relative levels of damage caused by each agent and the interactions between the agents must be understood before a new agent is released (Paterson *et al.*, 2014a).

Chapter 3: Interactions between *Phenrica guerini* and *Catorhintha schaffneri*, two biological control agents for *Pereskia aculeata* in South Africa

3.1. Introduction

3.1.1. Insect-insect interactions

Insect species represent about 60% of all the species on earth and about 30% of these are herbivorous insects (Schoonhoven *et al.*, 1998). The interactions between insects and plants they feed on have important ecological consequences (Kaplan and Denno, 2007) and are the basis of biological control programmes. These interactions play a role in structuring insect and plant populations (Stout *et al.*, 2006). The way insects interact may have an impact on their population size, population growth rate, as well as individual fitness and the host plants' performance (Abrams, 1987). It is necessary to understand the interactions between species or populations to predict ecological processes taking place between them (Abrams, 1987). Interactions between herbivorous insects may either be direct (involving pair wise interactions between two species) or indirect (including interactions mediated by a host plant) (Petersen and Sandström, 2001; Kaplan and Denno, 2007).

Direct insect-interactions do not require another mediator species such as a host plant (Abrams, 1987; 1995; Menge, 1995; Menge, 1997). A direct insect-interaction occurred between two biological control agents, *Rhinocyllus conicus* Frölich (Curculionidae) and *Urophora solstitialis* L. (Tephritidae), released against a nodding thistle, *Carduus nutans* L. (Asteraceae) (Groenteman *et al.*, 2007). Both agents often occur in the same capitula

simultaneously placing them in a potentially antagonistic situation (Groenteman *et al.*, 2007). The number of *U. solstitialis* found in each capitulum was decreased when *U. solstitialis* was released with *R. conicus* whereas numbers of *R. conicus* increased in the presence of *U. solstitialis* (Groenteman *et al.*, 2007). This reduction of *U. solstitialis* is predicted to be caused by *R. conicus* which preys on the *U. solstitialis* larvae because they supplied *R. conicus* with improved nutritional value than the receptacle tissue (Groenteman *et al.*, 2007).

Indirect interaction can be host plant-mediated or mediated by another organism (Masters and Brown, 1997; Fagundes *et al.*, 2005). An example of an indirect interaction occurred between two aphids, an underground root-feeding aphid, *Pemphigus betea* Doane and a leaf galling aphid, *Hayhurstia atriplicis* (L.) (Aphididae) mediated by the host plant, *Chenopodium album* L. (Amaranthaceae) (Moran and Whitham, 1990). The interaction between these aphid species resulted in a reduced population of root-feeding aphid in plants with leaves that were galled by the leaf galling aphid (Moran and Whitham, 1990). These two aphids have never interacted directly even though they both feed on the phloem sap of the plant and the feeding behaviour of the leaf galling aphid makes the phloem sap unavailable for consumption by the root-feeding aphid. This resulted in a reduction in the root-feeding aphid populations (Moran and Whitham, 1990).

Plants and insects are the most diverse and important organisms on Earth and their interactions have been extensively studied (Agrawal, 2004). The interactions can be spatial, temporal, and spatio-temporal (Kaplan and Denno, 2007; Milbrath and Nechols, 2014). Spatial interactions occur between two or more species that share a host plant at the same time but are targeting

different plant parts, for example, below-ground and above-ground insect herbivores (Blossey and Hunt-Joshi; 2003; Simelane, 2006). Insect herbivores that share a common niche during different times such as different seasons or different developmental stages of the plant are referred to as temporal interactions (Wold and Marquis, 1997). Spatio-temporal interactions occur between temporally separated insect herbivores that target different niches (Swope and Stein, 2012). All these interactions can have a negative or positive impact on the growth and performance of the insect herbivores and will often not be symmetrical because later-feeding species are likely to be influenced by previous feeders whereas the converse is unlikely (Masters and Brown, 1995).

The presence of one insect in some cases affects the survival and development of other insects (Persson, 1985). This is a negative interaction between insects and takes place when a common shared resource is exploited and not available for another individual (Petersen and Sandström, 2001). For example, there was an asymmetric interaction recorded when two foliar-feeding aphids *Melanocallis caryaefoliae* (Davis) and *Monellia caryella* (Fitch) both fed on the lower surface of pecan leaves, *Carya illinoensis* (Wangenh.) K. Koch (Juglandaceae), at the same time. *Melanocallis caryaefoliae* was affected by the presence of *M. caryella* whereas the presence of *M. caryaefoliae* had no negative effect on the survival and development of *M. caryella* (Petersen and Sandström, 2001).

The interaction between insects, or other herbivorous arthropods, that share a common host plant may sometimes result in complementary interactions where the presence of one individual may increase the performance of another individual (Caesar, 2003). Delfosse (1978)

conducted an interaction study between two biological control agents, *Orthogalumna terebrantis* Wallwork (Galumnidae) and *Neochetina eichhorniae* Warner (Curculionidae) which both feed exclusively on water hyacinth. The findings showed that there was a positive interaction between the agents because *N. eichhorniae* benefitted from the presence of *O. terebrantis* (Delfosse, 1978). The presence of *N. eichhorniae* was not advantageous for *O. terebrantis* but oviposition and feeding of *N. eichhorniae* increased when released with *O. terebrantis* (Delfosse, 1975; 1977; 1978). The interactions between herbivorous insects, therefore, can have a wide variety of different impacts on both the plant and insect population and this is important for biological control.

3.1.2. Multiple versus single biological control agents

When two or more biological control agents are released on the same target weed, one of the main concerns is how they will interact with each other and how their interaction will affect the target as well as the agents' populations (Myers, 1985). In many cases, weed biological control programmes require more than one biological control agent for success to be achieved (McEvoy and Coombs, 2000), but the number of biological control agents required to achieve complete control and sustain control of a target weed varies greatly between biological control programmes (Myers, 1985; Denoth *et al.*, 2002; Stilling and Cornellissen, 2005). Some programmes have detected competition between agents that shared a common host plant while others have complemented each other (Denoth *et al.*, 2002). The release of multiple agents increases the chances of finding the best agent (Ehler and Hall, 1982), but on the other hand, releasing multiple agents may reduce the possibility of successful biological control

programmes if the agents compete for a common resource (Ehler and Hall, 1982). Antagonistic interactions between multiple agents may lead to the elimination of an effective agent by an ineffective agent if it is a superior competitor (Ehler and Hall, 1982, Crowe and Bouchier, 2006).

Multiple agents may effectively control a weed due to the increased cumulative stress exerted on the plant (Hoffmann and Moran, 1998; Turner *et al.*, 2010). Successful biological control involving the use of multiple agents is therefore possible when the interaction between the agents results in a complementary interaction (Buccellato *et al.*, 2012). However, where successful biological control programmes involve multiple agents, the majority of damage is often inflicted by one agent (Denoth *et al.*, 2002; Jackson and Myers, 2008). In these biological control programmes, many of the agents have not played a role in suppressing the target weed, but by increasing the number of agents that are released there has been an increased chance of finding a successful agent (Myers, 1985; Julien and Griffiths, 1998; Crowe and Bouchier, 2006). This practice has been referred to as the lottery model (Myers, 1985).

It is extremely challenging to determine how effective an agent will be prior to release, so identifying which of the agents will result in the suppression of the weed before all the agents are released is difficult (McFadyen, 1998). With every new agent released, there is a possibility of finding a more effective agent but also the possibility that the agents will have an antagonistic interaction resulting in reduced levels of control. There is also always an innate risk when releasing a new agent, so the release of ineffective agents should be avoided (Myers, 1985, 2008; McEvoy and Coombs, 2000; Denoth *et al.*, 2002). When an agent has been released

and established, it may be extremely difficult or impossible to eradicate, so the release of a new agent should not be taken lightly (McEvoy and Coombs, 2000). The release of ineffective agents can also increase the chances of ecological knock-on effects because high population densities of the agent can be maintained without reducing the weed density (McClay and Balciunas, 2005; Balciunas and Smith, 2006). An effective agent will reduce weed densities and this will be followed by a decrease in the population of the agent, while an ineffective agent will not reduce weed densities and may therefore have consistently high populations which could be utilised as a food source for other organisms resulting in indirect ecosystem effects (Pearson and Callaway, 2006).

3.1.3. Classification of the effects of interactions between insects on their host plants

Evaluating the impact caused by multiple and single agents on a target weed is not straightforward. The evaluation of biological control agents' impact against a target weed involves determining how the agents interact with each other and the impact they have on the plant (Hatcher and Paul, 2001). Hatcher (1995) proposed four response categories that can be used to compare interactions between insects in relation to changes in plant productivity (Hatcher, 1995). The interactions may be: **1) Synergistic**: an interaction that occurs when the impact is significantly greater compared to the sum of the impacts achieved by two agents if released separately, **2) Additive**: an interaction between two or more agents that reduces plant growth more compared to the most damaging agent when released alone, **3) Equivalent**: an interaction where release of both agents results in an equivalent impact to that of the agent causing the highest damage, and **4) Inhibitory**: an interaction where both agents together have

significantly less impact than that caused by the least damaging agent when it is released alone (Myers, 1985, Hatcher, 1995). Turner *et al.*, (2010) modified the description of an additive interaction to be an interaction where the damage caused by multiple agents is greater than that of the most effective agent released alone but less than or equivalent to the added damage caused by each agent when released alone (Turner *et al.*, 2010).

An example of a synergistic interaction was observed in a biological control programme against *Lythrum salicaria* L. (Lythraceae) a perennial weed of Eurasian origin (Blossey, 1995). *Galerucella pusilla* Duft. and *Galerucella californiensis* L. (Chrysomelidae), two defoliating beetles, complemented each other when they were released in combination for the control of *L. salicaria* (Blossey, 1995). The interaction between these agents resulted in a significantly greater reduction of plants due to the greater decrease of average shoot lengths, seed productivity and suppressed flowering (Blossey, 1995). Out of five sites that were investigated, three showed that the damage inflicted by the combination of these beetles resulted in higher mortality and a greater decrease in average shoot lengths, reduced seed productivity, and suppressed flowering (Blossey, 1995).

There was an additive interaction between an undescribed leafhopper (Tribe Erythroneurini, formerly referred to as *Zygina* sp.) and rust fungus *Puccinia myrsiphylli* (Thuem.) Winter. (Pucciniaceae) released on *Asparagus asparagoides* L. Druce (Asparagaceae) (Turner *et al.*, 2010). In this example, there was a significantly greater reduction in the number of tubers, rhizome length and total tuber dry weight when both biological control agents were released together compared to when the most damaging agent was released alone (Turner *et al.*, 2010).

The interaction between two biological control agents of water hyacinth, *Orthogalumna terebrantis* Wallwork (Galumnidae) and *Eccritotarsus catarinensis* (Carvalho) (Miridae) resulted in an equivalent interaction (Marlin *et al.*, 2013) because the damage caused by the mite and miridon water hyacinth was equal to the impact of each biological control agent released alone (Marlin *et al.*, 2013). The impact to the plant, measured as mean lengths of the longest petioles, the total number of leaves produced, and change in wet biomass, was the same when each agent was alone as with both agents in combination (Marlin *et al.*, 2013).

An example of an inhibitory interaction was recorded between *N. eichhorniae* and *E. catarinensis* (Weyl and Hill, 2012). When these agents were released together, fewer feeding scars of *N. eichhorniae* were observed compared to when *N. eichhorniae* was present alone (Weyl and Hill, 2012). The percentage of feeding area of the mirid decreased as the number of *N. eichhorniae* increased (Weyl and Hill, 2012). The performance of the weevil, *N. eichhorniae* was reduced in the presence of *E. catarinensis* whereas *N. eichhorniae* did not affect the performance of *E. catarinensis* (Weyl and Hill, 2012).

There are no previous studies that have looked at the interactions between *P. guerini* and *C. schaffneri*, the two biological control agents of *P. aculeata* in South Africa. These biological control agents target two different plant parts; *P. guerini* chews the leaves and *C. schaffneri* is a stem-wilter that pierces and sucks from growing shoots. The difference in their feeding behaviour may result in a synergistic interaction because they are using different resources, however, it is important to investigate and understand the interactions between these two biological control agents. The aims of this study were to examine the effects of herbivory by *P.*

guerini and *C. schaffneri*, singly and in combination on the growth of *P. aculeata* and to investigate the influence of interactions between these two agents on the efficacy of the biological control programme.

3.2. Materials and methods

3.2.1. *Pereskia aculeata* potted plants

Three hundred *P. aculeata* cuttings measuring approximately 10cm in length and 2cm in diameter were collected from field sites around Grahamstown, Eastern Cape, South Africa. The cuttings were left to dry for three days after collection. The bases of the cuttings were then moistened slightly with water and dipped into Seradix B No. 2 root growth hormone powder (©Bayer (Pty) Ltd) to stimulate root growth. The cuttings were then grown in potting soil and fertilised with Multicote 6 controlled release fertiliser (Haifa Chemicals Ltd, Haifa™) according to the manufacturer's protocol. The potted plants were all checked daily and any unwanted organisms such as spiders and aphids were manually removed when necessary. All the potted plants were kept in a greenhouse at the Waainek Research Facility, Rhodes University, Grahamstown, South Africa and kept under the same water and fertilising regime.

3.2.2. Rearing of *Phenrica guerini*

Phenrica guerini adults were collected from a *P. aculeata* infestation in Port Alfred, one of the release sites where *P. guerini* was released between 1991 and 1997 (Klein 1999). The rearing of *P. guerini* was conducted in a laboratory at Rhodes University. The temperature of the laboratory varied between 25°C and 27°C with a 13-hour photoperiod. *Phenrica guerini* adults

were placed in an insect rearing cage (1.2m X 0.6m X 0.6m) with a potted *P. aculeata* plant. Eggs were collected and placed in Petri dishes with moist filter papers. The Petri dish lids were sprayed with a mist of water daily to prevent the eggs from desiccating. The eggs were monitored until hatching; the larvae were then transferred to plastic containers with perforated lids to undergo development. The larvae were ready for pupation after about 22 days when they were transferred to 2 litre containers filled with a moistened mixture of potting soil and sandy soil (50 % of each soil type). This mixture was filled up to a depth of 5cm to allow the larvae to dig deep enough to construct pupal chambers. Leaves and shoots of *P. aculeata* were inserted into moist florists foam to prevent them from wilting rapidly, and these were placed on top of the soil mixture. The damaged leaves were replaced with fresh ones when they were heavily damaged by the agent or if they were wilted. After pupation had taken place, the soil was sprayed daily with small amounts of water to reduce dehydration of the larvae. Overwatering was also avoided as this could have caused pupal chambers to collapse, resulting in pupal mortality. Adults emerged from the soil approximately nine days after pupation and were placed back into the cage to breed and produce eggs.

3.2.3. Rearing of *Catorhintha schaffneri*

A culture of *C. schaffneri* was maintained in a laboratory at Rhodes University. The facility temperatures were maintained at 23°C at night and 25°C during the day with a 12:12 hour light and the dark cycle. *Catorhintha schaffneri* culture was kept in insect rearing cages (1.2m X 0.6m X 0.6m) and provided with potted *P. aculeata* plants with the addition of *P. aculeata* shoots in floral foam when needed. Egg batches were collected from the *C. schaffneri* culture and kept

individually in Petri dishes until hatching. A fine paintbrush was used to transfer the newly emerged nymphs to plants in the insect rearing cages. The nymphs were monitored daily and a light mist of water was sprayed on the cages and plants daily to maintain humidity.

3.2.4. Experimental design

An experiment to investigate the interactions between *P. guerini* and *C. schaffneri* was conducted in a greenhouse situated at Waainek Research Facility, Rhodes University, Grahamstown, South Africa. Sixteen potted plants were used for each replicate of the experiment. These potted plants were set up inside the greenhouse a week prior to the inoculation of insects to allow them to acclimatise to the greenhouse environment. Larvae and nymphs of *P. guerini* and *C. schaffneri* were kept in Petri dishes with sprigs of *P. aculeata* until they were five days old before being used for the experiment. Waiting until the insects were five days old was necessary because one-day-old larvae and nymphs were too delicate to transfer to the plants and this resulted in mortality of both larvae and nymphs. Five days is a transitional stage between the first instar and the second instar for both agent species.

Experimental treatments were 1) control (with no insects), 2) a combination of *P. guerini* and *C. schaffneri* (both insects), 3) *P. guerini* only, and 4) *C. schaffneri* only. Four insect stocking densities of two, four, six and twelve individuals per plant were used (Table 3.1).

Table 3.1. The combinations of species and total number of insects released in each treatment of the experiment.

Stocking density	Treatments		
	<i>Phenrica guerini</i> only	<i>Catorhintha schaffneri</i> only	Both insects
2	2 larvae	2 nymphs	1 larva + 1 nymph
4	4 larvae	4 nymphs	2 larvae + 2 nymphs
6	6 larvae	6 nymphs	3 larvae + 3 nymphs
12	12 larvae	12 nymphs	6 larvae + 6 nymphs

Phenrica guerini larvae were placed on the fifth healthy leaf from the shoot tips while *C. schaffneri* nymphs were placed in open Petri dishes at the base of plants. *Catorhintha schaffneri* nymphs are more mobile than *P. guerini* larvae so they dispersed easily from the Petri dishes while *P.guerini* larvae typically feed immediately on the leaf on which they hatch and so needed to be placed on a leaf. The insect stocking densities were kept constant at all times by replacing dead insects on a daily basis and insects that were used for replacement were the same age as those that had died in order to control for differences in the level of damage caused by insects of different ages. Mortality of larvae and nymphs was recorded. The top lips of the pots were wrapped with fruit tree grease band (©STV International Ltd) to trap unwanted insects and to keep the experimental insects on the potted plants. Fruit tree grease band is a waterproof adhesive and stays effective for up to two months. The potted plants were labeled with wrap-around vinyl labels to prevent confusion when releasing insects onto the plants. The experiment was conducted for a period of ten days because, after ten days, the condition of the plants was found to be poor due to the feeding of the agents and new plants would be

required. The period of ten days used during this study is the same as the period used in Paterson *et al.*, (2014) who also reported that the condition of *P. aculeata* was poor after 10 days when first instar *C. schaffneri* at a stocking density of 10 was used. The plants were watered when necessary and the leaves were sprayed with a light mist of water daily. All the plants were kept under the same water regime and the experiment was replicated ten times.

The potted plants were arranged in a Latin Square Design (Fig. 3.1), in such a way that each treatment appeared once in each row and column (Rangaswamy, 1995). This meant that the potted plants from all the treatments were exposed to a similar range of environmental conditions (Quinn and Keough, 2002).

C	S2	P2	B2
S4	P4	B4	C
P6	B6	C	S6
B12	C	S12	P12

Figure 3.1 The Latin Square Design representing the four treatments by the letters C= Control, S= *Catorhintha schaffneri*, P= *Phenrica guerini* and B= Both insect in combination. Numbers 2, 4, 6 and 12 indicate different stocking densities.

3.3. Data collection

3.3.1. Parameters measured

Shoot lengths and the number of leaves were measured before and after the experiment. The number of leaves was determined by counting the leaves on potted plant and shoot lengths were measured from the shoot axils to the tip. Insect mortality was recorded on a daily basis when dead individuals were replaced. The leaves, shoots, roots and stems of *P. aculeata* were also harvested after the experiment to measure plant biomass. The plant parts were kept in labelled brown paper bags for two weeks and then oven dried at temperatures of 60°C for a week before they were weighed using a Mettler Toledo balance scale (Microsep (Pty) Ltd). Three samples were weighed daily until the plant biomass was constant three consecutive times. The plant parts weighed for plant biomass were leaves, roots and stems. These were weighed individually for each treatment and stocking density.

3.4. Data analysis

Data analysis was conducted using the statistical programme STATISTICA Version 13.0 (©Stat Soft, Inc., USA). All data were checked for normality. Data collected for leaves and shoot lengths were normally distributed and met the requirements of one-way analysis of variance (ANOVA). Plant biomass data and insect mortality were non-normally distributed and were therefore analysed by the generalized linear model (GLZ) with a normal distribution and a log function. Fishers LSD post hoc tests were conducted to identify homogenous groups after the GLZ and ANOVA.

3.5. Results

3.5.1. Numbers of leaves

There were significant differences in the change in the number of leaves between treatments at the end of the experiment ($F_{12,15}=16.69$, $p<0.05$; Fig. 3.2). Control plants had the greatest increase of 10.2 leaves ($SE \pm 1.05$) and this was significantly different to all other treatments (Fig. 3.2). The highest density of *C. schaffneri* alone resulted in the greatest number of leaves being lost, with a mean reduction of 11.7 leaves ($SE \pm 1.29$) over the 10-day period (Fig. 3.2). This treatment was significantly different to all other treatments, including *P. guerini* alone (Fig. 3.2). Leaf losses with *C. schaffneri* alone increased as the stocking density increased. For *C. schaffneri* alone at a stocking density of 2, the mean number of leaves increased by 1.8 ($SE \pm 1.78$) whereas, at a stocking density of 4, the mean number of leaves was reduced by 0.4 ($SE \pm 2.15$) (Fig. 3.2). Leaf losses at stocking densities of 2 and 4 were not significantly different from each other. *Catorhintha schaffneri* alone at a stocking density of 6 reduced the mean number of leaves by 4.7 ($SE \pm 1.89$), which was significantly greater than the number of leaves reduced by stocking densities of 2 and 4 (Fig. 3.2). The mean number of leaves for controls was significantly greater than *C. schaffneri* alone at stocking densities of 12 by 10.9 ($SE \pm 0.77$) leaves (Fig. 3.2).

Phenrica guerini alone was generally less effective at reducing the number of leaves than *C. schaffneri* alone (Fig. 3.2). Differences between *P. guerini* alone and *C. schaffneri* alone were significant at all stocking densities except the lowest stocking density of 2 (Fig. 3.2). *Phenrica guerini* alone was also less effective at reducing the number of leaves than both insects in combination. At a stocking density of 2, there was no significant difference between *P. guerini*

alone and the insects in combination but the difference between the stocking density of 4 and 6 was significant (Fig. 3.2). At a stocking density of 12, there was no significant difference between *P. guerini* and both insects in combination (Fig. 3.2).

Both insects in combination at stocking densities of 4, 6, 12 were more effective in reducing the number of leaves than the lowest stocking density of *C. schaffneri* alone as well as all stocking densities of *P. guerini* except for stocking density of 12 (Fig. 3.2). Both insects in combination at all stocking densities were significantly different from *C. schaffneri* alone at a stocking density of 12 which was the treatment that caused the greatest reduction in the number of leaves (Fig. 3.2).

In summary, the highest stocking density of *C. schaffneri* alone was more damaging than both agents and *P. guerini* alone at the same stocking density (Fig. 3.2). The damage done by *C. schaffneri* at a lower density of 6 was not significantly different from either both agents or *P. guerini* alone at the highest density of 12 but this was significantly different to all other treatments (Fig. 3.2). At lower stocking densities, *C. schaffneri* alone was as damaging as both insects in combination and more damaging than *P. guerini* alone. *Phenrica guerini* alone was always the less effective biological control agent in terms of reducing the number of leaves, at all the stocking densities (Fig. 3.2). *Catorhintha schaffneri* was always the best and was not significantly different from both insects in combination except at the highest stocking density of 12 which was the most damaging treatment and was significantly different to all other treatments. A summary of significant differences for the number of leaves measured at the end of the 10-day period at each stocking density is provided in Table 3.2.

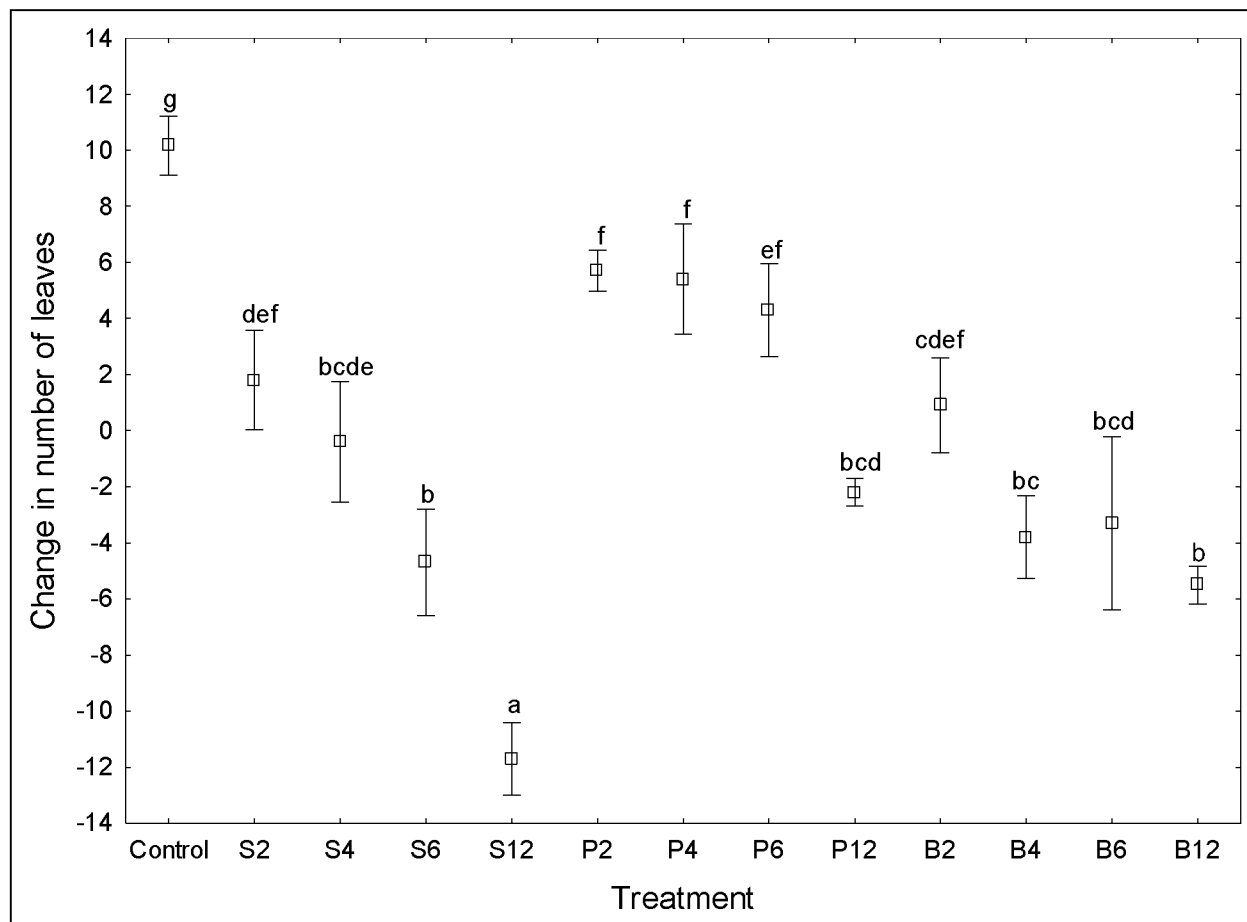


Figure 3.2 Mean ($\pm$ SE) changes in the number of leaves of different treatments at different stocking densities. Numbers 2, 4, 6, and 12 indicate different stocking densities. Treatments: S=*Catorhintha schaffneri*; P=*Phenrica guerini*; and B= both insects in combination. Different letters indicate significant differences ($p < 0.05$) according to Fisher's LSD test. Error bars indicate the standard error around each mean.

3.5.2. Shoot length

Controls had the greatest increase in average shoot length and were significantly different from all treatments for all stocking densities (Fig. 3.3). There were no significant differences between stocking densities of 2, 4, and 6 of *C. schaffneri* alone. The highest stocking density of *C. schaffneri* alone had the greatest reduction in shoot length relative to controls and this was statistically significant to all other treatments (Fig. 3.3). The impact on shoot length increased as

the insect stocking density increased for *C. schaffneri* alone (Fig. 3.3). The mean shoot length for *C. schaffneri* alone at stocking densities of 12 differed from control by 2.7cm (SE $\pm$ 2.53) (Fig. 3.3).

Phenrica guerini alone at all stocking densities were significantly better compared to the controls, but there was no significant difference between the stocking densities (Fig. 3.3). The shoot length of *P. guerini* alone at all stocking densities including the highest stocking density of 12 were not significantly different from *C. schaffneri* alone at stocking density 2, 4, and 6 (Fig. 3.3). *Phenrica guerini* alone at a stocking density of 12 resulted in increased shoot lengths whereas *C. schaffneri* alone at stocking density 12 resulted in reduced shoot lengths and this difference was statistically significantly (Fig. 3.3).

There were no significant differences for shoot length between stocking densities when both insects were in combination (Fig. 3.3). The combination of both insects at the highest stocking density caused a greater reduction in shoot length compared to the control, *P. guerini* alone at all stocking densities, and *C. schaffneri* alone at the stocking density of 2 and 4 (Fig. 3.3). The impact of both insects in combination at all stocking densities was not significantly different from that of *C. schaffneri* at a stocking density of 6 (Fig. 3.3). Stocking densities of 2, 4, and 6 of both insects in combination were not significantly different from *C. schaffneri* alone at stocking densities of 2 and 4, and *P. guerini* alone at all stocking densities (Fig. 3.3).

In summary, at the highest stocking density, *C. schaffneri* reduced shoot lengths more than *P. guerini* alone and both insects in combination (Fig. 3.3). *Phenrica guerini* had less impact on shoot lengths than *C. schaffneri* alone and both insects in combination (Fig. 3.3). The greatest

reduction in shoot length was 6.19cm (SE $\pm$ 0.5) for *C. schaffneri* at the highest stocking density followed by both insects at the highest stocking density with a reduction in shoot length of 0.59cm (SE $\pm$ 0.1). The changes in shoot length were significantly different between treatments ($F_{12,15}=11.31$, $p<0.05$; Fig. 3.3). A summary of significant differences for shoot lengths measured at the end of the 10-day period at each stocking density is provided in Table 3.2.

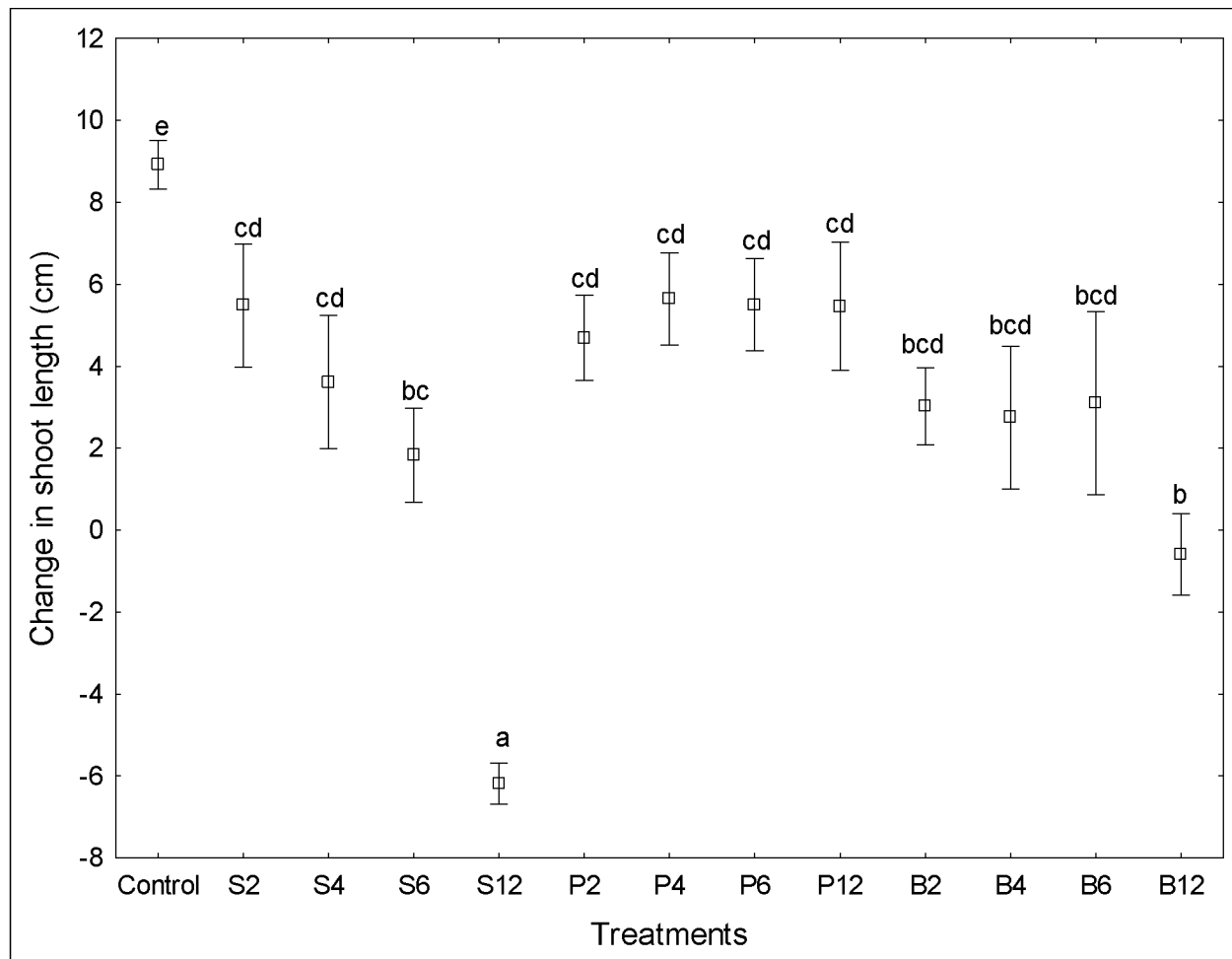


Figure 3.3 Mean ($\pm$ SE) changes in shoot lengths (cm) of different treatments at different stocking densities. Numbers 2, 4, 6, and 12 indicate different stocking densities. Treatments: S=*Catorhintha schaffneri*; P=*Phenrica guerini*; and B= both insects in combination. Different letters indicate significant differences ($p < 0.05$) according to Fisher's LSD test. Error bars indicate the standard error around each mean.

3.5.3. Plant biomass

There was no significant difference between treatments for plant biomass (Wald $\chi^2 = 6.36$; $p > 0.05$). Controls had the greatest plant biomass compared to all other treatments but this was not significant (Fig. 3.4). *Catorhintha schaffneri* alone at the highest stocking density of 12 had the lowest plant biomass compared to all other treatments but this was not statistically significant. A summary of differences for plant biomass measured at the end of the 10-day period at each stocking density is provided in Table 3.2.

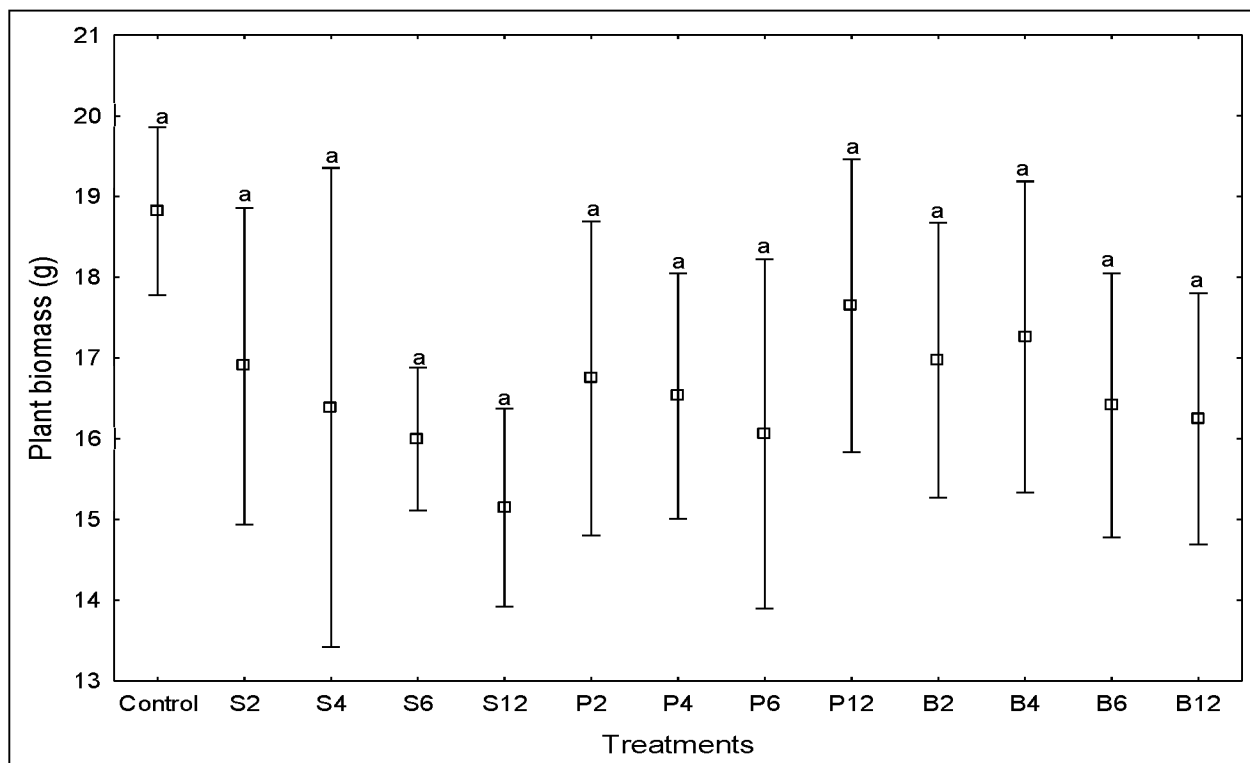


Figure 3.4 Mean ($\pm$ SE) plant biomass (g) after different release treatments at different stocking densities. Numbers 2, 4, 6, and 12 indicate different stocking densities. Treatments: S=*Catorhintha schaffneri*; P=*Phenrica guerini*; and B= both insects in combination. Letters indicate non-significant differences ($p < 0.05$) according to Fisher's LSD test. Error bars indicate the standard error around each mean.

Table 3.2. Means ($\pm$ SE) of *Pereskia aculeata* plant growth during a 10-day period when plants were exposed or not exposed to herbivory by a single or both agents in combination. Treatments: S=*Catorhintha schaffneri*, P=*Phenrica guerini*, B= both insects in combination. Letters given after the standard errors indicate significant differences ($p < 0.05$) according to Fisher's LSD.

Stocking density	Treatments	Change in the number of leaves	Change in shoot length (cm)	Biomass (g)
0	Control	10.15 ± 1.05^g	9.5 ± 2.49^e	18.8 ± 1.04^a
2	S	1.8 ± 1.78^{def}	5.48 ± 1.50^{cd}	16.9 ± 1.96^a
	P	5.7 ± 1.97^f	4.69 ± 1.03^{cd}	16.8 ± 1.95^a
	B	0.9 ± 1.69^{cdef}	3.02 ± 0.94^{bcd}	16.9 ± 1.70^a
4	S	-0.4 ± 2.15^{bcde}	3.6 ± 1.62^{cd}	16.4 ± 2.97^a
	P	5.4 ± 1.97^f	7.05 ± 1.45^{cd}	16.5 ± 1.52^a
	B	-3.8 ± 1.47^{bc}	2.75 ± 1.74^{bcd}	17.3 ± 1.93^a
6	S	-4.7 ± 1.89^b	1.83 ± 1.14^{bc}	16 ± 0.89^a
	P	4.3 ± 1.66^{ef}	5.81 ± 1.16^{cd}	16.1 ± 2.16^a
	B	-3.3 ± 3.08^{bcd}	3.1 ± 2.24^{bcd}	16.4 ± 1.63^a
12	S	-11.7 ± 1.29^a	-6.19 ± 0.5^a	15.2 ± 1.23^a
	P	-2.2 ± 0.49^{bcd}	5.47 ± 1.56^{cd}	17.7 ± 1.81^a
	B	-5.5 ± 0.67^b	-0.59 ± 0.99^b	16.3 ± 1.55^a

3.5.4. Insect mortality

Insect mortality of *Phenrica guerini* and *Catorhintha schaffneri* when each was released alone

There were significant differences between different stocking densities in the number of larvae that died when *P. guerini* was released alone (Wald $\chi^2 = 22.41$, $p = 0.00$, Fig. 3.5). *Phenrica guerini* at the stocking density of 2 had the lowest mean number of 1.43 (SE $\pm$ 0.29) larval deaths which were significantly different from the stocking density of 6 and 12 (Fig. 3.5). The mean number of larvae that did not survive at stocking density of 6 was 5.36 (SE $\pm$ 0.67) and this was significantly different from that of stocking densities of 2 and 12 (Fig. 3.5). The mean mortality of larvae at stocking density 12 was 10.9 (SE $\pm$ 2.19) and was significantly greater than mortality experienced by all other treatments, including all densities of *C. schaffneri* (Fig. 3.5). A summary of the total number of *P. guerini* larvae that experienced mortality measured at the end of the 10-day period at each stocking density is provided in Table 3.3.

When *C. schaffneri* was released alone, the stocking density of 2 had a nymphal mortality mean number of 0.8 (SE $\pm$ 0.36) nymph deaths and at stocking density 4, the nymphal mortality that did not survive was 1.2 (SE $\pm$ 0.97) nymphs and both of these were significantly lower than at the stocking densities of 6 and 12 (Fig. 3.5). The mean number of nymphs that died at the stocking density of 6 was 3.3 (SE $\pm$ 1.21) and this was not significantly different from that of stocking density 12 which was 3.9 (SE $\pm$ 0.86) nymphs (Fig. 3.5). A summary of the total number of *C. schaffneri* nymphs that experienced mortality at the end of the 10-day period at each stocking density is provided in Table 3.3.

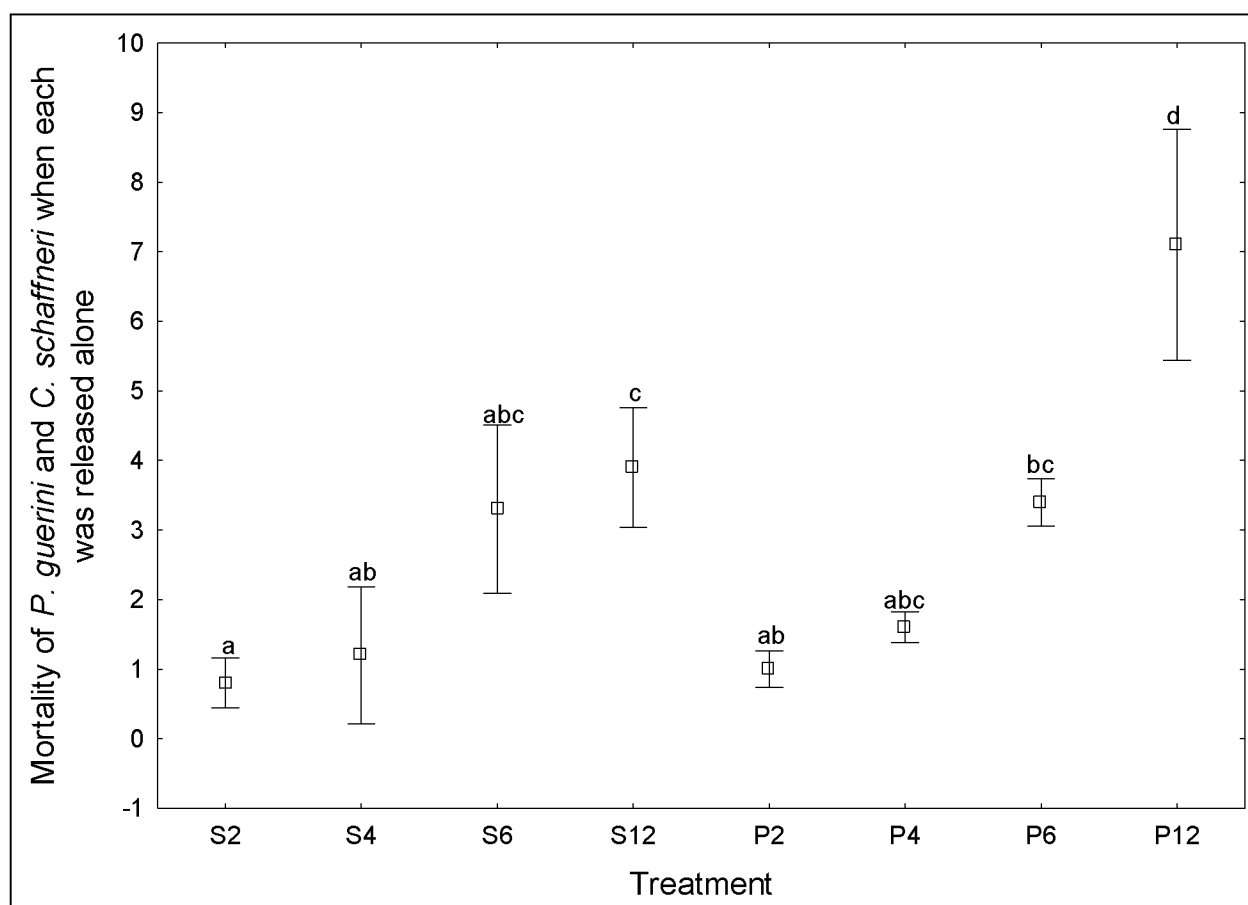


Figure 3.5 Mean ($\pm$ SE) mortality of *C. schaffneri* and *P. guerini* when each was released alone over a 10-day period at different stocking densities. Numbers 2, 4, 6, and 12 indicate different stocking densities. Treatments: S=*Catorhintha schaffneri* and P=*Phenrica guerini*. Different letters indicate significant differences ($p < 0.05$) according to Fisher's LSD test. Error bars indicate the standard error around each mean.

Insect mortality when in combination

There were significant differences in mortality when both insects were combined in a single cage (Wald $X^2 = 10.64$, $p < 0.05$; Fig. 3.6). When *C. schaffneri* and *P. guerini* were combined at low stocking densities of 2 and 4, they experienced the same mortality rates (Fig. 3.6). *Phenrica guerini* alone had the greatest mortality at its higher stocking density of 12 than all other treatments including *C. schaffneri* at the same stocking density (Fig. 3.6). This mortality was

higher than when *P. guerini* was released alone (Table 3.3). The mortality of *C. schaffneri* at a stocking density of 12 was not significantly different from *P. guerini* at the lower stocking density of 6 (Fig. 3.6). A summary of the total number of both *P. guerini* larvae and *C. schaffneri* nymphs that experienced mortality when in combination measured at the end of the 10-day period at each stocking density is provided in Table 3.3.

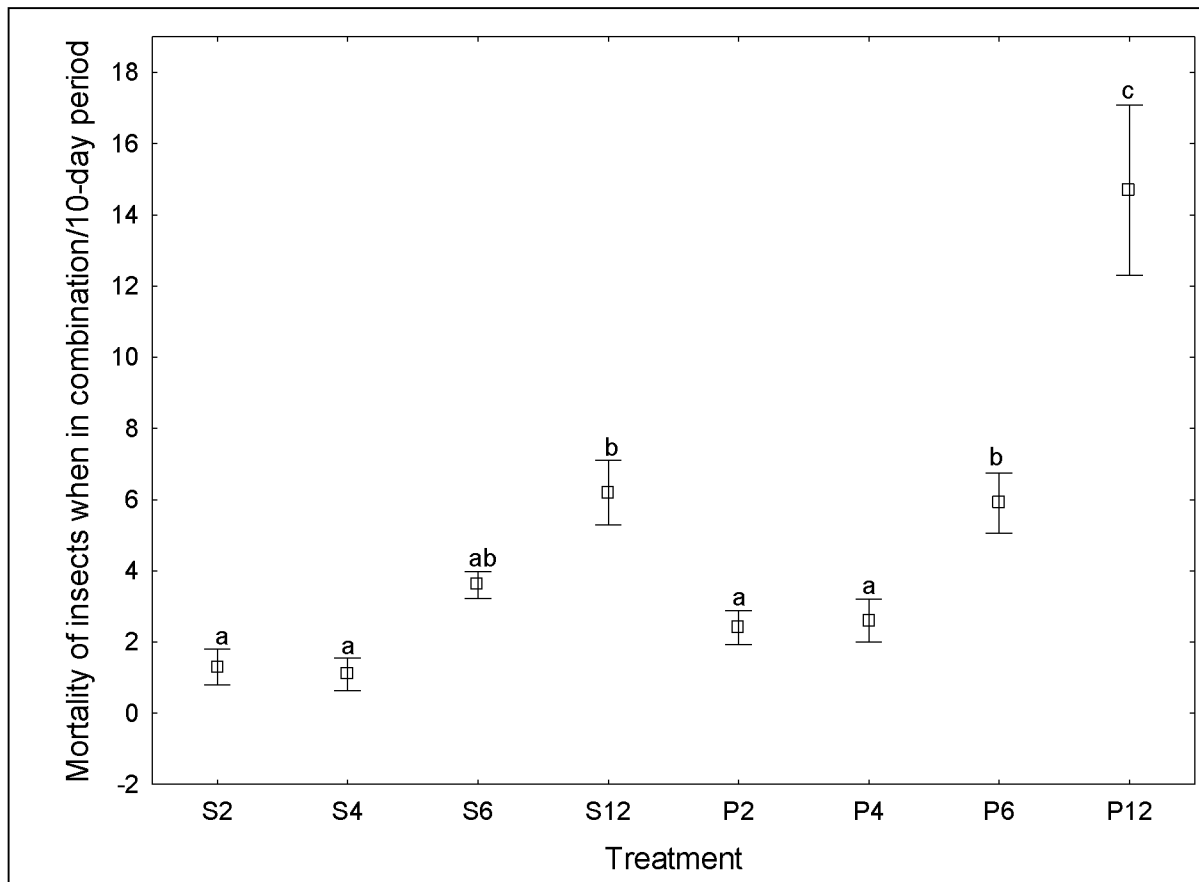


Figure 3.6 Mean ($\pm$ SE) mortality of *C. schaffneri* and *P. guerini* when both agents were released over a 10-day period at different stocking densities. Numbers 2, 4, 6, and 12 indicate different stocking densities. Treatments: S=*Catorhintha schaffneri* and P=*Phenrica guerini*. Different letters indicate significant differences ($p < 0.05$) according to Fisher's LSD test. Error bars indicate the standard error around each mean.

Table 3.3. Comparison of *P. guerini* larval mortality when released in combination with *C. schaffneri* nymphs or alone at various stocking density over a 10-day period. This table shows that *P. guerini* experienced higher mortality when in combination with *C. schaffneri* compared to when it is released alone.

Stocking density	<i>P. guerini</i> mortality for two treatments		Difference of <i>P. guerini</i> mortality when released with <i>C. schaffneri</i>
	Alone	Combined with <i>C. schaffneri</i>	
2	1 (SE $\pm$ 0.3)	2.4 (SE $\pm$ 0.5)	1.4 (SE $\pm$ 0.7)
4	1.6 (SE $\pm$ 0.2)	2.6 (SE $\pm$ 0.6)	1 (SE $\pm$ 0.5)
6	3.4 (SE $\pm$ 0.3)	5.9 (SE $\pm$ 0.8)	2.5 (SE $\pm$ 1.3)
12	7.1 (SE $\pm$ 1.7)	14.7 (SE $\pm$ 2.4)	7.6 (SE $\pm$ 3.8)

3.6. Discussion

The aim of this study was to investigate the interaction between the two biological control agents, *P. guerini* and *C. schaffneri* released against *P. aculeata*. The results have shown that *P. guerini* and *C. schaffneri* do not interact synergistically and their combination resulted in an antagonistic and inhibitory interaction. The number of leaves and the shoot length reduced by *C. schaffneri* alone was greater than when both insects were combined together. This could be due to the feeding mode of *C. schaffneri* because when *C. schaffneri* wilts the stems; the leaves are shed, leaving *P. guerini* with no suitable leaves to feed on. The wilting of the stem by *C. schaffneri* puts the agents in a one directional, asymmetric antagonistic situation because *C. schaffneri* is not affected by the presence of *P. guerini*.

The results of the study revealed that *P. guerini* larvae experienced significantly higher mortality than the *C. schaffneri* nymphs when both agents were together as well as when the

agents were separate. Mortality of *P. guerini* was also greater when exposed to *C. schaffneri* nymphs rather than *P. guerini* larvae at the same densities. This suggests that *P. guerini* has a higher rate of mortality than *C. schaffneri* and more importantly, that *P. guerini* mortality is increased by the presence of *C. schaffneri*. *Catorhintha schaffneri* may, therefore, outcompete *P. guerini* in the field, resulting in reduced densities of *P. guerini* populations at some sites. If this does occur, then there will not be an overall reduction in control of *P. aculeata* because *C. schaffneri* will be the only agent at the site and *C. schaffneri* alone is the most damaging treatment.

The damage inflicted by *C. schaffneri* alone, even at low densities, was always at least as high as the damage inflicted by both agents in combination and, at high densities, *C. schaffneri* was significantly more damaging than both insects in combination. *Catorhintha schaffneri* alone reduced the highest shoot length and this was expected because it is a stem-wilter, feeding directly on the shoots (Paterson *et al.*, 2014b). *Phenrica guerini* alone, even at the highest stocking density, had less impact on the number of leaves than *C. schaffneri* at the highest stocking density. This was unexpected because *P. guerini* targets the leaves of *P. aculeata* directly (Klein, 1999). The feeding by *C. schaffneri* caused greater than expected impact on the leaves of *P. aculeata*, making it more damaging to both shoot length and leaves, but this effect also appears to make the two agents incompatible. If both agents persist in the field at comparable densities, then it is possible that the level of control would be reduced compared to if *C. schaffneri* were present alone.

The findings of this study are in contrast with the cumulative stress model (Harris, 1991). In this case, releasing multiple agents that target the same host plant increases the likelihood that antagonistic interactions may occur between some agents (McEvoy and Coombs, 1999, 2000; Denoth *et al.*, 2002). *Phenrica guerini* was affected by the presence of *C. schaffneri*, but *C. schaffneri* was not affected by the presence of *P. guerini*. The interaction between these two biological control agents suggests that the stem-wilter is the superior competitor, with implications for the survival of *P. guerini*.

The biological control programme against *P. aculeata* is therefore an example of the lottery model described by Myers (1985). The model suggests that the release of many agents increases the chances of making a significant impact although not due to cumulative stress (Myers, 1985). This is contrary to the concept of minimising the number of biological control agents that are released (Myers, 1985, 2008; McEvoy and Coombs, 2000; Denoth *et al.*, 2002). Additional biological control agents should only be considered if an existing agent has failed to control the target weed, as argued by Denoth *et al.*, (2002). In this case, *P. guerini* had failed to control *P. aculeata* to appropriate levels (Chapter 2), so the release of *C. schaffneri* was justified. *Catorhintha schaffneri* had not been recorded as a natural enemy of *P. aculeata* at the time that *P. guerini* was released so there was little evidence to suggest that more damaging agents than *P. guerini* would be found in future.

The results of this study differ from those found in previous studies where agents that attack different plant parts resulted in cumulative stress. For example, the use of agents that attack different plant parts could account for the synergistic interaction that exists between the three

weevils, *Trichapion lativentre*, *Neodiplogrammus quadrivittatus*, and *Rhyssomatus marginatus* that resulted in the complete control of *Sesbania punicea* (Cav.) Benth. (Fabaceae) (Hoffmann, 1990; Hoffmann and Moran, 1998; Hoffmann and Moran 1999). The combination of these weevils was observed as having improved the biological control programme against *S. punicea* (Hoffmann, 1990; Hoffmann and Moran, 1999). Similarly, Buccellato *et al.*, (2012) noted a positive outcome of using multiple agents which attacked different plant parts. There was an additive interaction between a stem gall fly, *Procecidochares utilis* Stone (Tephritidae) and a leafspot pathogen, *Passalora argeratinae* Crous and A. R. Wood (Mycosphaerellaceae) in the biological control of *Ageratina adenophora* (Sprengel) R. M. King and H. Robinson (Asteraceae) (Buccellato *et al.*, 2012). The combination of these biological control agents led to a significant reduction of *A. adenophora* compared to when each was released alone (Buccellato *et al.*, 2012).

There are also examples of antagonistic relationships of agents that attack the different plant parts. In a study undertaken in Australia by Brieese (1997), two agents, a leaf-defoliating beetle *Chrysolina quadrigemina* Suffrian (Chrysomelidae) and a rootborer, *Agrilus hyperici* Creutzer (Buprestidae) were released for the control of *Hypericum perforatum* L. (Hypericaceae) (Brieese, 1997). *Chrysolina quadrigemina* was more damaging and a superior competitor to *A. hyperici* (Brieese, 1997). *Agrilus hyperici* was outcompeted by *C. quadrigemina* because *C. quadrigemina* had a faster rate of population increases and damaged large areas infested by *H. perforatum* leaving *A. hyperici* without food to consume (Brieese, 1997). This resulted in *C. quadrigemina* being the dominant and effective biological control agent (Brieese, 1997). A similar situation may occur if *P. guerini* and *C. schaffneri* were released at the same sites because *C. schaffneri* is a

better competitor and has a negative impact on *P. guerini* survival but is also the more damaging of the two agents.

Another example of an antagonistic relationship between agents attacking the same plant part is the two biological control agents, *Urophora affinis* and *Larinus minutus* which shared a common resource namely the flower heads of *Centaurea maculosa* (Crowe and Bouchier, 2006). The larvae of both *U. affinis* and *L. minutus* develop in the seed heads of *C. maculosa* and this resulted in a negative interaction between the biological control agents (Crowe and Bouchier, 2006; Seastedt *et al.*, 2007). The population and attack rates of *L. minutus* were decreased when released in combination with *U. affinis* (Crowe and Bouchier, 2006). *Urophora affinis* was not affected by the presence of *L. minutus* (Crowe and Bouchier, 2006). In this case, the interaction between biological control agents reduced the overall success of the spotted knapweed biological control programme (Crowe and Bouchier, 2006).

Although the two *P. aculeata* agents attack different plant parts, the relationship was not synergistic and did not result in cumulative stress. This suggests that even in cases where multiple agents attack different plant parts, it should not be assumed that the combined impact will result in cumulative stress to the target plant. Interaction studies are therefore required for all new agents, not only those that feed on the same plant part of the existing agent.

3.7. Conclusion

Catorhintha schaffneri alone should be the priority species for further mass rearing and releases for the control of *P. aculeata* according to the results of this study. Releases of *C. schaffneri* alone could enhance the chances of achieving effective biological control of *P. aculeata* in South Africa if it establishes in the field. This study, however, was conducted under greenhouse conditions and it is difficult to extrapolate these results to the field (Morin *et al.*, 2006; 2009). The efficacy of these biological control agents may differ during different times of the year and at different infestations of *P. aculeata* growing in different climates across South Africa. If *C. schaffneri* does not establish or reach suitable densities in the field to suppress *P. aculeata* infestations, then *P. guerini* could be a better agent, but a combination of both species at a single site is unlikely to be more damaging than either agent alone.

Chapter 4: General Discussion

The biological control programme for *Pereskia aculeata* in South Africa was initiated in 1991 with the introduction of *Phenrica guerini* followed by the release of *Catorhintha schaffneri* in 2014 (Klein, 2011; Paterson *et al.*, 2014b). Both of these biological control agents have now successfully established at several sites in the country. The damage inflicted by *C. schaffneri* in the field had not been determined and that of *P. guerini* was categorised as trivial, and the degree of control of *P. aculeata* as negligible (Klein, 2011), before this post-release evaluation study was conducted. *Phenrica guerini* does contribute towards the suppression of *P. aculeata* at Port Alfred, and possibly at other sites where it reaches similar densities, but the weed is not reduced below the damage threshold (Paterson *et al.*, 2011a) and there are very few sites in the country where agent densities were high enough to impact the weed (Chapter 2). The spread and negative threats of *P. aculeata* may, however, have been far greater than the current situation without *P. guerini*. The level of control provided by *P. guerini* is, therefore, greater than previously suggested but certainly not great enough to be classified as a complete success. The level of damage inflicted by *P. guerini* could be changed to 'considerable' but only at a very limited number of sites.

Although *P. guerini* has not resulted in a reduction of *P. aculeata* densities below the damage threshold, it is better than having no biological control agent at all. The reasons why it fails to reach high densities at most sites therefore deserve further investigation. It is possible that *P. guerini* numbers are still increasing and that its impact on *P. aculeata* has not yet been realised at some sites where it has not done sufficient damage. It may take several years before

biological control agents become damaging, and some authors have suggested that a period of ten or even 20 years should pass before post-release evaluations are conducted (McFadyen 1998). Although *P. guerini* was first released in South Africa over 25 years ago (Klein 1999), the majority of sites where the agent is established have only had populations of the agent for the last five years according to the release data provided by SASRI (Des Conlong. pers. comm.). It is, therefore, possible that the impact of *P. guerini* will increase at these sites over time. Increasing the mass-rearing effort could also improve control, or speed-up the population increase at sites where the agent is ineffective. Although it is possible that increased mass-rearing could improve control, there is little evidence to support this from the available data because sites where high levels of damage have been recorded, such as Port Alfred and Paradise Valley, have not had the greatest release efforts.

In order to reduce densities of *P. aculeata* below the 30% cover threshold calculated by Paterson *et al.*, (2011b), additional biological control agents are required, and one new agent, *C. schaffneri*, has been released and established at some sites (Paterson *et al.*, 2014b). Many biological control programmes involve the release of multiple biological control agents with the hope of finding the most effective agent, and the expectation that a combination of multiple agents will cause greater damage compared to one agent acting alone (Harris, 1991; Denoth *et al.*, 2002; Myers, 1985; Jackson and Myers, 2008). The interaction between several agents may result in complimentary or interference or neutral interactions, and this has an impact on the overall success of the biological control programme (Hatcher, 1995; Crowe and Bouchier, 2006; Groenteman *et al.*, 2007; Hoffmann and Moran, 1998; Turner *et al.*, 2010; Buccellato *et al.*, 2012). The success of a biological control programme is the result of the impact and damage

inflicted by an agent or agents, irrespective of the number of biological agents released against a target weed (Hoffmann and Moran, 1998). The interaction between *P. guerini* and *C. schaffneri* resulted in an antagonistic and inhibitory interaction which suggests that the impact of both agents in combination may be less than that of the new agent, *C. schaffneri*, alone (Chapter 3). These two biological control agents should not be released together at any infestations because of the risk of reducing the overall level of control that could be achieved.

The biological control programme against *P. aculeata* is better explained by a lottery model (Myers, 1985; 2008) rather than by cumulative stress (Harris, 1991). The release of *C. schaffneri* and *P. guerini* together does not increase the stress on *P. aculeata* but did result in the discovery of the more damaging agent. The interaction between these two agents suggests that the feeding by *C. schaffneri* results in the plant shedding its leaves, limiting the access of *P. guerini* to the food source and this had implications for the survival of *P. guerini* larvae. The number of leaves and shoot length reduced by *C. schaffneri* alone was also greater than both insects in combination with the same stocking density. This suggests that *C. schaffneri* is likely to be the more damaging biological control agent against *P. aculeata*.

The number of biological control agents released should be minimised in order to reduce releasing ineffective agents and non-target effects (Raghu *et al.*, 2006) so the decision to release the less damaging agent, *P. guerini*, should be questioned. Biological control agents should be released only if they are efficient, not because it is easy to rear them or because they are host specific (van Klinken and Raghu, 2006). Raghu *et al.*, (2006) proposed four filters based on the weed's ecology that can be used in evaluation and selection for possible biological

control agents. These filters include verifying the vulnerable parts of a plant's life cycle such as seed production, the kind of plant part that is at risk when exposed to particular herbivory such as leaves or seeds and which agents can damage the particular life cycle stage and plant part effectively (Raghu *et al.*, 2006). Retrospectively, it would be possible to predict that *C. schaffneri* would be the more damaging agent against *P. aculeata* and if this knowledge was available at the time of the first release of *P. guerini* then *C. schaffneri* would have been prioritised as the better agent. The existence of *C. schaffneri* as a natural enemy of *P. aculeata* was however not known at this time, so the release of *P. guerini* was justified given the available data at the time it was released.

Predicting the efficacy of biological control agents against a target weed prior to release is very challenging (McFadyen, 1998). The results of this interaction study suggest that the plant and insect parameters to be measured, insect stocking densities to be released and the duration of interaction studies should be standardised. The differences in these measurements may influence conclusions regarding the efficacy of biological control agents. For example, if the stocking densities of *P. guerini* were more than those used in this study, maybe the damage it inflicted on *P. aculeata* could have been better compared to *C. schaffneri*. The plant biomass could have been different also if the experiment was conducted for more than the 10-day period.

In most cases, the interaction studies between biological control agents are investigated under greenhouse or laboratory conditions (Raghu *et al.*, 2006; Morin *et al.*, 2009). It is important to take into consideration that these conditions may be over- or underestimated and they should

never be used to characterise field conditions because the results may be different (Morin *et al.*, 2009). *Phenrica guerini* did not damage a greater number of leaves compared to *C. schaffneri* under laboratory conditions but this may be different in the field. Ideally, interaction studies should be conducted in both the laboratory and the field. For example, the efficacy of the mealybug, *Hypogeococcus festerianus* (Lizer y Trelles) (Pseudococcidae) released as a biological control for *Harrisia cactus*, *Harrisia martinii* (Labour.) Britton & Rose (Cactaceae) was much greater in the field than what was predicted from laboratory-based studies (McFadyen and Tomley, 1981). The mass-rearing and release effort for *P. guerini* should therefore not be terminated until the impact of *C. schaffneri* in the field has been proven to be greater than the impact from *P. guerini*. If *C. schaffneri* fails to establish populations at high enough densities to damage *P. aculeata* then *P. guerini* may still be the more effective of the two agents.

The degree of control of *P. aculeata* is negligible at most sites and complete control has not been reached by the existing biological control agents. The new agent, *Catorhintha schaffneri*, was only released for the first time in 2014, so it is too soon to evaluate whether this agent may result in complete control. At present, however, infestations of *P. aculeata* are expanding in South Africa, and surveys for new biocontrol agents have been undertaken, and potential biological control agents considered include a stem-boring weevil, *Pereskiophaga brasiliensis* Anderson (Curculionidae) (Paterson *et al.*, 2014a; Anderson, 2015) which is currently being subjected to host specificity testing. It is important to take into account the interaction with the already established agents when selecting the new agents. This study has shown that the first two agents do not complement each other when released together on *P. aculeata*. The release of *C. schaffneri* or *M. chlorisalis*, together with *P. brasiliensis* or *A. machacalis*, were speculated

to be combinations which could enhance the biological control of *P. aculeata* and would probably reduce the potential antagonistic interaction which exists between two biological control agents targeting the same resource (Paterson *et al.*, 2014a). However, this study has shown that this is not always the case; the targeting of different plant parts by biological control agents does not always reduce competition between them.

Phenrica guerini is not compatible with *C. schaffneri*, but this should not suggest that it is an ineffective agent as it may interact synergistically with other potential biological control agents. Until a new biological control agent that is shown to be more damaging than *P. guerini* in the field is available, mass-rearing and release efforts of *P. guerini* should be maintained. The priority for the *P. aculeata* biological control programme should, therefore, be evaluating the impact of *C. schaffneri* in the field, in order to determine whether it will be a more effective agent than *P. guerini* and reduce *P. aculeata* densities below the damage threshold.

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