Potential impact and host range of *Pereskiophaga brasiliensis* Anderson (Curculionidae): a new candidate biological control agent for the control of *Pereskia aculeata* Miller (Cactaceae) in South Africa.

THESIS

Submitted in fulfilment of the requirements for the degree

MASTERS OF SCIENCE

At

Rhodes University

By

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Grahamstown

2018

Abstract

Pereskia aculeata Miller (Cactaceae) is a damaging invasive alien plant in South Africa that has negative impacts to indigenous biodiversity and ecosystem functioning. Mechanical and chemical control are not effective against *P. aculeata* so biological control is considered the only viable option. Two biological control agents, the leaf-feeding beetle *Phenrica guerini* Bechyne (Chrysomelidae) and the stem-wilting bug *Catorhintha schaffneri* (Coreidae), have been released in South Africa thus far. Post-release evaluations have indicated that *P. guerini* will not reduce *P. aculeata* densities to acceptable levels alone, while *C. schaffneri* was released very recently, so it is too soon to determine how effective that agent will be. Even if *C. schaffneri* is extremely damaging, it is likely that further agents will be required to reduce the densities of *P. aculeata* to acceptable levels within a reasonable time-scale. Additional agents should target the woody stems of *P. aculeata* which are not impacted by the damage of either of the released agents.

Pereskiophaga brasiliensis Anderson (Curculionidae) is a promising potential candidate agent that feeds on the thick woody stems of the plant in the larval stage. Climatic matching, genetic matching and field based host specificity observations all indicated that *P. brasiliensis* was a promising candidate. In this study, the impact of *P. brasiliensis* to the target weed, *P. aculeata,* was quantified under quarantine conditions to determine whether it was sufficiently damaging to warrant release. This was followed by host specificity testing to determine whether *P. brasiliensis* was suitably host specific for release in South Africa.

Impact studies indicated that *P. brasiliensis* was damaging to *P. aculeata* at insect densities that would be expected in the field. *Pereskiophaga brasiliensis* reduced the number of leaves of *P. aculeata* to a greater extent than it reduced shoot lengths, but both plant parameters were significantly reduced due to the feeding damage from the insect. This suggests that the damage from *P. brasiliensis* may be compatible with that of *C. schaffneri* which reduces shoot length to a greater degree than the number of leaves. *Pereskiophaga brasiliensis* is therefore sufficiently damaging to warrant release, and although interaction studies with the other agents would be required, it is expected that it should complement other existing agents.

Although *P. brasiliensis* is sufficiently damaging, at present the host specificity data indicates that it is not suitably specific for release in South Africa because oviposition and larval development to the adult stage was recorded on both indigenous and alien plant species within the families Cactaceae and Basellaceae. This non-target feeding was recorded during no-choice tests, which are very conservative, but significant non-target damage and development to the adult stage was recorded on an indigenous plant from a different family to the target weed. Further host specificity testing, including paired and multiple choice tests, are required to confirm the broad host range of *P. brasiliensis*.

Other biological control agents that damage the woody stems of *P. aculeata* should be considered. The stem-borer, *Acanthodoxus machacalis* (Cerambycidae) is considered the most promising of the other candidate agents as it can be sourced from a climatically matched region where genetically suitable *P. aculeata* plants are found, it is sufficiently damaging to the woody stems of *P. aculeata* and there is no evidence that the species has a broad host range. *Acanthodoxus machacalis* should be sourced from Rio de Janeiro, Brazil, and imported into quarantine in South Africa for host specificity testing.

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Acknowledgements

I would like to pay my gratitude to Dr Iain Paterson, I am so grateful to his inspiration, guardiance, support, patience, keen interest, scholarly comments and constructive suggestions, valuable advice, encouragement, assistance and polite behaviour throughout this study. I would like to thank my co-supervisor Prof Steve Compton for his valuable insights and output.

My thanks extend to Working for Water (department of Environmental Affairs) and National Research Foundation for funding this study.

I am especially grateful to Prof Martin Hill, Prof Julie Coetzee and Dr Grant Martin for welcoming me at the Centre for Biological Control (CBC) and giving me their proper guidance, inspiration and encouragement.

I am also highly thankful to Miss Jeanne van De Merwe for financial administration. To CBC group, Waainek Research Facility group and support staff, thank you so much for your contribution in my study.

To Nomoto Petela, Sandiso Mnguni and Zolile Maseko, thanks for assisting me to analyse my data

Where would I be without my mothers (Khungiwe Mdodana, Nosiseko Mandlana), my granny (Nozanele Mdodana), my dad (Mthetheleli Mandlana), my aunt (Nomonde Mqikela) and siblings especially my brother Asandile Mandlana who understood this journey and Nandipha Mdodana. Thank you for your support, prayers and for believing in me.

To all of my friends especially Tabisa Mdlangu and Samella Ngxande, you were more like my sisters and provided me with a great support and motivation. And my sisters in Christ, Nelisa Mpama, Dineo Molata, Zipho Maxwane, Sinoxolo Nombewu, Pamella Runqu and Kholiswa Malumko, thanks for the prayer sessions, support and motivation. Thanks to my very supportive friend, brother and motivator Sandiso Kahlana.

To my handsome and humble sons AMBESIWE and AYOLA MDODANA, if it wasn't for your presence in my life, I would have given up. You were the reason for me to wake up every morning and face challenges

To Almighty God, thank you for protecting me and giving me strength to achieve my masters. I never would have made without you. May you continue blessing me all the time. I know you will never leave me nor forsake me.

This is especially dedicated to my late grand father

Atwell Jaji Mdodana

Chapter 1: General Introduction

1.1 Introduction

Alien plant invasions are increasing in severity throughout the world (Richardson and Van Wilgen, 2004). Alien plants out-compete native plant species, dominating the ecosystems they invade, thus leading to reductions in native biodiversity and changes to the functioning of ecosystems (Palmer, 2009). In South Africa, *Pereskia aculeata* Miller (Cactaceae) is an invasive alien species that threatens indigenous biodiversity (Moran and Zimmermann, 1991b; Paterson *et al.*, 2011a). *Pereskia aculeata* covers and kills indigenous coastal and forest flora and can even cause large trees to collapse under its weight (Moran and Zimmermann, 1991b; Paterson *et al.*, 2011a). Chemical and mechanical control are ineffective and unsustainable, therefore biological control is considered the only effective, environmentally friendly, economically viable and sustainable method to control this alien invasive plant (Moran and Zimmermann, 1991b). In this study, research was conducted to develop a new candidate biological control agent for use against *P. aculeata* in South Africa.

This chapter consists of an introduction and literature review concerning invasive species, biological control of weeds, biological control of *P. aculeata*, and the new potential biological control agent, a weevil called *Pereskiophaga brasiliensis* Anderson (Coleoptera: Curculionidae).

1.1.1 Alien invasive species

Invasive species are considered one of the greatest threats to biodiversity on a global scale (Vitousek, 1992, Le Maitre *et al.,* 1996; Schmitz and Simberloff, 1997; Wilcove *et al.*, 1998; Pimentel *et al.*, 2001; Pauchard and Shea, 2006). International travel and the shipping of goods throughout the world is the main driver increasing the introductions of invasive species to new environments (Wilson *et al.*, 2009a, 2009b). Some invasive species are introduced intentionally into new environments while others were introduced accidentally (Wilson *et al.*, 2009a, 2009b). Many alien plant species have been shown to have negative impacts on human health and wealth by reducing native biodiversity and replacing native flora, which depresses the diversity and beauty of the landscape, disrupting natural fire regimes, reducing water quality and availability, reducing the value of agricultural land and sometimes impacting directly on human health by

causing allergies (Van Wilgen and Richardson, 1985; Richardson *et al.*, 1989; Van Wilgen *et al.*, 2001; Hill, 2003; Harminder *et al.,* 2005; Schooler *et al.*, 2006; Coetzee *et al.*, 2007a, Gooden *et al.*, 2009).

There are 379 alien invasive plant species that are currently listed in South Africa under the National Environmental Management: Biodiversity Act (NEM:BA), all of which are already problematic or could become problematic in future (Department of Environmental Affairs, 2016). There are about ten million hectares of South Africa covered by over 180 invasive alien plant species (Richardson and Van Wilgen, 2004). These invasions are extremely costly. The loss of fynbos due to invasive alien plants on the Agulhas plains of South Africa was estimated to be USD 3.2 billion and the cost to clear the country of invasive plants is estimated at USD 1.2 billion (Van Wilgen *et al.*, 2001). If components such as water production, wildflower harvesting, ecotourism, endemism and genetic storage are taken into account, the value of some ecosystems in South Africa are estimated to be well over 10 times greater without alien invasive plants (Van Wilgen *et al.*, 2001). This means that invasions in protected areas of fynbos alone could be costing the country over USD11.75 billion in 2001 currency values (Van Wilgen *et al.*, 2001).

Aquatic invasive alien plants have negative impacts in natural ecosystems as well as to economic activity (Coetzee *et al.*, 2007a; Gooden *et al.*, 2009). Aquatic alien plants directly impact water quality in aquatic environments by forming dense mats on the water that block sunlight, reduce oxygen concentrations and increase the nutrient load of waterbodies, thus degrading water quality and reducing biodiversity by eliminating native plants (Coetzee *et al.,* 2011a). Midgley *et al.,* (2006) conducted a study looking at the effect of water hyacinth, *Eichhornia crassipes* (Martius) Solms-Laubach (Pontederiaceae), on benthic biodiversity in two impoundments on the New Year's River, South Africa. The results indicated that the presence of *E. crassipes* mats had a detrimental effect on both the diversity and abundance of benthic invertebrates and algal biomass found on the substrate underneath these mats in the impoundments of the New Year's River (Midgley *et al.*, 2006). Other aquatic invasive alien plants such as water lettuce, *Pistia stratiotes* Linnaeus (Araceae), *Salvinia molesta* Mitchell (Salviniaceae), parrot's feather, *Myriophyllum aquaticum* (Vell.) Verdc. (Haloragaceae), and red water fern, *Azolla filiculoides* Lam. (Azollaceae) are also serious environmental problems (Cock *et al.*, 2000). They have extensively invaded the lakes and waterways of Africa, degrading water quality and reducing biodiversity by eliminating

native plants, blocking sunlight, altering and reducing access to the water by livestock (Coetzee *et al.,* 2011a). In the 1990s, there were huge infestations of *A. filiculoides* in South Africa which limited light penetration, impeded water flow by increasing the siltation rate of water bodies, and constrained the air diffusion into water which results in the water becoming anoxic (Raid and Munshi, 1979; Hill, 1999, Tellez *et al.*, 2008). The invasion of *A. filiculoides* nearly caused the extinction of the endemic fish *Sandelia bainsii* Castelnau (Anabantidae) before a successful biological control agent was released (Van Driesche *et al.*, 2010).

Terrestrial invasive alien plants significantly reduce biodiversity and reduce the carrying capacity of rangelands for wildlife and livestock (Moran and Zimmermann, 1991b; Richardson and Van Wilgen, 2004). They increase biomass and litter production which can result in changes to nitrogen fixation, litter chemistry and soil chemistry, thus affecting water quality (Van Wilgen and Richardson, 1985; Van Wilgen *et al.*, 2001; Brooks *et al.*, 2004). The increased biomass is also a major fire hazard and disrupts fire regimes resulting in further negative impacts on the ecosystem (Van Wilgen *et al.*, 2001). It is predicted that alien trees alone use 7% of South Africa's surface water and cost billions of rands every year in the loss of agricultural productivity and the resources spent controlling these weeds (Van Wilgen *et al.*, 2011). The scale of the problem and the negative impacts from invasive alien plants is therefore immense. Oerke *et al.* (1994), estimated that crop production was reduced by 16.6% by problematic plants and approximately 67% of the problematic plants found growing within crops are invasive alien species (Bromilow, 1995).

Invasive alien Cactaceae are among the most damaging of the invasive species in South Africa (Paterson *et al.,* 2011a; Kaplan *et al.*, 2017). Cactus species such as *Opuntia ficus-indica* (L.) Mill., *Opuntia aurantiaca* Lindley and *Opuntia robusta (*H.L.) Wendl. were introduced into South Africa as a source of fruit and fodder, and other species, such as *Pereskia aculeata*, were introduced as ornamental or barrier plants (Brutsch and Zimmermann, 1993, 1995, Paterson *et al.*, 2011a). The negative impacts of invasive Cactaceae are particularly evident within an agricultural setting. *Opuntia aurantiaca* invades disturbed areas and flourishes in overgrazed habitats. The main driver of this invasion are grazing animals which get the cladodes or joints of the plant stuck in their hides and transport the plants to uninfested areas (Robertson *et al.,* 2011). The damage of the spines of *O. aurantiaca* and other similar cactus species to livestock can result in direct economic losses, but there is also an indirect impact in that livestock will avoid areas of veld infested with

cactus species thereby reducing the carrying capacity of the land (Robertson *et al.*, 2011). There are also significant negative impacts from invasive alien cactus species to indigenous biodiversity. For example, *Opuntia stricta* (Haw.) Haw. (Cactaceae), was an extremely problematic cactus that reduced the space available for indigenous species in Kruger National Park (Robertson *et al.*, 2011). In 1953, it was reported that *O. stricta* had formed dense infestations across an area of approximately 35 000 ha in the Kruger National Park, so the negative impacts to biodiversity were on a large scale (Lotter, 1997).

Reducing the impacts of invasive alien plants include herbicidal control, mechanical control and biological control. The use of a combination of two or more of the control methods is required, but these different methods must therefore be implemented in a way that they are compatible. When control methods are combined it is known as integrated control (Zimmermann and Neser, 1999; Greathead, 2003; Zimmermann and Olckers, 2003).

Herbicidal control poses a threat to human health and to natural ecosystems. It also impacts non-target plant species, making this control method undesirable for infestations on a large scale or in ecologically sensitive areas (Moran and Zimmermann, 1991b; Klein, 1999; Hill, 1999). Chemical control also requires follow-ups which may need to be continued indefinitely, making this method expensive and unsustainable (Hoffmann *et al.*, 1999, Olckers *et al.*, 1999, Hill *et al.*, 1999). Mechanical control is labour intensive; it requires the physical removal of invasive alien plants using manual labour or machinery (Olckers, 1999; Hill, 1999; Cronk and Fuller, 2001; Caffrey *et al.*, 2010). Chemical and mechanical control may work for some invasive weeds but they are inappropriate for others, such as invasive alien vines. Vines become intertwined with other plants making them difficult to control without affecting all the other plants plant species present at the site. For most widespread weeds, and especially for invasive alien vines, mechanical and chemical control can only aim to control the target weed in a very limited area. Significant resources have been used in South Africa for the control of invasive alien plants, but the positive impacts from this expensive effort have been minimal (Van Wilgen *et al.*, 2011).

1.1.2 Biological control of invasive alien plants

Biological control, if carried out correctly, is a safe method of controlling invasive alien plants, because it is sustainable, environmentally friendly and not harmful to natural ecosystems

(McFayden, 1998). The greatest advantage of this method is sustainability because the agents are self-perpetuating, so if biological control is successful then control is permanent and no further interventions or follow-up treatments are required (McFayden, 1998).

Classical biological control is a method used to control terrestrial and aquatic invasive weeds by releasing exotic insects, mites or pathogens to provide permanent control (De Bach, 1964; Nordlund, 1996; McFayden, 1998). According to Wapshere *et al.,* (1989), classical biological control is defined as 'the introduction of host-specific exotic natural enemies that have co-evolved with exotic weeds'.

Classical biological control is widely utilised as a method of controlling invasive alien plants. Over 400 biological control agents have been released worldwide (Winston *et al*., 2014) and in South Africa alone, over 100 agent species have been released for the control of 59 invasive alien plants (Zachariades *et al.,* 2017). This form of biological control (as opposed to biological control of insects pests) was first implemented in southern India when a cochineal bug, *Dactylopius ceylonicus* (Green) (Dactylopiidae), was used to control the invasive cactus *Opuntia monocantha* (= *O. vulgaris*) Haw. (Cactaceae). Cochineal insects were used as a source of red dye and the introduction of this cochineal insect to southern India was intended as a way of starting an industry around the dye, rather than controlling the weed (Tryon, 1910). *Dactylopius ceylonicus* causes considerable damage through their toxic saliva, which poisons the *O. monocantha*, and within a few years the dense infestations were controlled (Sushilkumar, 2005). Following this success, many countries, including South Africa imported *D. ceylonicus* for the control of *O. monocantha* and these were the first intentional releases of biological control agents for invasive alien plants (Moran *et al.,* 2005).

One of the earliest and most successful biological control programmes was against another problematic cactus with the introduction of the cactus moth, *Cactoblastis cactorum* (Berg) (Pyralidae), which was introduced from Argentina to Australia in 1925 to control *Opuntia stricta* (Pemberton, 1995; Bennett and Habeck, 1995). There was 60 million acres (24 million hectares) of valuable farming land that was infested by *O. stricta* in Australia, causing an ecological and agricultural disaster (Dodd, 1940). By 1933, only a few years after the first release, the cactus

populations had collapsed and the plant is no longer considered a problem in Australia (Pemberton, 1995; Bennett and Habeck, 1995).

In South Africa, the first success using biological control was of cactus weeds in the 1930s, followed by the control of water weeds in the 1980s (Moran and Zimmermann, 1991b; Julien and Griffiths, 1998; McConnachie *et al.*, 2003). To quantify success of biological control on a broad, country-wide scale, Hoffmann (1995) proposed categories of determining biological control success by the amount of alternative control that is needed to reduce the target weed species to an acceptable level. This method of evaluation success has now been widely adopted by the biological control community of South Africa (Klein, 2011). There are three control categories: (1) complete control, when no other control measures are needed to reduce the weed to an acceptable level in the areas where the agents has established, (2) substantial control, when other control methods are needed to reduce the weed to an acceptable level but the amount of the alternate methods needed are reduced (for example less herbicide needed per area) (3) Negligible control, when control is completely reliant on alternative methods despite the implementation of a biological control programme (Hoffmann, 1995; Klein, 2011).

In the past 100 years of biological control in South Africa, 270 biological control agents have been evaluated in quarantine, of which 106 agents (39%) have been released to control invasive alien plants (Klein, 2011). Sixty-four agents (24%) were rejected and not released because of insufficient host specificity or efficacy (Klein, 2011). Fifty-seven agents (21%) were shelved for host-specificity testing and have not yet been released (Klein, 2011). Ten of the targeted weed species (21%) are considered to be under 'complete biological control', 18 species (38%) are under 'substantial control', in that conventional control measures are still needed, but at a reduced rate (Klein, 2011). Fourteen species (29%) are under 'negligible control' from agents because there has been virtually no reduction in the need for conventional control methods, despite the damage inflicted by agents (Klein, 2011). The release of five further agents (10%) is too recent to make any meaningful assessment (Klein, 2011).

1.1.3 Biological control of Cactaceae

Australia's prickle pear cactus (*O. stricta*) invasion is the most well-known example of successful classical biological control and set the benchmark for future cactus biological control programmes.

The vast infestations of prickly-pear covered 25 million hectares of pastoral land, forming dense monocultures, and the infestations has been permanently reduced by over 90%, primarily due to the action of the pyralid moth, *Cactoblastis cactorum* (Dodd, 1940). Following this success, biological control practitioners introduced *C. cactorum* into South Africa but the impact of the agent was far less spectacular (Paterson *et al.*, 2011a). Cochineal insects, as opposed to *C. cactorum*, have proven to be the more effective agents for controlling cactus weeds in South Africa (Paterson *et al.*, 2011a). In South Africa, the cochineal insect *Dactylopius ceylonicus* was the first biological control agent to be introduced against the invasive weed *Opuntia monacantha*, in 1913 (Lounsburry,1915). The density of the weed was reduced drastically and the impact from the weed to agriculture and biodiversity is now negligible. Since this early success, a further 14 biological control agent entities (species or biotypes) have been released for the control of 15 cactaceous weed species in South Africa. The agents were originally released against eight target species (including *O. monacantha*) but subsequently became established on seven additional cactus species (Zimmermann *et al.*, 2009).

One of the reasons for biological control of Cactaceae being so successful in South Africa is due to the lack of native Cactaceae in the Old World (Germishuizen and Meyer, 2003). *Rhapsalis baccifera* (J. Müller) Stern is the only species of Cactaceae that is considered native in southern Africa (Britton and Rose, 1919; Dyer, 1975). In a study conducted by Roland-Gosselin (1913) the old world *Rhipsalis* species were investigated and all the old world species were in fact representatives of species found in the Americas suggesting that while this species could be considered native, it is also cosmopolitan. Target weeds with an absence of native congeners in the introduced range are considered good targets for biological control because congener plant species are those most at risk from non-target effects from biological control agents (Pemberton, 2000). In addition the biology of the cactophogous insects has resulted in adaptations to Cactaceae's morphology and anatomy; as well as the taxonomic isolation which has resulted in almost no cactophagous insects being able to feed on any plant species outside of the family (Mann, 1967; Moran, 1980). Hence, oligophagous cactophages (species that feed on many species, but all within the family Cactaceae) has been used for biological control without any risks to non-target native species. The taxonomic isolation of Cactaceae in southern Africa makes it possible for generalist cactophagous species to be introduced as biological control agents provided cactus crop species and the disputed native *Rhipsalis baccifera*, are not threatened (Paterson *et al.* 2011a)

In South Africa, a variety of cochineal (*Dactylopius*) species and biotypes has provided effective biological control of many *Opuntia* species. Four cochineal species, two of which are comprised of two host-adapted biotypes for different *Opuntia* species, have been released in South Africa for the control of eight *Opuntia* species (Klein, 2011). In addition to these cochineal insects, a galling-mealy bug, *Hypogeococcus pungens* Granara de Willink (Hemiptera: Pseudococcidae) is an effective biological control agent associated with several species of columnar cactus (McFadyen, 1979; Paterson *et al.*, 2011a; Klein, 2011). The cochineal insects for the control of *Opuntia* species and close relatives, as well as *H. festerianus* for the control of columnar cacti, have resulted in a very high level of success for cactus weed biological control in South Africa (Zachariades *et al.*, 2017).

1.1.4 Biological control of *Pereskia aculeata.*

1.1.4.1 Biology of *Pereskia aculeata*

Pereskia is a genus of 17 species of neotropical trees, shrubs and vines which form a basal clade in the family Cactaceae (Leunenberger, 1986; Edwards *et al.,* 2005). Unlike other Cactaceae, *Pereskia* has plesiomorphic characteristics such as woody stems and branches, as well as fully developed leaves, hence it is not always recognised as cactus. The native distribution of this genus is from southern Mexico southwards through Central America and the eastern side of the Andes and northern Argentina, eastern Brazil, Venezuela, northern Uruguay and the Caribbean (Leuenberger, 1986).

Pereskia aculeata Miller (Cactaceae), also known as Barbados gooseberry, is a primitive creeping cactus, with short curved thorns on young growth, growing into a scrambling or climbing and vine-like plant with long branches (Fig1.1) (Leuenberger, 1986). It has white flowers which produce fruits which are yellow in colour, turning orange when ripe (Leuenberger, 1986). The fruit contains numerous flat, brown or black seeds (Campbell, 1988). The young growth of the plant is olive-green or reddish at the shoot tips which are about 4mm thick (Leuenberger, 1986). The shape of the leaves is very variable, from lanceolate to oblong or ovate and sometimes broadly ovate to nearly orbicular (Leuenberger, 1986). The size of narrow-leaves varies from 2.5-4cm x 8-11 cm (width x length) while the broad leaves are about 4.5- 7 cm x 1.5-5cm (Leuenberger, 1986). The thorns on the young shoots and stems are found in pairs and are hooked or claw like, while the

thorns on the older woodier stems are more typical of the Cactaceae, being clusters of straight long sharp spines (Leuenberger, 1986). *Pereskia aculeata* has whitish-green, fragrant flowers that range from 2.5-5cmx5-15mm and are cup-shaped to turbinate (Leuenberger, 1986).

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

Pereskia aculeata's native distribution is widespread and divided into distinct regions that are separated by a vast distance. The northern region includes the Caribbean, southern Central America and northern Venezuela, while the southern region includes southern Paraguay, southern and south-eastern Brazil and northern Argentina (Fig 1.2) (Leuenberger, 1986).

Pereskia aculeata is a polymorphic species and a number of different wild and garden varieties exists (Britton and Rose, 1919; Leuenberger, 1986). Plants from the northern and southern regions within its native distribution are also morphologically distinct (Leuenberger, 1986). The plants from the northern region of the native distribution have predominantly broad leaves while the plants from southern region have narrow leaves (Leuenberger, 1986). The fruit of the plants from

the north are spineless, while the ones from the southern region have spines which fall off the fruit when it ripens (Leuenberger, 1986). The most striking morphological difference between plants from the two regions of the native distribution is that the northern plants flowers have white stamen filaments whereas the southern plants have carmine-red stamen filaments (Leuenberger, 1986). Plants from South African populations exhibit characters from both regions with a variety of leaf shapes, fruits with spines and flowers with white stamen filaments (Paterson *et al.*, 2009).

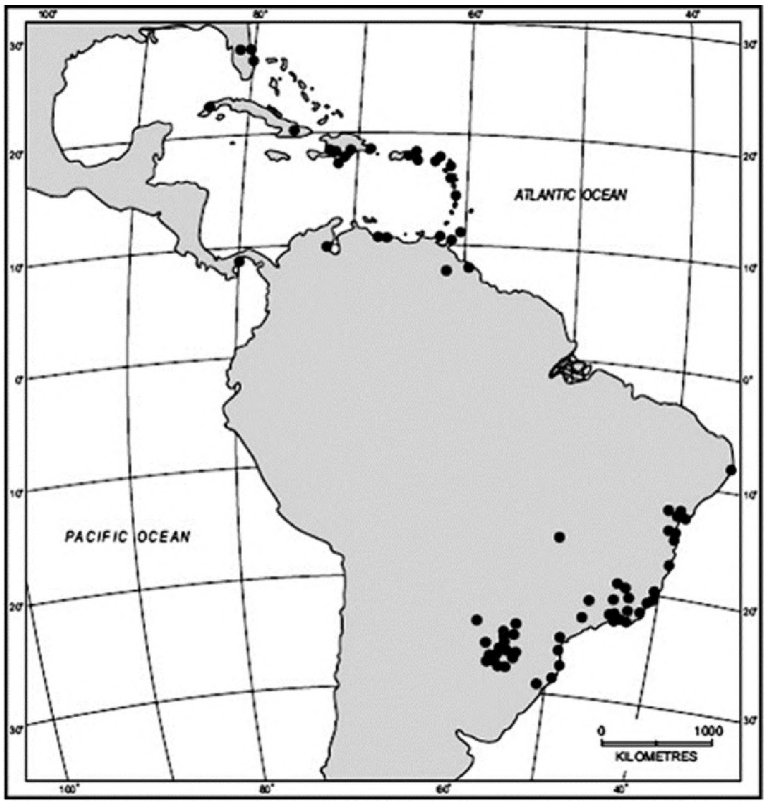

Fig 1.2 The native distribution of *Pereskia aculeata* (After Leuenberger, 1986). Black dots represent localities where the plant is found.

The large thorns on the older stems, and the hooked thorns on the younger shoots, make it difficult for livestock or humans to navigate through *P. aculeata,* so it is often used as a barrier hedge in South Africa (Bruton, 1981). In rural areas of Brazil where the plant is native, it is used as food for humans where the young shoots and leaves are cooked and eaten as a vegetable or as a pot herb (Leuenberger, 1986). In the Caribbean the fruits are eaten fresh (Britton and Rose, 1919) and the fruits can be used to make preserve (Moran and Zimmermann, 1991a).

Pereskia aculeata was first recorded in South Africa in 1858 in the Cape Town Botanical Garden (McGibbon, 1858). The plant was considered a weed of minor importance as recently as 1991 (Moran and Zimmermann, 1991b) but is now distributed in the provinces of Gauteng, Mpumalanga, Limpopo, Western Cape, North West and is abundant in the Eastern Cape and KwaZulu-Natal (Fig 1.3), where it invades coastal vegetation as well as forest habitats (Fig 1.3 Henderson, 1995).

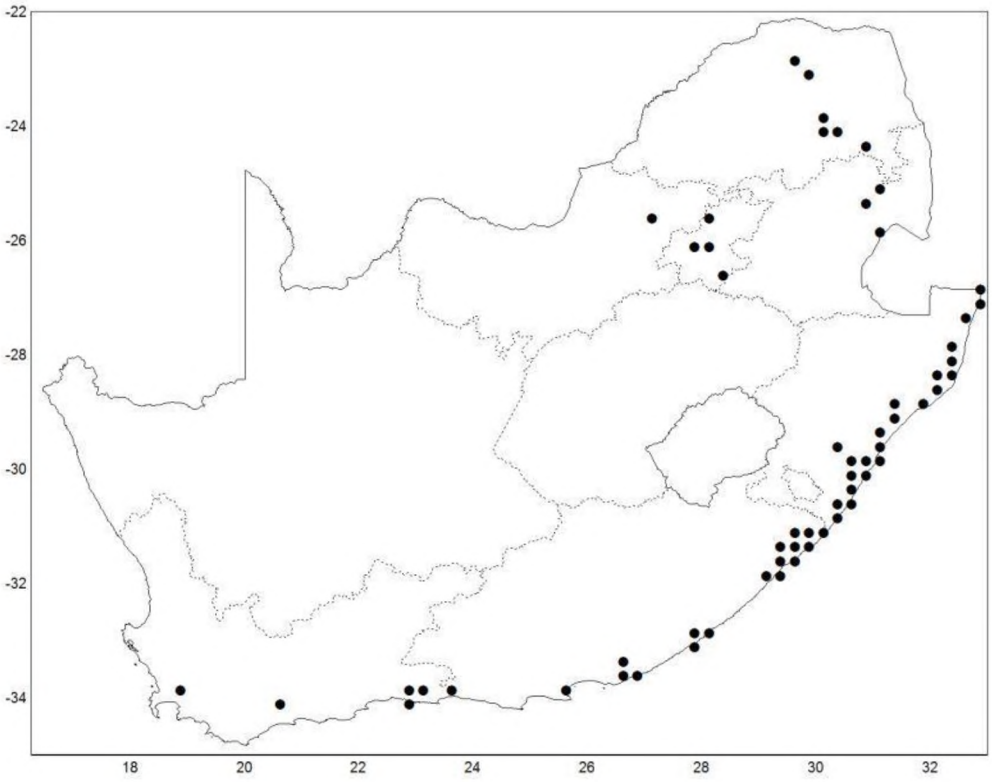

Fig 1.3 Distribution of *Pereskia aculeata* in South Africa. (Drawn by L. Henderson; data source: SAPIA database, ARC-Plant Protection Research Institute, Pretoria). Black dots represent quarter-degree squares where *P. aculeata* has been recorded.

In the 1970s, *P. aculeata* was declared a noxious weed (Proclamation. No. R35, 1979) having a negative impact on South Africa's biodiversity by invading indigenous plant habitats (Pickworth, 1972). It was also listed as a weed in South Africa under the Conservation of Agriculture Resources Act (1983). *Pereskia aculeata* is now listed in regulations in terms of the (NEMBA) National Environment Management: Biodiversity Act (2014) (Department of Environmental Affairs, 2016)

and is considered to be one of the major threats to indigenous biodiversity in the country (De Beer, 1988; Bruton, 1981; Moran and Zimmermann, 1991b; Paterson *et al.*, 2011a).

Pereskia aculeata reproduces both sexually and vegetatively. South African *P. aculeata* produce viable seeds that are spread by frugivorous birds and bats (Campbell, 1988). Invasive alien plants are considered to be either 'drivers' or 'passengers' of the invasion process. Drivers are those species that cause the disruption to the ecosystems and invade pristine ecosystems, while passengers are those that require disturbance, such as other invasive alien species, in order to get a foot-hold in new ecosystems (Thomas and Reid, 2007). *Pereskia aculeata* has the ability to invade pristine ecosystems, especially through the dispersal of seed that have been eaten by bats or birds, and is therefore considered a driver of the invasion process, invading pristine habitats but causing a disturbance that makes those habitats vulnerable to further invasions by other alien invasive plant species.

Pereskia aculeata has a negative impact on biodiversity by reducing plant species richness and biodiversity (Paterson *et al.,* 2011a). This reduction in plant species richness is likely to result in changes to other components of the fauna and flora of the ecosystems invaded by this weed, resulting in decreased diversity across all groups of plants and animals. A similar knock on effect to diversity of animals has been recorded for other cactus weeds (Robertson *et al.,* 2011). The mode of action by which *P. aculeata* reduces plant diversity is by growing-over and covering indigenous plants and outcompeting these plants for light and space (Paterson *et al.*, 2011b). Even large forest trees are killed with this mode of action as they collapse under the weight of the vine in the canopy, resulting in a light-gap in the forest that is dominated by *P. aculeata* (Moran and Zimmermann, 1991b; Paterson *et al.*, 2011b). A study conducted by Paterson, (2011b) at five sites along the coastal area of South Africa from Port St. Johns in the Eastern Cape Province, to Kosi Bay in northern KwaZulu-Natal, showed that when *P. aculeata* density increases, indigenous biodiversity was reduced (Paterson *et al.*, 2011a). The increase of *P. aculeata* density decreases the plant species richness, Shannon H diversity and Simpson's D diversity of indigenous plants. The *P. aculeata* also changed the functional group composition of the infested areas, changing it from a diverse flora composed of trees, shrubs, forbs and vines, to one almost completely dominated by *P. aculeata* and other vine species (Paterson *et al.*, 2011b). These changes in functional group composition and reduction in functional group diversity are likely to result in a

reduction of the ability of the ecosystem to provide ecosystem services, as functional group diversity is known as the mechanism whereby a reduction in biodiversity lead to a loss in ecosystem functioning (Loreau *et al.*,2001).

1.1.4.2 Biological control of *Pereskia aculeata* in South Africa

Pereskia aculeata has been surveyed for potential biological control agents in the weed's region of origin (Paterson *et al.*, 2014a). Promising agents were determined through climatic matching and genotype matching to the South African *P. aculeata* populations (Paterson *et al.* 2009; Paterson *et al.*, 2014a). Seventy-seven *P. aculeata* sites were surveyed for natural enemies in the native distribution (Paterson *et al.*, 2014a). During that field survey, 15 phytophagous natural enemy species were found associated with *P. aculeata,* including sampling at eight long term monitoring sites in Santa Catarina Province in Brazil (Table 1.1) (Paterson *et al.*, 2014a).

Five Coleoptera, four Hemiptera, three Lepidoptera, two Hymenoptera and one species of Diptera were found associated with the plant (Table 1.1) (Paterson *et al.*, 2014a). *Loxomorpha cambogialis* Guene (Pyralidae,), formerly placed under the genus *Epipagis,* and *Maracayia chlorisalis* Walker (Crambidae), were the only species that were recorded in the northern region of the native distribution and both species were also present in the southern native distribution.

Out of the 15 herbivore species found on *P. aculeata*, four species were eliminated as potential biological control agents due to their broad host ranges. These were *Aetalion reticulatum* (L) (Aethalionidae) which was reported as a pest of *Eucalyptus cloezian*a F. Muell. (Myrtaceae) in Brazil, Argentina and U.S.A (Ramoni-Perazzi, 2006; Menezes *et al.*, 2012). *Adetus analis* Haldeman (Cerambycidae) has been recorded as a pest of the Vegetable Pear or Chuchu (*Sechium edule* Jacq. (Curcubitaceae) which is grown as a minor crop in Brazil (De Souza Filho *et al.*, 2001). *Xyleborus affinis* Eichhoff (Scolytidae) which had been recorded on *Pinus* species in southern Brazil (Flechtmann *et al.*, 2001, Paterson *et al.*, 2014a). Finally, a leaf-tying moth, *L. cambogialis* Guene (Lepidoptera: Pyralidae), was rejected because its broad host range includes plants in the families Cactaceae, Portulacaceae and Basellaceae (Klein, 1999; Paterson *et al.*, 2011a).

Table 1.1 The phytophagous insect species found during survey for *P. aculeata* biological control agents and the current status of the species in terms of its potential use as a biological control agent.

Species	Imported	Status	Reason
Coleoptera			
Acanthodoxus machacalis (Cerambycidae)	No	Considered	Mode of damage
Pereskiophaga brasiliensis (Curculionidae)	Yes	Under consideration	Mode of damage
Adetus analis (Cerambycidae)	No	Rejected	Broad host range
Xyleborus affinis (Scolytidae)	No	Rejected	Broad host range
Phenrica guerini (Chrysomelidae)	Yes	Released	Mode of damage
Hemiptera			
Membracis sp.(Membracidae)	No	Rejected	Little impact
Bolbonata sp. (Membracidae)	No	Rejected	Little impact
Catorhintha schaffneri (Coreidae)	Yes	Released	Mode of damage, host specific
Aetalion reticulatum (Aetalionidae)	No	Rejected	Broad host range
Lepidoptera			
Porphyrosela sp. (Gracillariidae)	No	Rejected	Broad host range
Loxomorpha cambogialis (Pyralidae)	Yes	Rejected	Broad host range
Maracayia chlorisalis	Yes	Shelved	Mode of damage, difficult to rear
Hymenoptera			
Pseudopachylosticta subflavata (Cimbidae)	No	Rejected	Broad host range
Bruchophagus sp. (Eurytomidae	No	Rejected	Little impact
Diptera			
Asphondylia sp. (Gracillariidae)	No	Considered	Mode of damage

Bolbonata sp. (Membracidae) and *Bruchophagus sp.* (Eurytomidae*)* were not considered as promising potential biological control agents because of the kind of damage they cause (Paterson *et al.*, 2014a). For example, *Membracis sp.* and *Bolbonata sp* cause tiny puncture marks on the plant and do not have any noticeable impact on *P. aculeata* (Paterson *et al.,* 2014a). *Bruchophagus sp.* produces a gall on the plant, but does not have any obvious impact or effect on *P. aculeata* besides the small gall (Paterson *et al.*, 2014a). *Porphyrosela sp.* (Gracillariidae) was rejected due to broad host range because it was observed feeding on *Talinum paniculata* Gaertner (Portulacaceae) and *Anredera cordifolia* (Ten.) Steenis (Basellacaea). *Pseudopachylosticta subflavata* (Kirby) (Cimbicidae) was also observed developing on *T. paniculata* in Brazil, and was therefore not considered because it is likely that it could develop on native South Africa's *Talinum* species (Paterson *et al*., 2014a).

Maracayia chlorisalis Walker (Crambidae), *Asphondylia sp*., (Cecidomyiidae), *Acanthodoxous machacalis* Martins and Monne (Cerambycidae*)*, *Catorhintha schaffneri* Braisklovsky & Garcia (Coreidae) and *Pereskiophaga brasiliensis* Anderson (Curculionidae) which was previously referred to as *Cryptorhynchus sp.* (Paterson *et al.,* 2014a) and as an unknown species of Curulionidae in Paterson *et al* (2011a) were considered to be the most promising biological control agents in terms of climatic and genetic matching, mode of damage and inferred field host range (Paterson *et al.*, 2014a) .

Maracayia chlorisalis is a stem-boring moth that mines the stem and damages the young shoots, causing the shoot to die (Klein, 1999). *Asphondylia sp.* prevents the development of the seeds by producing galls on the ovaries of the flowers, thus reducing the spread of *P. aculeata* by seed (Paterson *et al.*, 2014a). *Acanthodoxus machacalis* mines the thick woody stems of the plant (Paterson *et al.*, 2014a). *Phenrica guerini* Bechyne (Chrysomelidae) is leaf feeding beetle that damages leaves and shoots (Klein, 1999). *Catorhintha schaffneri* is a stem-wilting bug that attacks the growing tips of shoots causing the plant to split, wilt and rot (Paterson *et al.*, 2014b). *Pereskiophaga brasiliensis* Anderson (Coleoptera: Cucurlionidae) is a stem miner that damages the structural tissue of the stems (Anderson, 2015).

At present, only two biological control agents have been released against *P. aculeata* in South Africa and a third potential agent, *P. brazilensis,* is under consideration in quarantine. These three insects will be discussed in greater detail below.

***Phenrica guerini* Bechyne (Chrysomelidae)**

The female *P. guerini* deposit eggs, which are red in colour, in groups of about 23 to 33 eggs on the under surface of *P. aculeata* leaves. The eggs hatch in 5-6 days after oviposition. The newly hatched larvae are reddish and become grey towards the end of the first instar. The second instar turns yellow and the third instar then turns grey. The first instar duration ranges from 5 to 6 days, second instar duration ranges from 4 to 7 days and the duration of the third instar is 11 to 24 days. During the third instar, a pre-pupal period also starts whereby the larva does not feed. Thereafter, the larvae drop off the host plants onto the soil to excavate a chamber where pupation will take place. The duration of pupation ranges from 9 to 14 days.

Both larvae and adults feed only on *P. aculeata*. The newly emerged first instar larvae feed on the leaves, forming shallow pits on the lower surface of the leaf around the egg clusters. As the larvae grow they move to the shoot tips to feed around the succulent young leaves' margins. Adults feed on young growth and on the leaf blades.

Phenrica guerini was the first biological control agent for *P. aculeata*, and was released in 1991 after host specificity testing indicated that it was completely monophagous and therefore safe to release in South Africa (Klein, 1999). In KwaZulu-Natal, *P. guerini* was released at ten sites, and in the Eastern Cape it was released at three sites between 1991 and 1997 (Klein, 1999), but it did not reduce the density of *P. aculeata* sufficiently and thus new biological control agents were needed (Klein, 1999; Paterson *et al.*, 2011a). A maximum of 300 beetles were released at each site, so a total of 1319 individuals were released at all sites throughout the country. The beetles established at Sezela Sugar Mill in KwaZulu-Natal and at Port Alfred in the Eastern Cape Province only (Klein, 1999). In 2009 and 2014 mass-rearing efforts were re-initiated and large numbers of the agent were realesed at multiple sites by the South African Sugar Research Institute (SASRI) but no post-release evaluation has been conducted. There were only three sites in 2013 that were known to have persistent populations and the impact of *P. guerini* was believed to be negligible (Klein, 2011). Between February 2009 and November 2014, a total number of 20625 individuals were released in 119 releases at 22 sites by SASRI and a recent post-release evaluation has indicated that *P. guerini* is damaging and reduces *P. aculeata* densities at sites where high population densities are reached (Mnqeta, 2017). The beetle only reaches these high densities at a

handful of sites in the country and the reduction in density of the weed is not great enough to result in a significant increase in indigenous biodiversity (Mnqeta, 2017; Paterson *et al.*, 2011b). This post-release evaluation suggests that while *P. guerini* may still play an important role in the biological control of *P. aculeata*, it is not sufficiently damaging and abundant to reduce the weeds densities to acceptable levels.

***Cathorhintha schaffneri* Brailovsky & Garcia (Coreidae)**

Catorhintha schaffneri deposit eggs in batches on the leaves or on the stems of the host plant. Each batch consists of an average of 15 eggs. The eggs take up to 15 days to hatch and nypmhs have five development stages (instars). The newly hatched nymphs are reddish in colour and turn to a black colour as they grow (Paterson *et al.*, 2014b). The nymphs are gregarious and the first instar nymphs are often found on the same shoot tips as older nymphs and the adults (Paterson *et al.*, 2014b). The first instar nymph takes an average of three days, the second nymph takes up to six days, and third instar last up to 13 days to ecdysis (Paterson *et al.*, 2014b). The final ecdysis then occurs at an average of 23 days since hatching (Paterson *et al.*, 2014b). The adults survive up to an average of 26 days (Paterson *et al.*, 2014b).

Catorhintha schaffneri was collected in May 2012 at coastal sites in Santa Catarina Province, Brazil, and imported into quarantine where host specificity testing and impact studies indicated that it was suitably specific for release as well as sufficiently damaging (Paterson *et al.*, 2014b). It was the second agent released against *P. aculeata* and was first released in October 2014. Four years after release, the agent was established at some sites in KwaZulu-Natal and in the Eastern Cape. Some preliminary data suggest that the agent may be effective but it is far too soon to determine whether this will be a damaging agent or not (ID Paterson, Rhodes University, Personal Communication 10/10/2017). Agents can take many years before they reach their full potential in terms of the damage that they inflict on the target weed, which has prompted some authors to suggest that a period of at least ten years should have passed since the first release of an agent before any post-release evaluation is conducted (McFadyen, 1998).

***Pereskiophaga brasiliensis* Anderson (Curculionidae)**

Fig 1.4 *Pereskiophaga brasiliensis*. 1) Dorsal habitus; 2) Ventral habitus; 3) Lateral habitus (taken from Anderson (2015)).

Pereskiophaga brasiliensis Anderson (Curculionidae) was collected from Santa Catarina, Brazil, and imported and cultured, in quarantine at Rhodes University, South Africa in 2012 (Fig 1.4). The species has been recently described and placed in a new genus (Anderson, 2015). *Pereskiophaga brasiliensis* have only been found feeding and developing on *P. aculeata* in native field host range studies (Paterson *et al.*, 2014a). This species was considered as a biological control agent of *P. aculeata* in South Africa because of its mode of damage, from climatic and genetic matching, and because it has a restricted field host range and was common and damaging at some sites in Santa Catarina, Brazil (Paterson, *et al*., 2014a).

Pereskiophaga brasiliensis is a stem miner that damages the structural tissue of stems often destroy the vascular tissue and causing the plant to die above the mines (Paterson *et al.*, 2014a). The adults feed on shoots and leaves, while larvae feed inside the stem tissue, feeding on the vascular tissue during the first instar and then hollowing out the whole stem over the duration of larval development (Paterson *et al.*, 2014a).

Pereskiophaga brasiliensis adults are medium-sized weevils, 3.8 - 5.0 mm long (Anderson, 2015). The length of the male ranges from 3.8 to 4.7 mm and it is 1.9 – 2.5 mm wide; and female length is from 4.2 to 5.0 mm with the width of 2.1–2.7 mm (Anderson, 2015). The body has a cuticle

that is covered sparsely with scales, with isodiametric to microstriate microsculpture (Anderson, 2015). As with most *Cryptorhynchinae,* the rostrum fits tightly into a deep sternal groove in the prosternum and mesoventrite.

Pereskiophaga brasiliensis is thought to be closely related to other weevils that are cactus-feeding species; these include *Gerstaeckeria sp.* Champion and *Eriocereophaga humeridens* O'Brien. *Eriocereaphaga humeridens* is the only described member of this genus and it is closely related to the genus *Gerstaekeria,* which are cactus feeding weevils that occur in the southern U.S.A., Mexico, Ecuador and Peru (O'Brien, 1976). *Eriocereophaga humeridens* was approved for introduction into Australia in 1976 as a biological control agent of *Harrisia martinii* (*Eriocerereus martinii*) (Cactaceae) (Mc Fadyen, 1979) but the agent did not establish (Winston *et al.*, 2014). All *Gerstaeckeria* species are known to breed on *Opuntia* cacti, although there has been little specific data published on their life histories. Although there are clear differences between the genera, *Pereskiophaga* and *Eriocereophaga* are closely related because they share a well-defined broad, glabrous, impunctuate band along their posterior margin of the protonal flanks. Their scales on the protonum are similarly clumped as well as their microsculpture surface (Anderson, 2015).

Newly emerged adults of *P. brasiliensis* are usually reddish in their first week, turning dark brown from the second week onwards. The larvae are white and attain a length of about 5 mm and live inside the stems, where pupation occurs. When the female is ready to oviposit, she removes a small piece of epidermal tissue from the stem by biting off the plant tissue and inserting the rostrum towards the inside, creating a hole. Once the rostrum has been inserted to its full length, the female removes it and slowly positions the tip of her abdomen to deposit an egg. Then she uses her abdomen to dab frass and exudate over the egg to cover and protect it. After three or four days, the eggs hatch, and the larvae develop inside the stem, feeding on the stem tissue. Pupation occurs by the third week, followed by adult eclosion in the fourth week when adults bore an exiting hole and come out of the stem. A pupation chamber is constructed inside the stem and made with woody plant fibers. Larval development from egg to adulthood takes at least four weeks including the pupal stage but developmental rate appears to be very variable, with some taking over seven weeks before eclosion.

The sex of the weevils can be determined by examining the rostrum. The females do not have scales along the rostrum because they use it to probe and excavate oviposition sites, thus rubbing

the scales off the front portion of the rostrum. Males have scales all the way to the end of the rostrum near the antennae. Naïve females may therefore not have probed an oviposition site but it appears that the behaviour is present in naïve females and most lose the scales very early in the adult stage, whether they have mated or not.

1.2 Objectives

Pereskiophaga brasiliensis is considered one of the most promising candidate agents for the control of *P. aculeata* in South Africa (Paterson *et al.*, 2011a; Paterson *et al.*, 2014a). If *P. brasiliensis* is suitable for release in South Africa it is likely to compliment already established agents because it feeds primarily on the older, woody, stems of the plant, rather than the leaves and shoots that *C. schaffneri* and *P. guerini* feed on. The purpose of this study was to determine if *P. brasiliensis* is sufficiently damaging and safe for release as a biological control agent of *P. aculeata* in South Africa by quantifying the impact it has on the plant under quarantine conditions and investigating the host range of the species.

Chapter 2: Quantifying the impact of *Pereskiophaga brasiliensis,* a new potential biological control agent for *Pereskia aculeata*

2.1 Introduction

Determining whether an agent will be effective at controlling its target weed is very difficult and has been referred to as the 'holy grail' of biological control of weeds (McFadyen, 1998). The difficulties associated with determining how effective an agent will be are primarily due to the fact that the work must be done within the confines of quarantine. Extrapolating quarantine based research into the field is notoriously difficult. It is, however, very important to understand the potential of an agent before it is released, because releasing ineffective agents must be avoided (McClay and Balciunas, 2005). Although host-specificity testing ensures that agents are safe for release, there is always a small intrinsic risk associated with the release of a biological control agent and this risk should only be taken if there is a good chance that the agent will be effective (Louda, 2000; McEvoy and Coombs, 1999; Sheppard, 2003; McClay and Balciunas, 2005). Assessing the impact of the candidate is therefore very important, although it has not been taken into account in many biological control programmes (Shea and Possingham, 2000; Wratten and Gurr, 2000). In this chapter, the impact of the potential biological control agent *P. brasiliensis* is quantified under quarantine conditions in order to determine whether it is capable of damaging the target weed, *P. aculeata*, sufficiently if it were to reach high enough population densities in South Africa.

2.1.1 The importance of releasing effective biological control agents

In the past 100 years of biological control programmes in South Africa, 270 agents have been considered as candidate biological control agents (Moran *et al.*, 2013). The agents that have been released have shown different impact levels on the targeted weeds species in South Africa**.** Of the 106 released agents, 71% (75) have established on 48 species from 14 families of invasive alien plants (Klein, 2011). Thirty-one percent of the targeted weeds have been extensively damaged

(Moran *et al.*, 2013). Twenty-six percent of the targeted weeds suffered considerable damage, while 19% showed only moderate damage. There was a trivial amount of damage to 13%, and an unknown amount of damage to 12% of the plants (Moran *et al.*, 2013).

The success rate of biological control on a worldwide scale is considered low by some authors but there is a good argument that success rates are reasonable or even greater than expected (Fowler *et al.,* 2000). Whether or not one considers the success rate of biological control of weeds acceptable, it is clear that increasing the level of success in biological control of weeds would be beneficial to the science as a whole as well as the ecosystems and societies that rely on it. One way to improve the success rate of biological control is to ensure that all the agents that are released are sufficiently damaging to the target plant.

Releasing ineffective agents has greater consequences than simply reducing the success rate of biological control of weeds. There is also an innate risk associated with the release of a new biological control agent that is only worth taking if the agent is likely to be effective (McClay and Balciunas, 2005). Other more direct consequences of releasing ineffective agents include ecological knock-on effects at multiple trophic levels (Carvalheiro *et al.*, 2008) and potential negative interactions with other agents (Ehler and Hall, 1982).

Biological control agents are intended to become part of the ecosystem into which they are introduced. The desired impact, is to reduce the densities of the target weed, and this is expected to have broad ecological implications, such as increasing biodiversity and ecosystem functioning. Since the agent is intended to become part of the ecosystem, it is also likely to have an impact on other trophic levels (Carvalheiro *et al.*, 2008). The agent itself will become prey to predators and parasitoids (Hill and Hulley, 1995) and could therefore influence populations of these predators and parasitoids; as well as the other species which these predators and parasitoids usually feed on (Carvalheiro *et al.,* 2008). All biological control agents, including successful agents, are likely to cause these multi-trophic impacts, but for successful agents the positive impacts of controlling the weed will greatly outweigh any negative ecological effects (Downey and Paterson, 2016). Ineffective agents are therefore likely to alter the food-web of the ecosystem into which they are released, but will not provide the benefits of controlling the weed.

Ineffective agents are also more likely to maintain large populations for prolonged periods. An effective agent will eventual result in a decline in weed populations and hence a decline in the

populations of the agent. Ineffective agents that establish may thrive on the target plant and build up to large populations that never reduce the populations of the target weed. This means that there is a permanent source of food for predators and parasitoids, so ecological knock-on effects are likely to be more severe and permanent (Carvalheiro *et al.*, 2008). About 19% of the established agents in South Africa are considered to cause moderate damage to the target weed, while 13% of these agents cause only trivial damage (Klein 2011; Moran *et al.*, 2013). These agents are permanently established in the country and are unlikely to ever be eradicated but some may be build-up to large populations and have undesired ecological impacts on multiple trophic levels. Pre-release impact studies could reduce the number of ineffective agents that are released by ensuring that agents are capable of damaging the target weed at agent densities that are likely to be found in the field after release. This would help avoid situations where agent populations build up to high numbers but do not damage the target weed sufficiently to result in control.

Ineffective biological control agents could also have negative impacts on other more effective agents for the same target weed. While in some cases multiple agents will interact synergistically, in other cases one agent can inhibit the other (Hatcher, 1995). For example, in the U.S.A. the two leaf-feeding agents that were released for the control of the invasive alien plant, *Lythrum salicaria* L. (Lythraceae) complemented each other after release and resulted in a greater level of control than having either agent alone (Blossey 1995a, 1995b). Another example of a synergistic relationship between agents is the three agents released for the control of *Sesbania punicea* (Cav.) Benth. (Fabaceae) (Hoffmann, 1990; Hoffmann and Moran, 1999). The three weevils released for the control of this plant result in complete control, but this can only be achieved if all the agents are present at a site because it is the cumulative stress from all the agents that results in control (Hoffmann, 1990; Hoffmann and Moran, 1999). If agents have an inhibitory relationship it can result in reduced control if the most effective of the agents is negatively impacted by others. For example, the leaf-defoliating beetles *Chrysolina quadrigemina* Suffrain (Chrysomelidae) and a root feeding bruprestid *Agrilus hyperici* Creutzer, which were both released for the control of *Hypericum perforatum* L. (Hypericaceae), had an antagonistic interaction that inhibited the success of biological control (Briese, 1997). The most effective agent was *C. quadrigemina* but it was outcompeted by *A. hyperici* and this resulted in a reduction in the overall level of control (Briese, 1997). Similarly, Weyl and Hill (2012), found that the very effective agent for water hyacinth, *E. crassipes*, the weevil *Neochetina eichhorniae* Warner (Curculionidae), was less damaging in the

presence of the less effective agent, the leaf-feeding bug, *Eccritotarsus catarinensis* (Carvhalo) (Miridae). It is therefore important that ineffective agents are not released, because they might disrupt the level of control already provided by other agents without damaging the target plant, or may establish and hamper future biological control agents that are discovered and released in future.

2.1.2 Impact studies in quarantine

It is extremely difficult to predict the effectiveness of an agent prior to release, but there are ways which can assist in selecting which of the agents is most likely to be effective. For instance, choosing the agents with a high number of generations per year and high fecundity have better chances of establishing and controlling the target invasive alien plants (Crawley, 1989). Also, climatic matching, genetic matching and mode of damage provides better chances of selecting an effective agent (Paterson *et al.*, 2014a). The level of damage in the native distribution is a good way to estimate the lowest level of damage that could be achieved in the introduced distribution because agents are likely to be suppressed by their own natural enemies, so population densities are likely to be greater in the introduced distribution if the climate is suitable (Hill and Hulley, 1995). Also, considering climate matching, genetic matchingand mode of damage can improve the chances of selecting an effective agent.

Although it is difficult to predict the potential range and abundance of an agent prior to release, evaluating the per-capita effect of the candidate agent can be done in the laboratory (McClay and Balciunas, 2005). Evaluating the impact of the agent using different densities of agents assists in determining the expectation of impact levels under different densities (McClay and Balciunas, 2005). It also determines whether the biological control agent densities will be likely to cause damage to the targeted weed in the field (McClay and Balciunas, 2005). These studies allow researchers to predict the effect of the candidate agents by providing evidence of the agent's potential to negatively affect the target weed growth parameters (Sheppard, 2003). However, the performance of the candidate agent on target weeds in the introduced range cannot be fully predicted, especially when challenged with new environmental conditions (Broughton and

Pemberton, 2008). Results from impact studies can be important in convincing reviewers of release applications that a given agent is significantly damaging to the target weed to warrant release.

Pereskiopahaga brasiliensis is considered one of the most promising of the potential agents for *P. aculeata* based on the level and mode of damage that was recorded in the native distribution (Paterson *et al*., 2014a). The level of damage was however, not quantified, and hence impact studies to quantify the damage of *P. brasiliensis* to *P. aculeata* are required. The aim of the study was therefore to assess the impact of the candidate biological control agent, *P. brasiliensis,* on the targeted weed, *P. aculeata,* under quarantine conditions and determine whether it is suitably damaging to the targeted weed to be considered for release in South Africa.

2.2 Materials and methods

All the plants used in this impact study were grown at the Waainek Research Facility at Rhodes University in Grahamstown, Eastern Cape, South Africa prior to being moved to quarantine. *Pereskia aculeata* plants were obtained from cuttings of plants growing naturally in the field in the Grahamstown area. *Pereskia aculeata* cuttings were rooted in the soil in a greenhouse, then transplanted to 30cm x 45cm plastic pots. Plants were allowed to grow for at least two months before being transported to quarantine and were all exposed to the same watering and fertilizing regime. One tablespoon of Multicote 8 fertilizer (13:6:20) was applied at the beginning of each trial and plants were watered once a week.

2.2.1 Experimental design

Pereskia aculeata plants were placed in 1.2 m x .06 m x 0.6 m insect cages in the Rhodes University Quarantine facility. Two mated female *P. brasiliensis* were placed on each plant and left for two weeks to allow oviposition and feeding. This simulated densities seen at sites with high levels of damage in Brazil (I. Paterson, Rhodes University, Pers. Com.). Any adults that died during the experiment were replaced daily. Two weeks was considered an appropriate length of time between inoculations because in preliminary trails most females had oviposited at least a single egg after this period. Plants were left for ten weeks to allow for larval development and damage from the insect to the plants to accrue. This allowed enough time for at least some of the first eggs that were ovipsoited to develop through to the adult stage. This experiment therefore focused on the impact of the larvae rather than the adults which could only feed on the plants for a two-week period. The larvae are substantially more damaging to the plant than the adult stage,

so assessing larval damage is appropriate. All plants were watered with an equal quantity of water whenever the soil was dry. The experiment was replicated ten times, with ten plants being exposed to the insect and ten control plants that were treated in exactly the same way but not exposed to the insect.

2.2.2 Data collection

The plant growth parameters measured in this study included shoot length, number of shoots and number of leaves. Measurements for shoot length (cm), number of shoots and number of leaves were taken at the start of the experiment prior to the weevils' inoculation and then at the end of the study. The change in plant parameters over the period of the study was compared between the treated and untreated plants. After the ten-week period of larval development, plants were dissected and inspected with the aid of a microscope. The number of larvae as well as the number of adult feeding scars were recorded.

2.2.3 Statistical analysis

Data were normally distributed so a T-test was used to determine differences between treated plants and controls. All analyses were conducted using STATISTICA ver. 11.

2.3 Results

Plants that were exposed to the two mated female *P. brasiliensis* had an average of 7.4 larvae (S.E. ± 0.52) at the end of the 12 week experiment. Adult feeding was recorded as feeding scars on the green parts of the plant including green stems, shoot tips and leaves. An average of 62 feedings scars (S.E. ± 5.56) were recorded on plants exposed to the insects.

Plants that were not exposed to any *P. brasiliensis* produced an average of 39.6 leaves (S.E. ± 8.0) over the 12-week period of the experiment (Fig. 2.1). Those exposed to the weevil did not produce any leaves, but lost an average of 5.7 leaves (S.E. ± 0.8) (Fig 2.1). The difference in the change in the number of leaves over the experimental period was significant (T test; $t = 4.27$, $p < 0.001$).

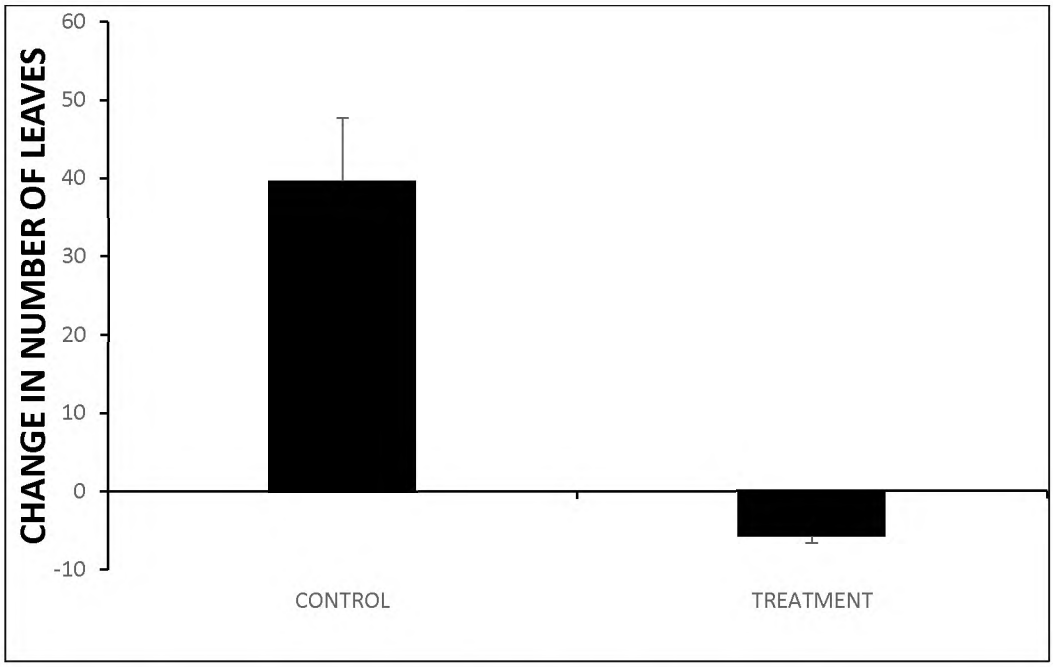

Fig 2.1 The change in number of leaves of plants exposed to *P. brasiliensis* (treatment) compared with those not exposed to *P. brasiliensis* (control) over a 12-week period. Control plants produced significantly more leaves during the course of the experiment (T test; $t = 4.27$, $p < 0.001$).

There was a significant change in the number of shoots produced by plants exposed to *P. brasiliensis* and those not exposed to the insect over the 12-month period (T test; $t = 8.29$, $p = 0.000$) (Fig 2.2). The number of shoots was reduced by an average of 0.6 shoots (S.E. ± 0.42) in those exposed to the insect, while control plants produced an average of 5.2 shoots (S.E. ± 0.40) (Fig 2.2).

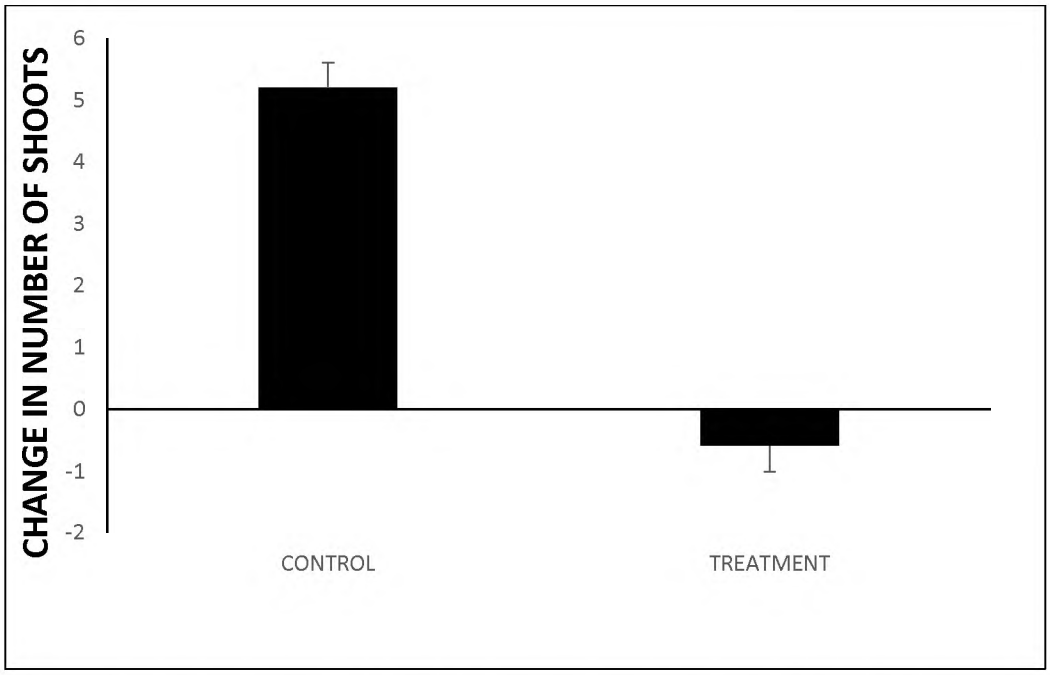

Fig 2.2 Change in the number of shoots over a 12-week period for plants with and without *P. brasiliensis*. Plants not exposed produced significantly more shoots during the experimental period (T test; $t = 8.29$, $p = 0.000$).

There was a significantly greater increase in shoot length for plants not exposed compared with plants that were exposed to *P. brasiliensis* (T test; $t = 4.98$, $p < 0.000$) (Fig. 2.3). Shoot length was on average reduced by 1.7 cm (S.E. ± 0.12) in plants exposed to the insect and increased by 10.74 cm (S.E. ± 0.2) on average for not exposed plants (Fig. 2.3).

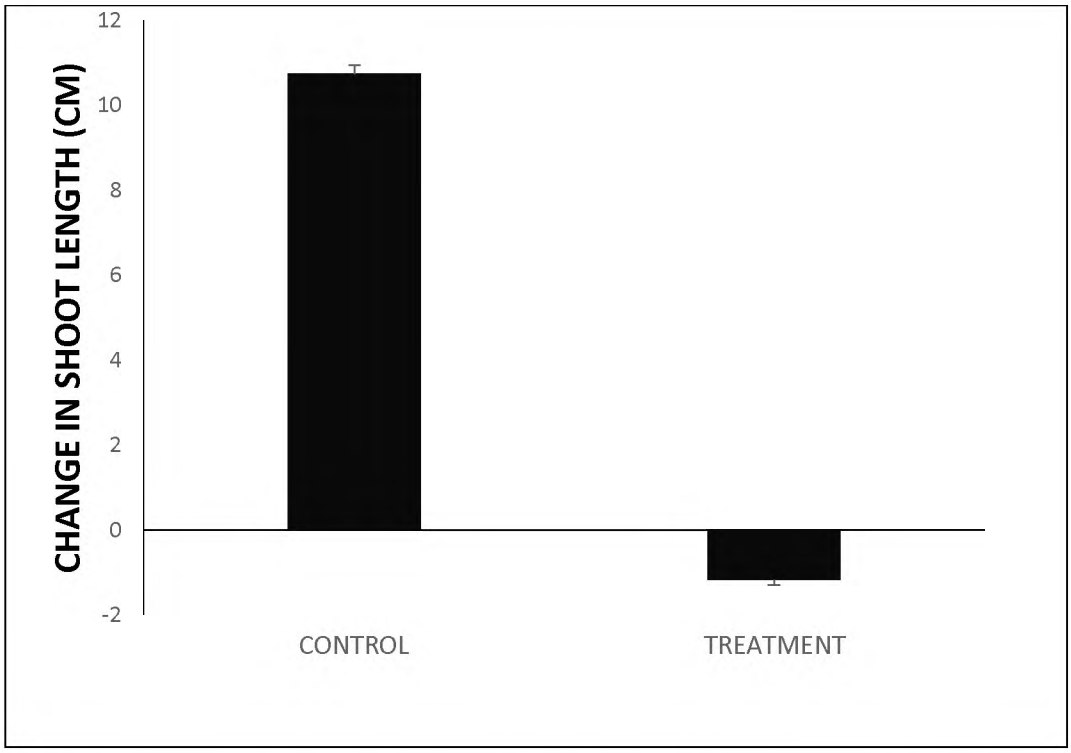

Fig 2.3. Changes in shoot length over a 12 week period of exposure to *P. brasiliensis* or no exposure to *P. brasiliensis*. Plants that were not exposed to *P. brasiliensis* produced significantly greater shoot lengths over the experimental period (T test; $t = 4.98$, $p < 0.000$).

2.4 Discussion

This study provides important information as to whether the proposed biological control agent, *P. brasiliensis*, is suitably damaging for release in South Africa by quantifying its impact on the invasive weed *P. aculeata*. The main purpose of this test was to determine how damaging *P. brasiliensis* is to *P. aculeata* by investigating its impact on shoot length, number of leaves and the number of shoots. All the plant growth parameters were significantly reduced by the presence of *P. brasiliensis*. The results of the experiment therefore confirm the observations made in the native distribution which suggested that the insect is damaging to *P. aculeata* (Paterson *et al.*, 2014a). Not only were there significant differences between treated and control plants, there was also a decrease in all plant parameters over the experimental period for all plants exposed to the insect and a large increase in all parameters for control plants. This confirms that *P. brasiliensis* can damage *P. aculeata* if comparable densities are reached in the field.

The reduction in the number of leaves and shoots caused by *P. brasiliensis* herbivory may reduce the shading by *P. aculeata* thus providing more light to native plants. The results revealed significant difference in shoot length between the controls and treated plants. The impact on the shoot length of the plants inoculated with *P. brasiliensis* indicates that the insect could reduce the plants ability to grow into the canopy, which could reduce its negative impacts on large trees in indigenous forests in South Africa (Moran and Zimmermann 1991b).

Although impact studies in quarantine are difficult to extrapolate to the field, there are a number of impact studies that have been beneficial for biological control programmes. Studies by Goolsby *et al.* (2009) conducted under quarantine conditions indicated that *Tetramesa romana* Walker (Eurytomidae) are damaging to potted plants of the target weed, *Arundo donnax* L. (Poaceae). This pre-release study was used to justify the release of the agent in U.S.A., where preliminary data have shown that the agent is effectively reducing the density of the target weed as predicted from pre-release studies (Goolsby *et al.,* 2009). This success has also depended on the agent reaching high densities in the field, so genetic suitability of the agent to the weed (the correct genotype of the agent being utilised) (Goolsby *et al.*, 2009) and presumably the climatic suitability of the agent to the introduced range has also played an important role.

Genotype and climate matching show that *P. brasiliensis* is likely to establish on the South African genotypes of *P. aculeata* and thrive in the South African climate (Paterson *et al*., 2009; 2014a). Although the results from quarantine indicate that *P. brasiliensis* could have significant impacts on the plant it is impossible to accurately predict success outside of the controlled quarantine conditions. Whether the agent will establish and populations will be become abundant enough to impact *P. aculeata* on a large scale are not known. It is, however, clear that if the candidate agent were to be released and did build up large populations, the damage to plants in South Africa would be significant. There is therefore little risk of releasing an ineffective biological control agent that could build up large populations and not impact the plant. This is important because agents that are not sufficiently damaging are more likely to result in undesirable multi-trophic ecological impacts (Carvalheiro *et al.*, 2008).

Paterson *et al.,* (2011b) conducted a pre-release assessment of *P. aculeata* infestations in South Africa and showed that a 50% reduction in *P. aculeata* cover is required before any recovery in indigenous plant biodiversity is likely to be achieved. Long-term post-release evaluation will be

required after the release of *P. brasiliensis* in order to determine the percentage of reduction by *P. aculeata* (Paterson *et al.*, 2011b). Whether the level of damage produced by *P. brasiliensis* in this study would result in the required level of damage is not known, but at the end of the experiment, control plants had far more than 50% greater numbers of leaves, shoots and shoot lengths, so it is clear that reducing the density of *P. aculeata* by 50% could be possible if *P. brasiliensis* reaches comparable densities in the field.

This study shows the potential of *P. brasiliensis* to reduce *P. aculeata* densities to below the damage threshold if high insect population densities are reached after release. *Pereskiophaga brasiliensis* impact should be compared with that of *P. guerini* and *C. schaffneri* so that the relative impacts of all the agents are known and also interaction studies should be considered between these three agents. The results from interactions between biological control agents of *P. aculeata* should be taken into account because potential negative interactions can result in reduction of biological control efficacy (Ehler and Hall, 1982; Crowe and Bourchier, 2006). If *P. brasiliensis* agent has a greater impact on *P. aculeata* than *P. guerini* and *C. schaffneri* and there is a possibility that negative interactions between all of these agents will reduce the efficacy of *P. brasiliensis*, then the release of all the agents at the same site would not be advised. By conducting impact studies using different combinations of biological control agents and *P. brasiliensis*, the most effective combinations could be determined.

An impact study was also conducted with *C. schaffneri* prior to the release of the agent in South Africa in October 2014 (Paterson *et al.*, 2014b). In this experiment, similar potted plants were used and exposed to 10 nymphs of *C. schaffneri* for a ten-day period (Paterson *et al.*, 2014b). The results are not directly comparable because the mode of damage of the agents is so different, the densities were different and period of exposure was different. It is however interesting that the average reduction in the number of leaves and shoot length from *C. schaffneri* was 9.33 leaves and 28.9cm while the equivalent in this study from *P. brasiliensis* damage was over 45 leaves and only about 11cm for shoot lengths. This suggests that *P. brasiliensis* is more damaging to the leaves, while *C. schaffneri* is more damaging to the shoots. The mode of damage for each agent, and hence the damage, is therefore focused on different parts of the plant, with *C. schaffneri* damaging the shoots and *P. brasiliensis* damaging the stems which reduced leaf production to a greater extent than shoot damage by *C. schaffneri* did. This suggests that the two agents could be compatible and work

in synergy to produce cumulative stress on *P. aculeata* but it is still essential that interaction studies are conducted if the agent were to be released in the country. While some agents that attack different parts of the plant have resulted in cumulative stress (Hoffmann and Moran, 1991), others have had antagonistic relationships that have resulted in reduced control of the target weed despite the fact that the agents attack different plant parts (Briese, 1997).

In conclusion, this study has provided evidence that *P. brasiliensis* could be a damaging biological control agent and significantly impact *P. aculeata* in South Africa. High population densities of the agent would need to be reached, but there is good evidence that the agent is climatically and genetically suitable for South Africa, so the agent is likely to build up to high numbers (Paterson *et al.,* 2009; Paterson *et al*., 2014b). Even though there is a possibility that *P. brasiliensis* could be a successful biological control agent, it is impossible to accurately predict success from studies conducted under controlled conditions in quarantine. While the impact of a potential agent is an essential component of pre-release studies, the most important pre-release study for a biological control agent is host specificity testing. This chapter provided evidence that *P. brasiliensis* is suitably damaging to be released in South Africa, but it can only be released if it is suitably host specific and will not harm indigenous plants or commercially important species in the country. The following chapter investigated whether *P. brasiliensis* is suitably specific to warrant release in South Africa.

2 Chapter 3: Host specificity of *Pereskiophaga brasiliensis*

3.1 Introduction

3.1.1 Host plant selection

Every herbivorous insect, to some degree, selects its food before it consumes and selects the plant as a site for oviposition. Selecting a host plants involves finding the right species of plant to support development, survival and feeding (Ramaswamy, 1988). There is a very wide variety of potential host plants in most environments, many of which are not suitable hosts and some of which could be poisonous. Therefore, the selection of a suitable host plant is not a trivial task, and for phytophagous insects, the task involves two stages: host finding and host acceptance (Ramaswamy, 1988).

3.1.2 Host finding

Phytophagous insects use visual cues and plant chemicals to locate and select a host plant from non-host plant in a diverse environment (Schoonhoven *et al.*, 2005; Fernandez and Hilker, 2007). Visual and chemical cues are combined to form a "host plant search image" which is used to allocate their host plants (Bernays and Chapman, 1994; Städler, 2002). Insects may be attracted to the host plants by the form and colour of the plant (Prokopy and Owens, 1978; Briscoe and Chittka 2001).

Insects also use odour to find hosts, an activity, which involves two stages: arousal followed by orientation (Bell, 1984; Carde, 1984). Arousal prepares the phytophagous insect to respond to other stimuli and results in the insect becoming mobile (Bell, 1984). The insect then uses the gradient of the odour to orientate and then locate the host plant (Visser, 1986). Volatile compounds emitted by host plants play an important role in host recognition and assist in guiding the herbivorous insects to their host plant (Honda, 1995; Bruce *et al.*, 2005). Odours can elicit a response from a greater distance than visual cues, with responses to odours being measured over distances of about 100m and those to visual cues only over about 10m (Schoonhoven *et al.*, 2005). Selection of host plants is therefore a combination of visual elements and olfactory behavior

although it is sometimes difficult to assess or identify which cue is more important (Scherer and Kolb, 1987).

3.1.3 Host acceptance

Once a phytophagous insect has reached a plant, it must either accept or reject it. Olfaction is still important at this stage, as well as contact, vision, mechanoreception and chemoreception (Prokopy, 1986; Bernays and Chapman, 1994). Over and above leaf odour, other physical properties that provide important cues in host acceptance are leaf surface texture, colour and size (Prokopy, 1986; Deboer, 1991). The waxy layers that cover the plant surface differ in chemistry from plant to plant, and many phytophagous insects respond to the chemical composition of the wax (Chapman and Bernays, 1989; Bell, 1984). Lastly, the internal constituents of the leaf, which includes phagostimulants and deterrents, are also involved in host acceptance (Deboer, 1991; Nielsen, 1989; Bernays, 1991; Jones, 1991).

3.1.4 Insect host ranges

There is a continuum of host range breadths in phytophagous insects from generalists to specialists. Generalists have broad host ranges meaning they feed on plants from a variety of different unrelated families while specialists are restricted to certain plant taxa (Marquis, 1991; Diniz and Morais 2002; Dyer *et al.*, 2007). *Myzus persicae* (Sulzer) (Aphidae), for example, is a generalist species of aphid which feeds on over 400 plant species in more than 40 families (Blackman and Eastop, 2000). Another aphid species, the cabbage aphid *Brevicoryne brassicae* L., is more specialised, feeding only on species within the family Brassicaceae (Embaby and Lofty, 2015). There are also specialist monophagous aphids which only feed on a single plant species, such as *Aphis asclepiadis* (Fitch), which only feeds on *Asclepius syriaca* (L.) (Asclepiadaceae) (Smith *et al.,* 2008).

Generalist insects have the ability to exploit many different plants and, given the wide variety of plants that are on offer in most environments, this seems to be an advantage, because a generalist insect herbivore should always have ample food (Gaston and Lawton, 1990; Novotny and Basset, 2005; Novotny and Weiblen, 2005). There are however many poisonous species of plant that could be detrimental to generalists, and in most cases generalists have to feed on multiple plant species in order to avoid the toxic effects of any one particular plant (Dussourd and Denno, 1994; Mooney *et al.,* 2012). By specialising in a particular plant species, the specialist insect does not have access

to most of the food resources in the environment but they are safe from consuming poisonous plants. The disadvantage of generalists is that they feed on different plants and must be able to tolerate, detoxify or metabolize an array of various chemicals that might have deleterious effects (Fox and Morrow, 1981). Therefore, generalist insects have costs due to their adaptation to a broad range of different chemicals in host species, but are less efficient for any particular host as compared to specialists because they feed only on specific host plants (Keese, 1998). Evidence that being a specialist is a good evolutionary tactic comes from the massive abundance for specialist herbivorous insects compared with generalists (Novotny and Basset, 2000; Diniz *et al.*, 2011). It is this abundance of specialist herbivores that is taken advantage of for the purpose of biological control.

Phytophagous insects are divided into three different categories: polyphagous, oligophagous and monophagous. Polyphagous insects are insects that feed on plant from more than one family (Bernays and Chapman, 1994). Although polyphagous insects consume a broad variety of plants, they are selective and will not consume every plant they come across (Schoonhoven *et al.*, 2005). This is the case with the desert locust, *Schistocerca gregaria,* Forsk. (Acrididae), which feeds on over 400 plant species but still rejects some. The amount consumed of those 400 plant species also varies (Nevo, 1996). The aphid species, *Aphis fabae* Scop., is another example of polyphagous insect species found on 33 to 39 genera (Tosh *et al.*, 2003). Another example is the caterpillar of the moth, Egyptian cotton leaf worm, *Spodoptera littoralis* Boisduval. (Noctuidae), which consumes 100 or more plant species from 49 different families (Magd El-din and El-Gengaihi, 2000; Azab *et al.*, 2001). Polyphagous insects are therefore numerous and taxonomically diverse, but far less abundant and diverse than specialist (Novotny and Basset, 2000; Diniz *et al.*, 2011). Polyphagous species are not appropriate for biological control and would feed on many of the close relatives of any plant that they were collected off and must therefore be identified as unsuitable candidates during host specificity testing.

3.2 Host specificity testing

Introducing an insect into a new environment where it is not native can be problematic because the insect is also an alien (even if it is a biological control agent) and if proper research is not conducted, the alien insect could become invasive. For this reason, host specificity testing is always conducted before releasing insect as a biological control agent (Blossey, 1995a, 1995b;

Heard, 2000). Host specificity testing is carried out in order to evaluate and provide sufficient information on the insect's host range to demonstrate that it poses no threat to any crops, indigenous or socially important plants and has been a core component of all biological control programmes for invasive alien plants since the 1970's (Wapshere, 1974; McFadyen, 1998). Host specificity tests determine the risks that might be posed by a candidate agent on non-target plant species when released in the new environment (McEvoy, 1996). This is done by testing the ability of the potential agent to survive on non-target plants compared with the target plant. Parameters such as feeding damage, survival, fecundity and duration of development are measured on the target plant and non-target plants (Maw, 1976).

The design of host specificity testing has two categories: no-choice tests and choice tests (Heard, 2000). All the experiments are conducted in isolated cages and the agents are exposed to selected non-target plants and the target plant (Heard, 2000).

3.2.1 No choice tests

No-choice tests are when the potential agents are confined with a single plant species and must either feed on and develop on that plant species or die. This can allow researchers to exclude any non-host plants that the agents are completely incapable of feeding or surviving on (Syrett and Emberson, 1997). No-choice tests are said to determine the agents "fundamental host range" or "physiological host range" (van Klinken, 2000). The physiological host range is the list of species that the agent can survive on, while the realised host range is defined as the subset of the physiological host range which the agents will utilise under natural conditions (van Klinken, 2000). Many of the plants within the physiological (or fundamental) host range are suitable hosts for development of the potential agent, but are inferior hosts to the primary host plant and would never be utilised when the primary host plant is present. No-choice tests are therefore very conservative and often predict a much larger host range than the realised host range (Heard, 2000).

The host plant must not only be suitable for supporting development, but must also enable females to find in order to oviposit (van Klinken, 2000). In addition, ecological and behavioural barriers such as host-finding, host-acceptance cues, habitat preference, niche location and life cycles synchrony should be overcome (van Klinken, 2000). Plants within the physiological host range may support development of larvae or nymphs, but will never be utilised as hosts under field

conditions if the adult females will not oviposite on them. This emphasises the importance of selecting the correct stage of the life-cycle when conducting host specificity testing.

3.2.2 Choice test

The choice test is a method whereby the insects are given many tests plants including the target weed to feed or oviposit on. It is used to reduce the rejection of potential biological control agents that arise from no-choice tests which produce false positives (feeding on plants that they will not feed on after release) (Withers *et al.,* 2000). The choice tests are designed to represent semi-natural conditions whereby the agents are exposed to both target and non-target plants presented simultaneously in a single large enclosure and the level of preference and performance on each test plant is measured (van Lenteren *et al*., 2003).

The mobility of the potential agent, and the way in which the host plant is selected, should be taken into account when conducting choice tests (Sutton *et al*., 2017). For example, *A. fabae* locates its host plants first using volatile chemical cues, followed by chemo-tactile cues like surface chemicals (Kennedy *et al*., 1959). Plant volatiles (secondary compounds) which are often used by insects to identify their host plant could be found in high concentrations within a small cage and can cause insects to oviposite on the incorrect host plant. If the test plant species is within the plants physiological host range then the potential agent may develop on the plant, producing a false positive result (Sutton *et al*., 2017).

The plants species that the potential agent fed, oviposited, or completed its development on during no-choice tests are the test plant species that are usually the focus of choice-testing. Choice tests can be designed in two ways: standard tests and choice minus control tests. Standard tests are those in which two or more plants species, including the target weed (control), are presented to the insect to measure feeding or ovipositional preference between these plants (McFadyen, 1983; Dunn *et al.*, 1989; Buckingham *et al*., 1991; Forno *et al*., 1992; Edwards, 1999). In choice minus control tests, insects are given a series of plants species excluding the target weed (control) in one cage or environment to measure the suitability of different host (Heard and van Klinken, 1998; Heard, 2000). The preferred stage of the life-cycle to conduct choice test is the mobile stage and the stage in which the choices are made (Marohasy, 1998).

3.2.3 Field based host specificity testing

Laboratory conditions will always influence the test organisms, so the best way to determine the realised host range of a potential agent is through experiments in the field. If possible, open-field or multi-choice test experiments are conducted in the country of origin to determine the test plants' acceptability (Clement and Cristofaro, 1995). These experiments are uncaged and conducted outdoors in a natural stand of the targeted or in the garden where untargeted test plants are grown in pots plants and are placed among the targeted weed species (Briese *et al.*, 1995; Briese, 1999). The field test is not always conducted by researchers because it is difficult to do. It requires the importation of test plants in the country of origin and these plants might become invasive themselves. Surveying of related plant species in the native distribution can be used to determine the realized host range of biological control candidates (Paterson *et al.*, 2014a). If a candidate agent fed on a test plant species during choice and no-choice tests but does not feed on the species in its native range, then this is strong evidence that the test plant is within the physiological host range and not the realised host range.

3.2.4 False negatives and positives

False positives refer to when an agent attacks a plant in host specificity tests that it will not attack in the field. False negatives are produced when agents do not attack the plant species during the test but will attack the plant in the field (Marohasy, 1998; Withers *et al.*, 2000). An over estimation of the host range can result in rejecting potentially effective agents that would be safe for release and the consequence of this could be that a damaging invasive alien plant is not controlled and continues to impact indigenous biodiversity or agriculture. The latter could potentially lead to the release of an unsafe biological control agent which may have negative effects on the environment and consequences for the reputation of biological control as a whole (Downey and Paterson, 2016). False positives can occur in no-choice tests because an insect that is not exposed to a suitable host may eventually attempt to feed on an unsuitable host plant due to starvation. False positive can also occur in choice tests because plant volatiles from the primary host, which are cues for insect feeding, may build up in the cage, or be absorbed by non-target plants, which could result in the agent feeding on the incorrect test plant (Thiery and Visser, 1986; Withers and Barton, 1998; Van Driesche and Murray, 2004; Sutton *et al.*, 2017). It is essential that the likelihood and implications of false negative and false positive results are understood when interpreting host range data (Murray *et al.*, 2010).

3.2.5 Time dependency

Time-dependent effects can also affect insect host selection. Time-dependent change is the change in response in relation to the time elapsed since the insects last feed or since the most recent oviposition (Papaj and Rausher, 1983). As the time passes by, the insect's response will increase from completion of oviposition or feeding on lower ranked plant species in the physiological host range (Withers *et al.*, 2000; Barton-Browne and Withers, 2002). It is also possible that time-dependency could result in false negatives. This could happen if a potential agent has a secondary host plant that it survives on and will feed on under natural conditions but will generally prefer to feed on the primary host plant. It might take longer for the potential agent to feed on, or oviposit on, the secondary host and, if the agent is not exposed for long enough for this to happen, and then a false negative may occur.

3.2.6 Learning and experience

Learning can be defined as a change in behaviour caused by prior experience. Some phytophagous insects such as Lepidoptera, Coleoptera and Orthoptera are known to have a learning ability (Prokopy and Lewis, 1993). The consequences of experience (i.e. learning, memory and forgetting) are important behavioural factors in the process of selection of a host plant species. These consequences may result in behavioural changes through mechanisms like associative learning, sensitization and habituation (Heard, 2000). In host specificity testing, naive (unfed or first instar larvae) as well as experienced insects are often used (Maw, 1976). If the newly emerged larvae commence their feeding on a certain plant species, it is likely that those larvae will not accept other plant species that might be accepted by inexperienced larvae (Maw, 1976). To avoid false positive or false negative results due to experience and induced preferences, naive insects are preferred when conducting host specificity tests, although they might not always be readily available.

3.2.7 Test plant selection

Wapshere (1974) proposed a testing strategy called the centrifugal phylogenetic testing method. This technique is based on selecting test plants based on the taxonomic relatedness to the target plant. Insects cue into structures or chemicals in plants that are more likely to be shared by closely related plant species (Wapshere, 1974). The centrifugal phylogenetic testing method involves the

exposing of the insect to a series of test plant species from those that are the most closely related to ones that are more distantly related to the target plant, until the fundamental and realised host ranges have been circumscribed (Wapshere, 1974; Briese, 2003). Insect herbivores show a strong phylogenetic conservatism of host associations (Briese, 1996; Briese and Walker, 2002). This pattern of strong phylogenetic conservatism in diet suggests the non-target plants of greatest risk are those closely related to known hosts (Futuyma, 2000), and this has been validated by recent reviews of non-target attack by biological control agents (Briese and Walker, 2002; Paynter and Flanagan, 2004; Pemberton, 2000; Barton, 2004). The greatest evidence for the validity of this technique is probably the excellent track record of biological control of weeds since the centrifugal phylogenetic testing method was adopted (Suckling and Sforza, 2014).

Host specificity testing is therefore a widely accepted and well-studied scientific approach that ensures that biological control agents are safe for release. There is always an innate risk in biological control but the risks are minimised through host specificity testing (Paynter *et al.*, 2015). The risks should be considered, in the form of a risk assessment, which considers both the risk of releasing the agent, as well as the risk of doing nothing and allowing the target weed to proliferate (Shaw *et al.*, 2011; Downey and Paterson, 2016).

In this study, we examined the host range of *P. brasilensis* the new candidate biological control agent for *P. aculeata*. The main purpose of this study was to determine if *P. brasiliensis* is a suitable candidate biological agent for the control of *P. aculeata* in South Africa by evaluating the host range of the potential agent through host specificity testing. Little was known about the specificity of this insect prior to this study, but observations of closely related plants during surveys in the native range of Santa Catarina, Brazil, suggested that the host range was likely to be restricted, making this a good candidate for further host specificity testing (Paterson *et al.*, 2014a).

3.3 Materials and methods

3.3.1 Insect culture

Pereskiophaga brasiliensis was cultured in quarantine at Rhodes University, Grahamstown, South Africa. Adults were reared in 2 litre plastic containers. Paper towels were used to line the containers so that excess water was absorbed. One large woody stem (16cm x 1.5cm), and two shoots of *P. aculeata* were placed in moist florist's foam, and put in each container. The two shoots were put in to feed the adults, which feed on the leaves and green stems, and the thick woody stems

were for female oviposition and for larvae to feed and develop inside. Five adult weevils were placed inside each container to feed and oviposit for a week. The adults were then moved to a new container. The shoots were changed every week and discarded while the old stems were kept in plastic jars sealed with plastic film to maintain high humidity. The old stems were kept for six weeks in order to allow the larvae within the stems to develop to the adult stage.

3.3.2 Test plants

All the test plants were grown at the Waainek Research Facility at Rhodes University, Grahamstown, Eastern Cape, South Africa prior to being moved to quarantine. *Pereskia aculeata* plants were collected from cuttings of plants that grow naturally in the field around Grahamstown. Test plants were obtained from local nursery stock and others were collected in the field from wild populations. The plants were transplanted into pots and grown until they reached the required size for host specificity testing in a green house.

3.3.3 Host specificity testing

3.3.3.1. Test-plant selection

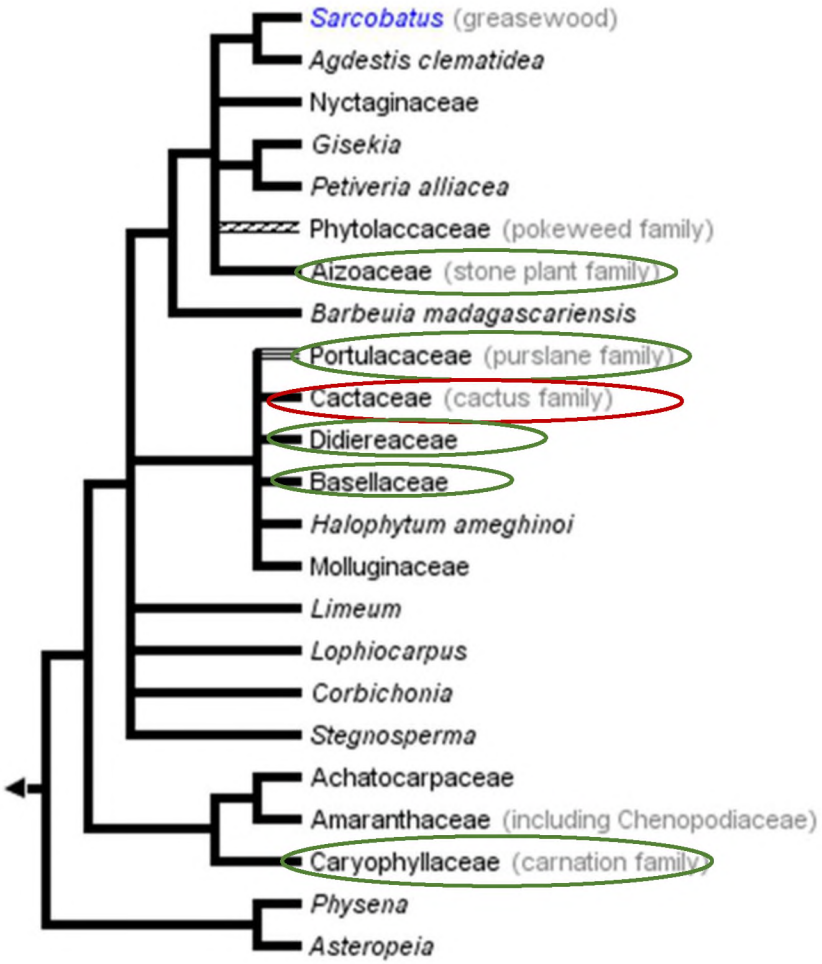

Fig 3.1 A phylogeny of the closely related families to the Cactaceae (From Cuenoud *et al.*, 2002). The circled families represent those tested during host-specificity trials.

Test plant species were selected according to the phylogenetic centrifugal method (Wasphere, 1974; Briese, 2003). The plant families Portulacaceae, Basellaceae and Didiriaceae are the closest related families to Cactaceae (Cuenoud *et al*., 2002; Downie *et al*., 1997) (Fig 3.1). There were 29 plant species tested. The majority of plants (69%) were from the families: Cactaceae, Portulacaceae, Basellaceae and Didieraceae. *Opuntia ficus-indica* and *Hylocerues undatus* are the only Cactaceous crop species found in South Africa and have economic importance in the country and were therefore included in testing (Brutsch and Zimmermann, 1993; Le Bellec *et al*., 2006).

Rhipsalis baccifera is the only known native cactus species in South Africa and hence it was included in the test plants species list. There are 51 species and 6 genera of Portulacaceae in southern Africa (Germishuizen and Meyer, 2003). All the species selected from Portulacaceae were native in South Africa except for *Talinum panucilatum* (Jacq.) Gaertn, (Germishuizen and Meyer, 2003). *Basella paniculata* Volkens is the only native species of Basellaceae in South Africa and was therefore also considered an important test plant species (Germishuizen and Meyer, 2003). Another member of this family, *Anredera cordifolia* (Tenore) Steenis (Basellaceae) is an invasive alien species in South Africa that is native to South America (Van der Westhuizen, 2011). This species was included because of the close taxonomic and phylogenetic relationship between the Basellaceae and the Cactaceae (Cuenoud *et al*., 2002; Downie *et al*., 1997). *Basella alba* L and *Basella rubra* L. are crop species within the family Basellaceae. *Basella rubra,* also known as red vine spinach, and *B. alba,* also known as Malabar spinach, are commonly used from Kenya through Uganda to Democratic Republic of Congo and West Africa as vegetables (Okulungu, 2003). Neither species is commonly utilised in southern Africa, but biological control agents could travel across borders and feeding or development outside of the family of the target plant would be important information on whether the plants are utilised in introduced range or not. The Didieriaceae is a family endemic to Madagascar and hence there are no indigenous species present in South Africa (Erbar and Leins, 2006). The species selected from this family, *Alluaudia procera* Drake is occasionally used as a garden plant in South Africa. Other more distantly related plant species (Order Saxifragales (Family: Crassulaceae), Order Asparagales (Family: Aspholdolaceae) and Order Malpighiales (Family: Euphorbiaceae)) with the same distribution and similar growth forms, such as indigenous succulent plant species, were also selected to represent the more distantly related relatives.

3.3.3.2 Adult no-choice testing (short-term exposure)

Each of the potted control and test plants were put in separate 1.2 m x .06 m x 0.6 m insect cages in quarantine. Two *P. aculeata* (control) plants were present at all times. Two female *P. brasiliensis* that had been left with males in order to mate were put on each test plant species as well as *P. aculeata* controls for a two week period. A two-week interval was selected to measure feeding and oviposition suitability based on the fact that in preliminary experiments the females generally oviposited over this period of exposure to *P. aculeata*. A fine mist spray of water was applied to each plant daily and plants were watered whenever required. It was not possible to have

the same water regime for all test plant species because some species required lots of water and would die without ample watering while others required very little water because they are from drier environments. After two weeks, the adult weevils were taken off the test plants and controls and the plants were kept for two weeks to allow the development of larvae. Any adult *P. brasiliensis* that died during this period were replaced on a daily basis. The two-week interval was chosen because it was assumed that the larvae would have started to develop by that time and would therefore be more visible than eggs. Five replications were conducted for all the test plants species including controls (*P. aculeata*). The plants were then dissected and examined under a microscope. The number of adult feeding scars, eggs and larvae on each plant were then recorded.

3.3.3.3 Adult no-choice testing (long-term exposure)

In most cases, mated female *P. brasiliensis* would oviposit on *P. aculeata* within a two week period so this was selected as a suitable period of exposure for the majority of test plant species. This method allowed for many species to be tested within a limited space in quarantine over a reasonable timeframe but there are, however, issues related to time-dependency (see 2.3.3 for more details). For example, if one of the test plant species is a suitable host for development but is less preferred than *P. aculeata* it is possible that oviposition will be delayed on the test plant species. This could result in false negatives, as the agent may be capable of development on one of the test plant species but did not oviposit because of the limited exposure time to the species. For this reason, some of the test plant species were selected for a longer period of exposure to the adult *P. brasiliensis*. The closest relative of *P. aculeata*, *P. grandifolia*, a representative of the Basellacaeae, *A. cordifolia*, and a representative of the Portulacaceae, *Portulacaria afra* Jacq., were selected for a trial where two *P. brasiliensis* adults were exposed to the test plants and controls for a period of 12 weeks. Five replicates of each of these plants were conducted for this trial along with five *P. aculeata* plants which were included as controls. Feeding scars from adult feeding, the number of eggs, larvae, pupae and adults were counted after the three-month period by dissecting the plants.

A further trial with a slightly shorter exposure period of 6 weeks but with five mated female *P. brasiliensis* was also conducted. Plants used in this experiment were included three replicates of the native cactus *R. baccifera*, two of the native Bacellaceae, *B. paniculata*, five of the native Portulacaeae, *P. afra*; as well as a single replicate of the close relative of *P. aculeata, Pereskia quisqueyana* and two replicates of *P. grandifolia,* and four *P. aculeata* controls. The uneven and sometimes low number of replicates for each species in this trial was due to the fact that some of the plant species are rare and difficult to grow. One important additional step for this experiment was that some of the larger larvae from test plant species that supported larval development were removed and reared in cut stems of the same test plant species to determine whether the test plant could support full development to the adult stage. There was not enough test plant material to keep all the larvae to the adult stage, so a few representative larvae were kept until they eclosed as adults. It was therefore not possible to compare proportions of adults from each plant species, but whether full development was possible was assessed.

3.3.3.4 Larval no-choice testing (one week interval)

Eight plant species were selected for larval no-choice testing from the Cactaceae, one species from the Portulacaceae, two species from the Basellaceae and one species from the Crassulaceae (Table. 3.4). Three 0.2- 0.3 cm holes were bored into different shoots or branches of each test plant using a drill with a small pin roller size. A second instar larva, taken from the culture which was maintained on *P. aculeata* cut stems, was placed inside each hole, so three larvae were placed on each test plant species. Second instar larvae were used because in trial experiments nearly all first instar larvae died during the process of transferring them to a new plant. The holes were covered with parafilm to prevent the insect escaping. The larvae were left for a week and then the plants were dissected. Mortality and frass (evidence of feeding) in the stems were recorded.

3.3.4 Statistical analysis

Data were analysed by calculating the means and standard errors of the parameters measured for each test plant species. The number of feeding scars on the different test plant species was analysed using a Kruskal-Wallis ANOVA because data were not normally distributed. All statistics were conducted in STATISTICA ver. 11.

3.4 Results

3.4.1 Adult no-choice testing (short-term exposure)

Feeding scars were only recorded on *P. aculeata*, *P. grandifolia*, *Portulacaria afra* and *Basella rubra* (Table 3.1). There were no signs of feeding or damage on the remaining test plants species other than these four species. Feeding damage was significantly less on *Pereskia grandifolia* (25 ±10.54), *Portulaca afra* (0.6 ±0.6) and *Basella rubra* (2.4 ±1.75) than that recorded on *Pereskia aculeata* controls (53.42 ±2.15) (Fig 3.2). *Pereskia grandifolia* is the closest relative and had the highest damage besides the target plant while very limited feeding was found on the two more distantly related species. Feeding by *P. brasiliensis* was significantly higher on *P. aculeata* than on *P. grandifolia, P. afra* and *B. rubra* (p= 0.00002). There was also a significant difference between *P. grandifolia* and other test plant species (*P. afra* and *B.rubra*) but no significant difference between *P. afra* and *B. rubra* (Fig 3.2).

Pereskiophaga brasiliensis oviposited on most of the *P. aculeata* controls (2.87 ±0.86 eggs) and there was only a single case where there were no eggs or larvae on a control. A single egg was oviposited on *R. cerescula* (0.2 ±0.2) but the egg was dead and desiccated when the plants were dissected and the larva had not hatched successfully (Table 3.1). No eggs were found on other test plants. Larvae were only found on *P. aculeata* controls (5.38 ±0.33), none were found on the other test plant species (Table 3.1). There was evidence of frass and mines meaning the larvae were feeding on *P.aculeata*.

Table 3.1 The average number of feeding scars, eggs and larvae by *P. brasiliensis* on various test plant species and *P. aculeata* controls after a two week period of exposure to two mated female weevils.

TEST PLANTS SPECIES (n) Native/Alien	**MEAN(±SE) FEEDING SCARS**	**MEAN(±SE) EGGS**	**MEAN(±SE) LARVAE**
Order Caryophyllales			
Cactaceae			
Pereskia acuelata (70) A	53.42 (±2.15)	2.87 (±0.86)	5.38 (±0.33)
Pereskia grandifolia (5) A	25(±10.54)	0	0
Pereskia quesquenyana (5) A	0	0	0

Rhipsalis baccifera (5) N	0	0	0
Rhipsalis cereuscula (5) A	0	0.2 (±0.2)	0
Rhipsalis sp. (5) A	0	0	0
Hylocereus undata (5) A	0	0	0
Opuntia aurantiaca (5) A	0	0	0
Opuntia ficus-indica (5) A	0	0	0
Portulacaceae			
Talinum caffrum (5) A	0	0	0
Talinum paniculata (5) N	0	0	0
Portulaca oleracea (5) N	0	0	0
Portulacaria afra (5) N	0.6(±0.6)	0	0
Anacampseros arachnoides (5) N	0	0	0
Anacampseros telephiastrum (5) N	0	0	0
Baselleceae			
Anredera cordifolia (5) A	0	0	0
Basella alba (5) A	0	0	0
Basella paniculata (5) N	0	0	0
Basella rubra (5) A	2.4 (±1.75)	0	0
Didieraceae			
Alluadia procera (5) A	0	0	0
Mesembryanthemaceae			
Carpobrotus deliciosus (5) N	0	0	0
Delasperma cooperi (5) N	0	0	0
Fauacaria tigrina (5) N	0	0	0
Glottiphyllum regium (5) N	0	0	0
Caryophyllaceae	0	0	0
Silene primuliflora (5) N	0	0	0
Order Saxifragales			
Crassulaceae	0	0	0
Crassula ovata (5) N	0	0	0

Cotlydon orbiculata (5) N	0	0	0
Order Asparagales			
Aloe arborescens (5) N	0	0	0
Order Malpighiales			
Euphorbia tirucalli (5) N	0	0	0

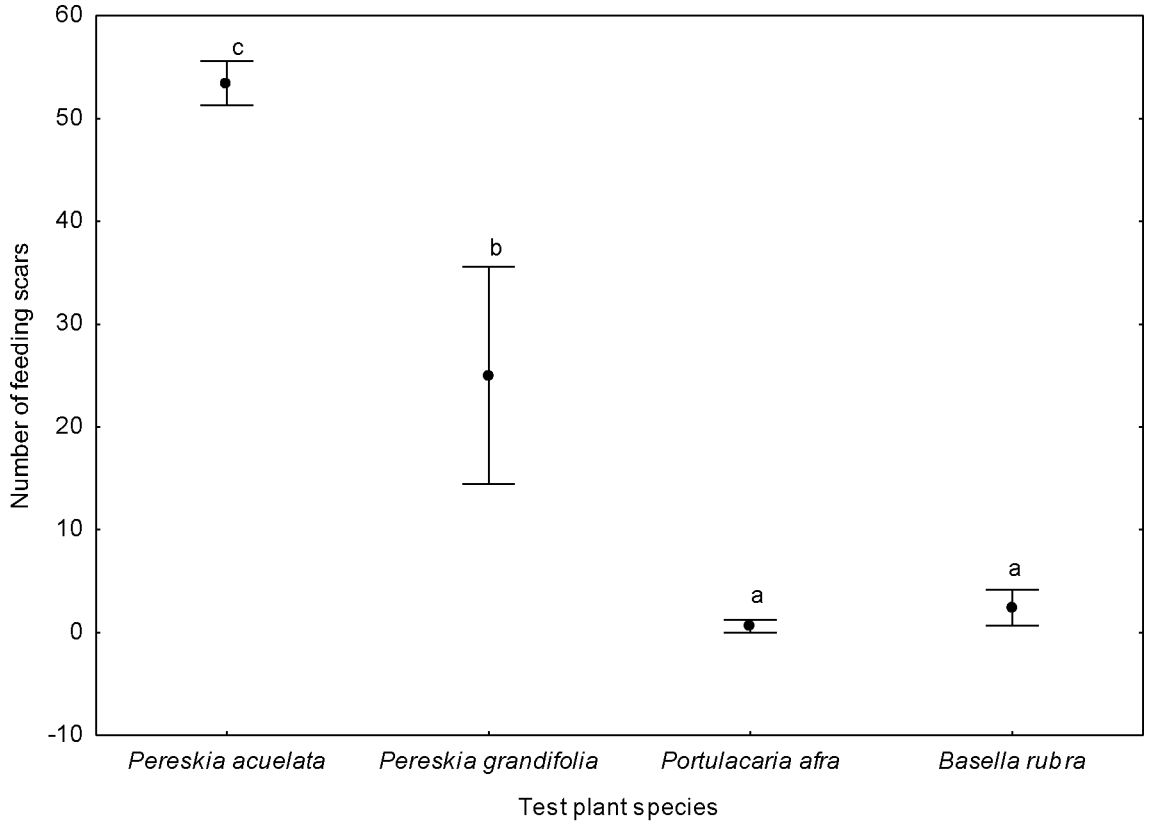

Fig 3.2 Feeding scars by adult *P. brasiliensis* on *P. aculeata* (control) and three test plant species. Different letters show significant differences (Kruskal-Wallis non-parametric ANOVA). Standard errors are represented by error bars (f = 26.6; $p<0.05$). Test plants that had no feeding recorded were not included in this graphic.

3.4.2 Adult no-choice tests (long-term exposure)

In the long-term exposure experiment over a 12-week interval, *P. brasiliensis* adults fed on *P. aculeata* (control), *P. grandifolia* and *A. cordifolia. Pereskia aculeata* had more feeding scars

(87.3 ±8.6) as compared to *P. grandifolia* (29 ±4.2) *and A. cordifolia* (37.2 ±0.91) (Table. 3.2). Out of the three plants species that had feeding scars, only two had larvae and eggs, these were *A. cordifolia* and the *P. aculeata* controls. *Pereskia aculeata* had more eggs per plant (5.3 ±2.7) than *A. cordifolia* had (1.5 ±0.66). The first instar larvae were found just underneath the bark of the stem, while second instar larvae were found continuously hollowing out its centre and travelling inside the stem in both directions in both *P. aculeata* and *A. cordifolia*. *Pereskia aculeata* had more larvae (4.3 ±0.4) than *A. cordifolia* (2.07 ±0.93) (Table 3.2). All the larvae found were alive and healthy, mining and destroying the inside of the stem on both plant species.

Table 3.2 The number of feeding scars, eggs and larvae after a longer-term exposure of two female *P. brasiliensis* for a 12-week period

Test Species (n) Native/Invasive	**Family**	**Feeding scars**	**Eggs**	**Larvae**
Pereskia aculeata (3) A	Cactaceae	87.3 (±8.64)	5.3 (±2.7)	4.3 (±0.4)
Pereskia grandifolia (5) A	Cactaceae	29 (±4.25)	0	1 (±0.89)
Portulacaria afra (5) N	Portulacaceae	0	0	0
Anredera cordifolia (5) A	Basellaceae	37.2 (±0.91)	1.5 (±0.66)	2.07 (±0.93)

The long-term exposure experiment over a 6-week interval using five mated *P. brasiliensis* females also resulted in non-target damage to members of the Cactaceae and Basellaceae (Table 3.3). In this experiment, the highest number of average feeding scars was on *P. grandifolia* rather than *P. aculeata*, although both species had very high numbers of feeding scars and there was a significant difference between *P. grandifolia* and other plants. The reason why *P. grandifolia* had such high numbers of feeding scars is probably due to the very young leaves growing on both plants used in these two replicates. No larvae or signs of oviposition were found on *P. grandifolia*. There were very few scars on the slightly more distantly related *P. quisqueyana* and also no oviposition or larval development. The indigenous cactus, *R. baccifera*, was not fed on by the adult weevils but was suitable for oviposition and for development of some larvae to the adult stage. The number of larvae found in *R. baccifera* was on average very similar to that on *P. aculeata*

(Table 3.3). *Basella paniculata,* the indigenous representative of the Basellaceae, was also fed on by adults, and even had a slightly greater number of feeding scars than the target plant (Table 3.3). *Basella paniculata* was also suitable for development, with 5 and 7 larvae being found in the two replicates, and these larvae did continue development to the adult stage. No feeding scars were recorded on *P. afra* and there were no signs of oviposition or larval development.

Table 3.3 The number of feeding scars from adult weevils, the number of larvae found developing in each test plant species and whether the larvae could develop fully to the adult stage after a 6-week period of exposure to five mated female *P. brasiliensis*.

Test species (n) Native/Alien	**Family**	**Feeding scars (± S.E.)**	**Larvae (± S.E.)**	**Development to adult**
Pereskia aculeata (control) (3) A	Cactaceae	240.5 (±40.41)	7.0 (±3.7)	YES
Pereskia grandifolia (2) A	Cactaceae	302.5 (±45.5)	0	NO
Pereskia quisqueyana (1) A	Cactaceae	14	0	NO
Rhipsalis baccifera (3) N	Cactaceae	0	6.6 (±5.7)	YES
Basella paniculata (2) N	Basellaceae	254 (±4.0)	6.00 (±1.0)	YES
Portulacaria afra (5) N	Portulacaceae	0	0	NO

3.4.3 Larval no-choice testing (one week interval)

After a week interval, the larvae survived on *P. aculeata* and one species of invasive cactus *Hylocerues undatus* (Haworth) Britton and Rose. No larvae survived on any of the other 10 test plants species. The survival on *P.aculeata* was (97.78%) and a single larva was recovered on *H. undatus* (6.67%) (Table 3.4). No mines or frass were found on other test plants except on *P. aculeata.* The presence of frass indicates that the larvae were feeding on *P. aculeata* and not on *H. undatus.* The single larva found on *H. undatus* was placed back in the same plant and re-examined after a second week when it was found dead and there was still no sign of the larva feeding on the test plant species.

Table 3.4 The percentage mortality and signs of feeding of *P. brasiliensis* larvae on control (*P. aculeata*) and 12 test plant species.

TEST PLANTS (n) **Native/ Alien**	**FAMILY**	**% SURVIVAL**	**FEEDING**
Pereskia aculeata (12) A	Cactaceae	97.78	+
Pereskia grandifolia (3) A	Cactaceae	0	_
Pereskia quesquayana (3) A	Cactaceae	0	_
Rhipsalis baccifera (3) N	Cactaceae	0	_
Rhipsalis cereuscula (3) A	Cactaceae	0	_
Hylocereus undatus (5) A	Cactaceae	6.76	_
Opuntia aurantiaca (3) A	Cactaceae	0	_
Opuntia ficus- indica (3) A	Cactaceae	0	_
Portulacaria afra (3) N	Portulacaceae	0	_
Anredera cordifolia (7) A	Basellaceae	0	_
Basella paniculata (3) N	Basellaceae	0	_
Crassula ovata (3) N	Crassulaceae	0	_

3.5 Discussion

The findings of this study suggest that the candidate biological control agent *P. brasiliensis* may not be suitably host specific to the target weed, *P. aculeata* for release in South Africa. If the results of adult no-choice tests for a two-week interval were the only information that could be used to determine the specificity of this insect, then it is likely that a decision that the species is host specific and safe for release would have been made. Although there were feeding scars found on the most closely related test plant species, *P. grandifolia,* in the two week trials, this would not be problematic in South Africa as it is a declared weed (Proclamation R.35, 1979) despite the fact that it was omitted from the NEMBA regulations (Department of Environmental Affairs 2016), so the limited feeding on this species would not have negative economic or environmental impact in the country. It was expected that *P. brasiliensis* would feed and develop on *P. grandifolia* as it is very closely related and has a similar internal structure within the stems. This is however based on visual observations and not a detailed analysis of the components that make up the internal parts of the stem. It is possible that there are fundamental differences to the stem structure that make *P. grandifolia* an unsuitable host for *P. brasiliensis.* The overall plant architecture is very different, with *P. grandifolia* being a tree with a single large trunk and *P. aculeata* being a scrambling vine, so some differences in the internal structure of the stems could be expected (Leuenberger, 1986).

Only three feeding scars were recorded on *P. afra* on a single leaf during the second week of the experiment but in the other four replicates, no feeding scars were found. This may be due to insect starvation or a need for water. The weevil is clearly incapable of feeding on *P. afra* as confirmed by the other two adult host specificity testing trials and the larval development trials. Similarly, *B. rubra,* which also had some exploratory feeding scars, is also not native in South Africa, but is a crop species grown in other parts of Africa. Based on the preliminary feeding from the trial with a short duration of exposure there is no threat to the *B. rubra* industries in Africa, but feeding of larvae in closely related species during the long-term exposure trials is concerning.

In the short-term exposure trial larvae were only found on *P. aculeata* and eggs deposited were significantly higher on *P. aculeata* than other plant speices, with only one dead egg being found on *R. cereuscula*. *Rhipsalis cereuscula* is also in the family Cactaceae but is not native in South Africa. So again, based on the short-term exposure alone, this non-target oviposition would be

considered an artefact of the no-choice conditions and not a concern in terms of the safety of the insect. In addition to these data, *R. cereuscula*, was included in field based host specificity tesing in Brazil. *Pereskia aculeata* and *R. cereuscula* were found growing together at a site in Santa Catarina, Brazil, and *P. brasiliensis* was found to be present on *P. aculeata* and not *R. cereuscula* after equal numbers of both species were dissected (Paterson *et al.* 2014a).

The results of the longer-term exposure experiments were contradictory to those of the short-term exposure experiments in that *P. brasiliensis* was not monophagous and could feed and develop on a few species in both the Cactaceae and Basellaceae. This highlights the importance of considering the impact of time dependant feeding when interpreting host specificity data (Barton-Browne and Withers, 2002). It is very surprising that the pattern of utilisation in this experiment did not follow the phylogenetic relationship of the test plant species (Briese, 2005). The closest relatives of the target weed, those within the genus *Pereskia*, were consistently poor hosts, but the native cactus species *R. baccifera* supported development, and even more surprisingly, two members of the Basellaceae supported development, incluing the native *B. paniculata*.

The contrasting results from the short and long-term exposure experiments could be due to time dependant changes due to *P. brasiliensis* not being exposed to its primary host plant (Barton-Browne and Withers, 2002). Hence, the insect fed and oviposited on *R. baccifera, A. cordifolia* and *B. paniculata* only after a period of at least two weeks without its primary host plant, *P. aculeata*. *Pereskia aculeata* is clearly the primary host plant for this species based on adult feeding damage and the larger number of larvae and egges found on *P. aculeata* compared to any of the other test plant species in all trials. After a period of time without the primary host plant, *P. brasiliensis* will accept less suitable hosts, such as other members of the Cactaceae and Basellaceae.

The feeding on *A. cordifolia* in the 12 weeks of exposure trial was considered problematic because, although *A. cordifolia* is a problematic invasive alien species that has even been targeted for biological control in South Africa (Van der Westhuizen, 2011), it is in a different family, which suggested that the host range of the insect was much more broad than the results of the short-term exposure experiment had indicated. This prompted further testing on the only indigenous members of the families Basellaceae and Cactacaeae, which proved that the two indigenous species, *R. baccifera* and *B. paniculata*, were suitable hosts for development of the larvae to the adult stage.

In addition to this, larvae of *P. brasilensis* have recently been recorded on *A. cordifolia* under field conditions in the native range (Prof. Vitorino; University of Blumenau; Brazil). The two indigenous species could be threatened if *P. brasiliensis* were released in South Africa. Both grow in similar environments and have overlapping distributions to *P. aculeata* in South Africa (Germishuizen and Meyer, 2003). Both species are the sole indigenous representatives of their families in South Africa's flora (Germishuizen and Meyer, 2003) and should therefore be considered an important part of South Africa's native biodiversity.

The larval no-choice experiments also contradicted the results of oviposition trials to some extent. Larvae are clearly capable of development on at least one other species in the family Cactaceae and at least two memebers of the Basellaceae, so one would expect larvae that were transferred to these species to survive after transferal. Seven replicates, which amounts to 21 individual weevils, that hatched and began development on *P. aculeata* died as soon as they were transferred to *A. cordifolia* but those that were transferred to *P. aculeata* all survived. This could be explained by changes to the insect's ability to feed on a plant due to learning or experience (Marohasy, 1998; Heard, 2000). The larvae of the weevil cannot be transferred to other species of host plant after feeding on the primary host, *P. aculeata*. This hypothesis is supported by the fact that larvae that developed in *R. baccifera* and *B. paniculata* in the long-term host specificity expeiments could be transferred back into cut stems of the same host plant and complete their development.

The fundamental host range is usually determined by no-choice tests (Syrett and Emberson, 1997). In this study, only no-choice tests were conducted, so it is not possible to determine with any certainty whether the non-target plants in the Cactaceae and Basellaceae would be included in the weevils realised or field host range. Paired choice tests, multiple choice tests and field host range are used for determining the field or realised host range of an insect (Syrett and Emberson, 1997; Pratt *et al.,* 2009). Conducting paired choice and multiple choice tests should be prioritised, but at present *P. brasiliensis* should be considered too oligophagous for release in South Africa.. Oligophagous biological control agents, such as *C. cactorum*, have been released for the control of cactus weeds in South Africa in the past (Paterson *et al.*, 2011a) but these are all at least restricted to the level of family. Non-target feeding on an indigenous species from a different family suggests that the host range of the agent is too broad for the purposes of biological control.

The fact that *P. brasiliensis* developed on plants in a different family to the target weed but could not develop on the closest relatives within the genus *Pereskia* is very surprising because insects are more likely to survive on closely related plants than plants distantly related to the target weed (Briese, 2005). *Pereskia* is a basal clade of the Cactaceae (Edwards *et al.* 2005) and species within this genus are therefore likely to share characteristics with closely related families, such as the Basellaceae, that may not necessarily be present in other members of the Cactaceae. This could explain why *P. brasiliensis* could develop on two members of the Basellaceae but not other cactus species, but it does not explain why it could not develop on other *Pereskia* species but could on the rather distantly related indigenous cactus, *R. baccifera*.

It is possible that morphology of the test plant species could be important in explaining these results. *Pereskiophaga brasiliensis* may be oligophagous, feeding on species of Cactacaea and Basellaceae but the four species that could support development in this study (*P. aculeata, R. baccifera, A. cordifolia* and *B. paniculata*) could be the only species that were tested that are suitable in terms of their morphology. Although this is a possible explanation, there are no specific morphological characters that clearly link the four plants that are suitable for development.

Pereskiophaga brasiliensis may be host specific in terms of its field or realised host range but it includes plants from other families in its fundamental or physiological host range. The fact that some of these plants, which can support the full development of the weevil, are quite distantly related, being in another family from the target weed, and because two of the plants that supported development are indigenous plants in South Africa, suggest that *P. brasiliensis* should not be released in South Africa at present due to the risk of non-target effects to indigenous plants in the country. Further testing under paired-choice and multiple choice conditions should be conducted in order to deterime the realised host range of the agent.

4 Chapter 4: General discussion

4.1 Introduction

The main focus of this study was to determine if *P. brasiliensis* is a suitable candidate for biological control of *P. aculeata* in South Africa. This was done by conducting impact studies to determine if it was suitably damaging to warrant release (Chapter 2) and then conducting host-specificity testing to determine whether it was suitably host specific (Chapter 3).

Impact studies are essential components of biological control programmes because only agents that are likely to be sufficiently damaging should be released. This reduces the chances of releasing ineffective agents, which should be avoided whenever possible (McClay and Balciunas, 2005). The impact of *P. brasiliensis* under quarantine conditions indicated that the agent is sufficiently damaging to warrant release in South Africa as it significantly reduced the number of leaves and shoots, as well as the shoot length of the target plant at reasonably low insect densities (Chapter 2). This impact data, in combination with the genetic matching (Paterson *et al*., 2009) and climatic matching data (Paterson *et al*., 2014a), suggest that if *P. brasiliensis* were to be released in South Africa it could effectively reduce *P. aculeata* densities.

Host specificity testing indicated that *P. brasiliensis* was unfortunately not suitably specific for release in South Africa. The fundamental host range, as determined in no-choice testing, included two indigenous species and two species (one indigenous and one alien) from another closely related family, the Basellaceae. The insect's ability to survive on plants outside of the family Cactaceae indicates that its host range is too broad to warrant release in South Africa. Although this is disappointing, it must be stressed that stringent host specificity testing is what has resulted in the excellent safety record of biological control of weeds worldwide (Suckling and Sforza, 2014). The rejection of an unsafe agent should therefore be seen as a success rather than a failure.

In this chapter, the general implications of this study to the field of biological control and host specificity in particular are discussed. This is followed by the more specific implications of the

study to the biological control program against *P. aculeata* and future research for the control of *P. aculeata* in South Africa.

4.2 Host specificity testing in biological control of weeds

Conducting host specificity testing and evaluating the impact of an agent on the targeted weed is important in biological control of invasive species. While impact studies can reduce the chances of releasing ineffective agents, host specificity testing plays a much more important role in ensuring that agents that are released are safe and will not feed on indigenous species and commercially important plants. The safety record of biological control of weeds on a worldwide scale is excellent, with no significant negative impacts to non-target species that were not predicted in host specificity testing (Suckling and Sforza, 2014). The safety record is much greater than that of biological control of insects because rigorous host specificity testing is conducted for all weed biological control agents (Downey and Paterson, 2016). The safety record in South Africa is also very high, with over 100 agents being released in the country over a period of more than 100 years and no non-target impacts being recorded (Klein, 2011; Zachariades *et al.*, 2017). On a worldwide scale, over 400 agents have been released spanning over 100 years and the only significant non-target effects that have ever been recorded were clearly predicted by host specificity testing (Suckling and Sfroza, 2014; Downey and Paterson, 2016). It is essential that this excellent safety record is maintained because any non-target impacts from a biological control agent could jeopardise the entire science and practice of biological control of weeds globally. There is pressure on researchers in biological control of weeds to release agents because funders desire quick and effective control of invasive alien plants, but the integrity of the science and practice is much more important and should not be compromised. The rejection of agents that are not safe for release should be regarded as a success as this practice maintains the safety of biological control.

In order to accurately determine the host range of a biological control agent it is important to take the mechanisms by which the agent selects its' host plant into account (Sutton *et al.* 2017). Understanding the mechanisms used to select host plants can help interpret host specificity testing data and can reduce false negative and false positive results (Sutton *et al.* 2017). Learning and time-dependency should also always be taken into account when conducting host specificity studies (Barton-Browne and Withers, 2002; Marohasy, 1998; Heard, 2000). In this study, the data

from host specificity testing with a short exposure period to the test plant species suggested that *P. brasiliensis* would be suitably specific for release in South Africa but this changed with a greater period of exposure (Chapter 3). If time-dependent changes to the host range were not considered a possibility, then a trial with longer exposure periods may not have been conducted, and an agent that is likely to have non-target impacts on indigenous plant species may have been released. This would not necessarily have been a trivial impact to indigenous plant species because *R. baccifera* was significantly damaged in some of the host specificity trials and is known to grow sympatrically with *P. aculeata* at many sites in the country (*Pers. Obs.*). Learning and behaviour is also an important aspect to consider in host specificity testing (Marohasy, 1998; Heard, 2000). In this study, larvae that had fed on *P. aculeata* could not be transferred successfully to other host plants despite the fact the some of those test plant species were suitable hosts for development. This was most likely due to past experience consuming a different host plant (Chapter 3).

In order to avoid the false negative results that could occur due to time-dependent changes to a potential agent's host ranges, researchers should allow test insects to be exposed to the test plants and controls until the insects die. This would prolong testing considerably and might not be logistically feasible in terms of time, quarantine space. Also no-choice tests like these creates abnormal situations in a cages and the longer these continue, the more likely the possibility of contradictory results. Some potential agents have a very long lifespan and could live for many years, even on plants that are not suitable for larval development. The life span of *P. brasiliensis* is not known, but some individual adult weevils have lived for well over a year in the quaratine facility (*Pers. Obs.*). So leaving *P. aculeata* on the test plant species until the adults died would have been impractical. Similarly, false negatives due to learning or behavioural changes could be reduced by not exposing the individual insects that are tested to any other host plants before testing. This would not have been possible for *P. brasiliensis* because eggs cannot be extracted from the oviposition sites without breaking them, so larvae, which start feeding immediately after hatching, had to be used in larval no-choice tests.

4.3 Implications for the biological control of *Pereskia aculeata*

The rejection of *P. brasiliensis* as a biological control agent against *P. aculeata* would be a setback to the biological control programme for this damaging environmental weed. The insect is very

damaging to the target weed and its mode of damage is different from the two agents that have already been introduced to South Africa, the leaf feeding *P. guerini* and the shoot-tip feeding *C. schaffneri* (Paterson *et al.*, 2014a,b). The combination of the two agents that feed on the green parts of the plant and a stem miner that feeds on the old, woody stems, would be very damaging in combination. Further testing using paired and multiple choice tests will be conducted, but at present it is evident that *P. brasiliensis* is not suitably specific for release in South Africa. Given the recent discovery of *P. brasiliensis* larvae in *A. cordifolia* in Brazil under field conditions, it seems very unlikely that paired or multiple choice tests in quarantine will not indicate that the agent feeds on members of the Basellaceae. The biological control programme must therefore either rely on the two biological control agents that have already been released or another agent should be considered.

Post-release evaluations of *P. guerini* has indicated that the agent is only damaging at a very limited number of sites in the country, and even at those sites, the damage is not suffcicent to completely control the weed (Mnqeta, 2017). *Phenrica guerini* may play a role in the future of the biological control programme, but it is very unlikely to ever result in full control of *P. aculeata*. *Catorhintha schaffneri* was only released in 2014 and it is therefore too soon to evaluate its impact in the field (Paterson *et al.*, 2014b). Even if *C. schaffneri* is extremely damaging, it would take many years of defoliating *P. aculeata* plants before the woody stems die. So even if *C. schaffneri* is very effective, an additional agent is desirable. There are a number of new agents that could be considered, and their mode of damage and the potential for them to interact synergistically with *C. schaffneri* should be considered the most important criteria on which they are selected.

Maracayia chlorisalis has been imported into South Africa in the past but is currently shelved as a biological control agent due difficulties with rearing the insect in quarantine (Klein, 2011). The mode of damage of *M. chlorisalis* is unlikely to be fully compatible with the damage by *C. schaffneri* because the same plant part is targeted by both insects. *Maracayia chlorisalis* larvae develop in the young shoots of *P. aculeata* and *C. schaffneri* also feeds on young shoots. Despite the different modes of damage, the same plant part is targeted and the insects are likely to have an antagonistic relationship.

The majority of the other natural enemies of *P. aculeata* that have been identified in the native range are not suitable candiates for biological control because of evidence of broad host ranges, or

the limited amount of damage inflicted on the plant (Table 1,1) (Paterson *et al.,* 2014a; Chapter 1). *Acanthodoxus machacalis* (Cerambycidae) and *Asphondylia sp.* (Cecidomyidae) are the only two known natural enemies that could be sufficiently damaging and are not known to have broad host ranges. *Acanthodoxus machacalis* mines the stems of the plant in a very similar way to *P. brasiliensis* and *Asphondylia sp.* galls the flowers and developing fruits, resulting in large galls instead of fruits with seeds (Paterson *et al.,* 2014a). Both of these natural enemies should be considered as potential biological control agents, but because vegetative reproduction is a major component of *P. aculeata's* success in South Africa, the stem-mining cerambycid should be prioritised. Very little is known about the host specificity of the agent, but there is no evidence that it has a broad host range and it is abundant in the climatically suitable region of Rio de Janeiro in southern Brazil (Paterson *et al.,* 2014a), where the closest genetic matches to the South African weed population of *P. aculeata* occur (Paterson *et al.,* 2009). This natural enemy should therefore be imported into quarantine in South Africa for host specificity and impact studies.

4.4. Conclusions

Pereskiophaga brasiliensis is suitably damaging to be considered as a biological control agent for *P. aculeata* but its fundamental host range includes members of a closely related family, the Basellaceae, and the current evidence therefore suggests that the agent should not be released at present. Further tesing, using paired and multiple choice tests in quarantine will be conducetd, but it is unlikley that these trials will not result in some development and damage to indigenous non-target plants. *Pereskia aculeata* is still a major environmental weed in South Africa and it is likely that new agents should be sourced to complement the two agents that have already been released. Future research should focus on the stem-mining Cerambycidae, *A. machacalis*, which should be imported into quarantine for host specificity testing and impact studies.

5 References

Anderson, R. (2015). *Pereskiophaga brasiliensis* Anderson, a new genus and species of Cryptorhynchinae (Coleoptera: Curculionidae) associated with primitive Cacti of the genus Pereskia Miller (Cactaceae) in Brazil. *The Coleopterists Bulletin* **69(4):**670-673

Azab, S.G., Sadek, M.M. and Crailsheim, K. (2001). Protein metabolism in larvae of the cotton leafworm *Spodoptera littoralis* (Lepidoptera: Noctuidae) and its response to three mycotoxins. *Journal of Economical Entomology* **30**: 817-823

Barton, J. (2004). How good are we at predicting the field host-range of fungal pathogens used for classical biological control of weeds? *Biological Control* **31**: 99-122.

Barton-Browne, L. and Withers, T.M. (2002). Time-dependent changes in the host-acceptance threshold of insects: Implications for host specificity testing of candidate biological control agents. *Biocontrol Science and Technology* **12**: 677-693.

Bell, W. (1984). Chemo-orientation in walking insects, pp. 93–106, in W.J. Bell, and R.T. Cardé (eds.). Chemical Ecology of Insects, Sinauer Associates, Inc., Sunderland, Massachusetts

Bennett, F.D. and Habeck, D.H. (1995). *Cactoblastis cactorum*: A successful weed control agent in the Caribbean, now a pest in Florida? *Proceedings of the VIIIth International Symposium on Biological Control of Weeds*, Canterbury, New Zealand, Pp. 21-26.

Bernays, E.A. (1991). Relationship between deterrence and toxicity of plant secondary compounds for the grasshopper *Schistocerca americana*. *Journal of Chemical Ecology* **17**: 2519-2526.

Bernays, E.A. and Chapman, R.F. (1994*). Host-plant Selection by Phytophagous Insects*. Chapman and Hall, New York.

Blackman, R.L. and Eastop V.F. (2000). Aphids of the world's crops: an identification and information guide, 2nd ed. Wiley, New York, NY.

Blossey, B. (1995a). A comparison of various approaches for evaluating potential biological control agents using insects on *Lythrum salicaria*. *Biological Control* **5**: 113-122.

Blossey, B. (1995b). Host specificity screening of insect biological control agents as part of an environmental risk assessment, Pp. 84-89 *In* H. T. Hokakanen and J. M. Lynch [eds.], Biological Control: Benefits and Risks. CUP, Cambridge.

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

Briese, D.D. (1999). Open field host-specificity tests: is 'natural' good enough for risk assessment? pp. 44-59. *In*: T. M. Withers, L. Barton, and J. Stanley [eds.], Host Specificity Testing in Australasia" Towards Improved Assays for Biological Control. CRC for Tropical Pest Management. Brisbane, Australia.

Briese, D.T. (1997). Biological control of St. John's wort: past, present, and future. *Plant Protection Quarterly* **12**: 73–80.

Briese, D.T. (2003). The centrifugal phylogenetic method used to select plants for host-specificity testing of weed biological control agents: can and should it be modernised? In: Improving the Selection, Testing and Evaluation of Weed Biological Control Agents. CRC Tech- no.l. Ser. No. 7, Pp. 23-33.

Briese, D.T. (2005). Translating host-specificity test results into the real world: the need to harmonise the yin and yang of current testing procedures. *Biological Control* **35**: 208-214.

Briese, D.T. and Walker, A. (2002). A new perspective on the selection of test plants for evaluating the host-specificity of weed biological control agents: the case of *Deuterocampta quadrijuga*, a potential insect control agent of *Heliotropium amplexicaule*. *Biological Control* **25:** 273-283.

Briese, D.T., Sheppard, A.W. and Reifenberg, J.M. (1995). Open field host-specificity testing for potential biological control agents of *Onopordum* thistles. *Biological Control* **5**:158-166.

Briscoe, A.D. and Chittka, L. (2001). The evolution of colour vision in insects**.** *Annual Review in Entomology* **46**: 471-510.

Britton, N.L., and Rose, J.N. (1919). The Cactaceae: Descriptions and illustrations of plants of the cactus family (Vol. 1, pp. 8–11). Washington: Carnegie Institution of Washington.

Broughton, A.J. and Pemberton, R.W. (2008). Efforts to establish a foliage-feeding moth, *Austromusotima camptozonale*, against *Lygodium microphyllum* in Florida, considered in the light of a retrospective review of establishment success of weed biocontrol agents belonging to different arthropod taxa. *Biological Control* **47**: 28-36.

Bromilow, C. (1995). Problem plants of South Africa. Briza Publications, Pretoria.

Brooks, M.L., D'antonio, C.M., Richardson, D.M., Grace, J.B., Keeley, J.E., Ditomaso, J.M., Hobs, R.J., Pellant, M. and Pyke, D. (2004). Effects of invasive alien plants on fire regimes. *BioScience* **54 (7)**: 677-688.

Bruce, T.J.A., Wadhams, L.J. and Woodcock, C.M. (2005). Insect host location: a volatile situation. *Trends in Plant Science* **10**: 269-274.

Bruton, M.N. (1981). Major threat to the coastal dune forest in Maputaland. *The Naturalist* **25**: 26-27.

Brutsch, M.O. and Zimmermann, H.G. (1993). The prickly pear (*Opuntia ficus-indica*) [Cactaceae]) in South Africa: Utilization of the naturalized weed, and of the cultivated plants. *Economic Botany* **47:**154–162.

Brutsch, M.O. and Zimmermann, H.G. (1995). Control and utilization of wild *Opuntias*. In: Barbera, G., Inglese, P. and Pimienta-Barrios, E. (Eds) Agro- ecology, cultivation and uses of cactus pear. FAO, Rome. 155-166.

Buckingham, G.R., Okrah, E.A. and Christian-Meier, M. (1991). Laboratory biology and host range of *Hydrellia balciunasi* (Diptera: Ephydridae). *Entomophaga **36***: 575-586.

Caffrey, J.M., Milliane, M., Evers, S., Moran, H. and Butler, M. (2010). A novel approach to aquatic weed control and habitat restoration using biodegradable jute matting. *Aquatic Invasions* **5**(2): 123-129.

Campbell, P.L. (1988). Seed germination of *Harrisia martini* and *Pereskia aculeata* with reference to their potential spread in Natal. *Applied Plant Science* **2**: 60-63.

Carde, R.T. (1984). Olfactory Guidance of Flying Insects. *In*: Chemical Ecology of Insects. Bell, W.J. and Carde, R.T., eds. Chapman and Hall, London/New York.

Carvalheiro, L.G., Buckley, Y.M., Ventim, R., Fowler, S.V. and Memmott, J. (2008). Apparent competition can compromise the safety of highly specific biocontrol agents. *Ecology Letters* **11**: 690–700.

Chapman, R. F. and Bernays, E. A. (1989). Insect behavior at the leaf surface and learning as aspects of host plant selection. *Experientia* **45**: 215-222.

Clement, S.L. and M. Cristofaro. 1995. Open-field tests in host-specificity determination of insects for biological control of weeds. *Biocontrol Science and Technology* **5**: 395-406.

Cock, M., Day, R., Herren, H., Hill, M.P., Julien, M.H., Neuenschwander, P. and Ogwang, J. (2000). Harvesters get that sinking feeling. *Biocontrol News and Information* **21**: 1-8.

Coetzee, J.A., Byrne, M.J. and Hill, M.P. (2007). Predicting the distribution of *Eccritotarsus catarinensis*, a natural enemy released on water hyacinth in South Africa. *Entomologia Experimentalis et Applicata* **125**: 237–247.

Coetzee, J.A., Hill, M.P., Byrne, M.J. and Bownes, A. (2011). A review of the biological control programmes on Eichhornia crassipes (C. Mart.) Solms (Pontederiacaeae), *Salvinia molesta* D.S. Mitch. (Salviniaceae), *Pistia stratiotes* L. (Araceae), *Myriophyllum aquaticum* (Vell.) Verdc. (Haloragaceae) and *Azolla filiculoides* Lam. (Azollaceae) in South Africa. *African Entomology* **19(2)**: 451-468.

Crawley, M.J. (1989). Plant life-history and the success of weed biological control projects. *Proceedings of the VII International Symposium on Biological Control of Weeds* (ed. E.S. Delfosse), 17-26. Instituto Sperimentale per la Patologia Vegetale (MAF), Rome.

Cronk, Q.C.B. and Fuller, J.L. (2001). Plant invaders: the threat to natural ecosystems. Earthscan Publications Ltd. London. UK. Pp 35-60.

Crowe, M.L. and Bourchier, R.S. (2006). Interspecific interactions between the gall-fly *Urophora affinis* Frfld. (Diptera: Tephritidae) and the weevil *Larinus minutus* Gyll. (Coleoptera: Curculionidae), two biological control agents released against spotted knapweed,

Centaurea stoebe L. ssp. *micranthos*. *Biocontrol Science and Technology* **16**: 417-430.

Cuénoud, P., Savolainen, V., Chatrou, L.W., Powell, M., Grayer, R.J., and Chase, M.W. (2002). Molecular phylogenetics of Caryophllales based on nuclear 18S rDNA and plastid rbcl, *atp*B and *mat*K DNA sequences. *American Journal of Botany* **89**:132-144

De Bach, P. (1964). The scope of biological control. Pp. 3-20 in De Bach (ed) Biological control of insect pests and weeds. Chapman & Hall, London, UK.

De Beer, H. (1988). Pereskia. Farming in South Africa, Weeds A. 13. Government Printer, Pretoria. 2pp.

Deboer, G. (1991). Effect of diet experience on the ability of different larval chemosensory organs to mediate food discrimination by the tobacco hornworm, *Manduca sexta*. *Journal of Insect Physiology* **37**:763-769.

De Souza Filho, M.F., Gabriel, D. and De Azevedo Filho, J.A. (2001). Caracterização Morfológica de Três Espécies de Broca-da-Haste em Chuchuzeiro (Coleoptera: Cerambycidae, Lamiinae). *Neotropical Entomology* **30**: 475-477.

Department of Environmental Affairs (2016) *Notice No. 864. National Environmental Management: Biodiversity Act, 2004 (Act No. 10 Of 2004). Alien and invasive species lists, 2016*, Government Gazette 40166, pp. 31–104.

Diniz, I.R. and Morais, H.C. (2002). Local pattern of host plant utilization by lepidopteran larvae in the Cerrado vegetation. *Entomotropica*. **17:** 115-119.

Diniz, I.R., Lepesqueur, C., Milhomen, M.S., Bandeira, F.C.S. and Morais, H.C. (2011). *Eloria subapicalis* (Lepidoptera: Noctuidae: Lymantriinae): rare and specialist species on *Erythroxylum* (Erythroxylaceae) in the *Cerrado*. *Zoologia* **28**: 58-62.

Dodd, A.P. (1940). The biological campaign against prickly pear. Commonwealth Prickly Pear Board Bulletin, Brisbane, Australia, 177 Pp.

Downey, P.O. and Paterson, I.D. (2016). Encompassing the relative non-target risks from agents and their alien plant targets in biological control assessments. *BioControl* DOI 10.1007/s10526-016-9744-1

Downie, S.R., Katz-Downie, D.S. and Cho, K.J. (1997). Relationships in the Caryophyllales as suggested by phylogenetic analyses of partial chloroplast DNA ORF2280 homolog sequences. *American Journal of Botany* **84:** 253-273.

Dunn, P.H., Rosenthal, S.S., Campobasso, G. and Tait, S.M. (1989). Host specificity of *Pterolonche inspersa* (Lep.: Pterolonchidae) and its potential as a biological control agent for *Centaurea diffusa*, diffuse knapweed, and *Centaurea maculosa*, spotted knapweed. *Entomophaga* **34**: 435-446.

Dussourd, D.E. and Denno, R.F. (1994). Host range of generalist caterpillars: trenching permits feeding on plants with secretory canals. *Ecology (Tempe)* **75**: 69-78.

Dyer, R.A. (1975). The Genera of South African Flowering Plants. Volume 1: Dicotyledons (pp. 386). Pretoria: Department of Agricultural Technical Services.

Dyer, L.A., Singer, M.S., Lill ,J.T., Stireman, J.O., Gentry, G.L., Marquis, R.J., Ricklefs, R.E., Greeney, H.F., Wagner, D.L., Morais, H.C., Diniz, I.R., Kursar, T.A. and Coley, P.D. (2007). Host specificity of Lepidoptera in tropical and temperate forests. *Nature* **448 (7154)**: 696-699.

Edwards, P.B. (1999). The use of choice tests in host-specificity testing of herbivorous insects, in *Host Specificity Testing in Australasia: Towards Improved Assays for Biological Control* (Withers, T.M., Barton Browne, L. & Stanley, J., Eds). Scientific Publishing, Department of Natural Resources, Brisbane, Australia, pp. 35-43.

Edwards, E.J., Nyffeler, R. and Donoghue, M.J. (2005). Basal cactus phylogeny: Implications of Pereskia (Cactaceae) paraphyly for the transition to the cactus life form. *American Journal of Botany* **92(7)**: 1177-1188.

Ehler, L.E. and Hall, R.W. (1982). Evidence for competitive exclusion of introduced natural enemies in biological control. *Environmental Entomology* **11**: 1-4.

Embaby, E.M. and Lofty, D.E. (2015). Ecological studies on Cabbage Pests. *International Journal of Agricultural Technology* **11(5):** 1146-1160.

Erbar, C. and Leins, P. (2006). Floral ontogeny and systematic position of the Didiereaceae. *Plant Systematics and Evolution* **261**: 165-185. doi:10.1007/s00606-006-0432-y

Fernandez, P and Hilker, M. (2007) Host plant location by Chrysomelidae. *Basic and Applied Ecology* **8**: 97-116.

Flechtmann, C.A.H., Ottati, A.L.T., and Berisford, C.W. (2001). Ambrosia and bark beetles (Scolytidae: Coleoptera) in pine and eucalypt stands in southern Brazil. *Forest Ecology and Management* **142**: 183-191. doi:10.1016/S0378-1127(00)00349-2.

Forno, I.W., Kassulke, R.C. and Harley, K.LS. (1992). Host specificity and aspects of the biology of *Calligrapha pantherina* (Col.: Chrysomelidae), a biological control agent of *Sida acuta* (Malvaceae) and *S. rhombifolia* in Australia. *Entomophaga* **37**: 409-417.

Fowler, S.V., Syrett, P. and Jarvis, P. (2000). Will the Environmental Risk Management Authority, together with some expected and unexpected effects, cause biological control of broom to fail in New Zealand? pp. 173-186. In Spencer, N. and R. Nowierski (eds.). *Proceedings of the Xth International Symposium on Biological Control of Weeds*. Bozeman, Montana, USA, July 4- 14, 1999. Montana State University, Bozeman, Montana, USA.

Fox, L.R. and Morrow, P.A. (1981). Specialization: species property or local phenomenon? *Science* **211**: 887-893.

Futuyma, D.J., 2000. Potential evolution of host range in herbivorous insects. In: VanDriesche, R.G., Heard, T.A., McClay, A., Reardon, R. (Eds.), Proceedings of Session: Host-Specificity Testing of Exotic Arthropod Biological Control Agents—The Biological Basis for Improvementin Safety. USDA Forest Service Forest Health Technology Enterprise Team, Morgantown, pp. 42–53.

Gaston, K.J. and Lawton, J.H. (1990). Effect of scale and habitat on the relationship between regional distribution and local abundance. *Oikos* **58**: 329-335.

Germishuizen, G. and Meyer, N.L. (2003). Plants of southern Africa: An annotated checklist. Strelitzia 14. Pretoria: National Botanical Institute.

Gooden, B., French, K., Turner, P.l. and Downey, P.O. (2009). Impact threshold for an alien plant invader, *Lantana camara* L., on native plant communities. *Biological Conservation* **142**: 2631-2641.

Goolsby, J.A., Spencer, D. and Whitehand, L. (2009). Pre-release assessment of impact on *Arundo donax* by candidate biological control agents *Tetramesa romana* (Hymenoptera: Eurytomidae) and *Rhizapidiotus donacis* (Hemiptera: Diaspididae) under quarantine conditions. *Southwestern Entomologist* **34(4)**: 359-376.

Greathead, D.J. (2003). Historical overview of biological control in Africa. In, Neuenschwander P, Borgemeister C, Langewald J. (Eds.) Biological control in PM systems in Africa. Chapter 2, pp 27-44. CAB] Publishers. Oxon. United Kingdom.

Harminder, P.S., Batish, D.R., Pandher, J.K. and Kohli, R.K. (2005). Phytotoxic effects of *Parthenium hysterophorus* residues on three *Brassica species. Weed biology and management* **5(3)**: 105-109.

Hatcher, P.E. (1995). Three-way interactions between plant pathogenic fungi, herbivorous insects and their host plants. *Biological Review* **70**: 639-694..

Heard, T.A. (2000) Concepts in insect host-plant selection behavior and their application to host-specificity testing. In: Van Driesche, R., Heard, T., McClay, A. and Reardon, R. (eds) *Proceedings of Session: Host Specificity Testing of Exotic Arthropod Biological Control Agents— The Biological Basis for Improvement in Safety, Proceedings of the X International Symposium on Biological Control of Weeds.* US Forest Service, Forest Health Technology Enterprise Team, Report FHTET-99-1, Morgantown, WV, pp. 1–10.

Heard, T.A. and van Klinken, R.D. (1998). An analysis of designs for host range tests of insects for biological control of weeds. Pest Management - Future Challenges, Sixth Australasian Applied Entomology Research Conference, Vol 1:539-546. Brisbane, Australia.

Henderson, L. (1995). Plant invaders of southern Africa. Plant Protection Research Institute Handbook No.5, Agricultural Research Council, Pretoria. 180pp.

Hill, M.P. (1999). Biological control of red water fern, *Azolla filiculoides* Lamarck (Pteridophyta: Azollaceae) in South Africa. *African Entomology Memoir No. I.* Pp 119-124.

Hill, M.P. (2003). The impact and control of alien aquatic vegetation in South African aquatic ecosystems. *African Journal of Aquatic Science* **28(1)**: 19-24.

Hill, M.P., Cilliers, C.J. and Neser, S. (1999). Life history and laboratory host range of *Eccritotarsus catarinensis* (Carvalho) (Heteroptera: Miridae), a new potential natural enemy released on water hyacinth, *Eichhornia crassipes* (Mart.) Solms-Laub.) (Pontederiaceae) in South Africa. *Biological Contro*l **14**: 127-133.

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

Hoffmann, J.H. (1990). Interactions between three weevil species in the biocontrol of *Sesbania punicea* (Fabaceae): the role of simulation models in evaluation. *Agriculture, Ecosystems and Environment* **32**: 77-87.

Hoffmann, J.H. and Moran, V.C., (1991). Biological control of *Sesbania punicea* (Fabaceae) in South Africa. *Agriculture Ecosystems Environment* **37**:157-173.

Hoffmann, J.H. (1995). Biological control of weeds: the way forward, a South African perspective. *Proc. BCPC Symp.* 1995, 64: Weeds in a Changing World. Pp. 77-89. Brighton: BCPC.

Hoffmann, J.H. and Moran, V.C. (1999). A review of the agents and factors that have contributed to the successful biological control of *Sesbania punicea* (Cav.) Benth. (Papilionaceae) in South Africa. *African Entomology Memoir* **1**: 75-79.

Hoffmann, J.H., Moran, V.C. and Zimmermann, H.G. (1999). Integrated management of *Opuntia stricta* (Haworth) Haworth (Cactaceae) in South Africa: an enhanced role for two, renowned, insect agents. In: Olckers, T. & Hill, M.P. (Eds) Biological Control of Weeds in South Africa (1990–1998). *African Entomology Memoir* **1**: 15-20.

Honda, K. (1995). Chemical basis of differential oviposition by lepidopterous insects. *Archives of Insect Biochemistry and Physiology* **30**: 1-23.

Jones, R.E. (1991). Host location and oviposition on plants. Pp. 108-138 in Bailey WJ, Ridsdill-Smith TJ (eds) Reproductive behaviour of insects. London: Chapman & Hall.

Julien, M.H. and Griffiths, M.W. (1998). *Biological Control of Weeds: A World Catalogue of Agents and Their Target Weeds, 4*th edn. CABI, Wallingford.

Kaplan, H., Wilson, J.R.U., Klein, H., Henderson, L., Zimmermann, H.G., Manyama, P. (2017). A proposed national strategic framework for the management of Cactaceae in South Africa, *Bothalia* 47(2), a2149. doi.org/10.4102/abc.v47i2.2149

Keese, M.C. (1998). Performance of two monophagous leaf feeding beetles (Coleoptera: Chrysomelidae) on each other's host plant: do intrinsic factors determine host plant specialization? *Journal of Evolutionary Biology* **11**: 403-419.

Kennedy, J.S., Booth, C.O. and Kershaw, W.J.S. (1959). Host finding by aphids in the field. II. *Aphis fabae* Scop. (gynoparae) and *Brevycoryne brassicae* L. with a reappraisal of the role of host-finding behaviour in virus spread. *Annuals of Applied Biology* **47**: 424-444.

Klein, H. (1999). Biological control of three cactaceous weeds, *Pereskia aculeata* Miller, *Harrisia martinii* (Labouret) Britton and *Cereus jamacaru* De Candolle in South Africa. In T. Olckers & M.P. Hill (eds), Biological control of weeds in South Africa (1990–1999). *African Entomology Memoir No.* **1**: 3-14.

Klein, H. (2011). A catalogue of the insects, mites and pathogens that have been used or rejected, or are under consideration, for the biological control of invasive alien plants in *South Africa. African Entomology* **19**: 515–549.

Le Bellec, F., Vaillant, F. and Imbert, E. (2006). Pitahaya (*Hylocereus spp.*): a new fruit crop, a market with a future. *Fruits* **61**: 237-249.

Le Maitre, D.C., Van Wilgen, B.W., Chapman, R.A. and McKelly, D.H. (1996). Invasive plantsand water resources in the Western Cape Province, South Africa: Modelling the consequences of a lack of management. *The Journal of Applied Ecology* **33**: 161-172.

Leuenberger, B.E. (1986). Pereskia (Cactaceae). Memoirs of the New York Botanical Garden. **41**:1-141.

Loreau, M., Naeem. S., Inchausti. P., Bengtsson, J., Grime, J.P., Hector, A, Hooper, D.U., Huston, M.A., Raffaelli, O., Schmid, B., Tilman, O. and Wardle, D.A. (2001). Biodiversity and ecosystem functioning: Current knowledge and future challenges. *Science* **294**: 804-808.

Lotter, W.D. (1997). Management Proposals for the Alien Aquatic Biological Invasions of the Kruger National Park. SAN Parks Internal Research Report No. 10/97.

Louda, S.M. 2000. Negative ecological effects of the Musk thistle biological control agent, *Rhinocyllus conicus*, pp. 215-243, In P.A. Follett and J.J. Duan [eds.]. Nontarget effects of biological control. Kluwer Academic Publishers, Boston/Dordrecht/London.

Lounsburry, C.P. (1915). Plant killing insects: the Indian cochineal. *Agricultural Journal of South Africa* **1**: 537-543.

Magd El-Din, M. and El-Gengaihi, S.E. (2000). Joint action of some botanical extracts against the Egyptian cotton leafworm, *Spodoptera* (Boised) (Lepidoptera: Noctuidae). *Egyptian, Journal of Biology* **10**: 51-56.

Mann, J. (1967). The Harrisia Cactus. Government Printer, Brisbane. Institute Inc., Cary, NC.

Marohasy, J. (1998). The design and interpretation of host-specificity tests for weed biological control with particular reference to insect behaviour. *Biocontrol News Inform.* 19, 13N-20N.

Marquis, R.J. (1991). Evolution of resistance in plants to herbivores. *Evolutionary Trends in Plants* **5**: 23-29.

Maw, M.G. (1976). Biology of the tortoise beetle, *Cassida hemisphaerica* (Coleoptera: Chrysomelidae), a possible biological control agent for bladder campion, *Silene cucubalus* (Caryophyllaceae), in Canada. *Canadian Entomologist* **108**: 945-954.

McClay, A.S. and Balciunas, J.K. (2005). The role of pre-release efficacy assessment in selecting classical biological control agents for weeds applying the Anna Karenina principle. *Biological Contro.* **35**: 197-207.

McConnachie, A.J, De Wit, M.P., Hill, M.P. and Byrne, M.J. (2003). Economic evaluation of the successful biological control of *Azolla filicuioides* in South Africa. *Biological Control* **28**: 25-32.

McEvoy, P.B. (1996). Host specificity and biological pest control. *BioScience* **46**: 401-405.

McEvoy, P.B. and Coombs E.M. (1999). Biological control of plant invaders: Regional patterns, field experiments, and structured population models. *Ecological Applications* **9**: 387-401.

McFadyen, P.J. (1979). A survey of insects attacking *Parthenium hysterophorus* L. (Compositae) in Argentina and Brazil. *Dusenia* **11**: 41-45.

McFadyen, P.J. (1983). Host specificity and biology of *Megacyllene mellyi* (Col.: Cerambycidae) introduced into Australia for the biological control of *Baccharis halimifolia* (Compositae).*Entomophaga* **28**: 65-71.

McFadyen, R.C. (1998). Biological control of weeds. *Annual Review of Entomology* **43**: 369-393.

McGibbon, J. (1858). Catalogue of Plants in the Botanic Garden, Cape Town, Cape of Good Hope. Saul Solomon, Cape Town, Pp 36.

Menezes, C.W.G. de., Soares, M.A., Assis Junior, S.L. de, Fonseca, A.J., Pires, E.M., and Santos, J.B. dos. (2012). New sucking insect (Hemiptera) attacking Eucalyptus cloeziana (Myrtaceae) in Minas Gerais, Brazil. *EntomoBrasilis* **5**: 246-248.

Midgley, J.M., Hill, M.P., Villet, M.H. (2006). The effect of water hyacinth, *Eichhornia crassipes* Solms-Laubach (Pontederiaceae), on benthic biodiversity in two impoundments on the new year's River, South Africa. *African Journal of Aquatic Science* **31**:25-30.

Mooney, K.A., Pratt, R.T. and Singer, M.S. (2012). The tri-trophic interactions hypothesis: interactive effects of host plant quality, diet breadth and natural enemies on herbivores. PLoS One 7: e34403.

Moran, V.C. (1980). Interactions between phytophagous insects and their Opuntia hosts. *Ecological Entomology* **5**: 153-164.

Moran, V.C., and Zimmermann, H.G. (1991a). Biological control of jointed cactus, *Opuntia aurantiaca* (Cactaceae) in South Africa. *Agriculture, Ecosystems and Environment* **37**:5-27.

Moran, V.C. and Zimmermann, H.G. (1991b). Biological control of cactus weeds of minor importance in South Africa. *Agriculture, Ecosystems and Environment* **37**: 37-55.

Moran, V.C., Hoffmann, J.H. and Zimmermann, H.G. (2005). Biological control of invasive alien plants in South Africa: necessity, circumspection, and success. *Frontiers in Ecolology and the Environment* **3**: 77-83

Moran, V.C., Hoffmann, J.H. and Zimmermann, H.G. (2013). 100 years of biological control of invasive alien plants in South Africa: History, practice and achievements. *South African Journal of Science* **109**: 1-6.

Mnqeta, Z., (2017) Interactions between biological control agents released on *Pereskia aculeata* Miller (Cactacaea), in South Africa. Rhodes University MSc Thesis. Rhodes University. Grahamstown. South Africa.

Murray, T. J., Withers T.M, and Mansfield, S. (2010). Choice vs. no-choice test interpretation and the role of biology and behavior in parasitoid host specificity tests. *Biological Control* **52**: 153-159.

Nielsen, J.K., Kirkeby-Thomsen, A.H. and Petersen, M.K. (1989). Host plant recognition in monophagous weevils: specificity in feeding responses of *Ceutorhyncus constrictus* and the variable effect of sinigrin. *Entomologia Experimentalis et Applicata* **53**: 157-166.

Nevo, D. (1996). The desert locust, *Schistocerca gregaria*, and its control in the land of Israel and the Near East in antiquity, with some reflections on its appearance in Israel in modern times. *Phytoparasitica* **24**, 7-32.

Nordlund, D.A. (1996). Biological control, integrated pest management and conceptual models. *Biocontrol News Information* **17**: 35-44.

Novotny, V. and Basset Y. (2000). Rare species in communities of tropical insect herbivores: pondering the mystery of singletons. *Oikos* **89**: 564-572.

Novotny, V. and Basset, Y. (2005). Host specificity of insect herbivores in tropical forests. Proceedings of the Royal Society B. **272**: 1083-1090.

Novotny, V. and Weiblen, G.D. (2005). From communities to continents: beta diversity of herbivorous insects. *Annales Zoologici Fennici* **42**: 463-475.

O'Brien, C.W. (1976). *Ericereophaga humeridens*, a new genus and species and potential biological control agent from Brazil [*Cryptorhynchinae, Curculionidae, Coleoptera*]. *The Coleopterists Bulletin* **30**: 303-307.

Oerke, E.C., Dehne, H.W., Schonbeck, F. and Weber, A. (1994). Crop Production and Crop Protection - Estimated Crop Losses in Major Food and Cash Crops. Elsevier, Netherlands.

Okulungu, C. (2003). *Selected Traditional Leafy Vegetable Recipes of the Democratic Republic of Congo* (DRC). Unpublished manuscript.

Olckers T. (1999). Introduction. *African Entomology Memoir* **1:** 1-2.

Olckers, T., Hoffmann, J.H., Moran, Y.C., Impson, F.A.C. and Hill, M.P. (1999). The initiation of biological control against *Solanum elaeagnifolium* Cavanilles and *S. sisymbriifolium* Lamarck (Solanaceae) in South Africa. *African Entomology Memoir* **1**: 55-63.

Palmer, M., Bernhardt, E., Chornesky, E., Collins, S., Dobson, A., Duke, C., Gold, B., Jacobson, R., Kingsland, S., Kranz, R., Mappin, M., Matinez, M.L., Micheli, F., Morse, J., Pace, M., Pascual, M., Palumbi, S., Reichman, O.J., Simons, A., Townsend, A. and Turner, M. (2009) Ecology of a crowded planet. *Science*. **304**: 1251-1252

Papaj, D.R. and Rausher, M.D. (1983) Individual variation in host location by phytophagous insects, in Herbivorous Insects: Host Seeking Behaviour and Mechanisms (Ahmad, S., Ed). Academic Press, New York, USA, Pp. 77- 124.

Paterson, I.D., Downie, D.A. and Hill, M.P. (2009). Using molecular methods to determine the origin of weed populations of *Pereskia aculeata* in South Africa and its relevance to biological control. *Biological Control* **48**: 84-91. doi:10.1016/j.biocontrol. 2008.09.012.

Paterson, I.D., Hoffmann, J. H., Klein, H., Mathenge, C.W., Neser, S. and Zimmermann, H.G. (2011a). Biological control of Cactaceae in South Africa. *African Entomology* **19**: 230-246.

Paterson, I.D., Coetzee, J.A., Hill, M.P. and Downie, D.D. (2011b). A pre-release assessment of the relationship between the invasive alien plant, *Pereskia aculeata* Miller (Cactceae), and native plant biodiversity in South Africa. *Biological Control* **57**: 59-65.

Paterson, I D., Vitorino, M.D., de Cristo, S.C., Martin, G.D. and Hill, M.P. (2014a). Prioritization of potential agents for the biological control of the invasive alien weed, *Pereskia aculeata* Miller (Cactaceae), in South Africa. *Biocontrol Science and Technology* **24**: 407-425.

Paterson, I.D., Mdodana, L.A., Mpekula, O., Mabunda, D.B.X. and Hill, M.P. (2014b). A promising biological control agent for the control of the invasive alien plant, *Pereskia aculeata* Miller (Cactaceae), in South Africa. *Biocontrol Science and Technology* **24(10)**: 1083-1095.

Pauchard, A. and Shea. K. (2006). Integrating the study of non-native plant invasions across spatial scales. *Biological Invasions* **8**: 399-413.

Paynter, Q. and Flanagan, G.J. (2004) Integrating herbicide and mechanical control treatments with fire and biological control to manage an invasive wetland shrub, *Mimosa pigra*. *Journal of Applied Ecology* **41**: 615-629.

Paynter, Q., Fowler, S.V., Gourlay, A.H., Peterson, P.G., Smith, L.A. and Winks, C.J. (2015). Relative performance on test and target plants in laboratory tests predicts the risk of non-target attack in the field for arthropod weed biocontrol agents. *Biological Control* **80**: 133-142. doi: 10.1016/j.biocontrol.2014.10.007

Pemberton, R.W. (1995). *Cactoblastis cactorum in* the United States: An immigrant biological control agent or an introduction of the nursery industry? *American Entomology* **41**: 230-232.

Pemberton, R.W. (2000). Predictable risk to native plants in weed biological control. *Oecologia* **125**: 489-494. doi:10.1007/s004420000477.

Pickworth, G. (1972). *Pereskia aculeata*. Natal Regional Newsletter 2 October 1972. Department of Agricultural Technical Services.

Pimentel, D., McNair, S., Janecka, J., Wightman, J., Simmonds, C., Connell, C.O. and Wrong, E. (2001). Economic and environmental threats of alien plant, animal, and microbe invasions. *Agriculture Ecosystems and Environment* **84**: 1-20.

Pratt, P., Rayamajhi, M., Center, T., Tipping, P. and Wheeler, G. (2009). The ecological host range of an intentionally introduced herbivore: A comparison of predicted versus actual host use. *Biological Control* **49 (2):** 146-153.

Proclamation R. 35. (1979) in terms of the Weed Act, 1937 (Act 42 of 1937). The declaration of certain plants to be weeds. Government Gazette of the Republic of South Africa, Vol. 165, No. 6323

Prokopy, R.J. (1986). Visual and olfactory stimulus interaction in resource finding by insects T.L. Payne, M.C. Birch, C.E.J. Kennedy (Eds.), Mechanisms in Insect Olfaction, Clarendon Press, Oxford (1986), pp. 81-89.

Prokopy, R.J. and Owens, E.D. (1978).Visual generalist-visual specialist phytophagous insects: host selection behaviour and application to management. *Entomologia Experimentalis et Applicata* **24**: 609-620.

Prokopy, R.J. and Lewis, W.J. (1993). Application of learning to pest management, pp. 308-342. *In*: Papaj, D. R. and A. C. Lewis (ed.) *Insect Learning: Ecological and Evolutionary Perspectives.* Chapman and Hall, New.

Raid, N. and Munshi, J.D. (1979). The influence of thick floating vegetation (water hyacinth: *Eichhornia crassipes*) on the physico-chemical environment of a fresh water wetland. *Hydrobiologia* **62 (1)**: 65-69.

Ramaswamy, S.B. (1988). Host finding by moths: sensory modalities and behaviours. *Journal of Insect Physiolog.* **34**: 235-249.

Ramoni-Perazzi, P. (2006). Primer registro de asociación entre *Aetalion reticulatum* (Linné) (Hemiptera: Aetalionidae) *Synoeca septentrionalis* Richards (Hymenoptera: Vespidae), *Entomotropica* **2:** 129-132.

Richardson, D.M. and Van Wilgen, B.W. (2004). Invasive alien plants in South Africa: How well do we understand the ecological impacts? *South African Journal of Scence* **100 (1)**: 45-52.

Richardson, D.M., Macdonald, I.A.W. and Forsyth, G.G. (1989). Reductions in plant species richness under stands of alien trees and shrubs in the fynbos biome. *South African Forestry Journal* **149**: 1-8.

Robertson, M.P., Harris, K.R., Coetzee, J.A., Foxcroft, L.C., Dippenaar-Schoeman, A.S. and van Rensburg, B.J. (2011). Assessing local scale impacts of *Opuntia stricta* (Cactaceae) invasion

on beetle and spider diversity in Kruger National Park, South Africa. *African Zoology* **46**:205–223. doi: 10.3377/004.046.0202.

Roland-Gosselin, M. (1913). Are the species of *Rhipsalis* discovered in Africa indigenous? *Torreya* **13**: 151-156.

Shaw. H., Tanner, R., Djeddour, D. and Cortat, G. (2011). Classical biological control of *Fallopia ja- ponica* in the United Kingdom – lessons for Europe. *Weed Research* **51**: 552-558

Scherer, C. and Kolb, G. (1987). The influence of color stimuli on visually controlled behavior in *Aglais urticae* L. and *Pararge aegeria* L. (Lepidoptera). *Journal of Comparative Physiology* **161**: 891-898.

Schmitz, D.C., and Simberloff, D. (1997). Biological invasions: A growing threat. *Issues of Science and Technology* **13**: 33-4

Schooler, S.S., McEvoy, P.B. and Coombs, E.M. (2006). Negative per capita effects of purple loosestrife and reed canary grass on plant diversity of wetland communities. *Diversity and Distributions* **12**: 351-363.

Schoonhoven, L.M., van Loon, J.J.A. and Dicke, M. (2005). Insect-Plant Biology, second ed. Oxford University Press, New York.

Shea, K. and Possingham H.P. (2000). Optimal release strategies for biological control agents: an application of stochastic dynamic programming to population management. *Journal of Applied Ecology* **37**: 77-86.

Sheppard, A.W. (2003). Prioritizing agents based on predicted efficacy: beyond the lottery approach. In: Jacob, H.S., Briese, D.T. (Eds.), Improving the Selection, Testing and Evaluation of Weed Biological Control Agents. CRC for Australian Weed Management, Glen Osmond, Australia, Pp. 11–21.

Smith, R.A., Mooney, K.A., and Agrawa, A.A. (2008). Coexistance of three specialist aphids on common milkweed, *Asclepias syriaca*. *Ecology* **89**: 2187-2196.

Städler, E. (2002). Plant chemical cues important for egg deposition by herbivorous insects. Pp. 171-197 in Hilker M, Meiners T (eds) Chemoecology of insect eggs and egg deposition. Berlin: Blackwell Publishing.

Suckling, D.M. and Sforza, R. (2014). What magnitude are observed non-target impacts from weed biocontrol? PLoS One 9, e84847.

Sushilkumar (2005). History, progress and prospects of classical biological control in India. *Indian Journal of Weed Science* **47(3)**: 306-320, 2015.

Sutton, G.F., Paterson, I.D. and Paynter, Q. (2017). Genetic matching of invasive populations of the African tulip tree, *Spathodea campanulata* Beauv. (Bignoniaceae), to their native distribution: maximising the likelihood of selecting host-compatible biological control agents. *Biological Control* **114**: 167-175.

Syrett, P. and Emberson, R. M. (1997). The natural host range of beetle species feeding on broom, *Cytisus scoparius* (L.) Link (Fabaceae), in Southwest Europe. *Biocontrol Science and Technology* **7**: 309-326.

Tellez, T., de Rodrigo López, E.M., Granado, G.L., Pérez, E.A., López, R.M and Guzmá, J.M.S. (2008). The Water Hyacinth, *Eichhornia crassipes*: an invasive plant in the Guadiana River Basin (Spain).

Thiery, D. and Visser, J.H. (1986). Masking of host plant odour in the olfactory orientation of the Colorado potato beetle. *Entomologia Experimentalis et Applicata* **41**:165-172.

Thomas, M.B. and Reid, A.M. (2007). Are exotic natural enemies an effective way of controlling invasive plants? *Trends in Ecology and Evolution* **22(9)**: 447-453.

Tosh, C.R., Powell, G., and Hardie, J. (2003). Decision making by generalist and specialist aphids with the same genotype. *Journal of Insect Physiology* **49**: 659-669.

Tryon, H. (1910). The wild cochineal insect, with reference to its injurious action on prickly pear (*Opuntia spp.)* in India, etc., and to its availability for the subjugation of this plant in Queensland and elsewhere. *Queensland Agricultural Journal* **25**: 188-197.

Van Der Westhuizen, L. (2011). Initiation of a biological control programme against Madeira vine, *Anredera cordifolia* (Ten.) Steenis (Basellaceae), in South Africa. *African Entomology* **19**: 217–222.

Van Driesche, R.G. and Murray, T.J. (2004). Overview of testing schemes and designs used to estimate host ranges. In: Van Dreische R, Reardon R ed. Assessing host ranges of parasitoids and predators used for classical biological control: a guide to best practice. United States Forest Service Publication, USA. Pp. 68-89.

Van Driesche, R.G., Carruthers, R.I., Center, T., Hoddle, M.S., Hough-Goldstein, J., Morin, L., Smith, L., Wagner, D.L., Blossey, B., Brancatini, V., Casagrande, R., Causton, C.E., Coetzee, J.A., Cuda, J., Ding, J., Fowler, S.V., Frank, J.f., Fuester, R., Goolsby, J., Grodowitz, M., Heard, T.A., Hill, M.P., Hoffmann, J.B., Huber, J., Julien, M., Kairo, M.T.K., Kenis, M., Mason, P., Medal, J., Messing, R., Miller, R., Moore, A., Neuenschwander, P., Newman, R., Norambuena, H., Palmer, W.A., Pemberton, R., Perez Panduro, A., Pratt, P.D., Rayamajhi, M., Salom, S., Sands, D., Schooler, S., Schwarzlander, M., Sheppard, A., Shaw, R., Tipping, P.W. and Van Klinken, R.D. (2010). Classical biological control for the protection of natural ecosystems. *Biological Control*. 54: S2-S33.

Van Klinken, R.D. (2000). Host-specificity testing: why do we do it and how we can do it better, in Host-specificity Testing of Exotic Arthropod Biological Control Agents: the Biological Basis for Improvement in Safety (van Driesche, R.G., Heard, T., McClay, A.S. and Reardon, R., Eds). USDA Forest Service Bulletin, FHTET-99-1, Morgantown, West Virginia, USA, Pp. 54-68.

Van Lenteren, J.C., Babendreier, D., Bigler, F., Burgio, G., Hokkanen, H.M.T., Kuske, S., Loomans, A.J.M., Menzler-Hokkanen, H.M.T., van Rijn, P.C.J., Thomas, M.B., Tommasini, M.G. and Zeng, Q.Q. (2003). Environmental risk assessment of exotic natural enemies used in inundative biological control. *Biological Control* **48**: 3-38.

Van Wilgen, B.W. and Richardson, D.M. (1985). The effects of alien shrub invasions on vegetation structure and fire behaviour in South African fynbos shrublands: a simulation study. *Journal of Applied Ecology* **22**: 955-966.

Van Wilgen, B.W., Richardson, D.M., Le Maitre, D.C., Marais, C. and Magadlela, D. (2001). The economic consequences of alien plant invasions: Examples of impacts and approaches to sustainable management in South Africa. *Environment, Development and Sustainability* **3**: 145-168.

Visser, J.H. (1986). Host odor perception in phytophagous insects. *Annual Review of Entomology* **31**: 121-144.

Vitousek, P.M. (1992). Effects of alien plants on native ecosystems. *In*: Stone CP, Smith CW, Tunison TJ (Eds.) Alien plant invasions in native ecosystems of Hawaii: Management and Research. University of Hawaii Press. Hawaii. Pp 29-41.

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

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

Weyl, P. and Hill, M. (2012). The effect of previous feeding on water hyacinth leaf acceptability by three water hyacinth biological control agents measured with a simple Y – tube olfactometer. *African Entomology* **20(1)**: 201-205.

Wilcove, D.S., Rothstein, D., Dubow, J., Phillips, A. and Losos, E. (1998) .Quantifying threats to imperiled species in the United States. *Bioscience* **48**: 607-615.

Wilson, J.R.U., Dormontt, E.E., Prentis, P.J., Lowe, A.J. and Richardson, D.M. (2009a). Biogeographic concepts define invasion biology. *Trends in Ecology and Evolution* **24**: 586.

Wilson, J.R.U., Dormontt, E.E., Prentis, P.J., Lowe, A.J. and Richardson, D.M. (2009b). Something in the way you move: dispersal pathways affect invasion success. *Trends in Ecology and Evolution* **24**: 136-144.

Winston, R.L., Schwarzlander, M., Hinz, H.L., Day, M.D., Cock, M.J.W. and Julien, M.H. (2014). Biological Control of Weeds: A World Catalogue of Agents and Their Target Weeds. 5th edition. USDA Forest Service, Forest Health Technology Enterprise Team, Morgantown, West Virginia. FHTET-2014-04. 838pp.

Withers, T.M. and Barton-Browne, L. (1998). Possible causes of apparently indiscriminate oviposition in host-specificity tests using phytophagous insects. In: *Sixth Australasian Applied Entomological Research Conference*. University of Queensland, Brisbane, Australia, Pp. 565-571.

Withers, T.M., Barton-Browne, L., Stanley, J., (2000). How time dependent processes can affect the outcome of assays. In: Van Driesche, R.G., Heard, T.A., McClay, A., Reardon, R. (Eds.), Proceedings of Session: Host-Specificity Testing of Exotic Arthropod Biological Control Agents—the Biological Basis for Improvement in Safety. USDA Forest Service, Forest Health Technology Enterprise Team, Morgantown, Pp. 27-41.

Wratten, S.D. and Gurr, G.M. (2000). Synthesis: the future success of biological control, Pp. 405-416. *In* G. Gurr and S. Wratten (eds.), Biological Control: Measures of Success. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Zachariades, C., Paterson, I.D., Strathie, L.W., Hill, M.P. and Wilgen, B.W.V. (2017). Assessing the status of biological control as a management tool for suppression of invasive alien plants in South Africa. *Bothalia* 47(2), a2142.

Zimmermann, H.G. and Neser, S. (1999). Trends and prospects for biological control of weeds in *South Africa. African Entomology Memoir*. **1**: 165-173.

Zimmermann, H.G. and Olckers, T. (2003). Biological control of alien plant invaders in Southern Africa. In, Neuenschwander P, Borgemeister C, Langewald 1. (Eds.) Biological control in IPM systems in Africa. Chapter 2, pp 27-44. CABI Publishers. Oxon. United Kingdom.

Zimmemann, H.G., Moran, V.C. and Hoffmann, J.H. (2009). Invasive cactus species (Cactaceae). *In*: Muniappan, R., Reddy, G.V.P. and Raman, A. (Eds) Biological Control of Tropical Weeds using Arthropods. 108-129. Cambridge University Press, Cambridge, U.K.