Post-release evaluation of the biological control programme against *Cereus jamacaru* De Candolle (Cactaceae), in South Africa

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Abstract

Cereus jamacaru De Candolle (Cactaceae) is an environment-transforming weed of both agricultural and wildlife habitats in South Africa. Weed infestations reduce carrying capacity of the land, and pose a risk to livestock, wildlife and human safety due to the plant's spiny thorns. The weed is considered to be under complete biological control in South Africa, due to its introduced control agent, *Hypogeococcus festerianus* Lizer y Trelles (Hemiptera: Pseudococcidae), although observational reports suggest that the level of success achieved is variable. In this thesis, a formal post-release evaluation of this biological control programme was conducted, specifically to determine the efficacy of *H. festerianus* as a biological control agent, and to identify factors which may limit or constrain the level of success achieved by the control agent. These data were collected with the intention of improving the control of *C. jamacaru* in South Africa.

A field-based study of *C. jamacaru* population demographics investigated the efficacy of *H. festerianus* as a biological control agent of the weed, by integrating weed growth, fecundity and survival metrics with *C. jamacaru* population dynamics and demographic patterns from 8 sites where *H. festerianus* was present and 14 sites where the control agent was absent. The findings indicated that *H. festerianus* significantly reduced weed fecundity, which resulted in fewer seedling recruits, and that levels of plant mortality were greater at sites where *H. festerianus* was present. The reduction in weed fecundity and survival translated into negative population-level consequences for *H. festerianus*. Weed-population age frequency distributions in the absence of *H. festerianus* demonstrated a "reverse J-shaped" distribution, indicative of high recruitment rates and population stability, while *C. jamacaru* populations infected with *H. festerianus* were described by bell-shaped distributions, and were typified by

limited recruitment, or a complete lack thereof. By constraining recruitment and inhibiting self-regeneration, *H. festerianus* appears to regulate populations of *C. jamacaru*.

Predation and parasitism of *H. festerianus* was believed to be a limiting factor for the biological control programme against *C. jamacaru* in South Africa, although no formal evaluation of this claim had been undertaken. Accordingly, the assemblage of natural enemies acquired by *H. festerianus* in South Africa was identified by field-collections of infected *H. festerianus* gall-material. Further, timed point-count surveys of natural enemies associated with *H. festerianus* were performed and integrated with the data on the impact of *H. festerianus* on weed population dynamics to assess the impact of two prominent predaceous taxa on *H. festerianus* efficacy as a biological control agent. Although *H. festerianus* had acquired a diverse suite of novel natural enemies in South Africa, this has not prevented the biocontrol agent from having an impact on *C. jamacaru* populations, although other subtler effects cannot be ruled out.

This study showed that biological control efforts employing *H. festerianus* for the management of *C. jamacaru* have been successful. Furthermore, these data demonstrated the utility of retrospective analyses in developing and improving the science of biological control, specifically how to improve candidate agent prioritisation, determining how many agents are required for successful biological control, and how to evaluate the success of biological control efforts. Improvements in our theoretical understanding of biological control will undoubtedly reduce costs of biological control programmes, improve success rates, and increase the predictability of biological control.

Table of Contents

Title page i

Abstract ii

Table of Contents iv

List of Figures vii

List of Tables xi

Acknowledgements xii

Chapter 1 – General Introduction 1

1.1. *Cereus jamacaru* De Candolle (Cactaceae) 2

1.1.1. Taxonomy 3

1.1.2. Biology and distribution 4

1.1.3. Invasion, impact and status 6

1.2. Biological control 7

1.2.1. Biological control of cactaceous weeds 10

1.2.2. Biological control of Cereus jamacaru 12

1.2.3. Biological control employing Hypogeococcus festerianus 14

1.3. Variable success in biological control 15

1.3.1. Plant-insect incompatibility 16

1.3.2. Climate suitability 19

1.3.3. Predation and parasitism 21

1.4. Aims and rationale 22

Chapter 2 - Evaluating the efficacy of *Hypogeococcus festerianus* as a biological control agent of *Cereus jamacaru* in South Africa 24

2.1. Introduction 24

2.2. Methods and materials..... 28
2.2.1. Field survey..... 28
2.2.2. Data collection..... 30
2.2.3. Statistical analyses..... 30
2.3. Results..... 32
2.3.1. Between region comparisons of control sites..... 32
2.3.2. Effect of infestation by Hypogeococcus festerianus..... 33
2.4. Discussion..... 46
2.5. Conclusions..... 52

Chapter 3 – Natural enemy acquisition by *Hypogeococcus festerianus* and its implications for biological control of *Cereus jamacaru*..... 54

3.1. Introduction..... 54
3.2. Methods and materials..... 58
3.2.1. Natural enemy assemblage associated with Hypogeococcus festerianus..... 59
3.2.2. Association between natural enemies and Hypogeococcus festerianus..... 61
3.2.3. Statistical analyses..... 62
3.3. Results..... 64
3.3.1. Natural enemy assemblage composition, structure and diversity.. 64
3.3.2. Predator assemblage..... 71
3.3.3. Parasitoid assemblage..... 72
3.3.4. Correlations between natural enemies and Hypogeococcus festerianus..... 73
3.4. Discussion..... 76
3.5. Conclusions..... 86

Chapter 4 – General Discussion..... 87

4.1. Defining success in weed biological control..... 88
4.2. Setting pre-defined management targets for environmental weeds..... 91
4.3. Developing the science of biological control..... 94

4.3.1. How many insect species are required for successful biological control? 95
4.3.2. Prioritisation of candidate control agents 99
4.4. Threat of exotic *Hypogeococccus* sp. populations to native cacti in the Americas 104
4.5. Implications for the biological control of *Cereus jamacaru* 107
4.6. Conclusions 109

References 110

Plate I 142

List of Figures

Fig. 1.1. *Cereus jamacaru*. (Drawn by Gill Condy, ARC-Plant Protection Research Institute, Pretoria, South Africa) (Henderson, 1995)..... 2

Fig. 1.2. Distribution of *Cereus jamacaru* in South Africa (Drawn by L. Henderson, 2016; South African Plant Invaders Database [SAPIA], ARC-Plant Protection Research Institute, Pretoria, South Africa). Filled black circles indicate the presence of *C. jamacaru* in a quarter-degree grid cell..... 6

Fig. 2.1. Fitted logistic regression curve which indicated that the probability of mature *Cereus jamacaru* individuals being reproductively active (*i.e.* producing fruit) was not independent of the abundance of its biocontrol agent *Hypogeococcus festerianus* (galls/plant^{-1}) ($\chi^2 = 76.62$; $P < 0.001$). The solid line represents the fitted logistic regression model, while histograms represent counts from the observed data..... 34

Fig. 2.2. Mean (± S.E.) number of fruits produced per mature *Cereus jamacaru* individual, at sites where *Hypogeococcus festerianus* was present (biocontrol) and where the biocontrol agent was absent (control), pooled between sample regions. Significant differences are indicated by columns followed by different letters (t-test; $P < 0.05$)..... 35

Fig. 2.3. Mean (± S.E.) proportion of mature *Cereus jamacaru* individuals fruiting (per 1000 m^{-2}), at field sites where *Hypogeococcus festerianus* was present (biocontrol) and where the biocontrol agent was absent (control), pooled between sample regions. Significant differences are indicated by columns followed by different letters (Mann-Whitney U test; $P < 0.05$)..... 36

Fig. 2.4. Mean (± S.E.) number of fruits produced by *Cereus jamacaru* per 1000 m^{-2}, at field sites where *Hypogeococcus festerianus* was present (biocontrol) and where the biocontrol agent

was absent (control), pooled between sample regions. Significant differences are indicated by columns followed by different letters (Mann-Whitney U test; $P < 0.05$)..... 37

Fig. 2.5. Relationship between the proportion of stems galled by *Hypogeococcus festerianus* and total fruit production ($Y_{[\log(\text{total fruit}+1)]} = 5.18 - 3.71x_{[\text{prop\_stems\_galled}]}$); Each point on the graph represents site means (per 1000 m^{-2}) of the respective variable. The solid line represents the fitted linear regression model..... 38

Fig. 2.6. Mosaic plot of individual *Cereus jamacaru* plant mortality frequencies (per 1000 m^{-2}), with respect to biocontrol employing *Hypogeococcus festerianus*. The area of each tile is proportional to the respective cell frequency of a corresponding 2 x 2 contingency table. The dashed (negative) and solid (positive) tile outlines indicate the sign of deviation from the expected frequencies of plant mortality. The shading of each tile is proportional to the standardised residual from the fitted model (values provided in figure legend). Significant deviations from expected cell frequencies were observed (Pearson's Chi-squared test: $P = 0.003$), and are indicted by darker-shaded tiles. Values given inside each tile represent the percentage of *Cereus jamacaru* individuals, pooled across sample sites, that were dead or alive with respect to the presence or absence of biocontrol..... 40

Fig. 2.7. Mean (± S.E.) number of *Cereus jamacaru* individuals per 1000 m^{-2}, partitioned into distinct age classes representative of all individuals: irrespective of age class (All), ≥ 8 years old (Mature; *i.e.* capable of reproduction), 3-8 years old (Pre-fruiting) and 1-2 years old (Seedlings). Columns within the same cage class followed by different letters are significantly different (t-test, $P < 0.05$)..... 41

Fig. 2.8. Age frequency distributions of *Cereus jamacaru* sample sites, where *Hypogeococcus festerianus* was absent (control sites indicated by grey-shaded bars), and where

Hypogeococcus festerianus was present (biocontrol sites indicated by black-shaded bars ■). Refer to Table 2.1. for site details..... 43

Fig. 3.1. A species accumulation curve (SAC) indicated that sampling additional *Cereus jamacaru* sites was unlikely to yield additional natural enemy species associated with *Hypogeococcus festerianus* during this study. The grey-shaded area indicates the standard deviation of the SAC through random permutation (Cotelli and Colwell, 2001)..... 65

Fig. 3.2. A non-metric multi-dimensional scaling ordination plot (nMDS) comparing natural enemy assemblages associated with *Hypogeococcus festerianus* infecting *Cereus jamacaru* in two different sampling regions (Groot Marico – GM and Rust-de-Winter – RW). Each data point in the nMDS plot represents the natural enemy community identified from a single sample site (see section 2.2.2. for further details). The Bray-Curtis dissimilarity scores were used to rank distances calculated using a community-abundance matrix. The stress of the nMDS plot was 0.11, which suggests that the representation of the data is appropriate.... 71

Fig. 3.3. Correlation between coccinellid predator abundance (individuals/site) and *Homalotylus* spp. abundance (individuals/site), recovered from emergence chambers filled with *Hypogeococcus festerianus* gall material..... 74

Fig. 3.4. Correlations between the number of *H. festerianus* galls (1000 m^{-2}), and (a) Predaceous beetle density (individuals/gall) ($Y_{[\text{predaceous beetle density}]} = 0.594 + 0.007_{[H.\ festerianus\ \text{galls}]}$; $\rho = 0.24$, $P = 0.582$) and (b) *Autoba costimacula* density (individuals/gall) ($Y_{[Autoba\ costimacula\ \text{density}]} = 0.090 - 0.001_{[H.\ festerianus\ \text{galls}]}$; $\rho = -0.50$, $P = 0.216$)..... 75

Fig. 3.5. Correlations between the proportion of *Cereus jamacaru* stems galled (1000 m^{-2}), and (a) Predaceous beetle density (individuals/gall) ($Y_{[\text{predaceous beetle density}]} = 0.794 + 0.356_{[\text{proportion stems galled}]}$; $\rho = 0.13$, $P = 0.754$) and (b) *Autoba costimacula* density (individuals/gall) ($Y_{[Autoba\ costimacula\ \text{density}]} = 0.117 - 0.081_{[\text{proportion stems galled}]}$; $\rho = -0.48$, $P = 0.227$)..... 76

Fig. 3.6. Schematic overview of probable trophic relationships between organisms that are associated with the *Hypogeococcus festerianus* – *Cereus jamacaru* biocontrol system. Directional arrows indicate, (➡) potential for weed biocontrol, (➡) potential for biotic interference, and (▪▪➡) potential protection from biotic interference of *H. festerianus*… 83

List of Tables

Table. 2.1. Summary information for *Cereus jamacaru* field sites sampled during the current study..... 29

Table. 3.1. Natural enemy assemblage associated with *Hypogeococcus festerianus* in its native distribution (Argentina). Surveys for natural enemies associated with *H. festerianus* in Argentina have been limited primarily to *Harrisia* spp., and not *Cereus jamacaru*..... 60

Table. 3.2. Natural enemy assemblage associated with *Hypogeococcus festerianus* in its introduced range (South Africa). Species abundance per site equates to the number of individuals emerged per collection chamber. Region refers to whether species were collected from emergence chambers in both sampling areas (Groot Marico and Rust-de-Winter), or only in a single region (GM or RD). Sites indicates the percentage of sites (n = 8) that at least one individual of a particular species was recovered from during this study. An additional natural enemy was collected from *H. festerianus* gall material on an *ad hoc* basis in the Kwa-Zulu Natal province, and is included in the table for completeness (region - KZ)..... 66

Table. 3.3. Mean (± S.E.) species richness, and Shannon *H* diversity and Simpson's *D* diversity indices of site-wise natural enemy assemblages associated with *Hypogeococcus festerianus* in South Africa (see section 3.2.3 for formulae)..... 69

Table. 4.1. Examples of weed impact groups (and species that may fall into each respective impact group), the impact each species has on the natural ecosystem, and accordingly an appropriate, ecologically-derived measure by which to evaluate biocontrol success is then provided..... 96

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Chapter 1

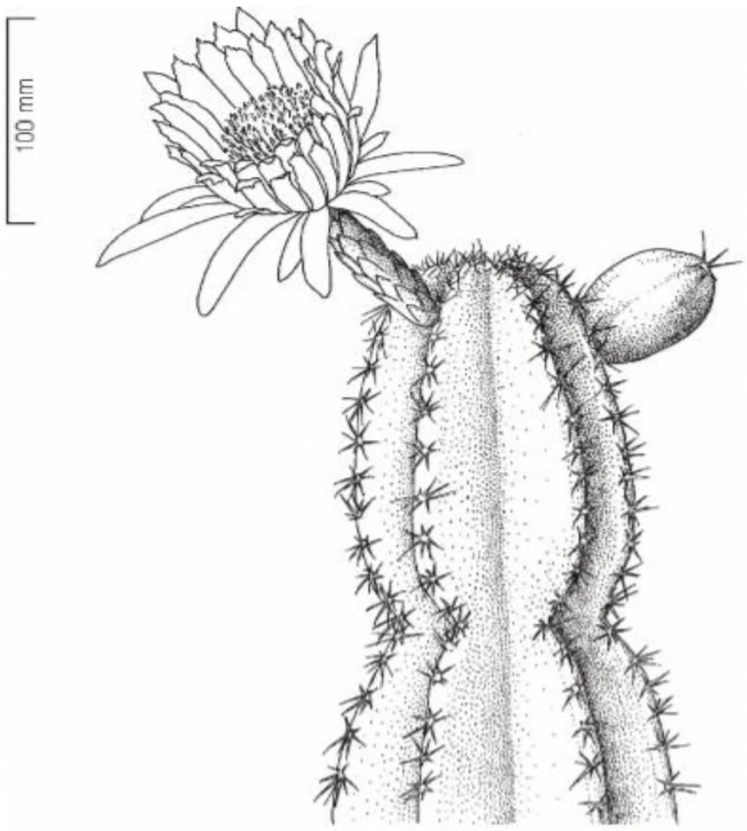
General Introduction

Invasive alien plant species constitute a major global threat to native biodiversity, ecosystem functionality and agricultural productivity (Mooney, 2005). Biological control (hereafter 'biocontrol') is an effective means by which to control alien invaders (McFadyen, 1998; McEvoy et al., 1999).

Cactaceous weeds have been particularly problematic in South Africa (Pettey, 1948; Zimmermann et al., 2009) and biocontrol has proven to be the most effective method of dealing with many of these species (Hoffmann and Moran, 2008; Klein et al., 2011; Paterson et al., 2011a). One of these species is *Cereus jamacaru* De Candolle (Cactaceae). This weed is considered to be under complete biocontrol (i.e., no additional management interventions are required against the target weed) in South Africa due to the action of its biocontrol agent *Hypogeococcus festerianus* (Lizer y Trelles) (Hemiptera: Pseudococcidae). However, no formal post-release evaluation of this biocontrol programme has been conducted (Klein, 2011). This thesis evaluated the efficacy of *H. festerianus* as a biocontrol agent of *C. jamacaru* in South Africa.

1.1. *Cereus jamacaru* De Candolle (Cactaceae)

Cereus jamacaru is a large, tree-like cylindrical cactus (Fig. 1.1.; Plate 1) (Anderson, 2001; Taylor and Zappi, 2004). The plant comprises a short, woody trunk and many upright stems (Britton and Rose, 1919). The stems comprise approximately 6 lateral ribs, which support groups of 6 – 8 spines on areoles. Flowers are long, funnel-shaped, white in colour, and nocturnal, giving the species its common name – 'Queen of the Night' cactus (Anderson, 2001). Upon wilting of its flowers the ovary will enlarge to produce a large, red to pink fruit which provides an edible, white, fleshy pulp containing many small, black seeds (Klein, 2002a).



(Drawn by G. Condy, National Botanical Institute, Pretoria; commissioned by Plant Protection Research Institute.)

Fig. 1.1. *Cereus jamacaru*. (Drawn by Gill Condy, ARC-Plant Protection Research Institute, Pretoria, South Africa) (Henderson, 1995).

1.1.1. Taxonomy

"There is little doubt that *Cereus* is one of the least understood genera of the tribe Cereeae, perhaps of the entire cactus family." – Edward Anderson (*The Cactus Family*, 2001)

Cereus Miller (Cactaceae: Cereeae) is one of the oldest genera of cactaceous plants, described by Philip Miller in 1754 (Anderson, 2001). The name derives from the Greek and Latin term *cereus*, meaning torch-like, in reference to the candelabrum-esque structure of the lectotype species, *Cereus hexagonus* (L.) Miller 1768 (Britton and Rose, 1919). Specimens belonging to the genus are characterised by: a tree-like structure, frequently obtaining great heights, multiple stems that contain large areoles which display large spines, large and funnel-form nocturnal flowers, and a predominantly naked pericarpel (Britton and Rose, 1919; Anderson, 2001). The genus is distributed from Argentina in the south, through the eastern parts of South America and extending into the Caribbean Islands in the north (Britton and Rose, 1919; Anderson, 2001).

Britton and Rose (1919) originally proposed *C. peruvianus* (L.) Miller as the lectotype of the genus, which was later amended to *C. hexagonus* as the illustration by Matthias de L'Obel used to describe *C. peruvianus*, was not recovered from the materials used by Miller in 1754 to describe the genus (Anderson, 2001). This original description typifies the taxonomic confusion pertaining to the genus *Cereus* still to this day, with the genus having contained species from most cactaceous genera at some point throughout history (Britton and Rose, 1919). The original monograph of the genus by Karl Schumann (1897-1899), containing 140 species (originally 104 species, and later amended to include an additional 36 species) is regarded by many as "artificial and complex" (Britton and Rose, 1919). The genus would later be amended by Berger (1905) and Riccobono (1909), with

Berger's treatment of the genus inflated with the inclusion of several additional genera (see Britton and Rose, 1919; and references therein). Benson (1982) proposes that even with the knowledge acquired since the previous genus classification, "there is still insufficient information for the adequate classification of *Cereus*." For this thesis, I follow the nomenclature and classification of Anderson (2001), recognising 34 species within the genus *Cereus*.

Cereus jamacaru is a highly polymorphic species, comprising two sub-species (*C. jamacaru jamacaru; C. jamacaru calcirupicola*), a monstrose form and many ornamental spineless variants (Anderson, 2001; Oliviera et al., 2013). Based on a lack of morphological differentiation, *C. jamacaru* is typically treated as a member of the *C. hexagonus* species complex (Winter et al., 2011). The *C. hexagonus* complex comprises 5 species: the type-species *C. hexagonus*, the two sub-species of *C. jamacaru* (*C. j. jamacaru*; *C. j. calcirupicola*), and two sub-species of *C. hildmannianus* K. Schum. (*C. h. uruguayanus*; *C. h. hildmannianus*) (Winter et al., 2011). All of the *C. hexagonus* complex constituent species are believed to be present in South Africa, barring the *C. j. calcirupicola* sub-species, although only *C. h. uruguayanus* and *C. j. jamacaru* are considered naturalised and/or invasive (Winter et al., 2011).

1.1.2. Biology and distribution

Cereus jamacaru occupies a diverse array of habitats from coastal vegetation to arid inland deserts, and is distributed from the states of Maranhao and Para in Northern Brazil to Minas Gerais in the South-East parts of the country (Britton and Rose, 1919; Taylor and Zappi, 2004). Congeneric *C. h. uruguayensis* is distributed throughout Southern Brazil (Santa Carina and Rio Grande do Sul), Uruguay and into Argentina (Winter et al., 2011), which suggests

that these two naturalised species in South Africa occupy different habitats. Nevertheless, *C. jamacaru* is most common in the "Caatinga" forest habitat (Bezerra et al., 2013). The "Caatinga" is a tropical, dry forest exclusively found in Brazil, which is characterised by soils with little nutrients and low rainfall (Santos et al., 2012).

This cactus can reproduce vegetatively, with any disconnected stems able to establish themselves by taking root, although the primary method of propagation is believed to be through seed set (Klein, 2002a). The fruits of cactaceous plants are usually fleshy and appealing to animals, containing a vast quantity of seeds embedded in the flesh (Taylor and Zappi, 2004). The fruits are important nutritional resources for many animals, which ingest, transport and disperse these seeds at great distances from the mother plant (Janzen, 1986). Klein (2002a) proposed that the appealing colour of *C. jamacaru* fruit attracts birds and primates in South Africa, which consume the flesh, and in doing so ingest many seeds, which they will excrete and disperse. This method of dispersal results in many seedlings being observed under trees and adjacent to fences (Klein, 2002a), as they act as perching sites for the birds, such as Dark-capped Bulbul (*Pycnonotus tricolor* Hartlaub) which has been observed consuming *C. jamacaru* fruits (Taylor and Walker, 1984). This phenomenon has been observed for other cactaceous plants, whereby *O. ficus-indica* (L.) Miller seeds are dispersed by the Cape Crow (*Corvus capensis* Lichtenstein) and the Pied Crow (*Corvus albus* Statius Müller) (Dean and Milton, 2000).

In South Africa, *C. jamacaru* populations were initially restricted to the drier, northern provinces of the country (de Beer, 1987). Populations have since been recorded in all nine provinces of South Africa (Fig. 1.2.), although the weed remains most problematic in the northern provinces, such as the Gauteng, Limpopo and the North-West Provinces. In these regions, the plant can become prolific, with Taylor and Walker (1984) estimating

densities of approximately 40 000 plants per hectare in the Moloto-Witnek region (25.28 S; 28.37 E), which is one of the most problematic infestations in South Africa.

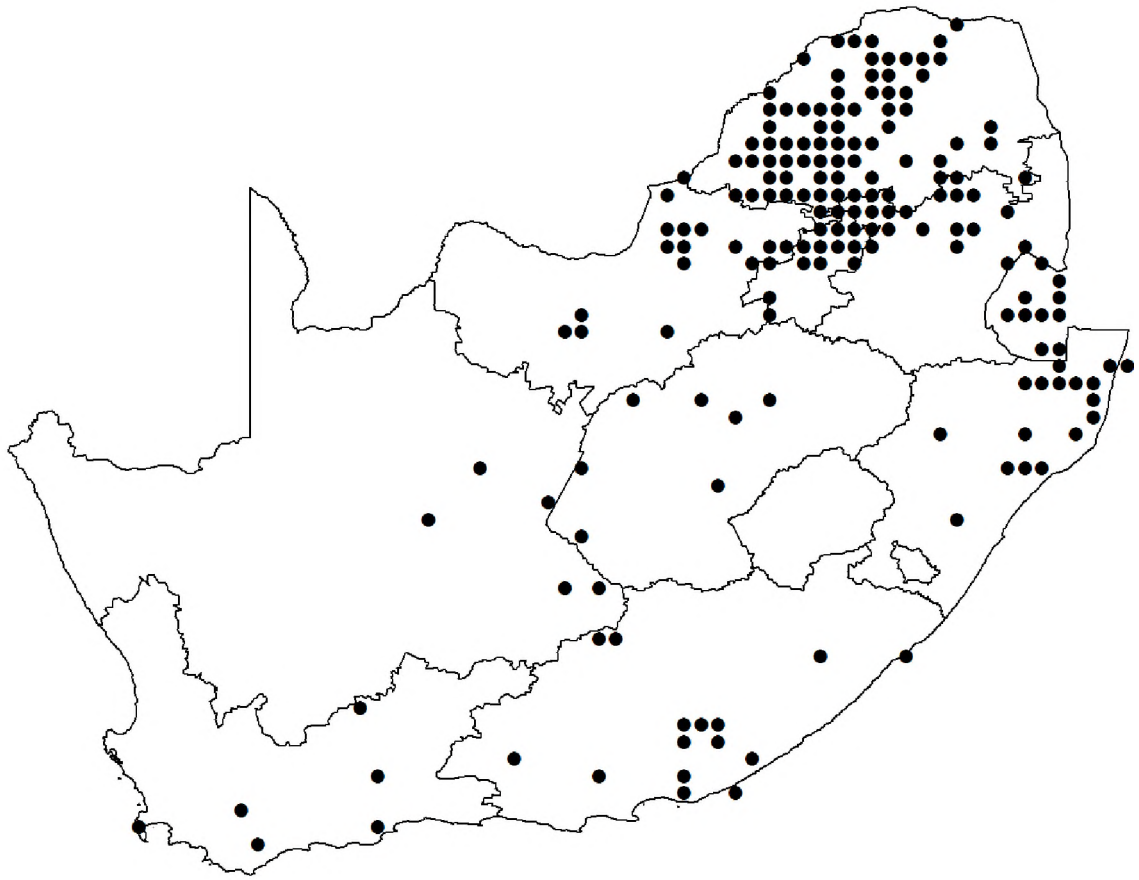


Fig. 1.2. Distribution of *Cereus jamacaru* in South Africa (Drawn by L. Henderson, 2016; South African Plant Invaders Database [SAPIA], ARC-Plant Protection Research Institute, Pretoria, South Africa). Filled black circles indicate the presence of *C. jamacaru* in a quarter-degree grid cell.

1.1.3. Invasion, impact and status

The history and route of invasion of *C. jamacaru* in South Africa is largely unknown, although the earliest published record dates to a single, ornamental individual in a private

garden in the Witnek region *circa* 1939 (Gauteng Province) (Klein, 1999). Its ornamental value is well appreciated, with records of planted *C. jamacaru* in the West Indies and surrounding islands almost a century ago (Britton and Rose, 1919). The cactus was probably introduced into South Africa by succulent collectors due to its aesthetically pleasing shape, white flowers and edible fruits (Klein, 2002a). Unknowingly, collectors and gardeners have likely aided the invasion of *C. jamacaru* in South Africa, as ornamental plants can disperse into the surrounding vegetation via frugivorous seed dispersal (Klein, 2002a; Winter et al., 2011). In doing so, *C. jamacaru* invades and transforms the natural landscape, which results in reduced grazing potential of the land, and poses a health risk to both humans and livestock due to its large spines (Klein, 2002a).

Due to the weed's impact on the native environment and loss of arable land to this species, the plant was proclaimed as a weed under the Conservation of Natural Resources Act of 1983 in South Africa (Klein, 2002a), and is also considered a category 1b invasive alien plant species by the National Environmental Management: Biodiversity Act (10/2004).

1.2 Biological control

Invasive plant species are important invasive taxa due to the negative impact they can have in their introduced range (McFadyen, 1998; Mack et al., 2000). Invasive plant species constitute a major threat to biodiversity (Richardson et al., 1989; Pimentel et al., 2001; Vilà et al., 2011), reduce the availability of arable land/grazing (Moran and Zimmermann, 1991; Klein, 1999) and are economically costly to control (Pimentel et al., 2005; Williams et al., 2010). The management of invasive plants has traditionally been performed using mechanical and chemical control methods (Williams et al., 2010). Mechanical control involves the physical removal of individuals which is labour-intensive, costly and unsustainable (Olckers et al.,

1999). Herbicidal spray treatments have been the primary choice to manage invasive plant species, although the utility and applicability of herbicides is dwindling due to their negative environmental impacts (Carson, 1962; Relyea, 2005), incompatibility with other control methods (e.g., biocontrol) (Klein, 1999) and because long-term repeated applications are often required to be effective (i.e., to manage recruitment from soil seedbanks) which makes their use unsustainable and expensive (Hoffmann et al., 1998; Hoffmann and Moran, 1999; Paynter and Flanagan, 2004).

Classical weed biocontrol is a cost-effective and sustainable management strategy for practitioners aiming to control invasive species (McFadyen, 1998; Fowler et al., 2000; Clewley et al., 2012). Biocontrol involves the use of a natural enemy of an invasive plant species, sourced from the weeds native distribution, to reduce the weeds density and impact in its adventive range (De Bach, 1964; Harley and Forno, 1992; Müller-Schärer and Schaffner, 2008; Van Driesche et al., 2010). One of the primary assumptions of biocontrol is that exotic plants become invasive by acquiring enemy-free status in their adventive range, and that by reuniting plant populations with their natural enemies, biocontrol limits or reduces weed invasiveness (Keane and Crawley, 2002). This assumption highlights the importance of top-down regulation by introduced herbivores during biocontrol programmes (Blossey and Notzold, 1995; Keane and Crawley, 2002).

A largely underappreciated aspect of top-down regulation by introduced herbivores is that they can act in concert, oppose, or demonstrate no interaction with bottom-up effects (Room and Thomas, 1985; Heard and Winterton, 2000; Jamieson et al., 2012). For example, resource availability can increase the tolerance to herbivory demonstrated by a weed (Hawkes and Sullivan, 2001; Lesica and Hanna, 2003). The relative strengths of top-down and bottom-up forces are highly variable across plant communities (Hillebrand et al., 2007), therefore it is unsurprising that the impact of biocontrol agents can be highly variable amongst weed

populations (Shea et al., 2010; Boag and Eckert, 2013). Research which elucidates the factors that mediate the interaction between top-down and bottom-up forces may be beneficial in determining plant invasion success, improving predictability of biocontrol success and tailoring weed management strategies (Miller, 2008; Jamieson et al., 2012).

The definition of biocontrol success has proven problematic (McFadyen, 1998; Syrett et al., 2000; Hoffmann and Moran, 2008; Carson et al., 2008), although it is generally accepted that an effective programme should reduce the density of the invasive species below a pre-determined threshold, where the significant negative consequences associated with the weed are ameliorated (Hulme, 2006). A reduction in plant size may be favourable in biocontrol efforts (McEvoy and Coombs, 1999), as this is likely to reduce the competitive strength of the invasive species which may allow for native species to re-establish (Clewley et al., 2012), while a reduction in seed production may impede the spread of the weed (Hoffmann and Moran, 1999), and reduced weed densities may allow for greater pasture production and grazing potential (Lockett et al., 2012; Liebman et al., 2016). It is believed that through reducing the density of the target invasive species, a biocontrol agent will allow for an increase in native species diversity (DeBach and Rosen, 1991), which is a desired result of many biocontrol programmes (Van Driesche et al., 2010).

A total of 106 biocontrol agents have been accepted for release in South Africa, against 48 invasive plant species (Klein, 2011). Of these, seventy-five (71 %) of the agents are established in the field, whereby the majority of weed species (59%) are considered to be under substantial or complete control due to the action of biocontrol agents (Klein, 2011). These results demonstrate the utility and effectiveness of biocontrol agents in managing invasive plant populations in South Africa.

1.2.1. Biological control of cactaceous weeds

There are approximately 1500 – 1800 species that belong to the family Cactaceae, which are predominantly succulent species, defined by their leafless, spine-bearing and photosynthetic stems that are adapted to survive and proliferate in harsh, xeric conditions (Gibson and Nobel, 1990; Zimmermann et al., 2009). The visually appealing structural characteristics and low water requirements of these species has led to many cacti being cultivated as ornamental plants from as early as the 15th century (Howard and Touw, 1981), while other species are cultivated for their fruits, as supplementary fodder, and as hedge plants (Casas and Barbera, 2002). *Opuntia ficus-indica* has been widely cultivated to mass-produce the carmine cochineal *Dactylopius coccus* Costa (Hemiptera: Dactylopiidae), which produces a commercially viable dye (Chávez-Moreno et al., 2009).

Due to the widespread cultivation of several species, many cacti species have become invasive outside of the New World (Novoa et al., 2015), with several cactaceous species constituting the most problematic plant invaders worldwide (Weber, 2003). Some of the earliest recorded invasive plants are cactaceous species belonging to the genus *Opuntia* (Tryon 1910; cited in: Zimmermann et al., 2009). The invasiveness of the cacti is attributed to the fact that they: (a) are tolerant of drought periods due to mass-retention of moisture in their succulent stems and undergoing crassulacean acid metalbolism (CAM) photosynthesis, (b) are more competitive than native species under high disturbance conditions and (c) can reproduce both sexually and asexually (Gibson and Nobel, 1990; Edwards et al., 2005; Zimmermann et al., 2009). Furthermore, there are no indigenous cacti outside of the New World (Zimmermann and Granata, 2002), with the possible exception of *Rhipsalis baccifera* (J.C. Mueller) Stearn (Cactaceae) (Wallace and Gibson, 2002; but see Rebman and Pinkava, 2001). The implications of this are that the lack of phylogenetically related plants in the Old

World posits that cactaceous species are unlikely to support a significant insect fauna, and as such are likely to acquire enemy-free space and proliferate accordingly in their adventive range, following the enemy-release hypothesis (Keane and Crawley, 2002). Conversely, the taxonomic isolation of the Cactaceae outside of the New World results in biocontrol being easier to implement against cactaceous weeds than for the majority of weed taxa, as biocontrol practitioners can safely use oligophagous, rather than monophagous herbivores, increasing the pool of candidate biocontrol agents significantly (Paterson et al., 2011a).

Biocontrol against invasive cacti has a long history, dating back over 150 years (Zimmermann et al., 2009). Indeed, the first herbivore released for biocontrol purposes was *D. ceylonicus* (Green) for the control of *O. vulgaris* (= *O. monacantha* Haw.) Miller in Sri Lanka during 1863 (Tryon, 1910; cited in De Lotto, 1974). Similarly, the first biocontrol programme initiated in South Africa involved the release of *D. ceylonicus* against the cactaceous weed *O. monacantha* (Lounsbury, 1915). Of the 49 recognised invasive cactaceous species (including problematic plants within their native distribution; see Moran and Zimmermann, 1984), 23 species have been targeted in biocontrol programmes worldwide, employing 19 species of insects and mites (Zimmermann et al., 2009). In rating the success of these programmes, Moran and Zimmermann (1984) stated that 48 % (11/23) of biocontrol efforts have brought the target cactaceous weed under complete control and 30 % (7/23) have provided a substantial level of control, while only 22 % have not provided control over the target weed.

In South Africa, a total of 15 different biocontrol agents (species or lower host-specific taxa) have been released for the management of eight cactaceous weeds, although these agents have been able to establish populations on 15 weed taxa, to date (Paterson et al., 2011a). Klein (2011) noted that 27 % of the invasive cacti in South Africa are under complete

control and 53 % are under substantial control, while the three remaining weeds (20 %) are either considered under negligible control or have not yet been evaluated.

Many of the more recent biocontrol programmes against invasive cacti in Australia and South Africa have targeted species that are found within the Cereinae (columnar cacti), most notably the genera: *Harrisia*, *Acanthocereus* and *Cereus* (Zimmermann et al., 2009). Extensive field surveys in the native range of these emerging cactaceous weeds between 1964 and 1985 resulted in the selection and importation of several candidate biocontrol agents for release against *Harrissia martinii* (Labouret) Britton (McFadyen and Fidalgo, 1976; McFadyen, 1979; McFadyen and Tomley, 1980; Moran and Zimmermann, 1991; Klein, 1999). Inadvertently, these collections would produce two suitably host-specific biocontrol agents for biocontrol efforts against *C. jamacaru* in South Africa (Klein, 1999; Paterson et al., 2011a).

1.2.2. Biological control of Cereus jamacaru

Cereus jamacaru is not considered to infest large tracts of land, nor be of significant prominence, in comparison to the majority of terrestrial, environmental weeds in South Africa (van Wilgen et al., 2012). However, approximately R 57 million has been spent by the Working for Water programme on clearing this species between 1995 and 2008 (van Wilgen et al., 2012). As with many invasive plant species, control efforts employing mechanical and chemical methods against *C. jamacaru* have been vastly expensive and unsustainable (van Wilgen et al., 2012). Biocontrol is viewed as the preferable method of controlling *C. jamacaru* in South Africa (Klein, 2002a).

Mechanical control of this species has proven largely ineffective, other than when performed for small plants that would fail to maintain biocontrol agents (Klein, 2002a; van

Wilgen et al., 2012). The uprooting of *C. jamacaru* has most likely increased the abundance of this species, as any intact plant part incorrectly disposed of can act as a source of infestation given the ability of *C. jamacaru* to propagate through vegetative measures (Klein, 2002a).

The chemical herbicide monosodium methanearsone (MSMA) has been used against *C. jamacaru* (Masmar L2032, Act 32/1947) (Vermeulen et al., 1996). Spray applications and stem-injections of larger specimens have proven effective in killing *C. jamacaru* individuals (Moran and Zimmermann, 1991; Klein, 1999). The success of early MSMA spray applications prompted the Department of Agriculture (DoA) to undertake a large-scale chemical control programme against *C. jamacaru* in the Gauteng and North-West Provinces of South Africa in the late 1990's (Klein, 1999). They assisted private landowners in selling the herbicide MSMA at a substantially reduced price (Klein, 1999). However, due to the unsustainable nature of chemical control and the apparent effectiveness of early biocontrol efforts against *C. jamacaru*, the state subsidy on MSMA was ended and biocontrol pursued as the control method of choice (Klein, 1999).

The biocontrol programme against *C. jamacaru* was a spin-off of the programme against *H. martinii* in South Africa, which in itself was the product of a collaboration with Australian biocontrol practitioners who initiated biocontrol against *H. martinii* in the 1970's (Klein, 1999; Paterson et al., 2011a). The mealybug *Hypogeococcus festerianus*, often referred to as *H. pungens* Granara de Willink (Hemiptera: Pseudococcidae), was first released against *H. martinii* at Muden, Kwa-Zulu Natal Province, South Arica, during 1983 (Moran and Zimmermann, 1991). The mealybug performed well against *H. martinii* and has subsequently established at many sites throughout the country (Klein, 1999). In 1993, three years after the release of *H. festerianus* against *H. martinii* at Kameeldrift (Pretoria, Gauteng

Province, South Africa), an abundance of mealybug was recorded on *C. jamacaru* plants (Klein, 1999).

A second biocontrol agent was introduced against *H. martinii* at the Kameeldrift site in 1990 (Moran and Zimmermann, 1991), in the stem-boring beetle *Nealcidion cereicola* (Fisher) (Coleoptera: Cerambycidae), previously known as *Alcidion cereicola*). *Nealcidion cereicola* was found to be highly damaging to *C. jamacaru* three years after the initial release against *H. martinii* in 1990 (Klein, 1999). Most *N. cereicola* were released on *H. martinii* but 20 larvae were inoculated onto a *C. jamacaru* plant in 1990 (Klein, 1999). Biocontrol implementation efforts employing *N. cereicola* have been scarce (Klein, 1999), with very few established populations countrywide, although the agent appears extremely damaging where it establishes significant population numbers (Paterson et al., 2011a).

1.2.3. Biological control employing *Hypogeococcus festerianus* (Lizer y Trelles) (Hemiptera: Pseudococcidae)

The biocontrol programme against *C. jamacaru* has been employed primarily by utilizing *H. festerianus*, which has been actively redistributed throughout the country (Paterson et al., 2011a). Upon establishment, the mealybugs congregate on meristems where they are believed to inject a toxin during feeding which results in deformed plant growth (McFadyen, 1979), although no mature plant tissue is affected (Klein, 2002b). The deformed growth patterns of *C. jamacaru* following herbivory, creates a gall structure which provides *H. festerianus* with a sheltered, protected environment to reside in (Klein 2002b; Plate I).

The mealybug is believed to be able to kill mature plants in approximately four years (Paterson et al., 2011a), although evidence of this in the field is somewhat lacking (Jordaan and Mantji, 2012). However, *H. festerianus* can significantly reduce fruiting and therefore

seed production by large, mature individuals, while any seedlings that do germinate are readily colonized and killed (Paterson et al., 2011a; Plate I). The regulatory effect of *H. festerianus* on *C. jamacaru* entails that the weed is not able to expand its invasive range, prompting Klein (2011) to propose that *C. jamacaru* be under complete control by *H. festerianus* in South Africa, where the agent is established. However, a formal post-release evaluation of the biocontrol programme against *C. jamacaru* has been not been conducted in South Africa (Paterson et al., 2011a).

1.3 Variable biological control success

Biocontrol programmes which do fail, primarily result from the agent failing to establish in its introduced range (Sheppard, 1992; Van Klinken et al., 2003), while agents that successfully regulate populations of exotic weeds can demonstrate a significant amount of variation in the magnitude of success observed (Denoth and Myers, 2005; Van Driesche et al., 2010; Boag and Eckert, 2013; Hovick and Carson, 2015). The mechanisms that underpin the variability in biocontrol agent establishment and success are poorly understood (Van Driesche et al., 2010), and may impose a significant constraint in future biocontrol endeavours against invasive species (McFadyen, 1998; Hovick and Carson, 2015). Clewley et al. (2012) argue that post-release quantification of biocontrol introductions may elucidate the factors that contribute to successful and/or failed biocontrol efforts, which will allow for the improvement of biocontrol implementation and success rates. As such, the scarcity of post-release evaluations of biocontrol introductions has been widely criticised, whereby most biocontrol programmes have focused on the identification, host-specificity determination and release of agents, and have largely failed to consider agent establishment and impact once released (McEvoy and Coombs, 1999).

There are several reasons to suspect that the biocontrol programme against *C. jamacaru* has achieved variable levels of success in South Africa. This is unsurprising given that this programme was a by-product of the biocontrol of *H. martinii*, which itself was implemented at low-cost and with minimal effort (Klein, 1999). In the following section, the reasons why such variability is suspected is highlighted, and how an understanding of these factors may contribute to the improvement of biocontrol programme against *C. jamacaru* in South Africa is discussed.

1.3.1. Plant-insect incompatibility

It is well documented that the introduction of biocontrol agents that are incompatible with the target weed has accounted for several failed biocontrol programmes against invasive cacti (Zimmermann et al., 2009). The early biocontrol practitioners in India and Sri Lanka dating back to the 19th century noted that *D. ceylonicus*, which was effective in clearing stands of *O. monacantha* was largely ineffective against other *Opuntia* weeds, most notably *O. dillenii* (Ker Gawler) Haworth. These observations led Burkhill (1911) (cited in Zimmermann et al., 2009) to note that these efforts were, "… a waste of money in fruitless attempts to destroy *Opuntia* weeds with inappropriate [species of cochineal] insects."

In subsequent years, the importance of introducing not only the correct species of cochineal insect, but rather host-adapted biotypes of cochineal would become an important factor in the successful biocontrol of cactaceous weeds in South Africa. The cochineal insect *D. opuntiae* (Cockerell) has been used to effectively manage *O. stricta* (Haw.) in Australia (Dodd, 1940) and *O. ficus-indica* in South Africa (Annecke and Moran, 1978). Surprisingly, *D. opuntiae* failed to control *O. stricta* in South Africa. In an attempt to elucidate the confounding factor in the success of biocontrol of *O. stricta* between the two countries, a

consignment of *D. opuntiae* was sourced from *O. stricta* in Australia (Volchanksy et al., 1999), as historical records indicated that the original consignment of *D. opuntiae* was likely a distinct strain of cochineal, originally collected from *O. streptacantha,* which is more closely related to *O. ficus-indica* than *O. stricta* (Zimmermann, 1997). Volchansky et al. (1999) demonstrated that there were indeed two biotypes of *D. opuntiae* through host-specificity testing, with the original consignment of *D. opuntiae* sent to South Africa performing well on *O. ficus-indica* but poorly on *O. stricta* (referred to as the 'ficus' biotype) and the latter consignment of cochineal collected on *O. stricta* in Australia performing well on *O. stricta* but poorly on *O. ficus-indica* ('stricta' biotype). A similar situation was later unravelled for the biocontrol of *Cylindropuntia* spp. in South Africa (Mathenge et al., 2010), with two host-specific biotypes of *D. tomentosus* (Lamarck) differentiated, which are host-adapted for either *C. fulgida* (Engelmann) F.N. Knuth var. *fulgida* (Engelmann) F.M. Knuth (*D. tomentosus* 'Cholla' biotype) or *C. imbricata* (DC.) F.M. Knuth (*D. tomentosus* 'Imbricata' biotype'). Notwithstanding, species and/or biotype matching is not always required for successful biocontrol of cactaceous weeds. For example, *Phenrica guerini* Bechyne´ (Coleoptera: Chrysomelidae) performs equally across several cultivars of *Pereskia aculeata* Miller from its native distribution and introduced range (Paterson et al., 2012).

There has been much confusion with regards to the taxonomic delineation of the *Hypogeococcus* sp. introduced into Australia and South Africa for biocontrol purposes. McFadyen (1979) noted that a consignment of *H. festerianus* was collected from *Eriocereus martinii* (= *H.* martinii) in Formosa Province, Argentina in 1972, which would later be released in Australia for biocontrol of several *Harrisia* spp. Specimens from this consignment would later be identified as *H. pungens* (Williams and Granara de Willink, 1992), as these specimens displayed three circuli on their ventral surface (as is diagnostic for *H. pungens*), and not a single circuli (as is diagnostic for *H. festerianus*). However, Aguirre et al. (2016)

found marked biological differences between *H. pungens sensu stricto* and the consignment of *H. pungens* exported to Australia, where *H. pungens s.s.* does not include any Cactaceae in its fundamental host-range and can undergo parthenogenesis, which contrasted with *H. pungens* exported to Australia. As such, the true identity of the *Hypogeococcus* sp. introduced in Australia and South Africa, which is a vital component of successful biocontrol programmes (Rosen, 1986), remains unresolved. Furthermore, the consignment of *H. pungens* collected for biocontrol purposes originates from *H. martinii*, and therefore may be somewhat incompatible with *C. jamacaru* and suggests that a more suitable taxonomic entity may be present in the native distribution of *C. jamacaru*. This view is supported in that the sedentary life-history of *H. festerianus* predisposes it to becoming specialised on individual plant genotypes due to reduced levels of gene flow between insect populations and having multiple generations on an individual plant (Karban, 1989). As a conclusive overview of *Hypogeococcus* sp. systematics has not been produced, the biocontrol agent in Australia and South Africa is referred to as *H. festerianus* in this thesis, as per Klein (2011) to avoid further confusion.

In addition to taxonomic confusion regarding control agent taxonomy and systematics, it is widely accepted that there are many taxonomic irregularities and vast amounts of unknown variation and complexity within the systematics of the Cactaceae, which has long troubled entomologists working with cactaceous plants (Anderson, 2001). Zimmermann et al. (2009) proposed that a thorough understanding of the target cactus species taxonomy and systematics are required for biocontrol success. Moran et al. (1976) and Annecke and Moran (1978) provided detailed accounts of how taxonomic errors and uncertainties regarding *O. aurantiaca* and *O. ficus-indica* systematics have negatively impacted on the successful management of these invasive species in South Africa.

An observational account of increased tolerance by *C. jamacaru* populations in the Kwa-Zulu Natal Province, South Africa to herbivory by *H. festerianus* has been reported (R. Brudvig, pers. comm; cited in Paterson et al. 2011a). Paterson et al. (2011a) notes that the increased resistance to *H. festerianus* may be due to the taxonomic identity of the host plant differing from other *C. jamacaru* populations in South Africa. However, it is argued that the broad host range of the oligophagous *H. festerianus* (McFadyen, 1979; McFadyen and Tomley, 1980) is unlikely to explain the weed's tolerance to herbivory regardless of host identity (Paterson et al., 2011a), although other plausible explanations are available. For example, *C. hildmannianus* tissue is vastly mucilaginous, in contrast to *C. jamacaru*, which may impede the activity and reduce the performance of *H. festerianus* on *C. hildmannianus*. This may explain the perceived tolerance of the Kwa-Zulu Natal population if they were indeed identified as *C. hildmannianus* (L. Henderson, pers. comm).

1.3.2. Climatic suitability

In order to be successful, biocontrol agents are required to establish and proliferate in their adventive range (Robertson et al., 2008). One of the reasons that biocontrol introductions fail is climatic incompatibility between the native distribution of a candidate control agent (i.e., where the agent consignment was sourced from) and the region in which it will be released (i.e., where biocontrol is to be implemented) (Wapshere, 1983). Cullen (1996) found that for 25 biocontrol introductions, climate played an important part in explaining the variable success of more than half of the case studies considered. As such, control agents that are sourced from similar climatic conditions to that of the introduced range are more likely to establish, proliferate and be effective control agents (Hoelmer and Kirk, 2005).

The importance of climatic suitability for successful biocontrol is two-fold. Firstly, climatic matching has been used to prioritise regions in a weed's native distribution to source biocontrol agents that may be more climatically suitable for release in the introduced range (Goolsby et al., 2003; Dhileepan et al., 2006). Secondly, climatic matching has been employed as a tool to identify climatically suitable regions to release biocontrol agents in the introduced range (Byrne et al., 2002; Senaratne et al., 2006).

While little is known of the biology of *H. festerianus*, notably that of its thermal requirements and tolerances, it is possible that climatic incompatibility may constrain the efficacy of this control agent in South Africa. As above, the *H. festerianus* consignment present in South Africa originates from the original collection performed in Formosa Province, Argentina in 1972 (McFayden, 1979). As such, an important climatic factor to consider may be the lower minimum temperatures experienced by *H. festerianus* in South Africa, than it would experience in its native distribution. *Hypogeococcus festerianus* reproduction may be constrained if the lower developmental threshold is not exceeded for a sufficient duration of time to allow for insect populations to successfully overwinter in South Africa, whereby the lower developmental threshold is defined as the critical temperature below which reproduction ceases. Furthermore, the abundance of *H. festerianus* may not be sufficient to effect enough damage to regulate *C. jamacaru* populations, which may be the product of climatic incompatibility if the thermal requirements of *H. festerianus* are not in accordance with the abiotic conditions experienced by the insect in South Africa. If this is indeed the case, a more climatically suitable consignment of *Hypogeococcus* sp. could be sourced from the weeds native distribution, which should also consider plant-insect incompatibility by collecting insects from *C. jamacaru* individuals, in addition to conducting collections in a climatic region most similar to that which the control agent will encounter in South Africa (Goolsby et al., 2006a).

1.3.3. Predation and parasitism

Predators (Sebolt and Landis, 2004; Denoth and Myers, 2005; Ding and Blossey, 2005; Hunt-Joshi et al., 2005) and parasitoids (Goeden and Louda, 1976; Hill and Hulley, 1995; Paynter et al., 2010) have been cited as limiting the efficacy of several biocontrol agents, and therefore impeding the level of success achieved by these biocontrol programmes. There are several examples of predation negatively impacting the survival, proliferation and efficacy of cactaceous biocontrol agents in South Africa including: egg predation by ants on *Tucamania tapiacola* (Dyar) (Lepidoptera: Pyralidae) against *O. aurantiaca* (Hoffmann, 1981) and on *Cactoblastis cactorum* Berg (Lepidoptera: Pyralidae) against *O. aurantiaca* and *O. ficus-indica* (Robertson, 1985; 1988). The effects of parasitism are usually subtler than predation (Goeden and Louda, 1976), which was evident in low field mortality of *C. cactorum* ascribed to the parasitic wasp *Bracon hebetor* Say (Hymenoptera: Braconidae) (Pettey, 1948).

Moran and Zimmermann (1991) noted that *Exochomus* spp. (Coeloptera: Coccinellidae) have been recorded feeding on *H. festerianus*, while *Chilochorus* spp. (Coleoptera: Coccinellidae) have been observed to prey on *H. festerianus* infecting *H. martinii* (Danninger, 2002). The suite of natural enemies associated with *H. festerianus* may have important implications for the success of this biocontrol agent following the enemy release hypothesis (Keane and Crawley, 2002). This hypothesis posits that in the introduced range, an exotic plant will experience reduced top-down regulation (herbivory), allowing it to proliferate and expand its range (Keane and Crawley, 2002). However, introduced biocontrol agents may also benefit from release from their own suite of natural enemies (Hawkins et al., 1999), implying that the co-introduction of control agents and natural enemies, or host-range extension by native enemies in the control agents' introduced range (Hill and Hulley, 1995), may limit the regulatory impact of control agents. The impact of the suite of predators

and parasitoids associated with *H. festerianus* in South Africa, may therefore be an important consideration with regards to the success of this biocontrol agent.

1.4. Aims and rationale

The primary aims of this thesis were to: (1) conduct a post-release evaluation of the biocontrol programme against *C. jamacaru* in South Africa, and in doing so (2) investigate factors that may contribute to the purported variable success achieved by biocontrol efforts employing *H. festerianus* against *C. jamacaru.* An understanding of factors that limit *H. festerianus* efficacy as a biocontrol agent of *C. jamacaru* will allow practitioners to tailor and improve the biocontrol programme against this weed, and will contribute to the theory and practise of biocontrol, by highlighting important considerations when implementing a successful biocontrol programme.

The variable success of biocontrol agents within and among target weed populations is well documented (Dodd 1940; Lesica and Hanna, 2004; Denoth and Myers, 2005; Boag and Eckert, 2013; Hovick and Carson, 2015), although the mechanisms explaining such variation are poorly understood (Van Driesche et al., 2010). The presence and abundance of the biocontrol agent *H. festerianus*, and the level of biocontrol success achieved against *C. jamacaru* was determined by a field-based monitoring study of weed populations with and without biocontrol (Chapter 2).

Introduced biocontrol agents are expected to support a suite of predators and parasitoids (Cornell and Hawkins, 1993; Hill and Hulley, 1995), which have been implicated in significantly reducing the efficacy of several cactaceous biocontrol agents in South Africa (Hoffmann, 1981; Robertson, 1985; 1988). The structure and abundance of the natural enemy assemblage associated with *H. festerianus* remains unstudied, and the effect of any acquired

natural enemies on the efficacy of *H. festerianus* as a biocontrol agent is unknown in South Africa (Paterson et al., 2011a). As such, the natural enemy assemblage associated with *H. festerianus* was identified and the effect of these acquired natural enemies on *H. festerianus* density and biocontrol efficacy were estimated by field-based collections and surveys (Chapter 3).

These data were discussed in the context of tailoring and improving the biocontrol programme against *C. jamacaru* in South Africa, as well as the implications of this study for the sustainable, long-term use of biocontrol as a weed management tool (Chapter 4).

Chapter 2

Evaluating the efficacy of *Hypogeococcus festerianus*, as a biological control agent of the cactaceous weed *Cereus jamacaru* in South Africa

2.1 Introduction

Understanding the interactions between host-specific natural enemies and plant population dynamics is important in the context of weed biocontrol (Halpern and Underwood, 2006; Garren and Strauss, 2009). The ubiquitous nature of plant-insect interactions in ecological communities suggests that herbivores (e.g., biocontrol agents) have great potential to affect plant population dynamics (Maron and Gardner, 2000; Miller et al., 2009), although whether and with what magnitude these effects are realised remains contentious (Hairston et al., 1960; Harper, 1977; Louda and Potvin, 1995; Maron and Crone, 2006). A growing body of literature suggests that herbivores may indeed regulate their host plant populations in a biocontrol setting (Caltagirone, 1981; McFadyen, 1998; Bellows, 2001; Van Driesche et al., 2010; Klein, 2011; Clewley et al., 2012). However, this is not always the case, as the introduction of control agents has proven unsuccessful in effecting control over the target weed in many instances (Julien and White, 1997; Callaway et al., 1999; Louda and Stiling, 2004; Thomas and Reid, 2007). A recent meta-analysis of 61 classical weed biocontrol agents released since 2000 found that approximately 48 % of these introductions resulted in effective suppression of the target weed (Clewley et al., 2012), while other authors have reported success rates of up to 80 % (Fowler et al., 2000).

Post-release evaluation of biocontrol is essential for the long-term success and sustainability of weed management (Carson et al., 2008). These evaluations may lend support for the active redistribution of effective agents (Nordblom et al., 2002), which can significantly reduce costs of sourcing additional, unnecessary additional species (Paynter, 2005), or if the management of a target weed is found to be unsuccessful, additional agents can be prioritised (Denoth et al., 2002). Furthermore, these evaluations may also provide important data to improve the science that underpins biocontrol (Paynter, 2006), and contribute to our understanding of plant-herbivore interactions (Briese, 2004).

However, accurately determining the efficacy of introduced biocontrol agents is not a trivial undertaking. For example, equating control agent establishment and subjective measures of agent abundance with weed suppression may be erroneous (Harris, 1973; Cullen and Snowball, 1979; Radford et al., 2001; McClay and Balciunas, 2005), if the control agent does not reach high enough population densities to exert enough pressure to suppress the target weed (Sheppard, 1992). Even if control agents are able to proliferate and reach high population densities they still may not have an impact on the weed if the level of damage does not exceed a threshold value under which individual plant performance is affected or if the impact of the agent is negated through compensation (Denoth and Myers, 2005; Jamieson et al., 2012).

Similarly, even when biocontrol agents successfully establish and elicit injurious effects at the individual-plant level, this may not result in weed suppression if individual-level effects do not translate into concomitant effects at the population-level (Carson et al., 2008; Morin et al., 2009). This may arise from the control agent not affecting critical life-history transitions (Paynter and Rees, 1997; McEvoy and Coombs, 1999; Denoth and Myers, 2005; Raghu et al., 2006; Shea et al., 2010; Dauer et al., 2012), or population-level compensatory mechanisms such as: life-history strategy (Crawley, 1989; Kelly and Dyer,

2002), density-dependent processes (Louda and Potvin, 1995; Maron and Simms, 2001), constant final-yield (Garren and Strauss, 2009; Swope and Parker, 2010), compensatory recruitment (Ortega et al., 2012), and the strength of microsite versus seed-limitation (Louda, 1982), which may negate the impact of herbivory. For example, Hoffmann (1990) demonstrated through simulation modelling that the combined impact of three biocontrol agents was required to reduce *S. punicea* seed production by > 99 %, which appeared to be the threshold above which negative population consequences would be observed for the weed. The 84% reduction in seed set associated with *Rhyssomatus marginatus* Fåhraeus (Coleoptera: Curculionidae) and the 98 % reduction associated with *Trichapion lativentre* (Bèguin-Billecocq) (Coleoptera: Curculionidae) was not found to result in reduced *S. punicea* population densities (Hoffmann, 1990). The impact of a third weevil *Neodiplogrammus quadrivittatus* (Olivier) (Coleoptera: Curculionidae), in concert with *R. marginatus* and *T. lativentre*, significantly reduced plant densities and brought *S. punicea* under complete biocontrol (Hoffmann and Moran, 1998; Klein, 2011). The complex nature of weed-agent interactions and their potentially dynamic outcomes highlights the need for quantitative, population-level evaluations of biocontrol agent efficacy (Hoffmann and Moran, 1999; Denoth et al., 2002; Carson et al., 2008; Morin et al. 2009).

It is possible to investigate the effect of biocontrol at the weed population-level by examining plant population age structure and resulting population dynamics (Hoffmann, 1990; Parker, 2000; Paynter, 2005). The age structure of a population may be represented by an age frequency distribution, which is obtained by plotting the number of individuals in each age class (e.g., 1 year age classes) (Paynter et al., 2003). Under optimal conditions, long-lived perennial plant populations have a constant recruitment rate (Mendoza and Franco, 1998), while their mortality rate is either constant or decreases with age (Agren and Zackrisson, 1990). The age structure of such populations will demonstrate a "reverse J" shape (Hett and

Loucks, 1976), which is indicative of active self-regeneration and therefore stable or expanding plant populations. However, an array of abiotic (e.g., precipitation) and biotic factors (e.g., herbivory) may influence recruitment and mortality rates, causing population age structures to depart from the "reverse J" shape, becoming uniform or bell-shaped (Bullock et al., 1996). By comparing weed population age structures between sites with and without biocontrol agents, this approach may be useful as a tool for post-release evaluation of long-lived weeds, as practitioners may be able to infer retrospective influences on population recruitment, and subsequently predict what the consequences will be for future weed population dynamics (Bullock et al., 1996), such as its regeneration capacity and population persistence (Agren and Zackrisson, 1990).

Cereus jamacaru is a long-lived, perennial plant (Klein, 1999). It primarily propagates through an extensive seed set, which has allowed it to successfully invade and proliferate across the northern parts of South Africa (Klein, 1999), and more recently the sub-tropical Kwa-Zulu Natal Province. The weed has been targeted for biocontrol using the mealybug *H. festerianus* (Moran and Zimmermann, 1991; Klein, 1999), which appears to reduce fruit production and therefore seed output (Paterson et al., 2011a). As such, *C. jamacaru* is considered to be under complete control in South Africa (Klein, 2011), although this is based on anecdotal evidence and expert opinion, as no formal post-release evaluation of the biocontrol programme against *C. jamacaru* has been conducted (Paterson et al., 2011a).

Demonstrating that *H. festerianus* has a negative impact on *C. jamacaru* would provide strong support for the active redistribution of this agent to uninfected weed populations across the country (e.g., Paynter, 2005), while additional control agents or alternative management strategies could be prioritised if *H. festerianus* demonstrates little impact on the target weed (e.g., Hoffmann and Moran, 1998). Accordingly, the objective of

this study was to evaluate the efficacy of *H. festerianus* as a biocontrol agent of *C. jamacaru* in South Africa. A field-based comparative study was performed, comparing individual and population-level *C. jamacaru* performance, weed age structures and resulting population dynamics, between sites where *H. festerianus* was present (biocontrol sites) and sites where the agent was absent (control sites). These findings provided valuable insights into how to tailor and improve the management strategy for *C. jamacaru* in South Africa, and provided further support for the long-term sustainability and utility of weed biocontrol.

2.2. Materials and methods

2.2.1. Field survey

Four regions were sampled across South Africa between February and May 2016, as this time period coincides with peak fruiting and flowering. As is the case with many biocontrol programmes, baseline data prior to biocontrol was unavailable (Carson et al., 2008). The sampling design implemented during this study may somewhat account for this limitation (Hovick and Carson, 2015), as sites were sampled that contained biocontrol agents (biocontrol sites) and sites that were clear of *H. festerianus* (control sites) (Morin et al., 2009), over a diverse range of ecological and climatic conditions (van Klinken et al., 2003).

A total of 22 sites were surveyed across the four regions, including eight biocontrol and 14 control sites (Table 2.1.). The Groot Marico (GM) and Rust-de-Winter (RD) regions contained both biocontrol and control sites, while in the Kwa-Zulu Natal (HL – Hluhluwe district and MK – Mkuze district) and Eastern Cape (EC) regions sites were clear of *H. festerianus*, and thus were all effectively control sites.

To confirm that any observed differences in *C. jamacaru* performance and population dynamics during the current study were due to the impact of biocontrol, and not underlying abiotic variation between sampling regions (e.g., climate), all weed parameters were

Table. 2.1. Summary information for *Cereus jamacaru* field sites sampled during the current study.

Region (Site)	Site code	GPS locality	*Hypogeococcus festerianus* biocontrol (present/absent)
Groot Marico			
Woodbine	GM1	25.490° S, 26.321° E	Present
Pokoje	GM2	25.485° S, 26.343° E	Present
Bergvliet Farm #1	GM3	25.440° S, 26.371° E	Present
Bergvliet Farm #2	GM4	25.445° S, 26.369° E	Absent
Bosveld	GM5	25.522° S, 26.391° E	Present
Rust-de-Winter			
Leeukraal Farm #1	RD1	25.149° S, 28,752° E	Present
Leeukraal Farm #2	RD2	25.162° S, 28.764° E	Present
Farm GR_120	RD3	25.194° S, 28.771° E	Absent
Pienaarsrivier	RD4	25.167° S, 28.626° E	Present
Rooikoppies	RD5	25.235° S, 28.560° E	Present
Leeukraal Farm #3	RD6	15.188° S, 28.679° E	Absent
Kwa-Zulu Natal			
Mzinene River	HL1	27.904° S, 32.365° E	Absent
Sana	HL2	27.754° S, 32.466° E	Absent
Bushlands Siding	HL3	28.081° S, 32.290° E	Absent
Mpempe	HL4	27.771° S, 32.442° E	Absent
Mbazwana	HL5	27.571° S, 32.482° E	Absent
Kwanyamazane	MK1	27.217° S, 32.557° E	Absent
Mseleni	MK2	27.367° S, 32.526° E	Absent
Mkuze #1	MK3	27.613° S, 32.042° E	Absent
Ubombo Dam	MK4	27.612° S, 32.043° E	Absent
Eastern Cape			
Alexandria	EC1	33.542° S, 26.389° E	Absent
Fort Brown	EC2	33.137° S, 26.621° E	Absent

compared between control sites in Groot Marico/Rust-de-Winter and Kwa-Zulu Natal/Eastern Cape.

2.2.2. Data collection

At each *C. jamacaru* site, a 100 m x 10 m (1000 m^{-2}) transect was erected. The age and mortality status (whether each plant was "alive" or "dead") of each plant was recorded within each transect. Plant density was recorded as the number of individuals within each transect. The age of *C. jamacaru* individuals can be measured due to the columnar, constricted growth form of each plant stem (Taylor and Walker, 1984). Each stem constriction is representative of the end of a growing season, and therefore the maximum number of constrictions counted for each individual plant gives the plant's age. Seedlings were considered to be individuals ≤ 2 years old, after which plants become multi-stemmed (Taylor and Walker, 1984).

The reproductive fitness of *C. jamacaru* was determined by recording the number of mature plants (plants are able to produce fruit at ≥ 8 years old) (Taylor and Walker, 1984), and their reproductive status (whether each plant was "fruiting" or "non-fruiting") for each site, and the number of fruits per plant, which was then summed over each site to provide a site-wise measure of fruit production.

The abundance of *H. festerianus* was recorded as the number of galls observed on each plant at each sample site, as the agent forms a white, woolly gall upon establishment (Klein, 2002).

2.2.3. Statistical analyses

All statistical analyses were performed using the statistical software R ver. 3.1.2, in the integrated development environment R Studio© ver. 2.15.3 (The R Foundation for Statistical

Computing 2013). Unless otherwise stated, all statistical functions used in subsequent analyses can be found in the 'stats' package (R Core Team, 2013).

The consequence of *H. festerianus* infestation for weed reproductive capacity was analysed by fitting a logistic regression model, which incorporated a binomial error structure, to the reproductive status of each mature adult plant capable of reproducing (plants ≥ 8 years old), with respect to mean *H. festerianus* abundance (no. of galls/plant^{-1}). The fit and appropriateness of the fitted model was explored by calculating the G^2 statistic (deviance) and the dispersion parameter using the 'rms' package (Harrell, 2015). A two-sample t-test was employed to evaluate whether individual plant fecundity was affected by biocontrol, where mean fruit production (plant^{-1}) was the dependent variable and biocontrol (biocontrol/control) was fitted as a grouping factor.

To evaluate whether impacts associated with *H. festerianus* herbivory at the individual-plant level translated into reduced *C. jamacaru* fitness at the population-level, two components of reproductive fitness were analysed. Firstly, the arcsine-transformed proportion of mature adult plants actively reproducing was compared between biocontrol and control sites by employing a Mann-Whitney U-test. Secondly, total fruit production (per 1000 m^{-2}) was compared between biocontrol and control sites by a two-sample t-test. Linear regression analysis was performed to investigate the interaction between the number of stems galled by *H. festerianus* and fruit production per 1000 m^{-2}.

To evaluate whether *H. festerianus* could kill *C. jamacaru*, as has been proposed previously in the literature (Klein, 1999; Paterson et al., 2011a), a 2x2 contingency table was computed containing the status of all *C. jamacaru* individuals sampled during this study ("alive" or "dead"), with biocontrol (biocontrol/control) fitted as a grouping factor. The contingency table was subjected to a Pearson's Chi-squared test to test the null hypothesis that the frequency of *C. jamacaru* mortality was independent of the presence of *H.*

festerianus. Standardised residuals were calculated to investigate which tabulated cross-classifications deviated most from the expected values, which was then statistically analysed by calculating modified Wald's odds ratios using the 'vda' package (Meyer et al. 2016). Deviations from expected *C. jamacaru* mortality frequencies were explored by representing the data using mosaic plots (Friendly, 1994; Gotelli and Ellison, 2004).

The effect of biocontrol on weed density was investigated by comparing plant densities between sites with and without *H. festerianus* by two-sample t-tests. This comparison was made for overall plant density (all ages), seedling density (1-2 years old), pre-fruiting adult density (3-8 years old) and mature adult density (8+ years old). Plant densities were log(n + 1) transformed prior to analysis to meet the assumption of normality, where required.

The influence of individual-level impacts associated with *H. festerianus* on *C. jamacaru* population dynamics and resulting demographic patterns was investigated by comparing age structures between sites with and without *H. festerianus* (Hoffmann, 1990; Paynter, 2005). The age structure of a population may be represented by an age frequency distribution, which is obtained by plotting the number of individuals in each age class against each successive age class (e.g., 1 year age classes). Age structures were compared and contrasted by visual inspection.

2.3 Results

2.3.1. Between region comparisons of control sites

The number of fruits produced per *C. jamacaru* individual was not significantly different between sampling regions (t = 1.16, d.f. = 4.42, P = 0.305), although individual plants did produce slightly fewer fruits/plant^{-1} at control sites in Groot Marico/Rust-de-Winter (23.67 ± 7.06; mean ± S.E.) than at control sites in Kwa-Zulu Natal/Eastern Cape (39.56 ± 8.87).

Similarly, no significant difference was observed for fruit production per site (per 1000 m^{-2}) between control sites in these two sampling regions (t = 1.84, d.f. = 4.19, P = 0.135).

A two-sample t-test indicated that plant densities at control sites were comparable between the two sampling regions (t = -1.31, d.f. 9.68, P = 0.218). Control sites in Groot Marico/Rust-de-Winter contained approximately 25.33 ± 3.17 plants, while sites in Kwa-Zulu Natal/Eastern Cape contained 32.70 ± 4.59 plants. Irrespective of sampling region, control sites were described by reverse J-shaped age frequency distributions, typified by high seedling numbers and diminishing proportions of mature adult individuals. Plant populations described by a reverse J-shaped distribution are stable or expanding populations, and can self-replace and persist indefinitely.

As such, *C. jamacaru* reproductive capacity and resulting population dynamics in the absence of *H. festerianus* was not significantly different between sampling regions (i.e., comparing control sites between Groot Marico/Rust-de-Winter and Kwa-Zulu Natal/Eastern Cape sampling regions). This finding suggests that abiotic variability (e.g., climate) between sampling regions was unlikely to account for observed differences between control and biocontrol sites. Therefore, it was deemed appropriate to pool *C. jamacaru* sites in the two sampling regions, which resulted in a more robust dataset which included a large amount of spatial variation (Hovick and Carson, 2015).

2.3.2. Effect of infestation by Hypogeococcus festerianus

Hypogeococcus festerianus had a deleterious effect on the reproductive status and resulting fecundity of *C. jamacaru*. The probability of a mature plant being reproductively active (i.e., producing fruit) was significantly influenced by the presence and abundance of *H. festerianus* biocontrol (χ^2 = 76.62, d.f. = 1, P < 0.001). As *H. festerianus* gall abundance increased, the

probability of observing *C. jamacaru* reproduction decreased significantly (Fig. 2.1.). The odds-ratio of the relationship between *H. festerianus* abundance and the reproductive status of *C. jamacaru* was 0.482, which is indicative of a ~ 52 % decrease in the probability of reproduction with each unit increase of *H. festerianus* abundance. A few *C. jamacaru* individuals could tolerate, and continue to actively reproduce even when infected with up to 7 *H. festerianus* galls, above which the probability of reproduction was minimal (Fig. 2.1.).

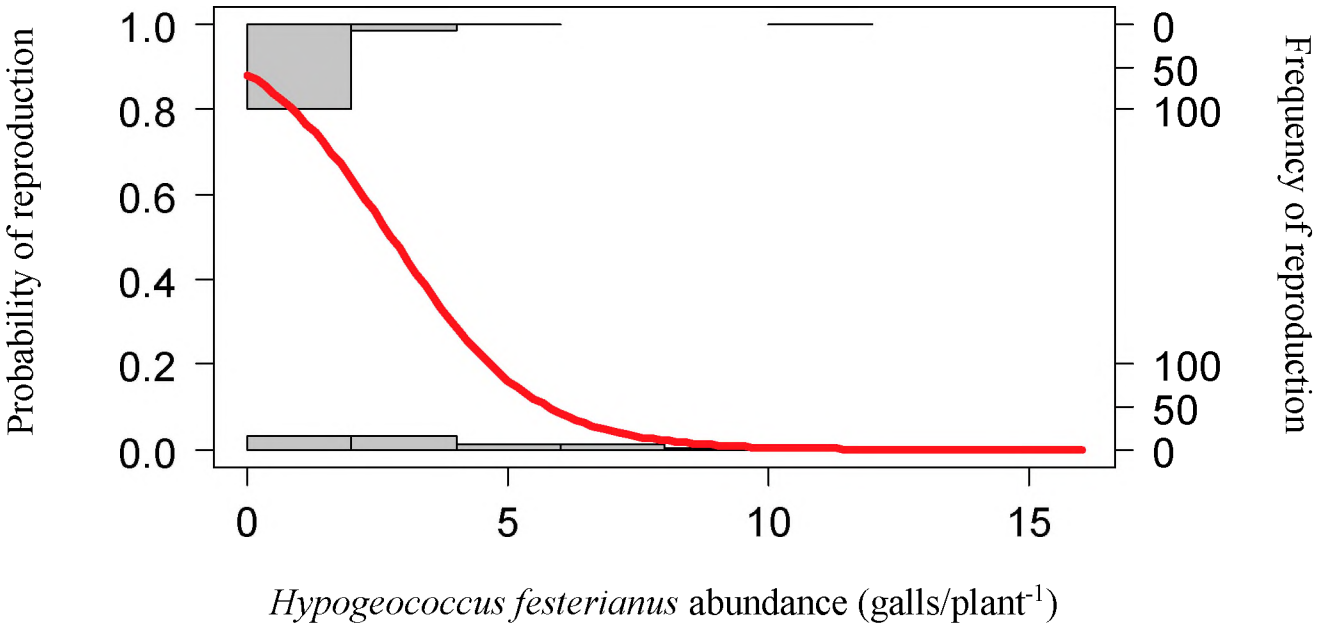


Fig. 2.1. Fitted logistic regression curve which indicated that the probability of mature *Cereus jamacaru* individuals being reproductively active (i.e., producing fruit) was not independent of the abundance of its biocontrol agent *Hypogeococcus festerianus* (galls/plant^{-1}) ($\chi^2 = 76.62$; $P < 0.001$). The solid line represents the fitted logistic regression model, while histograms represent counts from the observed data, as suggested by Smart et al. (2004).

Mature plants produced approximately 91 % fewer fruits/plant^{-1} when infected with *H. festerianus* (3.08 ± 1.20) than mature plants that were uninfected (36.15 ± 7.25) (t = 7.51; d.f. = 20; $P < 0.001$) (Fig. 2.2.).

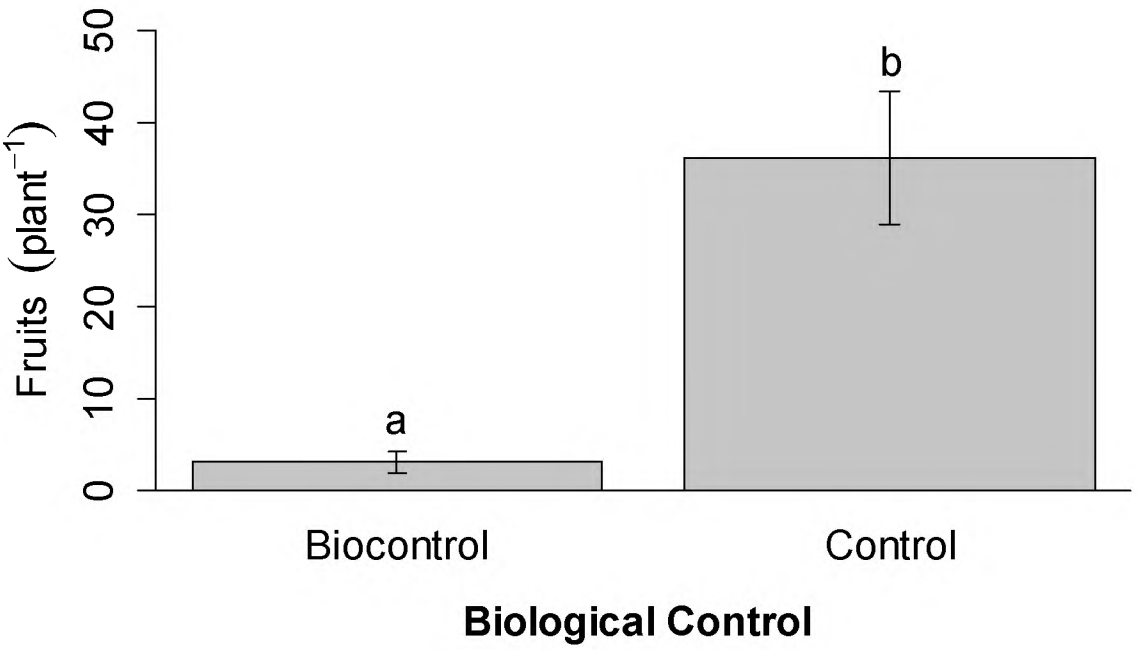


Fig. 2.2. Mean (± S.E.) number of fruits produced per mature *Cereus jamacaru* individual, at sites where *Hypogeococcus festerianus* was present (biocontrol) and where the biocontrol agent was absent (control), pooled between sample regions. Significant differences are indicated by columns followed by different letters (t-test; $P < 0.05$).

The deleterious effect of *H. festerianus* at the individual-plant level translated into population-level consequences for *C. jamacaru* fitness. Proportionately fewer mature plants were reproductively active at biocontrol sites ($U = 8.00$, $P < 0.001$), with only 35 % of mature plants producing fruit, while 100 % of mature plants at control sites actively produced fruit (Fig. 2.3.).

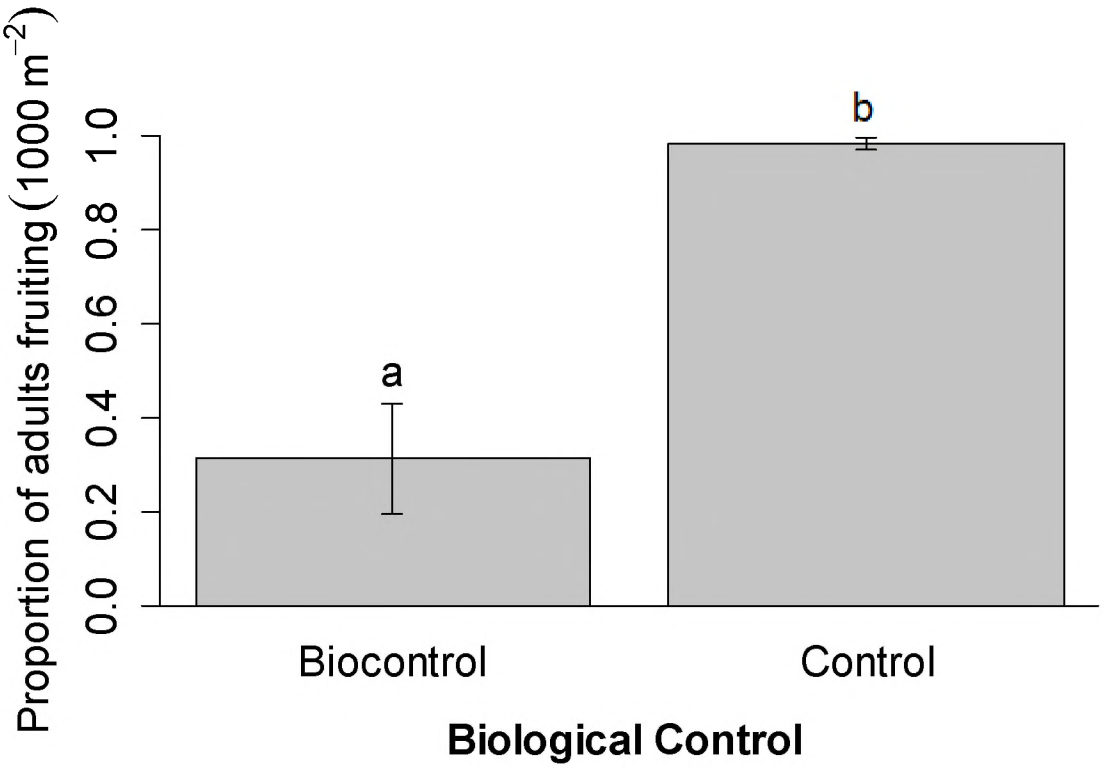


Fig. 2.3. Mean (± S.E.) proportion of mature *Cereus jamacaru* individuals fruiting (per 1000 m^{-2}), at field sites where *Hypogeococcus festerianus* was present (biocontrol) and where the biocontrol agent was absent (control), pooled between sample regions. Significant differences are indicated by columns followed by different letters (Mann-Whitney U test; $P < 0.05$).

By limiting the number of fruiting individuals and reducing individual-plant fecundity at biocontrol sites, *H. festerianus* significantly reduced fruit production at the site-level, whereby biocontrol sites produced approximately 59 % fewer fruits per 1000 m^{-2} (80.42 ± 28.43) than control sites (220.07 ± 52.58) (U = 100, P = 0.003) (Fig. 2.4.).

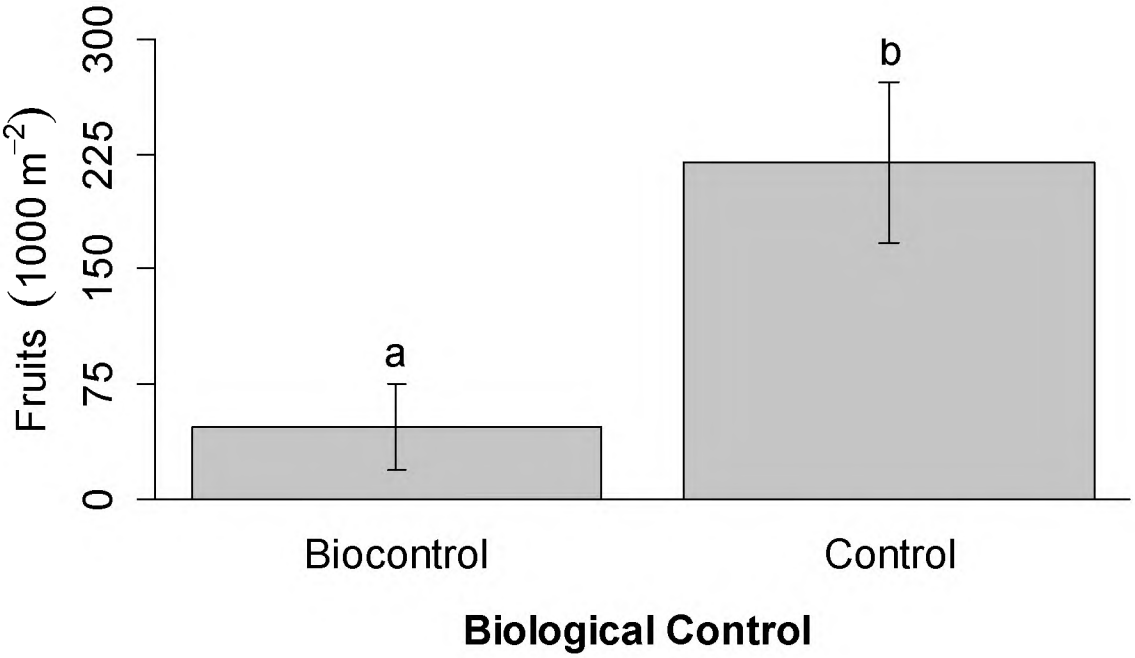


Fig. 2.4. Mean (± S.E.) number of fruits produced by *Cereus jamacaru* per 1000 m^{-2}, at field sites where *Hypogeococcus festerianus* was present (biocontrol) and where the biocontrol agent was absent (control), pooled between sample regions. Significant differences are indicated by columns followed by different letters (Mann-Whitney U test; $P < 0.05$).

A linear regression between the proportion of *C. jamacaru* stems galled by *H. festerianus* and fruit production per site demonstrated a strong, negative correlation (linear regression: $Y_{[\log(\text{total fruit} + 1)]} = 5.18 - 3.71x_{[\text{prop\_stems\_galled}]}$, $t_{20} = -6.72$, $P < 0.001$). The fitted regression model showed that the proportion of stems galled by *H. festerianus* per site explained approximately 68 % of the variation in *C. jamacaru* fruit production between sites (Fig. 2.5.).

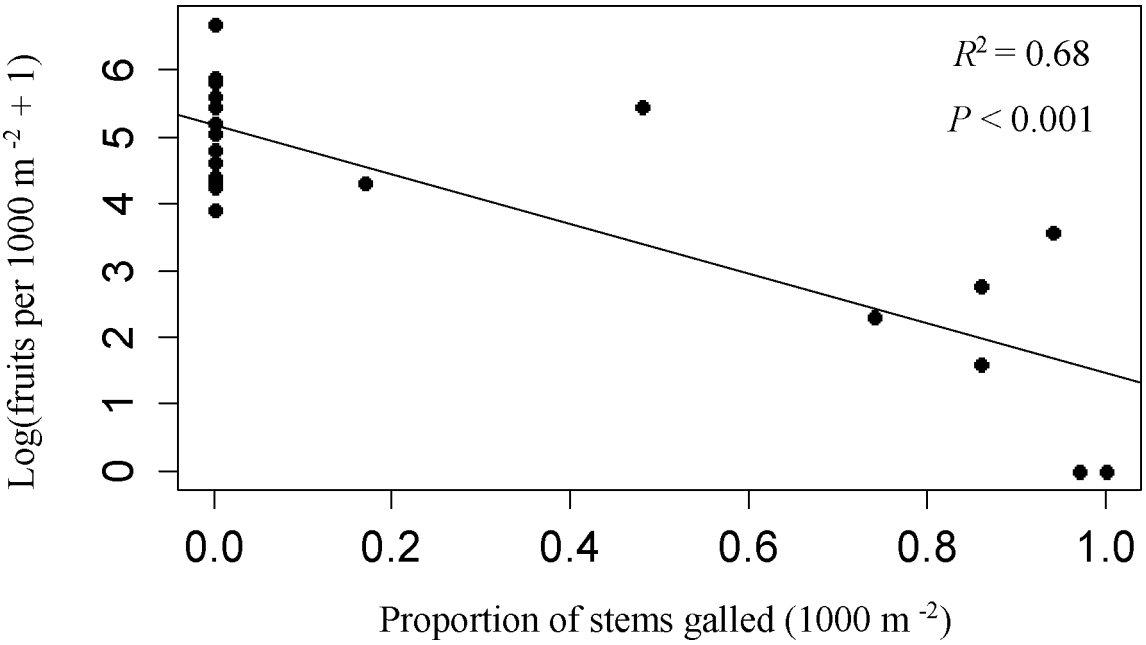


Fig. 2.5. Relationship between the proportion of stems galled by *Hypogeococcus festerianus* and total fruit production ($Y_{[\log(\text{total fruit} + 1)]} = 5.18 - 3.71x_{[\text{prop\_stems\_galled}]}$); Each point on the graph represents site means (per 1000 m^{-2}) of the respective variable. The solid line represents the fitted linear regression model.

The proportion of dead *C. jamacaru* individuals at each sample site was not independent of *H. festerianus* biocontrol (χ^2 = 8.82, d.f. = 1, P = 0.003). Inspection of standardised residuals indicated that there were fewer live plants at sites where *H. festerianus* was present, and more live plants at control sites, than would be expected if there were no association between *H. festerianus* and plant mortality (Fig. 2.6.). The odds of encountering a dead *C. jamacaru* individual were significantly higher at sites where *H. festerianus* was present than at control sites (Wald's modified odds ratio 95 % CI: 1.14-1.95).

Weed density was approximately 51 % lower at sites where *H. festerianus* was present (t = 3.17; d.f. = 17.01; P = 0.005). Control sites contained 31.36 ± 3.40 individuals (mean ± S.E.), while sites infected by the biocontrol agent contained 15.25 ± 3.76 individuals (Fig. 2.7.). This finding was largely explained by an 80 % reduction in seedlings (t = 5.15; d.f. = 19.49; P < 0.001), and a 37 % reduction in pre-fruiting plant density in association with *H. festerianus* biocontrol, albeit this latter reduction was not statistically significant (t = 1.69; d.f. = 11.13; P = 0.119). Mature plant density was comparable between biocontrol and control sites (t = 0.01; d.f. = 12.63; P = 0.991) (Fig. 2.7.).

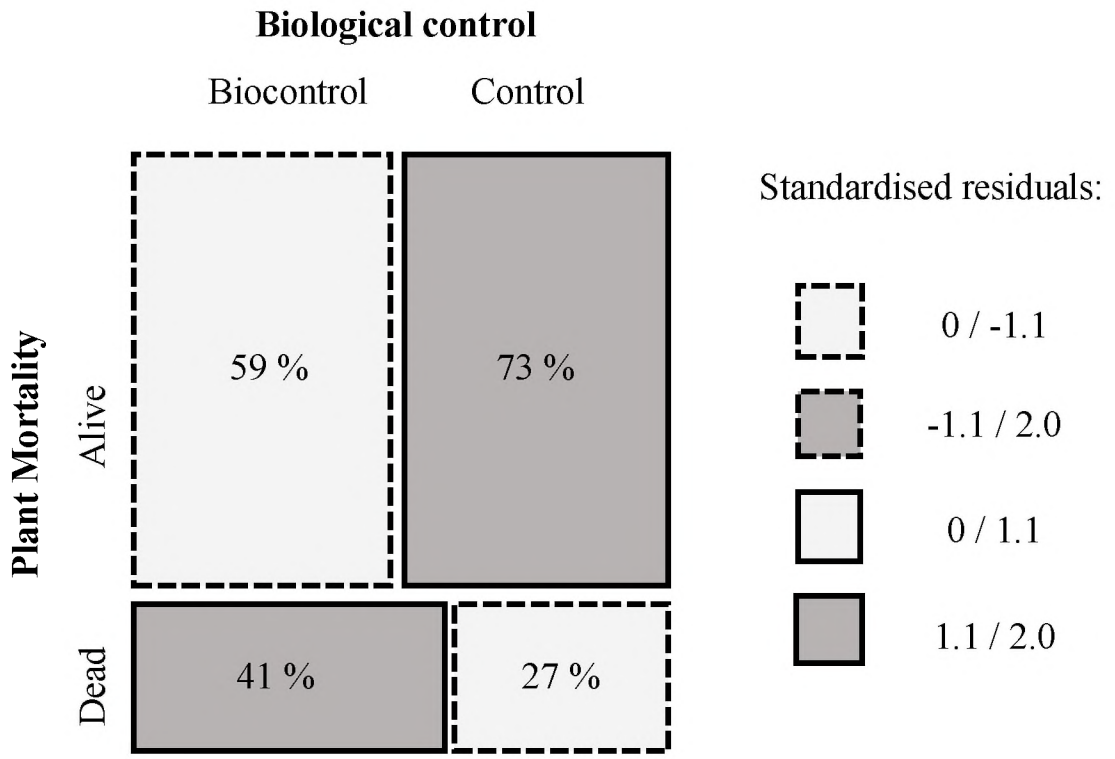


Fig. 2.6. Mosaic plot of individual *Cereus jamacaru* plant mortality frequencies (per 1000 m^{-2}), with respect to biocontrol employing *Hypogeococcus festerianus*. The area of each tile is proportional to the respective cell frequency of a corresponding 2 x 2 contingency table. The dashed (negative) and solid (positive) tile outlines indicate the sign of deviation from the expected frequencies of plant mortality. The shading of each tile is proportional to the standardised residual from the fitted model (values provided in figure legend). Significant deviations from expected cell frequencies were observed (Pearson's Chi-squared test: $P = 0.003$), and are indicted by darker-shaded tiles. Values given inside each tile represent the percentage of *Cereus jamacaru* individuals, pooled across sample sites, that were dead or alive with respect to the presence or absence of biocontrol.

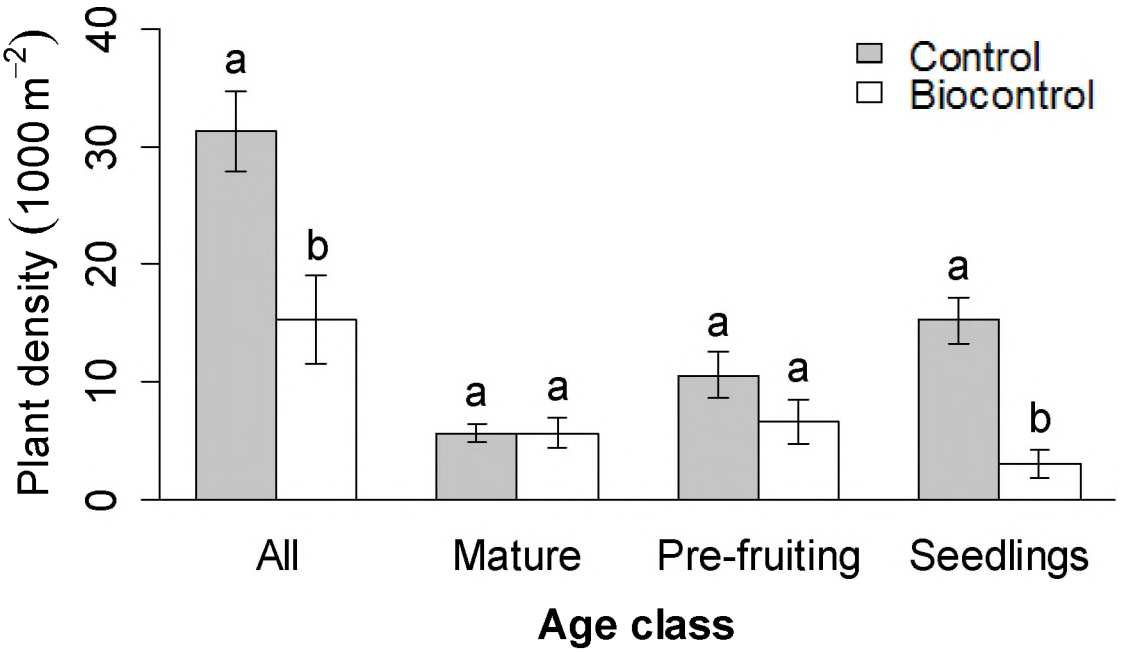


Fig. 2.7. Mean (± S.E.) number of *Cereus jamacaru* individuals per 1000 m^{-2}, partitioned into distinct age classes representative of all individuals: irrespective of age class (All), ≥ 8 years old (Mature; i.e., capable of reproduction), 3-8 years old (Pre-fruiting) and 1-2 years old (Seedlings). Columns within the same cage class followed by different letters are significantly different (t-test, $P < 0.05$).

Upon inspection of site-based *C. jamacaru* age structures, it was evident that *H. festerianus* influenced weed age structures and resulting population dynamics. Age structures in the absence of *H. festerianus* were dominated by seedlings and contained diminishing proportions of mature individuals (e.g., Fig. 2.8. RD3, RD6, HL1, MK1, MK2, MK3). In contrast, many sites associated with *H. festerianus* demonstrated more uniform age-structure distributions, which were typified by a lack of seedling recruits and consisted mainly of mature individuals (e.g., Fig. 2.8. GM1, GM3, RD1, RD4, RD5). However, several age structures deviated from the expected age frequency distribution associated with either control or biocontrol sites. For example, site GM2 (biocontrol site) demonstrated a similar age structure distribution and level of recruitment to stands where *H. festerianus* was absent, while a few of the control sites were described by age frequency distributions that were intermediate in appearance between typical biocontrol and control sites or more representative of biocontrol sites than would be expected (e.g., Fig. 2.8. GM4, HL2, HL5, EC2).

There was great variability with regards to age frequency distributions of *C. jamacaru* sites in the absence of *H. festerianus*, and between sites where *H. festerianus* was present, both between and within sampling regions. Firstly, a few control site age frequency distributions were more representative of intermediate site age structures, typified by fewer seedlings than would be expected for age structures of typical control sites, however declines in pre-fruiting and mature individuals observed at biocontrol sites were not apparent. This finding suggests that factors other than herbivory may somewhat limit the proliferation and abundance of *C. jamacaru*. Secondly, biocontrol sites in the Rust-de-Winter region appear to have been suppressed by *H. festerianus*, with an almost complete lack of successful recruitment over the preceding four years at three sites and diminishing proportions of adult plants (e.g., Fig. 2.8. RD1, RD4, RD5), while similar indications of weed suppression were

not as evident in the Groot Marico region (e.g., Fig. 2.8. GM2, GM3, GM5). Biocontrol sites in Groot Marico were typified by intermediate age frequency distributions, whereby the number of seedlings counted were generally lower than at control sites, however plant density in older age classes does not appear to have diminished despite the presence of *H. festerianus*. Lastly, age structures were variable within regions, as was evident in Rust-de-Winter, where at many biocontrol sites *C. jamacaru* appears to have been effectively suppressed by *H. festerianus* (e.g., Fig. 2.8. RD1, RD4, RD5), however one site within the region (Fig. 2.8. RD2) does not appear to have been suppressed to the same extent.

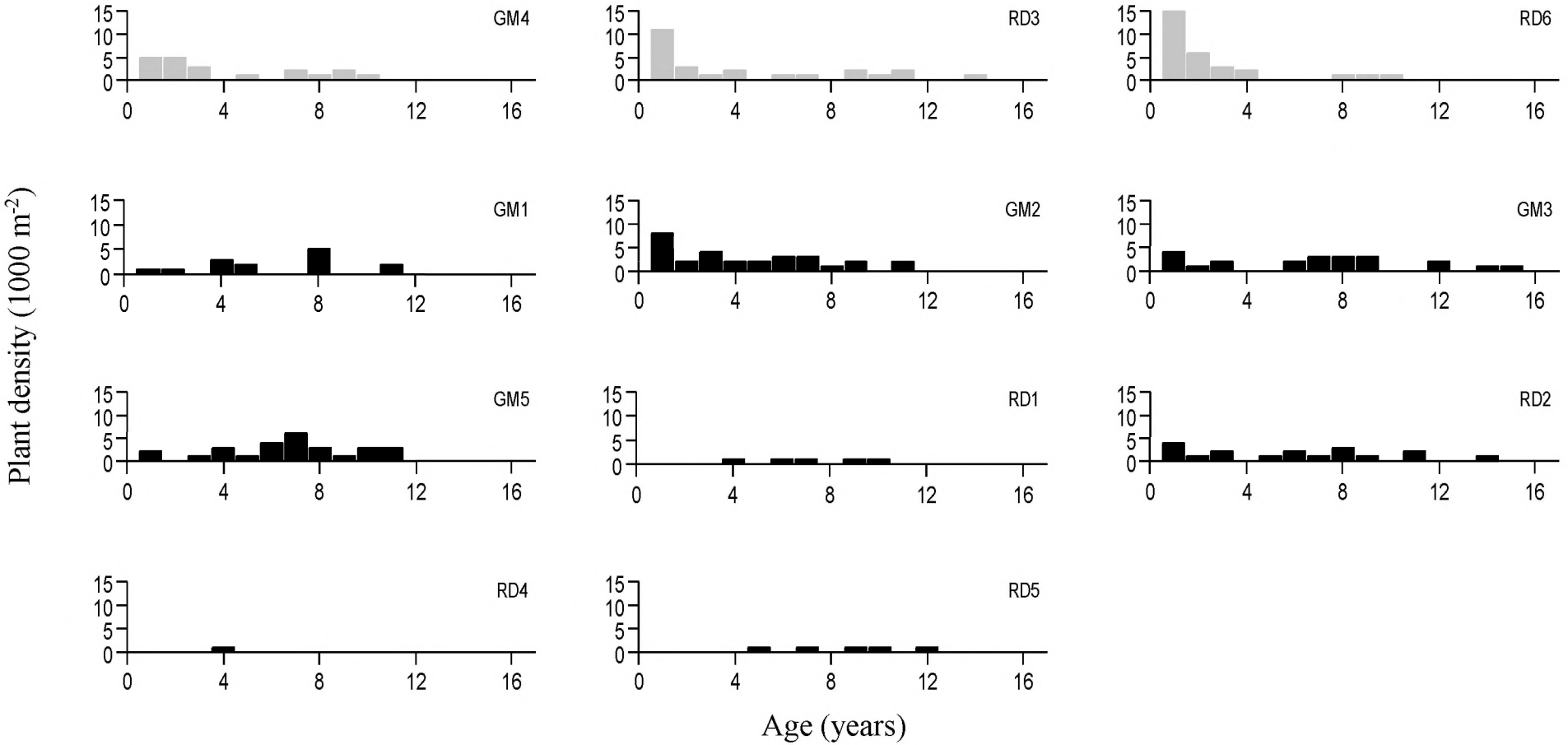


Fig. 2.8. Age frequency distributions of *Cereus jamacaru* sample sites, where *Hypogeococcus festerianus* was absent (control sites indicated by grey-shaded bars) and where *Hypogeococcus festerianus* was present (biocontrol sites indicated by black-shaded bars). Refer to Table 2.1. for site details.

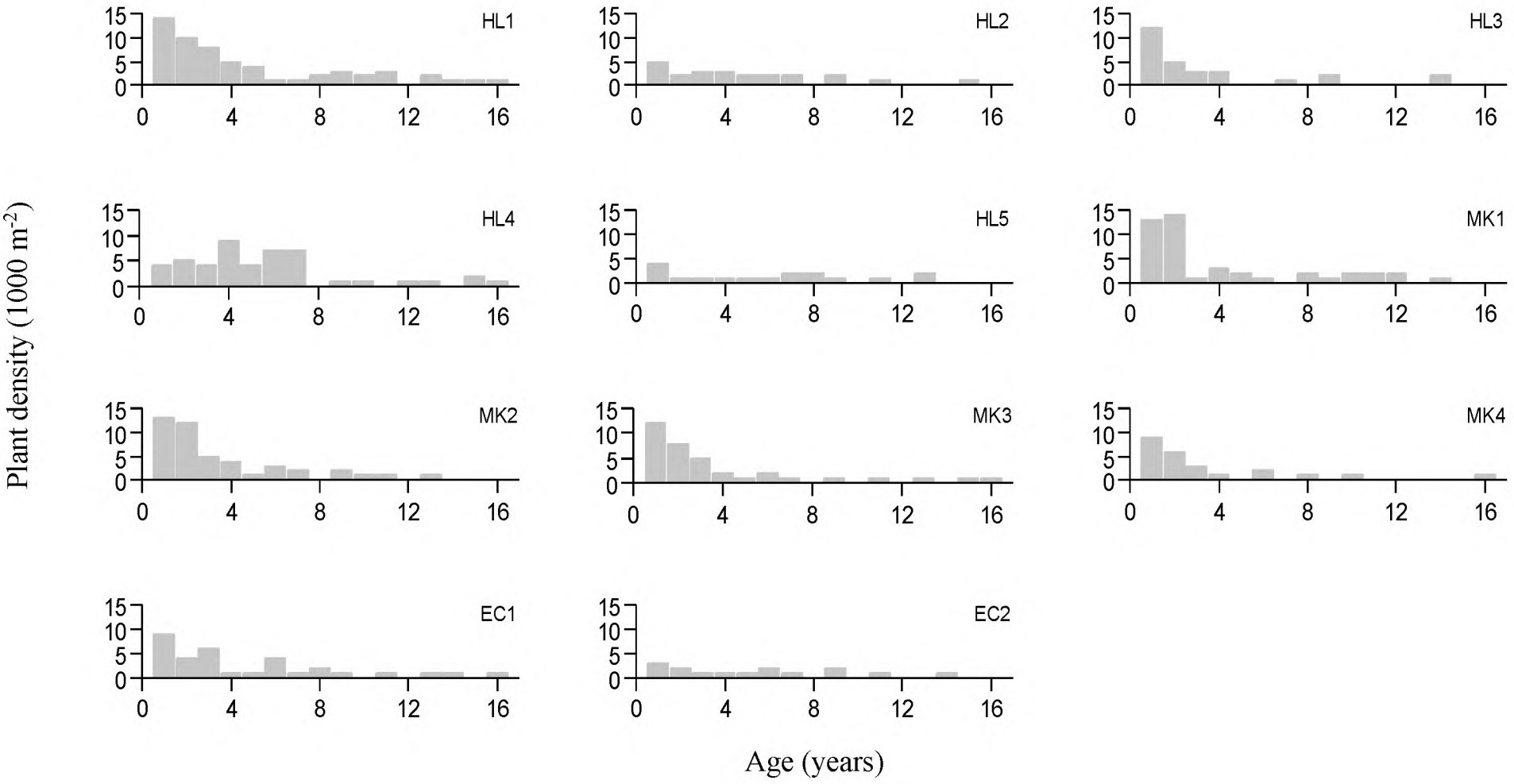


Fig. 2.8. continued.

2.4 Discussion

The findings from this study suggest that *H. festerianus* has a negative impact on *C. jamacaru* performance in South Africa, as indicated by fewer fruits produced and greater levels of plant mortality at sites where *H. festerianus* was present in comparison to control sites. Comparisons of *C. jamacaru* populations at control sites, between sampling regions, indicated that underlying abiotic conditions were unlikely to explain a significant amount of variation in *C. jamacaru* performance, and as such any observed differences in population dynamics were attributed to the impact of *H. festerianus* biocontrol. The findings confirm that *H. festerianus* can reduce *C. jamacaru* fruit production and kill individual plants (Paterson et al., 2011a). The reduced numbers of fruit recorded at biocontrol sites stemmed from individual-level effects associated with *H. festerianus*, whereby plants that were infected with the biocontrol agent produced fewer fruit and increasing control agent loads decreased the probability of *C. jamacaru* individuals being reproductively active. These findings are consistent with the literature, which has accumulated a wealth of studies which indicate that herbivory can elicit injurious effects at the individual-plant level (reviewed by Crawley, 1989).

Of greater consequence was whether *H. festerianus* could negatively impact *C. jamacaru* at the population-level, which is a requirement for successful biocontrol (Carson et al., 2008). Indeed, the negative impact of *H. festerianus* on weed fecundity and survival had a concomitant effect on *C. jamacaru* at the population-level. Plant densities were significantly lower at sites where *H. festerianus* was present, with respect to control sites. Further, age structures in the absence of *H. festerianus* demonstrated a reverse J-shaped age frequency distribution, being typified by high seedling numbers. Plant populations described by a reverse J-shaped distribution are considered to self-regenerating or expanding populations (Alvarez-Buylla and Martinez-Ramos, 1992), and as such are expected to persist or

proliferate under current conditions. In contrast, age structures of *C. jamacaru* populations where *H. festerianus* was present deviated from those observed at control sites. These age structures demonstrated a more uniform or bell-shaped age frequency distribution, and were typified by fewer or a complete lack of seedlings, than sites where biocontrol agents were present. Uniform age frequency distributions of *Sesbania punicea* (Cav.) Benth. (Fabaceae) (Hoffmann, 1990) and *Mimosa pigra* L. (Mimosaeae) (Paynter, 2005) have been interpreted as those weed populations, which due to a lack of recruitment, may ultimately be brought under complete biocontrol. These findings suggest that as mature individuals senesce and/or are killed by *H. festerianus*, the lack of recruitment associated with biocontrol appears to limit the ability of weed populations to self-replace. In doing so, *C. jamacaru* population persistence diminishes, and gradual population declines are observed, given sufficient time.

While it was clear that *H. festerianus* had a negative consequence on *C. jamacaru* population dynamics, the magnitude of impact associated with biocontrol herbivory was variable and may be mediated by other abiotic and biotic factors. The finding of variable biocontrol success in the current study is consistent with biocontrol literature which suggests that substantial variation in biocontrol success may be the norm, rather than the exception (Denoth and Myers, 2005; Grevstad, 2006; Jamieson et al., 2012; Boag and Eckert, 2013). A possible explanation for the observed variability in age structures, which were used as an indicator of biocontrol success, between regions and possibly even within regions, exists in the time that *H. festerianus* has been present at each sample site. Hoffmann (1990) demonstrated through simulation modelling of *S. punicea* biocontrol, using two weevils, that at least four years of biocontrol was required for any impact of herbivory on *S. punicea* recruitment to be observed, and approximately 12 years of sustained biocontrol pressure was required before the weed was visibly suppressed, as indicated by declines in plant density. While little data exists as to when and with what effort *H. festerianus* was released at sites

sampled during this study, there are unpublished records of the control agent being released by the Department of Agriculture in Rust-de-Winter since 1993, and in Groot Marico by Working for Water since 1998 (H. Klein, pers. comm). Thus, it is speculated that the between-region variability observed during this study may be due to *H. festerianus* being established for a longer period at sites in Rust-de-Winter (where weed suppression was more apparent) than in Groot Marico (where weed suppression was less apparent). The landowner of Bergvliet Farm (sites GM3 and GM4 in Groot Marico) noted that *H. festerianus* was only released at the former site during the summer of 2006, which indicates that the control agent has only been present, at least at this sample site, for a maximum of 10 years.

The time required for *H. festerianus* to have an observable impact on *C. jamacaru* populations is likely mediated by the presence and abundance of a soil seed bank. The infection of a mature *C. jamacaru* individual with *H. festerianus* arrests fruiting almost immediately (Klein, 1999), while the current study suggests that the effects of biocontrol on weed population dynamics take longer to become apparent ($\geq$ 10 years). Little is known about the presence and abundance of a seed bank within exotic *C. jamacaru* populations, however Taylor and Walker (1984) suggested that some form of seed dormancy may exist for *C. jamacaru* seeds, following their inability to stimulate germination in greenhouse trials. Therefore, an investigation in the presence and role of a seed bank in the context of successful biocontrol of this species may be warranted, as both modelling (Maron and Gardner, 2000) and experimental studies (Swirepik et al., 2008) have highlighted the requirement of depleting a soil seed bank for successful plant population management.

The importance of allowing sufficient time to elapse between control agent releases and evaluation studies is well documented (Hoffmann, 1995; Syrett et al., 2000). This led McFadyen (1998) to propose that at least 10-20 years should elapse between agent introduction and evaluation. However, this is not always the case, as control agents can

impact and suppress weed populations over much shorter time-periods (McConnachie et al., 2003). The magnitude of fruit reduction observed in Groot Marico suggests that, given sufficient time, *H. festerianus* is predicted to suppress weed populations, although other potentially confounding abiotic and biotic factors that may limit the magnitude of *C. jamacaru* biocontrol in this area cannot be ruled out at present.

There is a wealth of literature which demonstrates that recruitment and the population dynamics of many cactaceous species is dependent on favourable abiotic conditions, including: sufficient rainfall (Pierson and Turner, 1998; Godínez–Álvarez et al., 2003; Arroyo-Cosultchi et al., 2016) and soil type (Taylor and Walker, 1984), and biotic interactions including: the presence and abundance of nurse-plants (Mandujano et al., 2002) and interspecific competition (Burger and Louda, 1995, 1994; Reyes-Aguero et al., 2006). Nurse-plants are perennial shrubs and trees which provide enough canopy to facilitate seedling establishment for other plant species, specifically by proving a protective microhabitat suitable for germination (e.g., nurse-plant canopies provide shade which decreases temperatures and levels of photosynthetically active radiation experienced by seedlings) (Turner et al., 1966). It is now well appreciated that seedling recruitment and survival of many cactaceous species is positively correlated with sufficient moisture, whereby recruitment events are largely limited to years of high rainfall (Mandujano et al., 2001). While the present study did not evaluate the influence of abiotic conditions on *C. jamacaru* population dynamics, the observation that control site age structures were similar in composition and seedling numbers, indicated that recruitment was indeed possible in both Groot Marico and Rust-de-Winter (e.g., Fig. 2.8. GM4, RD3, RD6). This suggests that climatic conditions, at least at a regional scale, appear to have been favourable for recruitment and seedling survival in the last four years. As such, prevailing climatic conditions may not be a good predictor of the variable biocontrol success observed between

regions during this study, although the influence of micro-climatic variation between sites may be a contributing factor.

Taylor and Walker (1984) found that *C. jamacaru* (= *C. peruvianus*) recruitment was a function of soil type and the density of nurse-plants, whereby a negative correlation was observed between percentage sand (%) and seedling recruitment, while a positive correlation was observed between nurse-plant density and *C. jamacaru* density. Biotic interactions between *C. jamacaru*, dominant grasses and nurse-plants may therefore influence the magnitude of biocontrol success (e.g., Houston, 1963; Gilreath and Smith, 1988; Burger and Louda, 1994, 1995). The variable age frequency distributions and population dynamics of *C. jamacaru* exposed to *H. festerianus* may be explained by variation in sample site vegetation density and structure (G.F. Sutton, pers. obs.). The age structure of site GM2, where 74 % of *C. jamacaru* stems were galled by *H. festerianus*, was more representative of a control site than it was of other sites where *H. festerianus* was present and was more populous than any other biocontrol site. Given that the proportion of stems galled at this site was higher than several sites where age structures conformed to the expected uniform age frequency distribution associated with biocontrol sites, it appears that herbivore load may not explain the apparent lack of biocontrol impact at this site (Francks et al., 2006). It is possible that *H. festerianus* has not been present at this site for long enough to allow for the impact of herbivory to manifest for this weed population. Alternatively, the vegetation at this site was denser than at any other site sampled during this study (G.F. Sutton, pers. obs.), which could result in an increased availability of microsites and therefore increase recruitment rates, which may explain the lack of expected impact at site GM2.

Conversely, sites RDW4 and RDW5 were dominated by native grasses and contained very few nurse-plants within the sampled area, which may have contributed to the lack of recruitment and suppression of *C. jamacaru* at these sites. This is supported by Taylor and

Walker (1984) who proposed that interspecific-competition from native grasses would limit or prevent the establishment of *C. jamacaru* in Rust-de-Winter.

The successful biocontrol of *C. jamacaru* is primarily dependent on the impact of *H. festerianus*, although it appears that the magnitude of impact associated with herbivory is mediated somewhat by abiotic and biotic factors (e.g., Paynter, 2005). This is consistent with current biocontrol literature which posits that the factors that contribute to the variability of biocontrol success are poorly understood (Van Driesche et al., 2010). The long-term monitoring of *C. jamacaru* biocontrol efforts would be significantly improved by including measurements of both abiotic and biotic factors that may assist or constrain the biocontrol of this weed, and could provide valuable information to improve the management strategy for *C. jamacaru* and therefore the predictability of biocontrol success for this weed (McClay and Balciunas, 2005).

Mature *C. jamacaru* densities were independent of biocontrol employing *H. festerianus*, yet seedling densities and pre-fruiting densities were 80 % and 37 % lower at sites where *H. festerianus* was present. A possible explanation for this is that *H. festerianus* was not present when these older individuals were recruited into the population and that older individuals require an extended period of sustained herbivory before succumbing to *H. festerianus* (H. Klein, pers. comm.). Older, mature *C. jamacaru* individuals undoubtedly have far greater nutrient reserves than smaller plants, which is likely to make them more resistant or tolerant to herbivory than seedlings and pre-fruiting individuals (Franks et al., 2006; H. Klein, pers. comm.). However, given that mortality within the mature plant age-class was higher at biocontrol sites (~ 32 %) than at control sites (< 5 %), yet comparable plant densities were observed for this age-class with contrasting levels of biocontrol, suggests that the lack of a difference in plant densities may merely reflect differing pre-biocontrol site densities. Indeed, given that this study did not employ a randomized design of agent releases,

it is likely that releases of *H. festerianus* were focused on higher density sites, where the need for biocontrol was more pronounced. For example, more dense infestations of *C. jamacaru* have been recorded in the drier northern regions of South Africa (Taylor and Walker, 1984), where *H. festerianus* has been extensively redistributed, than in Kwa-Zulu Natal and the Eastern Cape, where the weed has become naturalised more recently and has not been actively targeted for biocontrol, to date.

2.5. Conclusion

This study indicated that *H. festerianus* had a negative impact on *C. jamacaru* populations, whereby weed density was significantly reduced and the capacity of the weed to regenerate appeared to be constrained by herbivory associated with *H. festerianus*. The magnitude of *H. festerianus* impact on *C. jamacaru* appeared to be mediated by both abiotic (e.g., time) and biotic factors (e.g., inter-specific competition, availability of nurse-plants), although the relative importance of these factors was secondary to the regulatory impact of *H. festerianus*. The level of success that has been achieved by this biocontrol programme is difficult to evaluate due to the lack of pre-defined management goals (Briese et al., 2002; Müller-Schärer and Schaffner, 2008; Paterson et al., 2011b), and a study is warranted to determine an ecologically relevant set of criteria with which to empirically evaluate the success of biocontrol against *C. jamacaru*.

Although multi-site comparisons have been proposed as an appropriate way to conduct post-release monitoring of biocontrol programmes (Morin et al., 2009), this approach provided correlative rather than causal support for the efficacy of *H. festerianus* biocontrol. In order to demonstrate a causal relationship between *H. festerianus* biocontrol and *C. jamacaru* population declines observed during the current study, (1) long-term monitoring of this biocontrol programme should be continued, and (2) experimental releases of *H.*

festerianus should be conducted and monitored in the Kwa-Zulu Natal and Eastern Cape provinces, where the agent is not yet established, using the demographic data collected during this study as a pre-release benchmark with which to evaluate the effect of *H. festerianus* (Evans and Landis, 2007). The long-term success of this biocontrol programme will be dependent on careful long-term evaluation of the impacts of *H. festerianus* herbivory on *C. jamacaru* populations (i.e., are additional biocontrol agents required for successful *C. jamacaru* biocontrol?), and how the level of biocontrol success is mediated by both abiotic and biotic factors (i.e., improving the implementation of biocontrol employing *H. festerianus*).

Chapter 3

Natural enemy acquisition by the biocontrol agent *Hypogeococcus festerianus* in South Africa

3.1. Introduction

The establishment, proliferation and efficacy of weed biocontrol agents in their introduced range, may be influenced by an array of biotic and abiotic factors, including the accumulation of natural enemies (Semple and Forno, 1987; Cornell and Hawkins, 1993; Simberloff and Von Holle, 1999; Norman et al., 2009). Biotic interference by natural enemies results primarily from acquired predators, and to a lesser extent parasitoid accumulation, with both assemblages generally composed of both specialist and generalist species (Goeden and Louda, 1976; Pratt et al., 2003; Sebolt and Landis, 2004). Candidate control agents are screened in quarantine facilities during the process of importation to ensure that their natural enemies are reared out (Beirne, 1975; Reimer, 1988; Knutson and Coulson, 1997). Care is taken during this procedure as introduced control agents are more likely to successfully establish and control the target weed if they acquire "enemy-free space," by escaping top-down regulation from their natural enemies (Keane and Crawley, 2002; Boughton et al., 2012). However, biocontrol agents may acquire novel native predators and parasitoids in their introduced range, ensuring that they do not experience "enemy-free space" upon introduction for biocontrol purposes (Cornell and Hawkins, 1993; Hill and Hulley, 1995).

Goeden and Louda (1976) estimated that approximately 50% of introduced weed biocontrol agents are attacked by acquired predators and parasitoids in their adventive range.

However, natural enemy acquisition may not necessarily translate into biotic interference and reduced agent efficacy (Van Klinken and Burwell, 2005; Chacon et al., 2008; Tipping et al. 2013, 2016). For example, Morrison (1984) found that although two *Dactylopius* spp. were attacked by a coccinellid predator *Exochomus flaviventris* Mader (Coleoptera: Coccinellidae), this predation was unlikely to explain the disappearance of *D. coccus* in South Africa after successful establishment, or limit the efficacy of *D. austrinus* on *O. aurantiaca*, which is considered to be under partial control (Klein, 2011). However, this is not always the case, with two reviews estimating that approximately 20% of classical biocontrol programmes have failed due to biotic interference (Stiling, 1993; Kimberling, 2004). McFadyen and Spafford Jacob (2003) proposed that due to under-reporting, the logistical difficulties of assessing natural enemy impact, and variable outcomes and relative magnitudes of biotic interference (Denoth and Myers, 2005), it is likely that the prevalence of biotic interference is greater than currently believed. Despite a growing body of literature highlighting the role of biotic interference in a biocontrol context, studies evaluating the potential for biotic interference and the influence it may have are lacking (Hunt-Joshi et al., 2005) and could significantly improve control agent selection (Hill and Hulley, 1995; Paynter et al., 2010), implementation of biocontrol (Hight et al., 1995), and thus the predictability of successful weed management (Chacon et al., 2008)

Practitioners have attempted to prioritise candidate agents on various criteria, in order to improve the probability of introducing successful herbivores (Goolsby et al., 2006a; Van Klinken and Raghu, 2006), including: plant response to herbivory (Harris, 1973; Dhileepan et al., 2006;), native distribution impact assessments (Wapshere, 1985), field host-range (Syrett and Emberson, 1997; Dhileepan et al., 2006; Paterson et al., 2014), climatic compatibility (Rafter et al., 2008; Robertson et al., 2008), and genotypic matching (Kniskern and Rausher, 2001; Goolsby et al., 2006b; Wardill et al., 2005; Paterson et al., 2009). An additional

criterion which has not received as much attention has been to evaluate whether and how candidate control agents could be prioritised according to their risk to top-down biotic interference from acquired natural enemies in their introduced range (Strong et al., 1984; Myers et al., 1989; Hill and Hulley, 1995; Van Klinken and Burwell, 2005). On one hand, practitioners have argued that herbivores that are kept rare in their native range may be more likely to establish, proliferate and successfully control their target host (Hunt-Joshi et al., 2005; Strong et al., 1984), or because they are kept rare in their native distribution they may be more effective control agents as the target weed has evolved little resistance against the herbivore (Myers et al., 1989). On the other hand, Cornell and Hawkins (1993) found a correlation between parasitoid accumulation in the region of origin and in the region of introduction. This finding suggests that herbivores that are attacked and kept rare by a suite of natural enemies in their native distributions, are likely to accumulate similar natural enemy loads in their adventive range, which may limit their efficacy as control agents. These contrasting hypotheses highlight the uncertainty with regards to whether candidate agents can and/or should be prioritised with regards to their relative susceptibilities to top-down regulation in their native distribution.

Understanding the factors that increase the susceptibility of control agents to biotic interference may be vital to consider when attempting to prioritise candidate agents (Hawkins et al., 1997; Van Klinken and Burwell, 2005). Firstly, feeding biology (endophagous vs ectophagous feeding) may play a major role in determining the susceptibility of control agents to attack by natural enemies (Cornell and Hawkins, 1995; Hill and Hulley, 1995). The studies by Cornell and Hawkins (1995) and Hill and Hulley (1995) lend strong support to endophagous agents being more heavily attacked than ectophagous species, which is likely explained by ectophages being more mobile (Askew and Shaw, 1986). Secondly, the taxonomic delineation of a candidate agent has been found to be a significant predictor of

susceptibility to biotic interference (Hill and Hulley, 1995). Hill and Hulley (1995) found that hemipteran agents suffered lower levels of attack than coleopteran, dipteran and lepidopteran agents, although caution should be exercised as to inferring any generalisations due to a low sample size. Thirdly, the presence of an ecological analogue (a native insect taxonomically related to the control agent at the superfamily level, and shares similar ecological characteristics and feeds on the same plant host as the agent) may significantly increase the risk of candidate control agents accumulating natural enemies in their adventive range (Paynter et al., 2010). Ecological analogues may be an important source of native natural enemies that may be capable of extending their host-range to colonize novel hosts in the form of introduced control agents (Paynter et al., 2010).

The biocontrol agent *Hypogeococcus festerianus* has achieved variable levels of biocontrol success against the cactaceous weed *Cereus jamacaru* in South Africa (Chapter 2). Observational accounts suggest that the variability of biocontrol success associated with *H. festerianus* may be linked to the acquisition and impact of at least two predaceous coccinellid beetles which attack the agent in the field (Moran and Zimmermann, 1991; Klein, 1999; Paterson et al., 2011), although no formal evaluation of this proposal has been conducted. The primary objectives of this study were therefore to: (1) identify the assemblage of predators and parasitoids associated with *H. festerianus* in South Africa; and (2) evaluate the association between acquired natural enemy densities and *H. festerianus* abundance and performance (potential for biotic interference).

Furthermore, *H. festerianus* is kept rare in its native range (Argentina) (McFadyen & Fidalgo 1974 cited in: McFadyen, 1979; Claps and de Haro, 2001), by an ecologically and taxonomically diverse assemblage of predators and parasitoids, including two coccinellid beetles, at least nine hymenopteran parasitoids and a single dipteran predator (Table 3.1.). It must be noted that the data on the natural enemy assemblage associated with *H. festerianus* in

its native distribution was collected from *H. festerianus* gall material on multiple *Harrissia* spp., and not *C. jamacaru* as in the current study. The natural enemy assemblage data from the native distribution was used to evaluate whether and how candidate control agents could be prioritised, with respect to biotic interference, using *H. festerianus* as a case-study. This was done by asking: (3) Is the richness and structure of the natural enemy assemblage attacking *H. festerianus* in its native distribution a good predictor of natural enemy acquisition in its adventive range?; (4) Do current predictions regarding taxonomic delineation, feeding guild and presence of an ecological analogue hold for natural enemy acquisition of *H. festerianus* in South Africa?; and (5) Is the scarcity of *H. festerianus* in its native distribution a good predictor of biocontrol success? These findings have important practical implications with regards to improving the success rate of *H. festerianus* biocontrol, and conceptually this study provides valuable information as to whether and how candidate biocontrol agents should be prioritised according to their vulnerability to biotic interference from acquired natural enemies (Hill and Hulley, 1995; Raghu et al., 2006).

3.2. Methods and Materials

Surveys for natural enemies associated with *H. festerianus* were conducted at eight sites where *C. jamacaru* was infected by *H. festerianus* (see Table 2.1. for sample site details). Sample sites were located in Groot Marico and Rust-de-Winter (e.g., sites GM and RD in table 2.1), as only sites within these two regions contained *H. festerianus*. The survey for natural enemies was conducted by employing two approaches: (1) the natural enemy community associated with *H. festerianus* was identified by collecting predators and parasitoids from emergence chambers filled with *H. festerianus* gall material; and (2) a timed point-count approach was adopted to estimate the relative abundance of two prominent predaceous taxa that were believed to negatively impact *H. festerianus* populations. These

data were used to evaluate the influence that biotic interference may have on *H. festerianus* performance and thus its efficacy as a biocontrol agent.

3.2.1. Natural enemy assemblage associated with Hypogeococcus festerianus

The assemblage of natural enemies attacking *H. festerianus* in South Africa was identified by collecting 10 *H. festerianus* galls per sample site and placing them in custom-made emergence chambers (50 cm x 40 cm x 25 cm). Emergence chambers were constructed from plastic boxes fitted with a plastic funnel on one end, which ended in a glass collection vial. Arthropods were attracted to the glass collection vial as this was the sole source of light in the emergence chambers. The sampling design resulted in eight emergence chambers being filled with 80 *H. festerianus* galls.

The collection vials of each emergence chamber were checked every 2-3 days, and any arthropods removed from the vials before being replaced. This was repeated on five occasions (i.e., the last sampling event was 15 days after initial collection of *H. festerianus* material). Coccinellid predator populations rapidly proliferated under similar artificial conditions when left for longer than their average life-cycle (~ 26 days) (Danninger, 2002). As such, sampling was terminated after 15 days to ensure that beetle abundance was not overestimated. Upon completion of the fifth sampling event, each emergence chamber was sampled destructively, whereby each *H. festerianus* gall was visually inspected, and any natural enemies encountered were removed from the wax with fine forceps.

Table. 3.1. Natural enemy assemblage associated with *Hypogeococcus festerianus* in its native distribution (Argentina). Surveys for natural enemies associated with *H. festerianus* in Argentina have been limited primarily to *Harrisia* spp., and not *Cereus jamacaru*.

Order (family)	Species	Reference
Coleoptera		
Coccinellidae	*Hyperaspidius trimaculatus (L.)*	McFadyen (1979)
Coccinellidae	*Diomus* sp.	McFadyen (1979)
Hymenoptera [a]		
Encyrtidae	*Anagyrus* sp.	McFadyen (1979)
Encyrtidae	*Anagyrus* sp. near. *psuedococci* (Girault)	McFadyen (1979)
Encyrtidae	*Anagyrus cachamai* Triapitsyn, Logarzo & Aguirre	Triapitsyn et al. (2014a)
Encyrtidae	*Anagyrus quilmes* Triapitsyn, Logarzo & Aguirre	Triapitsyn et al. (2014a)
Encyrtidae	*Leptomastidea* sp.	Triapitsyn et al. (2014b)
Encyrtidae	*Anagyrus lapachosus* sp. near.	Triapitsyn et al. (2016)
Encyrtidae	*?*	McFadyen (1979)
Encyrtidae	*?*	McFadyen (1979)
Signiphoridae	*Signiphora* sp.	McFadyen (1979)
Diptera		
Cecidomyidae	*Kalodiplosis floridana* Felt [b]	McFadyen (1979)

[a] An unidentified encyrtid infested *H. festerianus* populations under quarantine conditions in Australia, with no apparent impact (McFadyen 1979)

[b] Eradicated *H. festerianus* populations under quarantine conditions in Australia (McFadyen 1979)

Specimens were processed by sorting individuals into distinct morphospecies, and stored in 95% ethanol. All specimens were submitted to the Biosystematics Division at the Agricultural Research Council – Plant Protection Research Institute (ARC-PPRI), Pretoria, South Africa for further identification. Specimens are housed at the South African National Collection (ARC-PPRI) and are referred to by Rhodes University (RH) accession numbers.

3.2.2 Association between natural enemies and Hypogeococcus festerianus

A timed point-count approach was employed (Grevstad, 2006), to determine the presence and relative abundance of four predaceous beetles (hereafter 'beetles') and a lepidopteran predator *Autoba costimacula* Sallmüller (Erebidae). The inclusion of these two taxa and not any other natural enemies, in the timed-point count surveys was based on prior information from a study of natural enemies attacking *H. festerianus* on *H. martinii* in South Africa (Danninger, 2002), and the inconspicuous nature of parasitoids suggests that a point count approach would not have been appropriate to measure their relative abundances. Predaceous beetles have been cited as being sufficiently abundant on *H. festerianus* that they may impact the efficacy of this control agent (Danninger, 2002). These taxa were scored as a combined taxon ('beetles'), rather than on a species-by-species basis, as the larval stages could not be accurately identified in the field. *Autoba costimacula* was included as it was recorded from *H. festerianus* on a regular basis and actively predated upon female mealybugs and crawlers (Danninger, 2002).

The point-count approach was adopted at the scale of individual transects (see Chapter 2), so that measures of beetle and *A. costimacula* density could be compared with *H. festerianus* abundance and measures of efficacy as a biocontrol agent, at an equivalent spatial-scale. Counts were conducted by recording the total number of beetles and *A. costimacula* individuals on a single, randomly selected gall over a 30-second observation

period, for each individual plant that had been infested with *H. festerianus*, within each transect.

3.2.3. Statistical analyses

A species-accumulation curve was computed to determine whether sampling effort was sufficient to provide a representative account of the natural enemies associated with *H. festerianus* during this study. As advised by Gotelli & Ellison (2001) the standard error of the curve was estimated via random permutation.

Standard non-parametric measures of community composition and diversity were calculated for site-wise enemy assemblages, including: species richness, Shannon *H* diversity index and Simpson's *D* diversity index (Schooler et al., 2006). Species richness was defined as the number of morphospecies present in each emergence chamber. The choice to use both diversity indices was based on the emphasis of the respective indices, whereby the Shannon *H* index emphasizes the presence of rare species while Simpson's *D* index emphasizes common species (Magurran, 2004). The Shannon *H* and Simpson's *D* indices were calculated using the following formulae:

$$\text{Shannon } H \text{ Diversity Index} : H = -\sum_i p_i log_b p_i$$

$$\text{Simpson's } D \text{ Diversity Index} : D = 1 - \sum {p_i}^2$$

Where p_i is the proportional abundance of species *i* and *b* is the base of the logarithm.

To compare the structure of the natural enemy assemblages associated with *H. festerianus,* Bray-Curtis dissimilarity scores were computed (a measure of β-diversity) (Bray and Curtis, 1957). The Bray-Curtis index is calculated from a community-matrix of natural enemy species presence and abundance, and produces a matrix of pairwise comparisons of assemblage dissimilarity between samples (e.g., sample sites). The index takes values ranging from 0 (two sites share the same species) to 1 (two sites do not share any common species). To evaluate whether the natural enemy community composition varied between regions, an analysis of SIMilarity (ANOSIM; Clarke, 1993) was conducted on pairwise dissimilarity scores. This analysis was performed as a one-way design, whereby region (Groot Marico / Rust-de-Winter) was fitted as a factor, and was based on 999 permutations. Additionally, a two-sample t-test was performed to evaluate the influence of spatial scale on community assemblage, by comparing dissimilarity scores within and between regions. The Bray-Curtis dissimilarity matrix was ordinated by non-metric multidimensional scaling (nMDS). The resulting nMDS plot allows for graphical representation of complex community assemblage data, with the aim of identifying meaningful relationships between samples (in this case the natural enemy assemblages collected from different sample sites). The validity of the nMDS plot to accurately represent the Bray-Curtis dissimilarity matrix was inferred by calculating a stress value, whereby a value > 0.20 would be an invalid representation of the data, < 0.20 would be deemed a suitable representation of the data, while a stress value of < 0.10 would provide a good depiction of the data (Clarke, 1993). All community data analyses were implemented in the 'vegan' package (Oksanen et al., 2013).

To determine whether there was an association (i.e., density-dependent response) between predaceous taxa density and *H. festerianus* gall density, and *H. festerianus* establishment, Spearman's rank correlation analyses were performed on site-wise mean beetle and *A. costimacula* densities with respect to: (i) the number of *H. festerianus* galls per

1000 m^{-2} and (ii) the proportion of *C. jamacaru* stems galled per 1000 m^{-2}. Spearman's rank correlation analysis was chosen as these data failed to satisfy the requirement of normality. The assumption was that significant correlations between predaceous taxa densities and *H. festerianus* gall density would indicate association between *H. festerianus* and its predators, and significant negative correlations would be indicative of potential biotic interference. Similarly, a significant negative correlation between predaceous taxa densities and the proportion of *C. jamacaru* stems galled by *H. festerianus* would suggest that control agent establishment may be limited by natural enemies. Caution should be exercised when interpreting these data as association does not provide conclusive evidence that a trophic relationship exists between taxa (Christensen et al., 2011).

3.3 Results

3.3.1. Natural enemy assemblage composition, structure and diversity

A total of 269 individual arthropod specimens emerged from collection chambers containing *H. festerianus* galls, which comprised 15 different species (Table. 3.2.). Six species were recovered at 25% or fewer of samples sites and/or in low abundance (< 5 total individuals pooled across sites) and were therefore not considered to be associated with *H. festerianus*. As such, nine natural enemy species were considered sufficiently abundant and widespread to potentially be associated with the control agent (Table. 3.2.). A species-accumulation curve indicated that sampling additional sites was unlikely to yield additional natural enemy species associated with *H. festerianus* during this study (Fig. 3.1.).

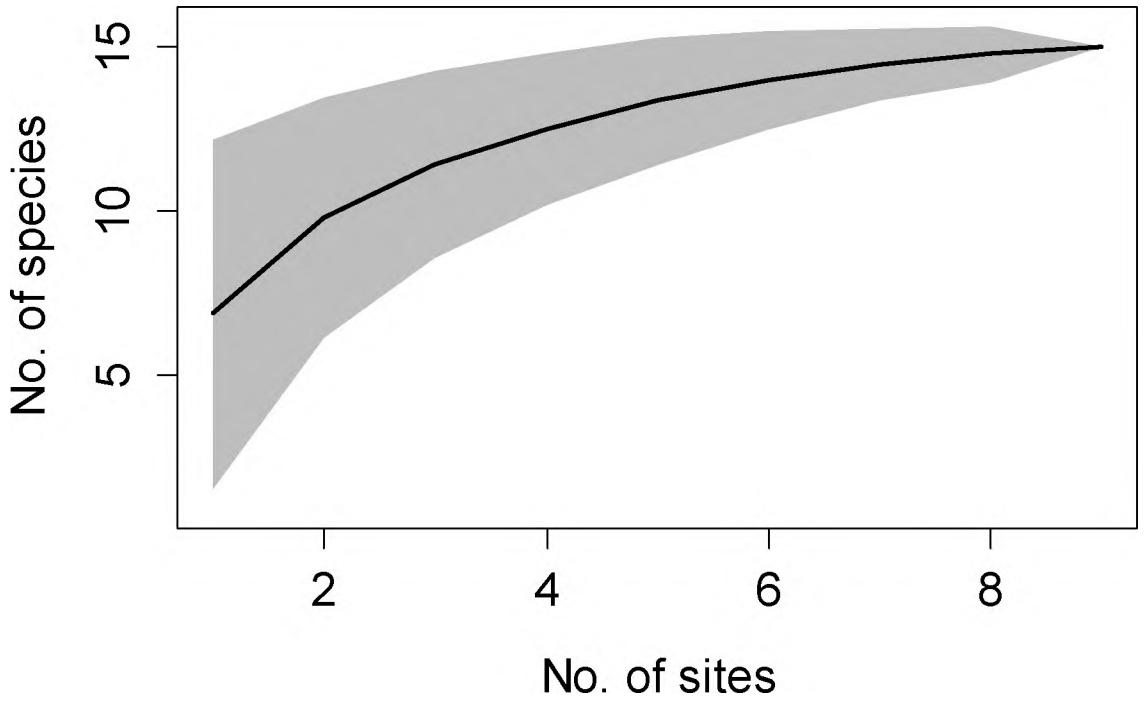


Fig. 3.1. A species accumulation curve (SAC) indicated that sampling additional *Cereus jamacaru* sites was unlikely to yield additional natural enemy species associated with *Hypogeococcus festerianus* during this study. The grey-shaded area indicates the standard deviation of the SAC through random permutation (Gotelli and Ellison, 2004).

Table. 3.2. Natural enemy assemblage associated with *Hypogeococcus festerianus* in its introduced range (South Africa). Species abundance per site equates to the number of individuals emerged per collection chamber. Region refers to whether species were collected from emergence chambers in both sampling areas (Groot Marico and Rust-de-Winter), or only in a single region (GM or RD). Sites indicates the percentage of sites (n = 8) that at least one individual of a particular species was recovered from during this study. An additional natural enemy was collected from *H. festerianus* gall material on an *ad hoc* basis in the Kwa-Zulu Natal province, and is included in the table for completeness (region - KZ).

			Species abundance								Distribution	
Order	Species (Family)	RH Accession No.	GM1	GM2	GM3	GM5	RW1	RW2	RW4	RW5	Region	Sites (%)
Coleoptera	*Chilocorus* prob. *nigrita* Fabricius (Coccinellidae)	1084	1	16	18	1	3	21	3	2	Both	100
	Exochomus sp. (Coccinellidae)	1085	0	26	25	9	18	4	0	8	Both	75
	Nephus sp. (Coccinellidae)	1086	0	7	1	0	0	1	0	0	Both	37,5
	beetle_sp4	1092	0	0	0	0	0	7	4	2	Both	37,5
Hemiptera	hemipteran_sp. 1 (Reduviidae)	1082	0	0	1	1	0	0	0	0	GM	25

Lepidoptera	*Autoba costimacula* Sallmüller (Erebidae)	1072, 1073	1	2	2	8	6	1	2	1	Both	100
Araneae	arachnid_sp1	1087	1	4	0	2	0	2	1	1	Both	75
	arachnid_sp2	1088	3	0	0	0	0	1	0	0	Both	25
	arachnid_sp3	1096	1	0	1	0	1	2	0	1	Both	62,5
	arachnid_sp4	1097	0	3	2	0	0	0	0	0	Both	25
Diptera	diptera_sp1 (Drosophilidae)	1098	0	0	0	0	0	0	2	0	RD	12,5
	diptera_sp2 (Dolichopodidae)	1080	-	-	-	-	-	-	-	-	KZ	-
Hymenoptera	*Anagyrus* sp. 1	1076, 1077, 1093, 1094	1	3	2	0	4	6	5	3	Both	87,5
	Encyrtid sp. 1	1075	1	3	3	1	0	1	3	0	Both	75
	Hockeria sp.	1095	0	0	0	0	0	2	1	0	Both	25

Two sample t-tests indicated that indices of Shannon *H* diversity (t = -0.31, d.f. = 6, P = 0.764) and Simpson *D* diversity (t = -0.16, d.f. = 6, P = 0.875) were not significantly variable between sampling regions, while species richness demonstrated a similar relationship (t = -0.17, d.f. = 6, P = 0.864) (Table. 3.3.).

Table. 3.3. Mean (± S.E.) species richness, and Shannon *H* diversity and Simpson's *D* diversity indices of site-wise natural enemy assemblages associated with *Hypogeococcus festerianus* in South Africa (see section 3.2.3 for formulae).

Region (site)	Species richness	Shannon *D* diversity	Simpson's *D* diversity
Groot Marico	**7.5 ± 0.7**	**1.59 ± 0.11**	**0.72 ± 0.04**
GM1	7	1.83	0.81
GM2	8	1.69	0.76
GM3	9	1.46	0.68
GM5	6	1.36	0.64
Rust-de-Winter	**7.8 ± 1.2**	**1.66 ± 0.10**	**0.74 ± 0.05**
RD1	5	1.11	0.55
RD2	11	1.92	0.80
RD4	8	1.96	0.85
RD5	7	1.63	0.75

Bray-Curtis pairwise dissimilarity scores comparing natural enemy assemblage composition between sites ranged from 0.15 (majority of natural enemies shared between both sites) and 0.86 (few natural enemies were shared between both sites). ANOsim analysis of community structure indicated that the composition of natural enemy assemblages sampled during this study were independent of the region of sampling (R = 0.04; P = 0.407). Furthermore, natural enemy assemblages were as variable in composition within regions (0.59 ± 0.05) as they were between regions (0.61 ± 0.04) (t = -0.26, d.f. = 28, P = 0.799), based on pairwise comparisons of Bray-Curtis dissimilarity scores. These findings were corroborated and graphically depicted by nMDS analysis, which indicated that the composition of natural enemy assemblages associated with *H. festerianus* was independent of the region of sampling (Fig. 3.2.). A stress value of 0.11 was obtained for the nMDS plot, which suggests that the relationships depicted in the graphical representation of the data was valid.

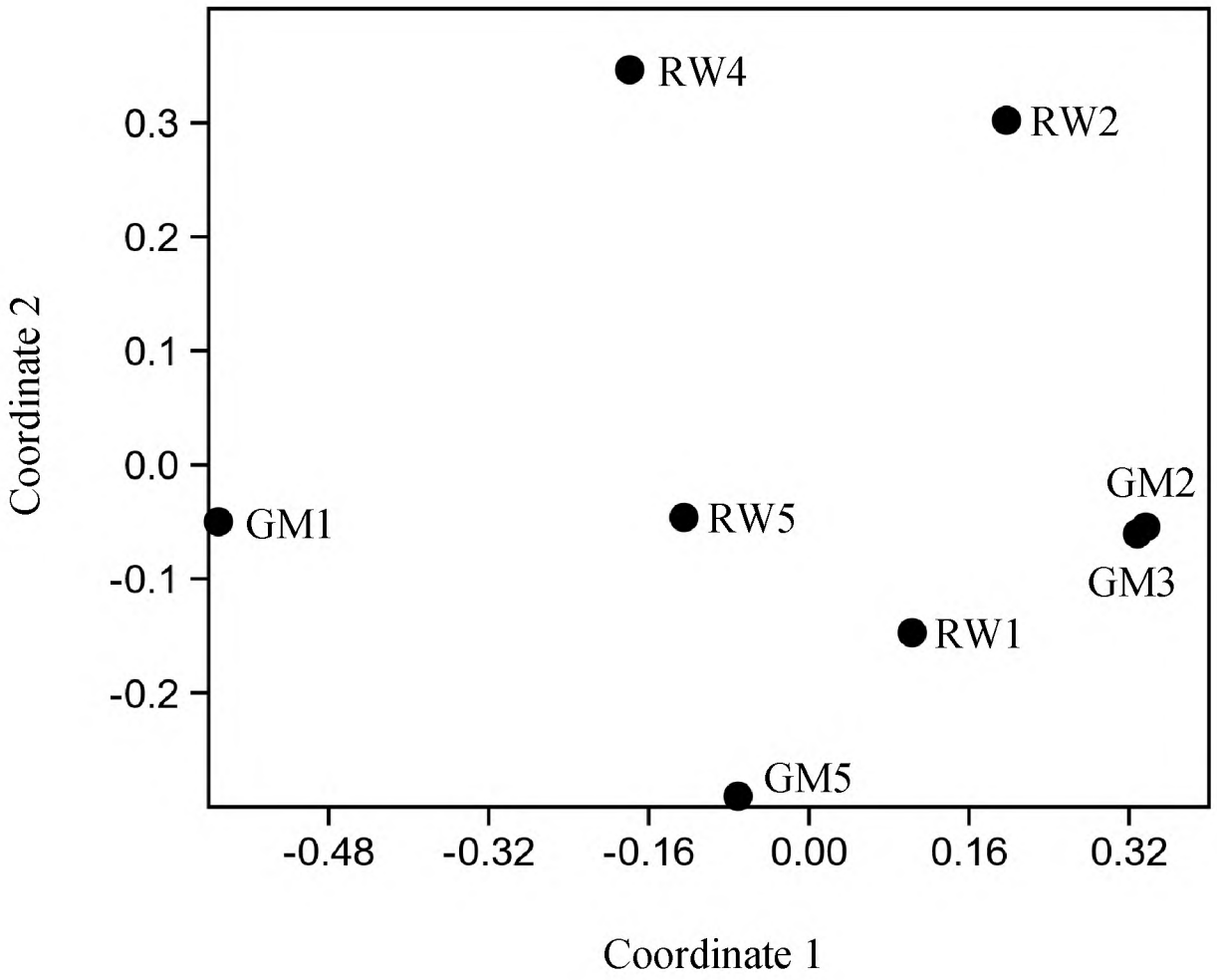


Fig. 3.2. A non-metric multi-dimensional scaling ordination plot (nMDS) comparing natural enemy assemblages associated with *Hypogeococcus festerianus* infecting *Cereus jamacaru* in two different sampling regions (Groot Marico – GM and Rust-de-Winter – RW). Each data point in the nMDS plot represents the natural enemy community identified from a single sample site (see section 2.2.2. for further details). The Bray-Curtis dissimilarity scores were used to rank distances calculated using a community-abundance matrix. The stress of the nMDS plot was 0.11, which suggests that the representation of the data is appropriate.

3.3.2. Predator assemblage

Eleven predaceous arthropod species were collected from emergence chambers filled with *H. festerianus* galls (Table. 3.2.), comprising four species of beetles, one hemipteran, one lepidopteran, one dipteran and four arachnids.

The most commonly recovered taxon was the Coleoptera, constituting 66 % (n = 177) of the total number of natural enemies that were collected from the emergence chambers, pooled between sites. *Chilocorus* prob. *nigrita* represented 24 %, and *Exochomus* sp. represented 33 % of the total number of natural enemies collected during this study, while *Nephus* sp. (3 %) and beetle_sp4 (4 %) were less common. *Chilocorus* prob. *nigrita* and *Exochomus* sp. were recovered at a rate of 0.81 ± 0.27 and 1.13 ± 0.33 individuals per *H. festerianus* gall, while *Nephus* sp. (0.11 ± 0.08) and beetle_sp4 (0.16 ± 0.08) were recovered less frequently. *Chilocorus* prob. *nigrita* was recovered from *H. festerianus* material from all eight sample sites (100 %), while *Exochomus* sp. was recovered from six sites (75 %), and *Nephus* sp. and beetle_sp4 were each recovered from three sites (37.5 %).

A predaceous lepidopteran, *A. costimacula* was recovered from 100% of sample sites, although it was never common, only constituting 9 % of the total number of natural enemies associated with *H. festeirnaus*. The remaining predaceous taxa were generalist spiders making up 10 %, and singe hemipteran and dipteran species each constituting < 1 % of the total number of natural enemies collected during this study (Table 2). An additional predaceous dipteran (Dolichopodidae; n = 15 individuals) was collected on an *ad hoc* basis from *H. festerianus* galls on *C. jamacaru* in the Kwa-Zulu Natal region, which has a predatory adult life-stage, and may prey upon mealybugs such as *H. festerianus* (M. Mansell, pers. comm.; University of Pretoria, South Africa).

3.3.3. Parasitoid assemblage

Five species of hymenopteran parasitoids were collected from emergence chambers filled with *H. festerianus* galls (Table. 3.2.). Three of these parasitoids were believed to attack *H. festerianus*, although they represented only 14 % of the total number of natural enemies collected during this study. A total of 0.16 ± 0.13 (mean ± S.E.) parasitoids were collected per *H. festerianus* gall, averaged across species and sites. *Anagyrus* sp. 1. and Encyrtid sp. 1. were slightly more abundant (*Anagyrus* sp. 1.: 0.30 ± 0.20; Encyrtid sp. 1.: 0.15 ± 0.14) than *Hockeria* sp. (0.04 ± 0.07). *Anagyrus* sp. 1 was recovered from 87.5 % of sample sites, Encyrtid sp. 1. from 75 % and *Hockeria* sp. from only 25 % of sample sites.

The remaining two species of hymenopteran parasitoids collected during this study were identified as *Homalotylus africanus* and an additional *Homalotylus* sp. (Hymenoptera: Encrytidae). Members of the *Homalotylus* genus are known to be primary parasitoids of coccinellid larvae (Noyes 2011), and therefore are most likely associated with the coccinellid beetles preying on *H. festerianus* during the current study, and not the biocontrol agent itself. *Homalotylus africanus* emerged from *H. festerianus* gall material from all 8 sample localities, while *Homalotylus* sp. was recovered from 87.5 % of sample sites. When pooled across species, *Homalotylus* spp. abundance demonstrated a positive, albeit non-significant correlation with the total number of predaceous coleopteran individuals collected per site (R^2 = 0.68, t_6 = 2.29; P = 0.062) (Fig. 3.2.). *Homalotylus* spp. were recovered at a rate of 0.72 ± 0.12 (mean ± S.E.) individuals per coccinellid predator, indicating that these parasitoids were present in high numbers during the current study.

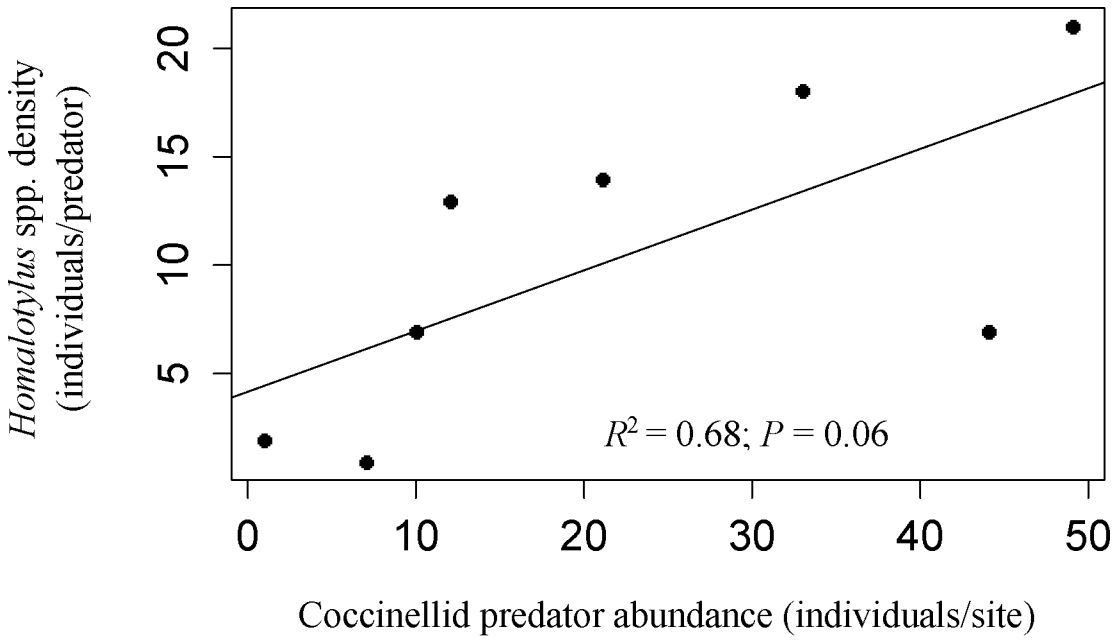


Fig. 3.3. Correlation between coccinellid predator abundance (individuals/site) and *Homalotylus* spp. abundance (individuals/site), recovered from emergence chambers filled with *Hypogeococcus festerianus* gall material.

3.3.4. Correlations between natural enemies and Hypogeococcus festerianus

There were no significant correlations between *H. festerianus* abundance and predaceous beetle density ($\rho = 0.24$; $S_6 = 64.00$; $P = 0.582$; Fig. 3.4.a), or *A. costimacula* density per 1000 m^{-2} ($\rho = -0.50$; $S_6 = 126.00$; $P = 0.216$; Fig. 3.4.b). Similarly, there were no significant correlations between the proportion of *C. jamacaru* stems galled by *H. festerianus* and predaceous beetle density ($\rho = 0.13$; $S_6 = 95.00$; $P = 0.754$; Fig. 3.5.a), or *A. costimacula* density per 1000 m^{-2} ($\rho = -0.48$; $S_6 = 124.48$; $P = 0.227$; Fig. 3.5.b). While no statistical association was found between both predaceous taxa and *H. festerianus*, direct observations of predation by *Chilocorus* prob. *nigrita*, *Exochomus* sp. and *A. costimacula* were observed in the field, which provides strong support that a trophic relationship exists between *H. festerianus* and these three acquired natural enemies (Christensen et al., 2011).

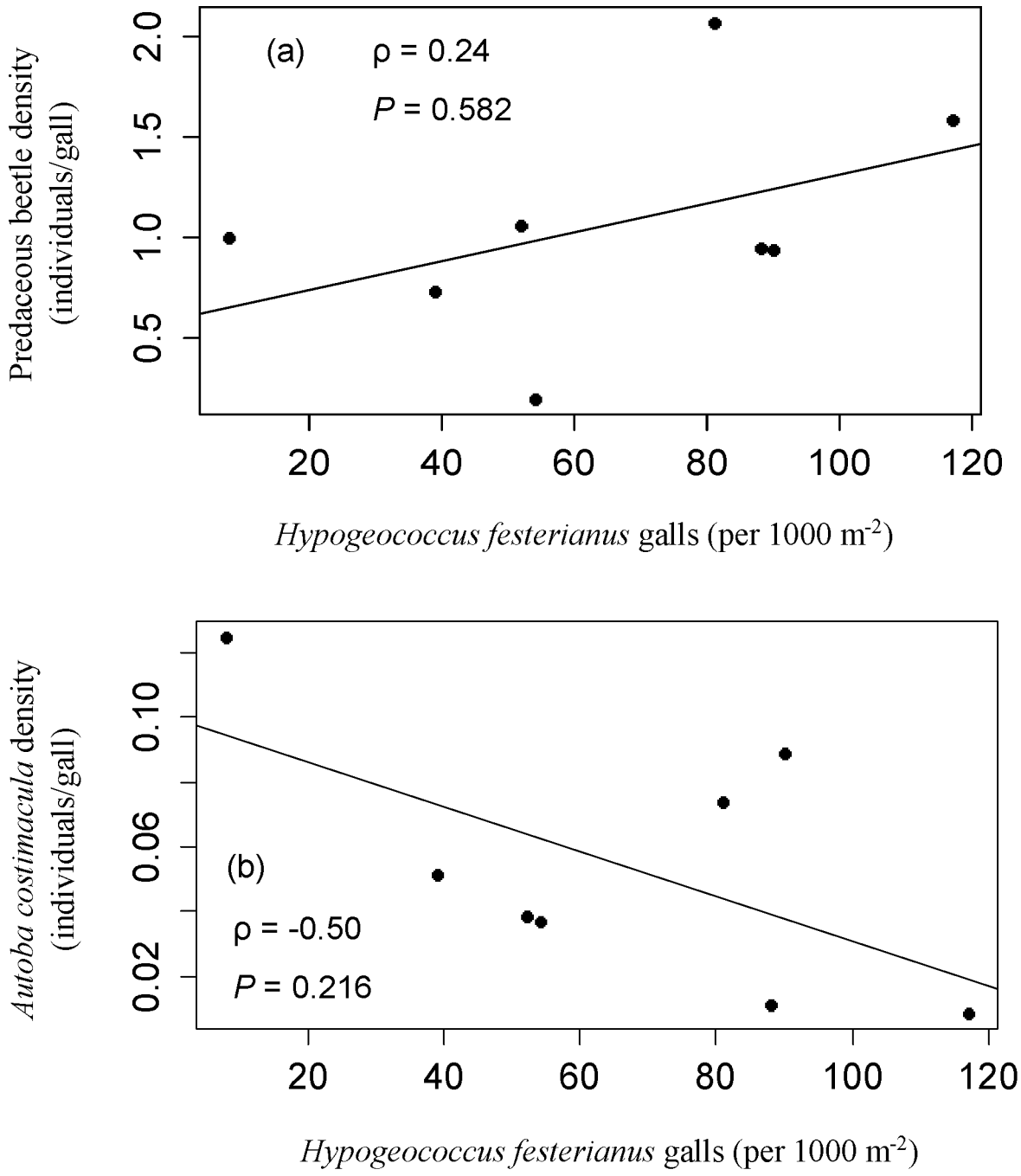


Fig. 3.4. Correlations between the number of *H. festerianus* galls (1000 m^{-2}), and (a) Predaceous beetle density (individuals/gall) ($Y_{[\text{predaceous beetle density}]} = 0.594 + 0.007_{[H.\ festerianus\ \text{galls}]}$; $\rho = 0.24$, $P = 0.582$) and (b) *Autoba costimacula* density (individuals/gall) ($Y_{[Autoba\ costimacula\ \text{density}]} = 0.090 - 0.001_{[H.\ festerianus\ \text{galls}]}$; $\rho = -0.50$, $P = 0.216$).

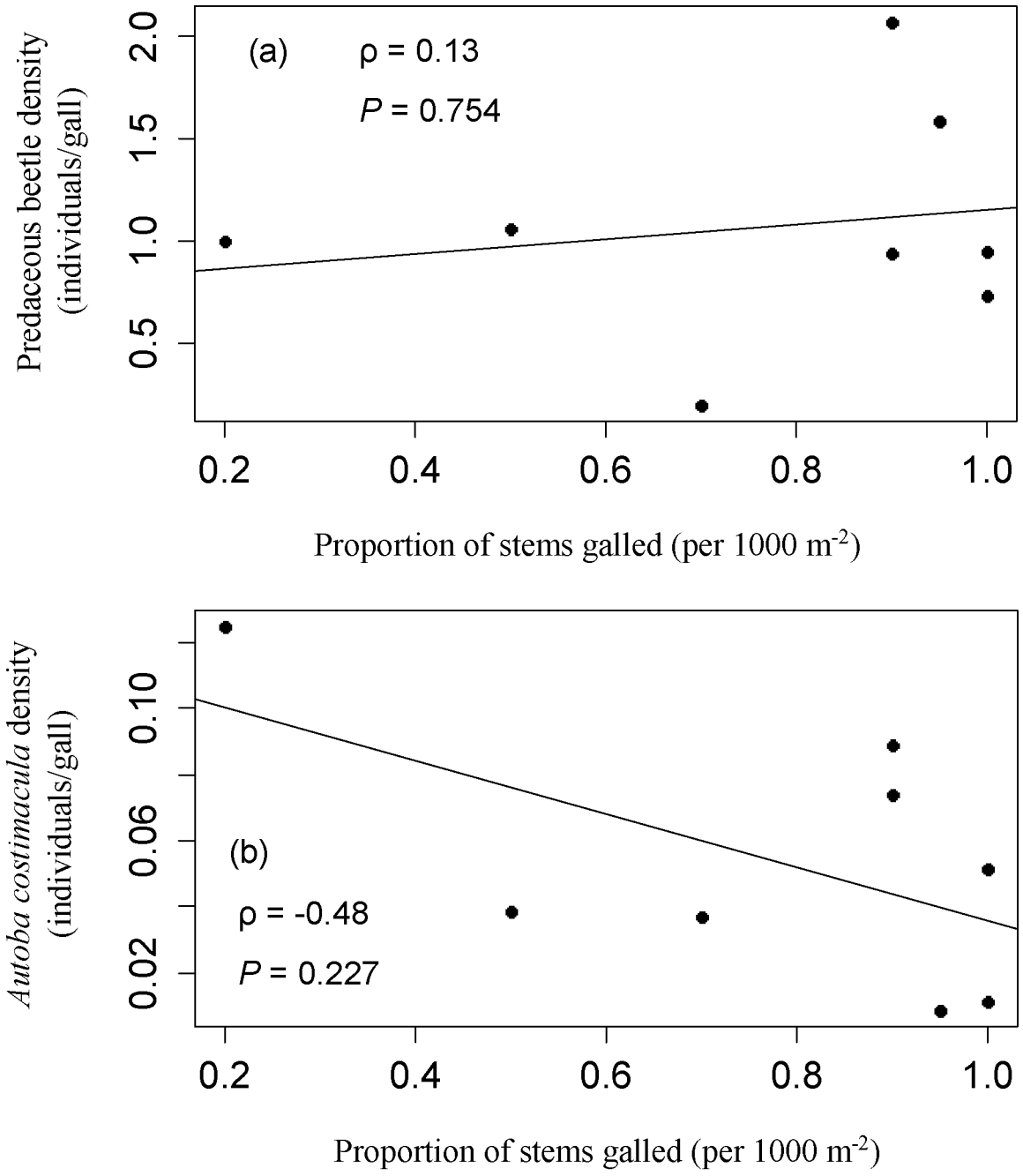


Fig. 3.5. Correlations between the proportion of *Cereus jamacaru* stems galled (1000 m^{-2}), and (a) Predaceous beetle density (individuals/gall) ($Y_{[\text{predaceous beetle density}]} = 0.794 + 0.356_{[\text{proportion stems galled}]}$; $\rho = 0.13$, $P = 0.754$) and (b) *Autoba costimacula* density (individuals/gall) ($Y_{[\textit{Autoba costimacula} \text{ density}]} = 0.117 - 0.081_{[\text{proportion stems galled}]}$; $\rho = -0.48$, $P = 0.227$).

3.4. Discussion

It is estimated that approximately 20% of introduced biocontrol agents have failed to establish and/or regulate their target weed host, due to biotic interference from acquired natural enemies (Stiling, 1993; Kimberling, 2004), with some authors suggesting that this figure may be an underestimate (McFadyen & Spafford Jacob, 2003). This study provides data which suggests that although *H. festerianus* has acquired a diverse natural enemy assemblage in South Africa, this has not prevented the biocontrol agent from having an impact on *C. jamacaru* populations. However, other subtler negative impacts on *H. festerianus* efficacy as a biocontrol agent cannot be ruled out (e.g., the agent may become more damaging if allowed to proliferate unconstrained by predation and parasitism).

Nine species of natural enemy emerged from emergence chambers containing *H. festerianus* gall material, including: four predaceous beetles, one lepidopteran, two arachnids and two hymenopteran parasitoids. This finding is in accordance with current theory which posits that introduced biocontrol agents can rapidly acquire natural enemies in their adventive range (Hill and Hulley, 1995; Paynter et al., 2010). Predators were more abundant than parasitoids in the *H. festerianus* study system constituting 86 % of the total natural enemy load. This finding conforms to current predictions of biotic interference, which suggests that predation is the more frequent mode of interference (Goeden and Louda, 1976; Dray et al., 2001). For example, Briese (1986) found that predation, more so than parasitism, decreased population densities of the biocontrol agent *Anaitis efformata* Guenèe in a semi-natural field experiment of exposed vs covered *Hypericum perforatum* L. (St John's Wort).

Only predaceous coccinellid beetles and the lepidopteran *A. costimacula* were considered sufficiently abundant and widespread to potentially impact *H. festerianus* populations. Coccinellid beetles have been implicated in biotic interference of several biocontrol agents (Nechols et al., 1996; Denoth and Myers, 2005; Ding and Blossey, 2005).

The two most common coccinellid predators associated with *H. festerianus* material were *Chilocorus* prob. *nigrita* and *Exochomus* sp. *Chilocorus nigrita* is native to the Indian sub-continent, and due to its regulatory impact on scale-insects has been introduced throughout the world as a biocontrol agent (Omkar and Pervez, 2003). It has been introduced into South Africa where it successfully regulates the citrus-pest *Aonidiella aurantii* Maskell (Hemiptera: Diaspididae) and *Asterolecanium miliaris* (Boisduval) (Hemiptera: Asterolecaniidae) (Samways 1989). The biocontrol efficacy of *Chilocorus nigrita* (Omkar and Pervez, 2003) and its abundance in the field during the current study highlights the possibility of biotic interference of *H. festerianus* by this species. A native coccinellid in South Africa, *E. flaviventris*, has been recorded in abundance on several species of *Dactylopius* (Hemiptera: Dactylopiidae) (Pettey, 1948; Annecke et al., 1969; Morrison, 1984), which were introduced from the New World for the biocontrol of *Opuntia* weeds (Moran, 1980). By reducing the numbers of *E. flaviventris* on *D. opuntiae* using low-dosage DDT sprays, Annecke et al. (1969) demonstrated that this predator limits the proliferation and efficacy of *D. opuntiae*. Reducing *E. flaviventris* populations resulted in a dramatic increase in *D. opuntiae* numbers, and concomitantly control of *O. ficus-indica* was improved significantly. Annecke et al. (1969) demonstrates the potential that *E. flaviventris* has with regards to initiating biotic interference for introduced control agents. The larvae of *A. costimacula* were observed to voraciously prey upon *H. festerianus* females and crawlers (Danninger, 2002). However, *A. costimacula* was not as abundant as the predaceous beetles associated with *H. festerianus* during this study, and as such is likely not present in high enough numbers to negatively impact *H. festerianus* populations.

Direct observations of predation on *H. festerianus* by these three predaceous species suggest that a trophic relationship may exist between these acquired natural enemies and *H. festerianus* (Christensen et al., 2011). While this finding, and previous observational accounts

(Moran and Zimmermann, 1991; Klein, 1999), indicated that there was great potential for biotic interference of *H. festerianus* in South Africa, the impact of acquired natural enemies has not prevented the biocontrol agent from having an impact on *C. jamacaru* populations. However, it is possible that natural enemies may have other subtler effects on *H. festerianus* populations, which may somewhat constrain or limit the magnitude of biocontrol success achieved against *H. festerianus* in South Africa. For example, predators and parasitoids may limit the impact of *H. festerianus* by slowing population growth rates, which may reduce the agents' potential to kill the host plant as there are fewer herbivores per plant, while fewer viable insects may restrict dispersal to new plants.

Given the abundance and potential for top-down regulation from predaceous coccinellid beetles and *A. costimacula* surveyed during this study, the lack of evidence for impacts on *H. festerianus* populations and biotic interference was unexpected. There are at least two plausible explanations for this, which need not be considered mutually exclusive. Firstly, the natural enemies attacking *H. festerianus* may themselves not have escaped their own natural enemies (Rosenheim, 1998). The proliferation and biocontrol success of *Chilocorus* prob. *nigrita* has been linked to an almost complete lack of top-down regulation from natural enemies (Omkar and Pervez, 2003). Indeed, only a single parasitoid, *Homalotylus* sp., has been recovered from *Chilocorus* prob. *nigrita*, and never in high abundance (Puttarudriah & Channa Basavanna 1953, cited in: Omkar and Pervez, 2003). During this study, the parasitoid *H. africanus* was recovered in relatively high numbers from emergence chambers filled with *H. festerianus* galls. *Homalotylus africanus* is a primary parasitoid of several genera of coccinellid predators, including: *Chilocorus*, *Nephus* and *Scymnus* (Hochberg and Ives, 2000). Several studies have shown that percentage parasitism of two closely related coccinellid predators, *C. renipustulans* (Scriba) and *C. bipustulatus* by congeneric *H. flaminius* ranges from 4-95 %, which can result in rapid and dramatic predator

population declines (see Stathas, 2001; and references therein). *Homalotylus flaminius* has previously been recovered from *H. festerianus* attacking *H. martinii* in South Africa, and as such it is believed that the enemy identified as *Homalotylus* sp. 1 in this study may in fact be *H. flaminius* (Danninger, 2002). It is probable that parasitism by both *Homalotylus* spp. may regulate coccinellid predator population densities associated with *H. festerianus*, and by doing so may act as a protector of the *H. festerianus*. This may explain the limited impact of the coccinellid predators on *H. festerianus* during this study. Secondly, the biology of *H. festerianus* may confer resistance against attack by natural enemies. Morrison (1984) found that predation on *Dactylopius* spp. in South Africa was largely mediated by the differential composition and physical properties of the waxy covering secreted by the different cochineal species. The author demonstrated that *D. coccus* produced a fine powder-like waxy covering which, by clogging the tarsi of *E. flaviventris*, conferred protection against predation, while *D. austrinus* and *D. opuntiae* produced a woolly waxy-covering, which was not as effective in deterring *E. flaviventris*. While little is known about the structure and composition of the waxy covering produced by *H. festerianus*, this may prove an interesting topic to explore further.

However, there are several limitations to the current study which may have prevented the realised effect of predators to be fully resolved. Firstly, it is suspected that some *H. festerianus* galls counted during the field-survey were dead and therefore did not contain any insects, which would not be expected to attract and harbour any predators or parasitoids. A reliable method by which to distinguish between galls with and without insects, would improve the accuracy with regards to the number of viable *H. festerianus* galls measured. Secondly, whether each individual stem was dead or dying back was not measured, which if measured, may have provided a better indication of the effect of predation on *H. festerianus* efficacy. Lastly, given that this data was collected during a single sample period, there was no

indication of the temporal variation and/or turnover of the natural enemy assemblage associated with *H. festerianus*, nor whether there was any temporal variation in predator and parasitoid abundances.

Several authors have highlighted their concerns with regards to the potential non-target effects associated with introducing exotic arthropods for the purpose of biocontrol, although these concerns are primarily for insect biocontrol (Simberloff and Stiling, 1996; Louda et al., 1997; Strong and Pemberton, 2000; Pearson and Callaway, 2003). However, an underappreciated indirect component of non-target effects for weed biocontrol agents exists if an introduced control agent impacts non-target species via common natural enemies (i.e., apparent competition (Morris et al., 2004; Willis and Memmott, 2005). While this topic has been given theoretical attention (see Holt, 1984; Bonsall and Hassell, 1997), field-based quantitative evaluations of such indirect non-target effects have been scarce (Morris et al., 2001, 2004; Willis and Memmott, 2005; Tipping et al., 2016). Settle and Wilson (1990) demonstrated that invasion by the exotic variegated leafhopper *Erythroneura variabilis* Beamer (Hemiptera: Cicadellidae) indirectly resulted in population declines of the native grape leafhopper *E. elegantula* Osborn (Hemiptera: Cicadellidae) in California, due to an increase in population numbers of their shared parasitoid *Anagrus epos* Girault (Hymenoptera: Mymaridae). Therefore, it appears that exotic herbivores (e.g., introduced control agents) may allow for rapid increases in native natural enemy populations (Schonrogge et al., 1996), which can have negative consequences for shared native hosts. Food web analyses provide a powerful tool by which practitioners may evaluate the presence and magnitude of both direct and indirect non-target effects (Henneman and Memmott, 2001; Morris et al., 2004). Although such analyses are challenging (Polis and Strong, 1996), due to factors such as: intra-guild predation (Arim and Marquet, 2004) and mutualisms/commensalisms (Deyrup et al., 2004), some authors have argued that introduced

agents can unpredictably influence food web interactions, and as such these types of study will be vital to evaluate the risk of indirect, non-target effects associated with biocontrol introductions (Tipping et al., 2013, 2016).

Hypogeococcus festerianus may be an important component of a large, dynamic food web consisting of multiple trophic-levels (a representation of the potential trophic interactions identified during this study is provided in Fig. 3.6.). The agent is associated with a suite of generalist predators and parasitoids, while food web complexity is increased with the addition of *H. africanus* and *Homalotylus* sp. 1. which are primary parasitoids of at least one of the predaceous coccinellid beetles, and an unidentified formicid, which actively prey upon *A. costimacula* larvae (G.F. Sutton, pers. obs.). It appears that these trophic interactions may dampen the effects of predation on *H. festerianus* (e.g., Finke and Denno, 2004), although detailed evaluation of the community-level outcomes of introducing *H. festerianus*, and more generally biocontrol agents, should be prioritised in the future (Tipping et al., 2016). However, it must be stressed that food web interactions are usually weak in natural ecosystems (Polis and Strong, 1996), and may be of little significance when weighed up against the negative consequences of leaving an alien invader unchecked (Headrick and Goeden, 2001).

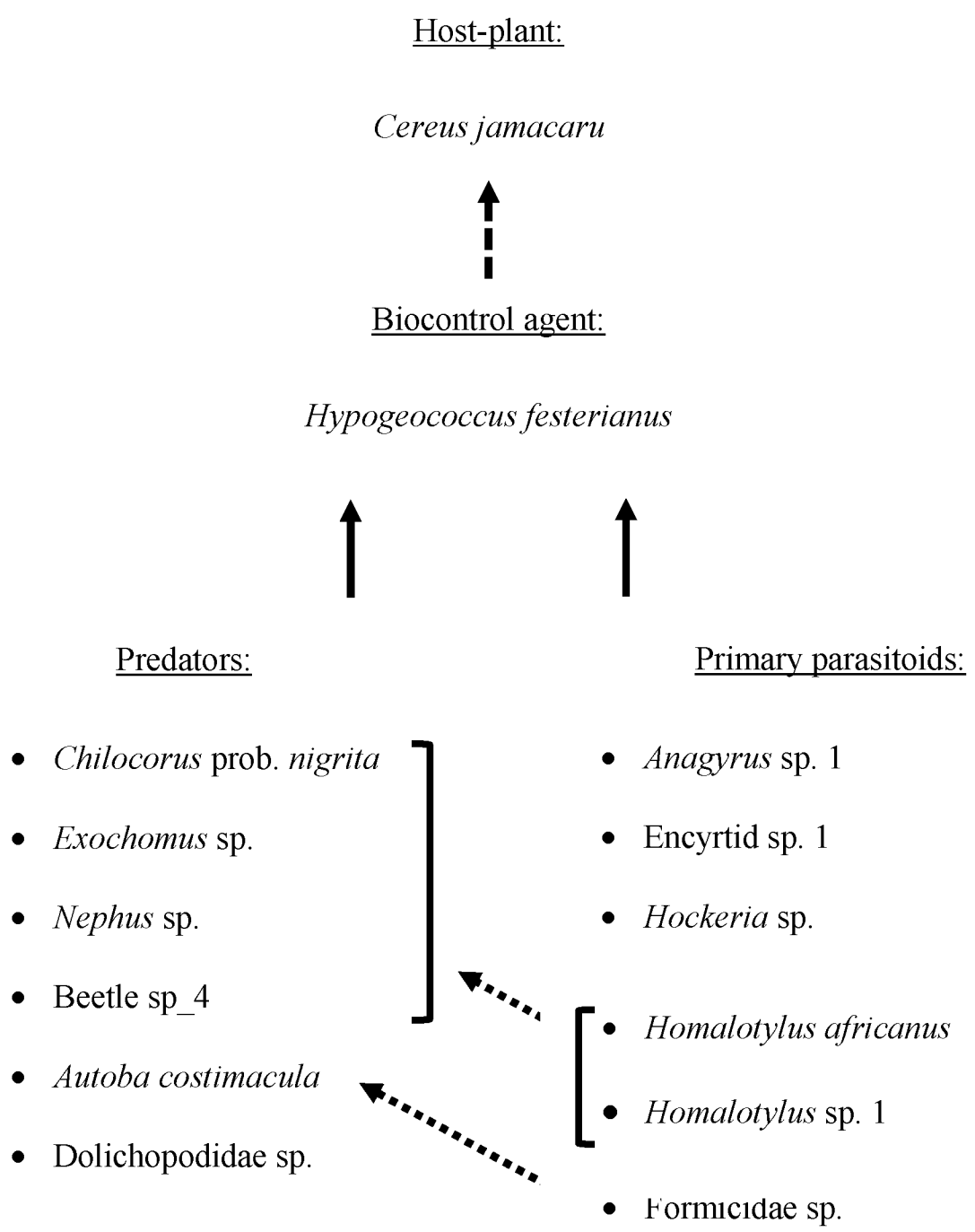


Fig. 3.6. Schematic overview of probable trophic relationships between organisms that are associated with the *Hypogeococcus festerianus* – *Cereus jamacaru* biocontrol system. Directional arrows indicate, (➡) potential for weed biocontrol, (➡) potential for biotic interference, and (▪▪➡) potential protection from biotic interference of *H. festerianus*.

Given that we now have a baseline understanding of the natural enemy assemblage abundance and structure associated with *H. festerianus* in South Africa, a carefully designed predator-exclusion experiment is advised to adequately evaluate the potential for biotic interference of *H. festerianus*. The importance of the basic ecological data collected during this study is illustrated with Hunt-Joshi et al. (2005) recommending that manipulating predator densities may provide an accurate account of the impacts of predation on biocontrol agent abundance and efficacy. However, this approach would likely not result in an accurate representation of predation by coccinellid beetles on *H. festerianus* due to the protective role of *H. africanus* and *Homalotylus* sp. 1 for *H. festerianus* populations. Therefore, a prospective field-experiment to evaluate the magnitude of biotic interference on *H. festerianus* should evaluate the establishment and proliferation of *H. festerianus* and subsequent *C. jamacaru* performance (i.e., measure reproductive status and fruit production) by performing caged vs uncaged releases of *H. festerianus* in order to exclude all predators and parasitoids from accessing *H. festerianus* galls. Alternatively, a step-wise approach could be adopted whereby *H. festerianus* is established on a stem before introducing beetles at several densities, and their impact monitored before introducing *Homalotylus* spp., and similarly monitoring their impact on the predaceous beetle population. Concurrently, monitoring protocols should be developed to evaluate the impact of *H. festerianus* on food web interactions (Carvalheiro et al., 2007). These experiments will allow for a more accurate determination of risk of biotic interference to *H. festerianus* populations, potentially improve the implementation of releasing and establishing *H. festerianus* in the field (i.e., if caged releases do indeed protect *H. festerianus* from generalist predation), and simultaneously scrutinise the presence, magnitude and implications of indirect, non-target effects associated with *H. festerianus* biocontrol (Willis and Memmott, 2005).

The findings from this study, when augmented with data pertaining to the efficacy of *H. festerianus* as a biocontrol agent of *C. jamacaru* (Chapter 2), suggested the majority of predictions derived from current ecological theory regarding the predictability and impact of biotic interference held true for *H. festerianus*. The structure and composition of the enemy assemblage attacking *H. festerianus* in its native range was a good predictor of natural enemy acquisition in South Africa. The richness of the enemy assemblage attacking *H. festerianus* in Argentina (n = 12; Table 3.1.) is comparable to the richness measured in South Africa during this study (n = 9; Table 3.2.), although it must be stressed that the sampling effort between regions differed significantly. However, the composition of the assemblages varied slightly between the native range of *H. festerianus* and in its adventive range, with the natural enemy assemblage comprised primarily of hymenopteran parasitoids in the native range, while the natural enemy assemblage primarily comprised generalist predators in its adventive range.

Additionally, it appears that the rarity of *H. festerianus* in its native distribution was a good predictor of biocontrol efficacy, although it is unclear whether this was explained by the control agent finding enemy free-space in the introduced range (Strong et al., 1984), or whether it was effective as *C. jamacaru* has undergone little selection for resistance against *H. festerianus* herbivory (Myers et al., 1989).

Furthermore, predictions derived from Paynter et al. (2010) who found that the correlation between parasitoid richness for a biocontrol agent in its native range and introduced distribution was contingent on the control agent possessing an ecological analogue in the introduced range also held true in this study. At least one true ecological analogue of *H. festerianus* is present in South Africa, a *Diaspis* sp. (Hemiptera: Diaspididae), while if the criteria for defining an ecological analogue are relaxed (Lawton, 1985), there are numerous mealybug species which would be suitable ecological analogues of *H. festerianus* in South Africa (H. Klein, pers. comm.). Therefore, the expectation was that *H. festerianus* would be

parasitized in South Africa, which was indeed the case during this study. Paynter et al. (2010) found that biocontrol agents possessing ecological analogues, and which were therefore heavily parasitized in their introduced distribution, were not considered to be successful biocontrol agents. In contrast, *H. festerianus* clearly deviated from this expectation as it was heavily parasitized in South Africa, yet still had a significant impact on *C. jamacaru* populations.

Predictions regarding natural enemy acquisition by *H. festerianus* according to its feeding biology were not in line with current theory. Hill and Hulley (1995) found that poorly concealed endophagous species were more susceptible to attack than well-concealed endophages or ectophagous herbivores. *Hypogeococcus festerianus* (ectophagous) was associated with three parasitoids during this study, which was far fewer than would be expected for the ectophagous feeding guild according to Hill and Hulley (1995), who showed that control agents of this feeding guild supported 8.67 ± 13.28 (mean ± SD) parasitoids. However, Hill and Hulley (1995) found that none of the seven hemipteran control agents surveyed during their study supported any parasitoids, which suggests that predicting natural enemy acquisition based on taxonomic delineation deviated from expectation in the case of *H. festerianus*. Hill and Hulley (1995) ascribed the lack of parasitoid accumulation observed during their study to the fact that five of the seven agents considered were cochineal insects (Dactylopiidae), which are not known to be attacked by parasitoids. It is believed that the carminic acid which the cochineal insects produce may be an effective parasitoid deterrent (Moran, 1980; Morrison, 1984). *Hypogeococcus festerianus* does not produce carminic acid, which may explain its unexpected susceptibility to parasitism and predation.

3.5. Conclusion

Hypogeococcus festerianus has acquired a diverse assemblage of natural enemies in South Africa, although it appears that at current natural enemy densities, biotic interference has not been sufficient in magnitude to prevent *H. festerianus* from having an impact on *C. jamacaru* populations. The control agent appears to be embedded in a potentially large and complex food web. Predaceous coccinellid beetles and the predatory moth *A. costimacula* were associated with *H. festerianus* in South Africa, however it appears that both taxa were attacked by their own suite of predators and parasitoids, which may have limited their impact on *H. festerianus* populations. The *H. festerianus* biocontrol system therefore provides an ideal opportunity to assess the indirect effects of introducing biocontrol agents on native food web dynamics (if any), and studies to this effect should be prioritised in future (Tipping et al., 2013, 2016).

The structure and richness of the natural enemy assemblage attacking *H. festerianus* in its native distribution and the presence of an ecological analogue were good predictors of natural enemy acquisition by *H. festerianus* in South Africa, in contrast to predictions derived from the control agents feeding biology and taxonomic delineation, which did not conform to expectations from the literature. Most importantly, the efficacy of *H. festerianus* as a biocontrol agent was not well predicted from current literature. Prioritising candidate biocontrol agents according to their relative susceptibility to biotic interference may be worthwhile (especially in conjunction with other prioritisation systems, see Goolsby et al., 2006b), although this approach will require further study to accurately identify factors on which to base prioritisation systems (e.g., native distribution enemy assemblage richness and rarity may be favourable traits for candidate control agents), and which factors may provide little predictive information (e.g., taxonomic delineation) (Van Klinken and Burwell, 2005).

Chapter 4

General Discussion

The research conducted for this thesis was performed to evaluate the efficacy of the biocontrol agent *H. festerianus* for managing exotic populations of the cactaceous weed *C. jamacaru* in South Africa, and identify to factors that may constrain the efficacy of this agent. This was done by performing a nationwide survey of weed populations with and without *H. festerianus*, in order to elucidate the impact of the biocontrol agent at the individual-plant level, and investigate whether these effects translated into meaningful *C. jamacaru* suppression at the plant-population level. The influence of top-down predation and parasitism and weed-agent incompatibility on *H. festerianus* performance and efficacy as a biocontrol agent were evaluated, and other potential limiting factors identified from survey data and field-observations.

In this chapter, the difficulty of defining success in weed biocontrol and a possible way in which evaluations of control agent efficacy could be improved are discussed. The importance of developing the science of biocontrol is then considered, specifically regarding the perennial debates over: (1) how many control agents are required for successful biocontrol? (Denoth et al., 2002) and (2) what is the best way to prioritise candidate agents? (Harris 1973; Goeden 1983). The risk to native cactaceous plants posed by pest populations of *H. festerianus* in Puerto Rico is then inferred. Lastly, the implications of this research for improving the biocontrol programme against *C. jamacaru*, and future research endeavours are discussed.

4.1. Defining success in weed biological control

Accurately evaluating whether an introduced biocontrol agent effects meaningful suppression over a target invader is vital to the long-term management of exotic weeds, provides data that can be used to improve the science of biocontrol and may lend support for the sustainable and continued investment in biocontrol as a weed management strategy (Syrett et al., 2000; Morin et al., 2009). In a few select cases, quantifying the impact and efficacy of biocontrol agents has been straightforward, with drastic reductions in plant density ascribed to the impact of the agents (Dodd, 1940; McConnachie et al., 2003). The spectacular early successes achieved by several biocontrol programmes (e.g., *Cactoblastis cactorum* against *Opuntia stricta* in Australia and *Chrysolina quadrigemina* (Suffrian) against *Hypericum perforatum* in California), has undoubtedly resulted in unrealistic expectations of future biocontrol programmes (Hoffmann & Moran, 2008).

In South Africa, biocontrol success is defined as the amount of alternative control required, in conjunction with biocontrol, to reduce the target weed below an acceptable threshold level (Hoffmann, 1995; Klein, 2011). The categories used to define biocontrol success are: (1) *complete control* – no additional control methods are required to reduce the weed to an acceptable level, (2) *substantial control* – additional control interventions are required to reduce the weed to an acceptable level, although biocontrol has reduced the amount of additional control required, (3) *negligible control* – despite the implementation of biocontrol, control of the target weed is solely reliant on alternative control methods, and (4) *undetermined* – the biocontrol programme has not been evaluated, or the release of the agent is too recent to allow for a representative evaluation of its efficacy (Hoffmann, 1995; Klein, 2011). This evaluation system ultimately provides a relative indication of the importance of biocontrol in relation to alternative control methods (Paterson et al., 2011b).

More generally, successful biocontrol has almost exclusively been defined by reductions in weed density or abundance associated with biocontrol introductions (DeBach, 1964; Andres and Goeden, 1971; Wilson and Huffaker, 1976). However, control agents often have less obvious effects on the target weed than what would be expected from the unprecedented benchmark set by the early control programmes. This has been detrimental to the implementation and sustainability of biocontrol as a management tool, as many of these programmes are deemed to have failed as weed densities do not decline rapidly despite the release of biocontrol agents (Hoffmann and Moran, 2008). Thus, a recent effort has been made to develop biologically-driven, quantifiable and realistic criteria to adequately evaluate the impact of control agents (Van Klinken and Raghu, 2006), and not simple rely on statistically significant reductions in weed density (which may have little biological relevance). However, the difficulty of this approach is to decide on an appropriate metric on which to evaluate biocontrol success, as weed invaders display a diverse array of impacts on native ecosystems (Brooks et al., 2004; Ehrenfeld, 2010).

In order to standardise the post-release evaluation component of biocontrol programmes, the impact of the weed on its invaded ecosystem should be used to inform the selection of an appropriate measure on which to evaluate biocontrol success. This approach highlights that the success of a biocontrol programme is the mitigation of the *impact* of a target weed invader, and not the mitigation of the weed itself. To adopt such an approach, weeds will be required to be categorised into groups depending on their impact on the native ecosystem, such as weed groups that: reduce biodiversity, reduce grazing potential, reduce agricultural productivity, reduce water quality and availability, and negatively impact on fire regimes (see Table. 4.1. for examples). Indeed, a weed may have multiple impacts on the ecosystem that it invades, and therefore not fit into a single category, which will require that the most appropriate measure of impact for each weed infestation be chosen, or a composite

of several measures of weed impact be applied, to evaluate biocontrol success. A standardised method of biocontrol programme evaluation could then be used to set pre-defined management goals with which to adequately evaluate the level of biocontrol success achieved (e.g., Paterson et al., 2011b).

Our current state of knowledge is limited with regards to the impacts that weed invaders have on their invaded ecosystems (Downey and Richardson, 2016). Very few studies have attempted to utilise data collected on the impact of alien invaders to set appropriate measures of weed impact on which to evaluate biocontrol success (e.g., Paterson et al., 2011b). Paterson et al. (2011b) demonstrated that *P. aculeata* densities of ≤ 30 % coverage were associated with native species biodiversity metrics that were not significantly different from if *P. aculeata* was absent from the ecosystem. Accordingly, Paterson et al. (2011b) proposed that an appropriate management goal for successful biocontrol of *P. aculeata* would be to maintain or reduce weed density below 30 % coverage. Gooden et al. (2009) demonstrated that native species richness associated with *L. camara* in wet schlerophyllous forests in Australia remained stable below *L. camara* percentage cover of approximately 75%, and could be used as an appropriate management target for *L. camara* biocontrol, although this was not the authors original intention. There is an array of studies which demonstrate the positive effect of weed removal on native biodiversity, and which could easily be contextualised to provide appropriate measures on which to evaluate biocontrol of environmental weeds. A caveat of this approach is reductions in weed density due to biocontrol may result in weed replacement, whereby the target weed is replaced by a secondary invader (Clewley et al., 2012), and therefore such studies should consider not only species richness and diversity, but also assemblage composition and interpreted accordingly. The challenge for biocontrol practitioners will be to develop and/or adopt appropriate methods and statistical analyses with which to identify appropriate management goals for

weeds belonging to other impact groups (e.g., weeds that reduce grazing potential/carrying capacity).

In the following section, a possible manner in which to identify biologically-driven pre-defined management targets for weeds that reduce grazing potential and/or carrying capacity of rangelands is discussed, as an example of how to determine appropriate management targets for weed biocontrol. This weed impact group was chosen as it is particularly relevant to the current study of the *C. jamacaru* biocontrol programme in South Africa, and indeed biocontrol of cactaceous weeds.

4.2. Setting pre-defined management targets for agricultural weeds

It is now well appreciated that exotic weeds, such as *C. jamacaru*, may significantly reduce the grazing potential of land used for livestock (DiTomaso, 2000), reduce habitat utilisation by wildlife (Belcher and Wilson, 1969), prove toxic to livestock and wildlife (McEvoy et al., 1991), hinder livestock passage and accessibility to water and shading (Lonsdale, 1988), and may pose a risk to livestock and wildlife safety (Briese et al., 2002; Jordaan and Mantji, 2012). For example, Olson (1999) demonstrated that the grazing capacity of rangeland infested by the invasive alien plants *Euphorbia esula* L. and *Centaurea* spp. was reduced by more than 50%. An appropriate measure by which to evaluate the biocontrol of weeds that reduce grazing potential/carrying capacity is to measure the carrying capacity of a tract of land. Carrying capacity is expressed as the number of large stock units (LSU's) carried per hectare, per year ($LSU.ha^{-1}.year^{-1}$), without deterioration of the grazing or soil (Edwards, 1981).

By calculating a carrying capacity, biocontrol practitioners may infer at what weed density the economic impact of the weed is not significantly different from if the weed were

absent from the ecosystem (complete biocontrol), or weed densities which provide a return on investment in implementing biocontrol (substantial biocontrol) (*sensu* Hoffmann, 1995; Klein, 2011). The LSU concept, and its derivatives (e.g., equivalents of agricultural LSU's which have been converted into indigenous ungulate LSU's), have been widely used in South Africa to estimate stocking rates of indigenous ungulates on nature reserves and game farms (see Boshoff et al., 2001; and references therein). However, the utility of the LSU concept to determine stocking rates has not been validated (Hardy, 1996), and can be difficult to conduct and interpret (Peel et al., 1998). As such, studies required to accurately determine the carrying capacity of wildlife habitat that has been invaded by a weed invader in comparison to habitats where the invader is absent, may be decidedly complex, and may require collaboration with personnel who possess the necessary skills, resources and experience to conduct such a study.

Alternatively, biocontrol practitioners could adopt a multi-pronged approach, which does not pose the logistical difficulties of studies estimating carrying capacity using LSUs, whereby several metrics are used to estimate the level of habitat usage and grazing pressure exerted by ungulates, with respect to levels of weed infestation (Trammel and Butler, 1995). The underlying assumption of this approach is that infestation of wildlife habitats by exotic weeds may decrease habitat usage, which may be inferred as decreased carrying capacity in a particular area, as the infested land either becomes obsolete as a food source (e.g., 0% utilisation by ungulates) or diminishes in its carrying capacity (e.g., utilisation values less than those obtained for a structurally and functionality comparable tract of uninfested land). The negative impact of the exotic weed on ungulate utilisation may be due to important graze/browse species being maintained at low population abundances, or completely lost from a particular habitat, due to exotic species being better competitors for nutrients and

mineral resources (Trammel and Butler, 1995), and/or avoidance of habitats infested by exotic weeds (Lym and Kirby, 1987).

This multi-pronged approach was adopted by Trammel and Butler (1995) who compared the number of faecal pellet and signs of twig damage, which were interpreted as measures of habitat utilisation and levels of browsing, within paired sample sites that were infested by the weed *E. esula*, and sites where the weed was absent. In doing so, Trammel and Butler (1995) demonstrated that habitat usage for bison (*Bos bison*), elk (*Cervus elaphus*) and deer (*Odocoileus* spp.) was significantly lower in rangeland-sites infested by *E. esula* than it was in paired sites where *E. esula* was absent.

A similar approach could be adopted for the purpose of determining *C. jamacaru* densities which are associated with significantly reduced levels of habitat usage and graze/browse production. As such, important measurements to take would include: the number of faecal pellets, number of individuals per key graze/browse species utilised, total number of key graze/browse species individuals and total biomass of key graze/browse species, or any other relevant metric for the specific study system. These measurements could then be compared between sites where *C. jamacaru* was present (invaded site) and where *C. jamacaru* was absent (control site), or preferably over a range of *C. jamacaru* densities (invaded sites) with respect to control sites. The result would be a measure of habitat utilisation by ungulates and grazing/browsing potential, relative to *C. jamacaru* densities. The threshold *C. jamacaru* densities above which habitat utilisation and grazing/browsing potential significantly differs from sites where *C. jamacaru* was absent, could then be used as a pre-defined management target, with which to adequately evaluate the success of biocontrol against *C. jamacaru* in South Africa.

The choice of appropriate metrics on which to evaluate the impact of a weed invader will obviously vary with the impact a particular weed exerts on the native ecosystem, and

may vary between populations of a particular weed species, and therefore will need to be carefully selected to best represent the impact of the weed within a particular habitat (see Table 4.1. for examples). By evaluating the success of biocontrol programmes in terms of the role biocontrol plays in mitigating the negative impact of a target weed, rather than rating success as a function of plant density, practitioners possess a more ecologically relevant tool to adequately evaluate biocontrol. This approach may significantly improve the level of biocontrol success against target weed invaders (e.g., if additional control agents are deemed necessary, or complimentary control strategies are employed, which mitigate the negative impact of the weed), which may in-turn increase investment and the confidence placed in the practise of biocontrol as a weed management tool. Additionally, it is possible that some biocontrol programmes which are seemingly successful are in fact not very successful (i.e., the weed is maintained at low densities but the threshold management target is not met due to biocontrol), or alternatively some biocontrol programmes may be wrongly considered to be unsuccessful (i.e., the weed remains in a habitat at high densities but is maintained below the threshold management target), whereby the determination and application of pre-defined management targets may provide a more relevant measure of biocontrol success.

4.3. Developing the science of biological control

Retrospective analyses, such as the studies conducted during this thesis (Chapter 2, 3), of biocontrol programmes provide a powerful tool by which to evaluate and develop the science, predictability and success of biocontrol (Louda et al., 2003). As such, a retrospective approach was adopted whereby the findings from the current study were evaluated with respect to two prominent points of contention in the current biocontrol literature, namely: (1) the need for single versus multiple agents for successful biocontrol of weeds (Denoth et al., 2002), and (2) whether and how candidate control agents could be prioritised, in order to

increase the likelihood of selecting the agent most likely to successfully control a target weed (Van Klinken and Raghu, 2006).

4.3.1. How many insect species are required for successful biological control?

A long-standing debate amongst biocontrol practitioners is that of "How many insect species are necessary for the successful biocontrol of weeds?" (Denoth et al., 2002). Harris (1985) proposed the "cumulative stress model," which indicates that multiple herbivores were required to successfully manage the target weed, as this maximises the level and diversity of stress experienced by the weed. Alternatively, numerous successful biocontrol programmes can be attributed to the impact of a single biocontrol agent, which has been formalised as the "lottery model," whereby releasing multiple agents maximises the chances of releasing the "correct" biocontrol agent (Myers, 1985).

In two meta-analyses, 81 % and 54 % of successful biocontrol programmes are considered to be due to the impact of a single control agent (Myers, 1985; Denoth et al., 2002). More recently, Myers (2008) found that ten additional biocontrol programmes were successful due to the regulatory impact of a single control agent. Nevertheless, Denoth et al. (2002) demonstrated that 46% of successful biocontrol programmes required the combined impact of multiple control agents, suggesting that determining the optimal number of control agents to release, in order to successfully manage a target weed, may not be a straight forward question to answer. Indeed, the number of successful control programmes ascribed to the impact of multiple control agents may be under-reported due to the monetary costs of introducing additional control agents, which may discourage practitioners to source additional control agents when the first agent is unsuccessful (H. Klein, pers. comm.).

Table. 4.1. Examples of weed impact groups (and species that may fall into each respective impact group), the impact each species has on the natural ecosystem, and accordingly an appropriate, ecologically-derived measure by which to evaluate 'complete' biocontrol success is then provided (Hoffmann, 1995; Klein, 2011).

Weed impact group (weed species)	Impact on natural ecosystem	Appropriate measure of biocontrol success	Reference
1. Native biodiversity			
Pereskia aculeata Miller (Cactaceae)	Reduces native plant biodiversity at > 30 % cover	Limit *P. aculeata* to ≤ 30 % cover	Paterson et al. (2011a)
Lantana camara L. (Verbanaceae)	Reduces native plant species richness at > 75% cover	Limit *L. camara* to ≤ 75 % cover	Gooden et al., (2009)
Eichhornia crassipes Mart (Solms-Laubach) (Pontederiaceae)	Reduces benthic maroinvertebrate diversity when *E. crassipes* form impeneterable mats	Define an *E. crassipes* percentage cover where maroinvertebrate biodiversity is not significantly different from if the weed were absent from the habitat	Coetzee et al. (2014)
2. Grazing capacity/habitat utilisation			
Euphorbia esula L. (Euphorbiaceae)	Reduces forage utilisation by cattle at ≥ 10 % cover	Limit *E. esula* to < 10 % cover	Hein and Miller (1992)
	Reduces livestock carrying capacity at ≥ 50 % cover	Limit *E. esula* to < 50 % cover	Lym and Messersmith (1987)
Opuntia stricta (Haworth) Haworth (Cactaceae)	Reduces wildlife forage and movement	Define densities of *O. stricta* where wildlife foraging levels are not significantly different from if the weed were absent from the habitat	Lotter and Hoffmann (1998)

Opuntia aurantiaca Lindley (Cactaceae)	Reduces carrying capacity and injurious to livestock when "heavy infestations" occur, which Serfontein (1961) describes as weed infestations containing "…large plants [> 5 cladodes] with numerous isolated cladodes, which are inclined to occur in clumps..."	Control success of *O. aurantiaca* may be defined as maintaining weed populations to containing few large plants, or preferably determine densitites of *O. aurantiaca* where grazing capacity/habitat usage is not significantly different from if the weed were absent from the habitat	Serfontein (1961) cited in: Zimmermann (1981)
3. Agricultural productivity			
Parthenium hysterophorus L. (Asteraceae)	Reduces crop yield	Define densities of *P. hysterophorus* where crop yield is not significantly different from if the weed were absent from the habitat	Khosla and Sobti (1979)
4. Fire regimes			
Bromus tectorum L. (Poaceae)	Increases fire frequency, by obtaining greater relative frequencies (percentage of 0.10 m^2 quadrats containing *B. tectorum*), from every 60-100 years to burning at intervals of less than 5 years	Limit *B. tectorum* relative frequencies to < 14 % of quadrats	Whisenant (1989)
5. Water quality/access to water			
Eichhornia crassipes	Increases levels of water transpiration	Define densities of *E. crassipes* where levels of transpiration are not significantly different from if the weed were absent from the habitat	Fraser et al. (2016)

Furthermore, the premise of single versus multiple agents in successful biocontrol may be dependent on the biology of the weed, which may skew the generalised findings from these studies. For example, annual weeds with short-lived seed banks may be effectively controlled by a single control agent (e.g., *Centaurea solstitialis* Linnaeus (Asteraceae) is effectively controlled by *Eustenopus villosus* (Boheman) (Coleoptera: Curculionidae); Denoth et al., 2002), while perennial weeds and especially those with long-lived seedbanks may require multiple agents which target several key life-history transitions to successfully regulate the target weed (e.g., *O. stricta* is controlled by the combined impact of *Cactoblastis cactorum* Berg (Lepidoptera: Pyralidae) and *D. opuntiae*; Denoth et al., 2002).

The results of the current study indicate that *H. festerianus* significantly reduces weed densities and alters population dynamics of *C. jamacaru* in Groot Marico and Rust-de-Winter, to the point where sustainable weed population regeneration is unlikely, and further population declines are expected after an approximately 15 year time lag, due to the long-lived life-span of this cactaceous weed (Chapter 2). This finding lends support to the "lottery model," proposed by Myers (1985), whereby a single control agent is considered sufficient to control a target weed invader. The efficacy of *H. festerianus* as a biocontrol agent of the perennial, long-lived *C. jamacaru* may result from its impact on both adult plant survival and weed fecundity, although the relative contributions of each life-history transition are unknown, at present.

If the "cumulative stress model," is to describe the successful biocontrol of *C. jamacaru*, the cumulative impact of at least both biocontrol agents already released against *C. jamacaru* (i.e., the combined impact of *H. festerianus* and *N. cereicola*), and/or combinations of additional agents from the native range would have been required to manage this weed in South Africa. It is unknown whether *H. festerianus* will have a similar impact on *C. jamacaru* populations in Kwa-Zulu Natal and Eastern Cape, and may be an important

consideration when attempting to describe the efficacy of *H. festerianus* as a biocontrol agent. Following Denoth et al. (2002), biocontrol programmes are urged to prioritise single-species introductions, and rigorously evaluate the impact of the agent at the weed population-level before sourcing and releasing additional control agents. Introducing fewer agents may reduce the costs of biocontrol programmes (McClay and Balciunas, 2005), and increase the efficiency of biocontrol as a management tool for alien weeds (Myers, 2008). The real challenge for biocontrol practitioners will be to identify candidate agents' pre-release that are most likely to be successful biocontrol agents (Myers, 1985).

4.3.2. Prioritisation of candidate control agents

Biocontrol practitioners have attempted to prioritise candidate control agents according to a variety of biotic and abiotic criteria, including: plant-insect incompatibility/genetic matching (Kniskern and Rausher, 2001; Giblin-Davis et al., 2001; Goolsby et al., 2003; Wardhill et al., 2005), damage to the target plant (Wapshere, 1985; Rayacchetry et al., 1998; Paynter et al., 2003), susceptibility to predation and parasitism (Lawton, 1985; Wapshere et al., 1989; Ozman and Goolsby, 2005), and climatic matching (Dhileepan et al., 2006; Senaratne et al., 2006; Rafter et al., 2008; Robertson et al., 2008). McFadyen (1998) suggests that the ability to predict and select the most effective candidate control agent may be considered the "holy grail" of biocontrol. Therefore, studies that identify factors which contribute to, or constrain, the potential of biocontrol agents being realised upon introduction may be vital, as they can inform which criteria are useful for agent prioritisation (and which criteria provide little predictive power), and therefore improve and optimise the agent selection process (Zalucki and Van Klinken, 2006). In doing so, an efficient means to prioritise candidate control agents may minimise the number of control agents released against a target invader (Zalucki and Van Klinken, 2006), which may: reduce the risk of non-target effects (Strong and Pemberton,

2000), increase the success rate of biocontrol programmes (McEvoy and Coombs, 1999) and reduces the costs of biocontrol (Denoth et al., 2002). Despite the perceived importance of an effective agent prioritisation method, only two studies have adopted a systematic approach to prioritisation of candidate control agents, by combining multiple characteristics which may be favourable for biocontrol into a formalised scoring system (Harris, 1973; Goeden, 1983), and their adoption by biocontrol practitioners has been lacking (van Klinken and Raghu, 2006).

Genetic matching of biocontrol agents to their host plants has been suggested as a prioritisation criteria for the selection of candidate control agents (Paterson et al., 2014). Plant-insect incompatibility has been proposed as a possible constraining factor of the biocontrol programme against *C. jamacaru* in South Africa (see Paterson et al., 2011a), however two lines of evidence suggest that this may not be a limiting factor. Firstly, the oligophagous nature of *H. festerianus*, which feeds extensively within the Cactoideae (McFadyen and Tomley, 1980), suggests that host-adaptation to possible distinct taxonomic entities of *C. jamacaru* or other members of the *Cereus* genus (e.g., *C. hildmannianus*), which may be naturalised in South Africa, is unlikely to constrain the biocontrol efficacy of *H. festerianus*. Secondly, observations of *H. festerianus* performance on potted *C. jamacaru* individuals from four regions across South Africa (see Table 2.1.) and individuals from two *Harrissia* spp. (*H. martinii* and *H. balansae* (K. Schum.) N. P. Taylor & Zappi) under greenhouse conditions suggest that host plant delineation and *C. jamacaru* provenance did not significantly constrain the ability of *H. festerianus* to establish on *C. jamacaru*. These plants were collected and grown in a greenhouse at Rhodes University where the efficacy of the *H. festerianus* on plants from different populations was to be compared by conducting bioassays. These bioassays were designed to determine whether genetic mismatching between *C. jamacaru* populations where the agent has established (Groot Marico and Rust-

de-Winter) and where *H. festerianus* has failed to establish, provide inadequate levels of weed suppression or has yet to be redistributed to, may explain the variable success of this agent. Unintentionally, and completely unaided, *H. festerianus* (which was present on two plants that grown in the outer most row of the potted plants in the greenhouse) was able to establish upon the majority of the potted plants in the greenhouse (including 18/23 potted plants from KZN populations) within one month of being potted. As regrowth began in early summer (August-September 2016), *H. festerianus* rapidly colonized all freshly growing shoot tips, and initiated shoot deformation for plants from all 12 *C. jamacaru* populations sampled and for both *Harrisia* spp. As such, there is at least preliminary data which suggest that genetic mismatching between *C. jamacaru* populations was unlikely to prevent establishment of, and impede the efficacy of *H. festerianus* as a biocontrol agent in Kwa-Zulu Natal, regardless of the taxonomic delineation of *C. jamacaru* in that region.

Climatic incompatibility between the native distribution and adventive range of a candidate control agent has been linked to their failure to establish, proliferate and effect meaningful control over target weeds, and therefore may be an important criterion by which to prioritise candidate agents (Dhileepan et al., 2006; Robertson et al., 2008). Little is known of the thermal biology of *H. festerianus*, both in its native distribution and in its introduced range. However, indications are that *H. festerianus* establishment is not constrained by climatic variability in South Africa, with the agent successfully established in the hot and dry conditions in Groot Marico and Rust-de-Winter, and in the sub-tropical climate of Kwa-Zulu Natal, although the latter observation is based on an admittedly small number of observations. The efficacy of *H. festerianus* as a biocontrol agent does not appear to differ significantly between *C. jamacaru* populations in Groot Marico and Rust-de-Winter, while there is a current lack of sufficient information to evaluate the possibility that *H. festerianus* efficacy may be limited in Kwa-Zulu Natal due to the sub-tropical climate (Paterson et al.,

2011a). The more humid, and wetter climate in Kwa-Zulu Natal has been purported as a possible reason why *C. jamacaru* appears more tolerant of *H. festerianus* herbivory in the region (R. Brudvig, pers. comm.; cited in Paterson et al. 2011a). An appropriate evaluation of this claim exists in conducting experimental releases of *H. festerianus* onto populations of *C. jamacaru* in Kwa-Zulu Natal, and carefully monitoring the establishment, population growth and impact *H. festerianus* on the host plant, over time. Nevertheless, climatic variation in South Africa does not appear to be a significant constraining factor of biocontrol against *C. jamacaru*, although it may play a less obvious role in limiting population growth or explaining subtle variations in *H. festerianus* performance between geographic regions.

Candidate biocontrol agents may potentially be prioritised by their relative susceptibility to biotic interference from acquired novel predators and parasitoids (Ozman and Goolsby, 2005). The current study found that several predictions derived from current theory with regards to the risk of *H. festerianus* to biotic interference from novel predators and parasitoids held true. Indeed, the richness, structure and diversity of the assemblage of predators and parasitoids associated with *H. festerianus* was well predicted by ecological theory (Chapter 3). More importantly, the efficacy of *H. festerianus* as a biocontrol agent of *C. jamacaru* in South Africa was relatively well predicted by current theory, whereby candidate agents that are kept rare in their native distribution are more likely to be effective biocontrol agents. However, not all of the predictions derived from the literature held true for *H. festerianus,* whereby hemipteran biocontrol agents in South Africa usually do not readily acquire parasitoids upon introduction (Hill and Hulley, 1995), which was in contrast to *H. festerianus* which was associated with at least three parasitoids during this study. This unexpected finding may stem from the fact that the majority of the hemipterans considered during the study by Hill and Hulley (1995) were cochineal insects, who produce carminic acid which is believed to be an effective parasitoid deterrent (Moran, 1980). Nevertheless,

prioritisation of biocontrol agents according to their relative susceptibility to biotic interference may indeed prove an appropriate manner by which to prioritise candidate control agents.

Nevertheless, some authors have argued that attempting to predict control agent efficacy prior to release in the adventive range may be a futile task (Simmonds, 1976), which may explain the current lack of a formalised scoring system by which to prioritise candidate control agents. This study suggests that plant-insect incompatibility, climatic matching and the impact of acquired natural predators and parasitoids may not have a significant effect on the efficacy of *H. festerianus* as a biocontrol agent in South Africa. However, the fact that consideration of all three of these criteria have been critical to the success of multiple biocontrol programmes worldwide (e.g., Goeden and Louda, 1976; Reimer et al., 1988; Volchanksy et al., 1999; Goolsby et al., 2004; Wardhill et al., 2005; Dhileepan et al., 2006; Senaratne et al., 2006; Rafter et al., 2008; Mathenge et al., 2009), suggests that predicting agent efficacy pre-release and therefore the development of a universal, broad-scale agent prioritisation system may indeed be challenging. The relative importance of each factor is likely to differ for each biocontrol agent-weed system, so prioritisation systems could be improved by considering the factors that increase or decrease the chances of success at a finer scale of resolution of the candidate agents. For example, candidate control agents that are sedentary or have poor dispersal capacities and complete a greater number of generations in relation to the target host may be more susceptible to differential host adaptation and therefore forming host-adapted races (Hanks and Denno, 1994). As such, biocontrol programmes targeting a weed where a candidate control agent displays a sedentary life-history (e.g., cochineal insects on *Opuntia* weeds), should consider genetic matching as a more important prioritisation criteria. Similar refinements could be put in place with regards to other criteria on which to prioritise candidate control agents. For example, only when

considering candidate control agents that are to be released in an area of extreme climatic conditions and/or variation would climatic matching studies prioritised, or only when considering leaf-mining candidate control agents would the risk to biotic interference be determined and used to prioritise control agents, as leaf-miners may be more susceptible to top-down regulation that other arthropod feeding guilds (Hill and Hulley, 1995). Studies that attempt to improve the prioritisation of candidate control agents and improve the theoretical understanding of factors which contribute to successful biocontrol should be prioritised and encouraged.

4.4. Threat of exotic Hypogeococccus sp. populations to native cacti in the Americas

The cactus moth *C. cactorum* has been employed as a biocontrol agent of several *Opuntia* spp. Around the world, being most well-known for dramatically reducing *O. stricta* densities in Australia, and to a lesser extent in South Africa (Dodd, 1940; Pettey, 1948). The oligophagous nature of *C. cactorum* has never been problematic for implementation of biocontrol against cactaceous weeds in the Old World, due to the lack of any familial representatives in this region. However, the discovery of *C. cactorum* in the Florida Keys in 1989 (Habeck and Bennett, 1990) has caused widespread concern over the safety of native *Opuntia* species in this region, where the moth is considered invasive (Bennett and Habeck, 1995). This concern is compounded by the rapid rate of spread of *C. cactorum*, which is estimated to disperse by up to 40 km per year (Johnson and Stiling, 1998), and has subsequently been recorded in the southwestern USA and may eventually reach Mexico, which is a hotspot for cactus biodiversity and commercial cultivation (Zimmermann et al., 2004). The highest diversity of *Opuntia* species is distributed throughout Mexico and the southwestern USA, where an estimated 60 endemic *Opuntia* species are found (Zimmermann et al., 2004). To date, all six native *Opuntia* species in Florida are attacked

by *C. cactorum* (Johnson & Stiling, 1998), however the long-term consequences of moth establishment and impact on *Opuntia* species diversity, persistence and conservation are unknown.

A demographic understanding of the effects *C. cactorum* may have on *Opuntia* spp. is vital for their conservation, and preservation of species diversity and ecosystem function, as it is expected that *C. cactorum*, due to its damaging nature, may lead to local extinctions of *Opuntia* spp. (Dodd 1940; Baker and Stiling, 2009). Several demographic studies of *Opuntia* spp. (Mandujano et al., 2001; Godinez-Alvarez et al., 2003; Miller et al., 2009), suggest that consumer effects at the individual-plant level may translate into devastating effects for the population dynamics of native and endemic cactaceous species, especially when the cumulative effects of native cactus-feeding insects are considered (Miller et al., 2009). The findings of the current study may provide further support for the demographic consequences of pest populations of cactus-feeding insects, highlighted by the dramatic impacts ascribed to *H. festerianus* herbivory on *C. jamacaru*, and the predicted deleterious impacts on resulting population dynamics of this cactus plant. While this may be a broad inference regarding the role cactus-feeding insects may play in determining population dynamics and structure of their host plants, the discovery of exotic populations of *Hypogeococcus* spp. on an array of columnar and other cacti (subfamily Cactoidea) in Puerto Rico in 2005 suggests that a similar situation to that of *C. cactorum* may have arisen (Zimmermann and Perez Sandi y Cuen, 2010), and to which the current studies' findings have a more direct application. It must be stressed that neither *C. cactorum* nor *H. festerianus* were intentionally introduced into these novel habitats for biocontrol purposes, as such neither case should be considered to be examples of non-target effects associated with biocontrol (Johnson and Stiling, 1998; Zimmermann and Perez Sandi y Cuen, 2010).

The *Hypogeococcus* sp. pest in Puerto Rico and the *Hypogeococcus* sp. introduced for biocontrol purposes in Australia and South Africa appear to constitute a cryptic species complex, following physiological, behavioural and observational studies of the biology of different *Hypogeococcus* spp. populations (Zimmermann and Perez Sandi y Cuen, 2010; Carrera-Martinez et al., 2015; Aguirre et al., 2016). As such, there are great taxonomic uncertainties with regards to which species of *Hypogeococcus* is present in Puerto Rico (and indeed Australia and South Africa), and following the rest of this thesis, will be referred to as *H. festerianus* to avoid further confusion, and any inferences made should be considerate of this.

Hypogeococcus festerianus has been recorded on species belonging to the *Althernathera* and *Acalypha* (Euphorbiaceae) genera, while extensive individual plant-level damage has been recorded for members of the Cactaceae, including: *Pilocereus royenii* (L.) Byles & G.D. Rowley, *Leptocereus quadricostatus* (Bello) Britton & Rose, *Melocactus intortus* (Miller) Urban and *C. hexagonus* in Puerto Rico (Zimmermann et al., 2010). The current situation may be more precarious than originally expected, with *H. festerianus* said to be proliferating and spreading rapidly in the region, which is compounded by the key ecological functions (Fleming et al., 2001) provided by the now inflated list of seven native cactaceous species attacked by *H. festerianus* in the dry forests of Puerto Rico (Carrera-Martinez et al., 2015). Two of these species, *L. quadricostatus* and *L. grantianus* are considered endangered and therefore are protected by federal law (see Carrera-Martinez et al., 2015), whereby even minor damage to these species may be fatal for their existence (Zimmermann and Perez Sandi y Cuen, 2010). The findings of this study and another recent study (Carrera-Martinez et al., 2015) suggest that the long-term future of the native cactaceous fauna on Puerto Rico may indeed be in jeopardy due to the presence and impact of *H. festerianus*. Mortality for several of the native cacti was exceedingly high when infected

with *H. festerianus*, most notably for *M. intortus* (Carrera-Martinez et al., 2015). The current study (Chapter 2) highlights the negative impact of *H. festerianus* on individual-plant fecundity and seedling recruitment for *C. jamacaru* populations, while similar deleterious consequences were observed for seedling survival in association with the *H. festerianus*, which suggests that both vegetatively-propagating species and species that propagate through seed set may be affected by *H. festerianus* in Puerto Rico. Thus, the fate of the cactaceous fauna of Puerto Rico appears unfavourable if *H. festerianus* is left unchecked, highlighting the need for immediate control interventions, such as biocontrol (Zimmermann and Perez Santi y Cuen, 2010).

4.5. Implications for the biocontrol of Cereus jamacaru

Post-release evaluations of biocontrol agent efficacy can provide practitioners with information that can be used to improve the level of biocontrol success achieved against target invaders (Morin et al., 2009). These evaluations are primarily used to determine whether additional control agents are required to affect control over the weed (Myers, 1985; Denoth et al., 2002), or whether alternative management strategies should be implemented, in the form of an integrated weed management programme (Paynter and Flanagan, 2004; Buckley et al., 2004). The finding that *H. festerianus* has a negative impact on *C. jamacaru* populations (Chapter 2) and the observations that chemical control appears to be antagonistic with biocontrol efforts employing *H. festerianus* (see Klein, 1999), suggests that an integrated management strategy for *C. jamacaru* in South Africa (which combines chemical and biocontrol), may be inappropriate.

The findings from the current study suggest that complimentary biocontrol agents may not be required for the management of *C. jamacaru* in South Africa, with the agent *H. festerianus* predicted to provide complete control over the invader, with time

(Chapter 2). However, this is not to say that greater control of *C. jamacaru* wouldn't be achieved upon introduction of additional, complimentary herbivores sourced from the native range. It is believed that long-lived perennials, such as *C. jamacaru*, are sensitive to reductions in mature plant survival (Silvertown et al., 1993). Given that *H. festerianus* infestation primarily results in mortality of seedlings, reduces reproductive capacity of *C. jamacaru*, and has a less marked impact on adult survival (Chapter 2), introducing a control agent that reduces adult growth and survival, may enhance the success of biocontrol against this weed.

The stem-boring beetle *N. cereicola* was released and subsequently became established at several sites near Pretoria (South Africa) during the 1990's (Klein, 1999). At the original release site, the beetle establish upon all adult plants within 7 years of release. Establishment was poor at two other release sites (Boekenhoutskloof and Rust-de-Winter, Gauteng Province, South Africa), with very few feeding and ovipositional scars observed on plants that had been infested, within 2 years of infestation with *N. cereicola* (Klein, 1999). The poor establishment of the beetle is believed to have been due to: herbicidal applications which are believed to be antagonistic to *N. cereicola* larval survival (Klein, 1999), and their long life-cycle and limited mobility (McFadyen and Fidalgo, 1976; H. Klein, pers. comm.). *Nealcidion cereicola* should be considered the primary candidate for re-introduction if additional, complimentary biocontrol agents are required for *C. jamacaru* management in South Africa, given the highly-damaging stem-boring nature of this species (Klein, 1999). With greater care taken in the mass-rearing, mass-release and monitoring of this agent upon re-introduction, the magnitude of biocontrol success against *C. jamacaru* may be greatly improved.

However, *H. festerianus* has a significant deleterious impact on *C. jamacaru* populations in South Africa (Chapter 2), and indications are that given sufficient time, this

biocontrol agent is able to limit the ability of *C. jamacaru* to recruit new individuals into its population, which leads to declines in population density. As such, the biocontrol programme against *C. jamacaru* in South Africa should: (1) prioritise the active redistribution of *H. festerianus* to all weed populations where the agent is absent (eg., Paynter, 2005); (2) conduct experimental releases of *H. festerianus* in the Kwa-Zulu Natal and Eastern Cape provinces, where the agent is not yet established, using the demographic data collected during this study as a pre-release benchmark with which to evaluate the effect of *H. festerianus* (Evans and Landis, 2007); and (3) continue the long-term monitoring of the *C. jamacaru* populations surveyed in Groot Marico and Rust-de-Winter initiated during the current study, as well as for any experimental releases conducted in the future.

4.6. Conclusions

This thesis indicated that biocontrol efforts employing *H. festerianus* for the management of *C. jamacaru* have been successful. Furthermore, these data demonstrated the utility of retrospective analyses in developing and improving the science of biocontrol, specifically how to improve candidate agent prioritisation and evaluate the success of biocontrol efforts. Improvements in our theoretical understanding of biocontrol will undoubtedly reduce costs of biocontrol programmes, improve success rates, and increase the predictability of biocontrol.

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Plate I: (top left) Healthy and reproductively active *Cereus jamacaru* individuals; (top right) Fatality of a mature *C. jamacaru* plant infected with *H. festerianus*; (bottom left) Typical *Hypogeococcus festerianus* gall structure and deformed tissue growth; (bottom middle) *Cereus jamacaru* seedling heavily infected by *H. festerianus;* and (bottom right) a fruit eaten out by frugivorous birds, which will disperse seed set.