Quantifying the impacts of cactus biological control agents in South Africa

THESIS

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ABSTRACT

Invasive alien cacti are prominent weeds that threaten indigenous biodiversity and have a negative impact on agricultural productivity in South Africa. These plants are problematic because they form dense thickets that reduce the carrying capacity of rangelands; restrict the movement of livestock and wildlife thus reducing access to shade and water sources; and are directly harmful to livestock, wildlife and people due to their sharp spines. Biological control is the most effective, affordable and environmentally friendly method to control invasive alien cacti and minimize their negative impacts. Cochineal insects (*Dactylopius* spp.: Dactylopiidae) and the gall-forming mealybug, *Hypogeococcus* sp. (Pseudococcidae) are used as biological control agents for cacti. The agents are however poor dispersers, so mass-rearing and augmentative releases are required in order to establish the agents at sites where they are needed. This study aimed to evaluate the mass-rearing and release efforts of cactus biological control in South Africa, quantify the impact of the biological control agents on cactus plant populations through long-term monitoring, and assess the benefits accrued due to the biological control agents through the perceptions of land-users.

An assessment of the effectiveness of the release effort for cactus biological control agents was conducted by comparing where biological control agents have been released with the known distribution of the target weeds. Only 26% of the quarter degree squares that are known to be occupied by invasive alien cacti have had biological control agents released in them. This indicated that the mass-rearing and release efforts in South Africa are inadequate and should be increased, especially in areas where few releases have been made and many cacti are present, such as the Limpopo Province.

The impact of the biological control agents on cactus plant populations was assessed by monitoring agent densities, target plant densities and target plant reproductive outputs before and after releases. Plant biomass and reproductive output were reduced by biological control agents for three of the target weeds that were assessed, while the duration of the study was too short to measure reductions for the fourth target weed.

Benefits to land-users were then quantified through a questionnaire survey. Land-users perceived biological control as an effective management option, with 81% of the land-users reporting that there was less invasive alien cactus after releasing biological control agents on their land. Forty-nine percent of the land-users believed that the negative impacts of the cactus had been reduced and that they benefited more from their land since control was achieved. Since land-users were only interviewed within four years of the releases being conducted, it is expected that the percentage of land-users who gained benefits from biological control will increase in future. Ninety-seven percent of the land-users stated that the agents were safe and had not fed on any other plants or had any detrimental impacts. These perceptions indicated that land-users regarded biological control as a safe and effective method of controlling invasive alien cacti.

This study confirms that biological control is an effective and safe way of controlling invasive alien Cactaceae. It is also the first to assess some of the benefits that land-users have accrued due to biological control of cactus weeds. It is however evident that a greater mass-rearing and release effort is required for South Africa to get the maximum benefits possible from the use of the biological control agents for cactus weeds that are available in the country.

UMONGO

Izityalo ze-cactus ezisuka kwamanye amazwe zilukhula olubalaseleyo, olwenza ingxaki kwintlobo ngeentlobo zezityalo nezilwanyana kwaye ezizityalo zinefuthe neziphumo ezingalunganga kwimveliso yezolimo eMzantsi Afrika. Ezi zizityalo ziyingxaki kuba zenza amatyholo ashinyeneyo athi anciphise umthamo wokusebenzisa umhlaba: zinqanda ukuhamba nogkukhululekileyo kwemfuyo nezilwanyana zasendle ngokwenza oko zingakwazi ukufikelela emthuzini xa kutshisa nakwimithombo yamanzi yokusela; zikwayiyo nengozi kwimfuyo, izilwanyana zasendle kunye nabantu ngenxa yamave abukhali afumaneka kwezizityalo. Ukulawula nokwehliza ubunzini nezinga lemigcipheko yezizityalo, kusetyenziswa indlela ekuthiwa yi-biological control. Lendlela yeyona ndlela isebenza ngokuphucukileyo, efikelelayo, nengenabungozi kokusingqongileyo. Izinambuzane ze-cochineal (i-*Dactylopius* spp.: Dactylopiidae) kunye ne-mealybug, i-*Hypogeococcus* sp. (Pseudococcidae) zisetyenziswe njengezixhobo ze-biological control ezinceda ukulawula ezizityalo ze-cactus zingafunekiyo. Ingxaki yazo ezizinambuzane zizixhobo ze-biological control zingentla kukuba azikwazi kuzisasaza ngokwazo ukuba zifikelele nakwizityalo ezikude ngoko ke ukukhuliswa nokukhutshwa ngobuninzi bazo kuyafuneka ukukhawulelana nalengxaki kunye nokwandisa amathuba wokuba zifikelele kuzozonke izityalo ze-cactus ekufuneka zizilawule. Olu phononongo lujolise ekuvavanyeni iinzame zokukhulisa ngobuninzi nokukhupha ezezinambuzane zizixhobo ze-bioloigical control zokulawula izityalo ze-cactus eMzantsi Afrika, ukujonga ubungakanani befuthe notshintsho elenziwa zezinambuzane zizixhobo ze-bioloigical control kwizityalo ze-cactus emva kwexesha elide lokuzijonga, kunye nokuvavanya inzuzo efunyenwe ngenxa yokulawula ezizityalo ze-cactus ngokwemibono yabasebenzisi bomhlaba.

Uvavanyo lweenzame zokukhutshwa kwezixhobo zezixhobo zezinambuzane ze-bioloigical control kwizityalo ze-cactus lwenziwa ngokuthelekisa iindawo apho izixhobo zezinambuzane ze-biological control zikhutshwe khona kunye neendawo apho izityalo ze-cactus kwaziwayo ukuba ziyafumaneka khona. Yi-26% kuphela yesikwere sekota eyaziwayo ukuba kukhona izityalo ze-cactus nalapho kukhutshwe khona kwezinambuzane ezizixhobo ze-biological control. Oku kubonisa ukuba iinzame zokukhulisa nokukhutshwa kwezinambuzane ezizixhobo ze-biological control eMzantsi Afrika azanelanga kwaye kufuneka zandiswe, ngakumbi kwiindawo apho kukho ukhutsho olumbalwa olwenziweyo kunye nezityalo ze-cactus ezininzi ezifumaneka khona, njengePhondo laseLimpopo.

Iimpembelelo yokukhutshwa kwezinambuzane ezizixhobo ze-biological control kwizityalo ze-cactus zavavanywa ngokujonga ubunizi bezinambuzane ezizixhobo ze-biological control, ukuxinana kwezityalo ze-cactus ekujoliswe kuzo kunye nemveliso yokuzala kwezityalo ze-cactus phambi nasemva kokuba kukhutshwe izinambuzane ezizixhobo ze-biological control. Ubungakanani bezityalo ze-cactus kunye nemveliso yokuzala ziye zacutheka emva kokukhutshwa kwezinambuzane ezizixhobo ze-biological control kwinzityalo ezintathu ze-cactus ebekujoliswe uvavanyo kuzo, ngelixa ixesha lophononongo lalifutshane kakhulu ukuvavanya unciphiso kwisityalo se-cactus sesine.

Izibonelelo zabasebenzisi bomhlaba zaye zavavanywa kusetyenziswa uhlobo lwemibuzo. Abasebenzisi bomhlaba balubona ukusetyenziswa kwezinambuzane ezizixhobo ze-biological control njengendlela yolawulo olusebenzayo, yi81% yabasebenzisi bomhlaba echaze ukuba izityalo ze-cactus zecuthekile emva kokukhutshwa kwezinambuzane ezizixhobo ze-biological control emhlabeni wabo. Amashumi amane anesithoba eepesenti zabasebenzisi bomhlaba bakholelwa ukuba impembelelo ezingalunganga ze-cactus zincitshisiwe kwaye baxhamle kakhulu kumhlaba wabo okoko lwaphunyenzwa. Oludliwano-ndlebe belwenziwe kubasebenzisi bomhlaba kwisithuba seminyaka emine emva kokuba kukhutshwe izinambuzane ezizixhobo ze-biological control, kulindeleke ukuba ipesenti yabasebenzisi bomhlaba abathe bafumana izibonelelo kwi-biological control lonyuke kwixesha elizayo. Amashumi alithoba anesixhenxe ekhulwini abasebenzisi bomhlaba bachaze ukuba izinambuzane ezizixhobo ze-biological control zikhuselekile kwaye khange zidle kuzo naziphi na ezinye izityalo ezingezi eze-cactus ebekujoliswe kuzo okanye zineempembelelo eyingozi. Ezi mbono ziyabonisa ukuba abasebenzi bomhlaba bayithatha i-biological control njengendlela ekhuselekileyo nesebenzayo yokulawula izityalo ze-cactus zamanye amazwe.

Olu phononongo luqinisekisa ukuba ulawulo olusebenzisa izinambuzane ezizixhobo ze-biological control yindlela esebenzayo nekhuselekileyo yokulawula iCactaceae yamanye amazwe. Ikwangolokuqala ukuvavanya ezinye zezibonelelo ezizuzwe ngabasebenzisi bomhlaba ngenxa ye-biological control yezityalo ze-cactus. Nangona kunjalo kucacile ukuba ukwandisa inzame zokukhulisa nokukhupha ngobuninzi izinambuzane ezizixhobo ze-biological control kuyafuneka ukuze uMzantsi Afrika ufumane izibonelelo eziphakamileyo enokubakho ngokusetyenziswa kwe zinambuzane ezizixhobo ze-biological control zokulawula ukhula lwe-cactus olukhoyo kweli lizwe.

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Lumka Mdodana, soze kwanele ukubulela kwam kuwe, awekho amagama endinowasebenzi abonakalisa nanele ukubulela kuwe. Wandinceda, wandibizela kwiDyunivesithi yaseRhodes wagqiba wandishiya, ayizange yafika nanina na kum ukuba ndingafunda ndifikelele kulendawo ndikuyo. Ukundikhwaza kwakho ngo 2013 kwandivulela amathuba Nkosazana! Ndiyabulela Nkosazana Lumka!

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THESIS DEDICATION

This thesis is dedicated to my late family members: my father, Thembalethu Winston Mnqeta, and my brother, Kwezi Dawn Mnqeta.

CHAPTER 1: GENERAL INTRODUCTION

Natural ecosystems provide humans with essential ecosystem services and, if an ecosystem is disturbed or imbalanced, the provisioning of these ecosystem services may be disrupted (Spangenberg et al. 2014). Most natural ecosystems have been negatively impacted by anthropogenic activities such as land degradation, pollution, overexploitation, and invasion by alien plants, resulting in a reduction in the benefits from ecosystem services (Vitousek et al. 1997; Walsh et al. 2016). Natural ecosystems will continue to degrade if no preventive and restorative measures are implemented to protect, conserve, and promote healthy ecosystems (Vitousek et al. 1997). The restorative measures should include controlling invasive alien plants (Bradshaw 2002).

Ecosystem services are both direct and indirect benefits that humans obtain for free from well-functioning ecosystems, and these services are essential for the growth, development, and survival of humans (MA 2005; DEWHA 2010; Lawton 2013; Wiborn 2013). The Millennium Ecosystem Framework classifies ecosystem services into four categories: **1) Provisioning services**: the essential products supplied by and obtained from ecosystems, such as food and freshwater **2) Regulating services**: benefits acquired from regulation and maintenance of ecosystem processes, such as regulating floods, climate and drought **3) Cultural services**: non-material benefits people get from ecosystems, such as religious, spiritual, recreational and tourism, and **4) Supporting services**: services required by the ecosystem to produce all other services such as soil formation and nutrient cycling (de Groot et al. 2010; Lawton 2013; Wiborn 2013). All four categories of ecosystem services can be negatively impacted by invasive alien plants when they become overabundant in natural and agricultural ecosystems.

1.1. Invasive alien plants

Invasive alien plants are plant taxa introduced outside their native range, that can establish and spread in the new areas, causing environmental and socioeconomic negative impacts (Richardson et al. 2000; Myers and Bazely 2003; Weber 2003; Catford et al. 2009). The invasion by these alien plant species threatens natural ecosystems, negatively impacts native biodiversity, decreases agricultural productivity, and disrupts the provisioning of ecosystem services provided by pristine ecosystems (Paterson et al. 2011a; Simberloff et al. 2013; Wilson et al. 2013). Due to their ability to outcompete and replace native species, the second leading cause, following habitat destruction, contributing to the loss of biodiversity globally is alien invasion (Byers et al. 2002; McGeoch et al. 2010; Vilà et al. 2011; Weber 2003).

Four stages occur before an alien species becomes invasive (Richardson et al. 2000; Blackburn et al. 2011): **1) Introduction**: introduced species occupy new areas outside their native range. **2) Establishment**: self-regulation and formation of mature plant infestations by the introduced species. **3) Naturalization**: the introduced species can sustain the existing plant population and reproduce more plants. **4) Spread**: dispersal to new areas that are not infested by the introduced species. The majority of alien species do not reach the stage when they spread, but alien plants that reach this stage are likely to become overabundant and problematic, and they are extremely difficult to manage (Pyšek et al. 2020). The mechanisms that influence biological invasion success are not well understood, and predicting which introduced plants will become invasive and problematic is a challenge

(Meyer et al. 2005). There are, however, some traits such as phylogeny, taxonomy, growth form, and mode of reproduction that can predict which species are likely to invade non-native ranges successfully and these traits can be used in risk analyses, thus preventing the introduction of potentially harmful alien plants into areas where they do not occur (Novoa et al. 2015a).

1.1.1. Review of impacts of invasive alien plants

Many invasive alien plants were intentionally introduced for beneficial attributes, such as economic benefits and ornamental purposes (Hanspach et al. 2008; Pyšek et al. 2020). These species are an increasing global threat that continues to have adverse effects on the economy, agricultural productivity, and water security of many countries (Pimentel et al. 2005; Pyšek, et al. 2020). Invasive alien species have resulted in economic losses worth $138 billion in the USA annually and approximately $14.45 billion in China over six years (Pimentel et al. 2005; Xu et al. 2006). It was reported that in Southeast Asia, the human health sector of the country alone had experienced an economic loss of US$1.85 billion from diseases associated with invasive alien plants, and this loss combined with agricultural loss to US$33.5 billion (Nghiem et al. 2013). Introduced plant species have detrimental effects on agricultural productivity; for example, in China, more than 1400 naturalized plant species pose a significant threat to agricultural yields (Xu et al. 2006). Massive water losses have been reported due to the invasion of invasive alien plants; for example, in the south-western USA, 1.4-3 billion cubic meters have been lost due to the presence of *Tamarix ramosissima* Ledeb. (Tamaricaceae) (Pejchar and Mooney 2009). This tree occurs along streams and uses large quantities of water compared to native plants, and this water could have been useful for drinking, irrigation, and hydropower (Pejchar and Mooney 2009). The growing global threat of invasion and the establishment of alien plant species indicates an urgent need to take action as this will help reduce the negative impact of invasive alien plants in all countries, including South Africa (Seebens et al. 2017).

1.1.2. Invasive alien plants in South Africa

Invasive alien plants are a severe threat to native biodiversity, the provision of ecosystem services, agricultural environments, economy, and human societies of South Africa (Richardson & van Wilgen, 2004; Wilson et al. 2013). About 900 alien plant species are naturalised and more than half of these species have invaded natural ecosystems (van Wilgen 2018). Invasive alien plants cover over 10 million hectares of South Africa, and the area that is invaded and the number of invading species continues to increase (van Wilgen and Lange 2011; van Wilgen 2018). Among these species, the most prominent and widespread species are alien trees, such as the European *Pinus* species, Australian *Acacia* species, *Lantana camara* L. (Verbenaceae) and succulent species in the family Cactaceae (van Wilgen 2018).

South Africa is a water-scarce country, and water is a valuable resource compared to many other countries (Percival and Homer-Dixon 1998; Bwapwa 2019). One of the significant negative consequences of invasive alien plants is high water consumption that reduces water security in the country (Chamier et al. 2012). For example, in South Africa, approximately 1.44 billion m^3 of water is used by alien plants annually (Colvin et al. 2016). The total of this water lost to invasive alien plants would be sufficient for approximately 3.38 million households of four members each, or enough to irrigate cropland of about 120 000 hectares (Colvin et al. 2016). Invasive alien plants such as *Acacia*, *Eucalyptus,* and *Hakea* have a higher annual total evaporation rate than indigenous plants, with annual total evaporation rate of the indigenous Fynbos biomes being 520mm/yr and that of the alien vegetation being 895mm/yr (Meijninger and Jarmain 2014).

Invasive alien plants do not only have a negative impact on water security, but these plants are also a significant threat to human well-being and animal health in South Africa (van Wilgen & de Lange 2011). Human beings and animal health are disrupted by invasive alien plants. For example, *Parthenium hysterophorus* L. (Asteraceae), known as famine weed, causes severe allergic reactions such as dermatitis, hay fever, and asthma when humans, livestock, and wildlife come in contact with it (Wise et al. 2007; Patel 2011; van Wilgen and Lange 2011; Strathie et al. 2021). Livestock avoids eating *P. hysterophorus*, but when they accidentally consume the weed, the meat becomes contaminated, with a direct economic loss for the livestock owner (Wise et al. 2007; Patel 2011; Strathie et al. 2021).

The plant invasions in South Africa by alien plants has negatively affected grazing and rangelands (van Wilgen and de Lange 2011). These plants decrease the ability of rangelands to support livestock and wildlife by displacing indigenous vegetation and forming impenetrable thickets, making it difficult for livestock to forage in affected areas (Richardson and van Wilgen 2004). For example, in arid regions, the invasive trees, *Prosopis* sp. (Fabaceae), forms dense stands that decrease the growth of herbaceous ground cover used for livestock grazing (Shackleton et al. 2014). Other invasive alien plants that have occupied and continue to grow and negatively impact rangelands include *Chromolaena odorata* (L.) R. M. King & H. Rob. (Asteraceae), *Solanum elaeagnifolium* Cavanilles (Solanaceae), and *Campuloclinium macrocephalum* (Less.) DC. (Asteraceae) (van Wilgen & De Lange, 2011).

1.2. Control methods for invasive alien plants in South Africa

The Working for Water Working (WfW) programme, currently housed under the Natural Resource Management (NRM) programmes of the Department of Forestry, Fisheries and the Environment (WfW: NRM: DFFE), was initiated in October 1995 to manage and reduce invasive alien plants in South Africa. The programme aims to reduce the negative effect of invasive alien plants and restore ecosystem services such as water provision, create employment opportunities, and empower poor rural communities through poverty relief projects (Zimmermann et al. 2004a; van Wilgen et al. 2012a; van Wilgen and Wannenburgh 2016). More than R600 million was spent annually to clear invasive alien plants over 10 million hectares of land in South Africa in the years 1995 to 2010 (de Lange and van Wilgen 2010; van Wilgen et al. 1998), and there has been significant growth in the funds allocated and area cleared by WfW in the past decade. The best way to halt invasions is by stopping the introduction of exotic plant species into the country, and there should be law enforcement of regulations to prevent alien plants from getting into the country (Pyšek et al. 2020; Seebens, et al. 2017), but once the invasive alien plant is established, control and eradication measures to reduce the negative impacts are required.

There are three methods of controlling alien invasive plants with naturalised populations, all of which have been invested in by the Working for Water (WfW) programme: 1) chemical control, 2) mechanical control, and 3) biological control. Each of these methods has disadvantages and advantages, which are discussed individually below. In many cases, integrated management is the most effective method of controlling invasive alien plants (van Wilgen and Lange 2011), but this must be done so that different methods complement each other and should be done on a case-by-case basis (Zachariades et al. 2017).

1.2.1. Chemical control

Herbicide applications can reduce the density of invasive alien plant infestations quickly and effectively, but the reduction in invasive alien plant density is often not sustainable and frequent follow-ups are required because propagules of the invasive alien plants remain in the ecosystem after treatments (van Wilgen et al. 2001). Some herbicides have non-target effects, killing non-target plant species or other organisms, and there are serious concerns about the hazards herbicides pose to the environment (van Wilgen et al. 2001). Herbicides are also costly and sometimes ineffective in controlling the target weeds, specifically when the funding to continue with clearing is not available, and regrowth in cleared areas is likely to occur (Thompson et al. 1991; van Wilgen and Lange 2011). WfW has spent a total of 3.2 billion rands (approximately 457 million US$) between 1995 and 2008; a large proportion of this was used on herbicides and the application of herbicides (van Wilgen et al. 2012b). Herbicides are harmful to people too, so it is vital that proper training and protective clothing should be provided and worn at all times when applying the herbicide (van Wilgen et al. 2001). However, herbicides are an essential part of integrated control strategies and appropriate for alien plant species, especially those that are not widespread but pose a significant risk of becoming more widespread and problematic in future (van Wilgen et al. 2001; Te Beest et al. 2017).

1.2.2. Mechanical control

Mechanical control of alien invasive plants usually refers to hand-pulling or physical removal of the target weed using tools or machines (van Wilgen et al. 2001). Tools that could be used are saws, slashers, axes, and machinery such as chainsaws and brush cutters (van Wilgen et al. 2001). This method includes cultural control, for example, the use of fire and over sowing with other species of plants. In some cases, biomass must be removed or burned on site. Mechanical control can be useful within small and accessible areas, mostly if plants are destroyed before seeds are produced (Benefield et al. 1999; Sheley et al. 1998; van Wilgen et al. 2001; Te Beest et al. 2017). This method is usually implemented as a follow-up method after chemical control because it succeeds in small infestations (van Wilgen and Lange 2011). However, it is labour-intensive, time-consuming, and very costly, so it is not suitable for dense infestations or on a landscape scale (Sheley et al. 1998; van Wilgen et al. 2001; van Wilgen and Lange 2011).

1.2.3. Biological control

Biological control involves the deliberate introduction of natural enemies such as insects and pathogens, which are referred to as biological control agents when released in the region where the invasive alien plant is a problem (McFadyen 1998). It is a management option that is environmentally safe and effective, but it is sometimes very slow (van Wilgen et al. 2013). The success rates of biological control agents in reducing invasive alien plants vary. It is hard to predict the level of success prior to the release of a new agent (Schwarzländer et al. 2018).

The main aim of biological control is to decrease the density and spread of a target weed to a level where it does not cause a significant negative impact on native biodiversity, economy, and agriculture (Hajek et al. 2016; Myers 1985; Seastedt 2015; van Wilgen et al. 2013). Biological control involves the collection of natural enemies (usually herbivorous insects, but sometimes herbivorous mites or pathogens) from the native range to areas where they are required to control problematic plants (McFadyen 1998). Biological control is founded on the Enemy Release Hypothesis (ERH), one of the many hypotheses used to explain the success of invasive alien plants (Mitchell and Power 2003). This hypothesis states that invasive alien plants can outcompete native plants because they do not have natural enemies with which they co-evolved in the introduced range and have a competitive advantage over indigenous plants, which will usually have co-evolved natural enemies. Biological control agents are introduced from the native range of the target weed to where the plant is a threat to reduce this competitive advantage (Keane and Crawley 2002; Liu and Stiling 2006). Understanding the ERH and factors contributing to plant invasion is essential to minimize future economic and environmental negative impacts through biological control (Keane and Crawley 2002).

There are two main approaches to implementing biological control of invasive alien plants: classical biological control and augmentative biological control (McFadyen 1998). Classical biological control is the foundation of biological control of weeds and involves the deliberate release of biological control agents and does not require multiple additional releases when they have been established in the field (Hajek 2004; van Driesche et al. 2008). Augmentative biological control is similar to classical biological control but requires mass-rearing of biological control agents, which are mass-released at periodic intervals to augment the populations of the agent in the field (van Lenteren and Bueno 2003). Biological control agents must be suitably host-specific for both approaches to damage and control the target invasive alien plant, without negatively impacting the indigenous plants or any plants valued by society.

Host specificity testing is conducted to determine if a candidate natural enemy has a suitably restricted host range before it is released as a biological control agent into the region where the invasive alien plant is problematic (McFadyen 1998). In South Africa, applications for releases of biological control agents are processed and administered by the National Biological Control Release Application Review Committee (NBCRARC), and releases can only be carried out after permission is granted by the relevant authorities in South Africa (Klein 2011).

Biological control is regarded as a safe and environmentally-friendly method, although some authors have raised questions and concerns about non-target impacts and the unintended consequences of biological control (Louda et al. 2005; Seastedt 2015; Simberloff 2012; Simberloff and Stiling 1996; Suckling and Sforza 2014). Three cases are recorded where biological control agents had significant negative impacts on non-target plant populations (Hinz et al. 2020). These cases occurred in the 1950s and 1960s when non-targets effects of biological control agents on indigenous biodiversity were not strictly considered before the releases (Hinz et al. 2020). The non-target effects caused by biological control introductions are considered minimal compared to the significant positive impact caused by reducing the target invasive alien plant populations associated with the release of these agents (Suckling and Sforza 2014; Hinz et al. 2019). Less than 1% of all agents released against weeds have had adverse effects (Suckling and Sforza 2014; Hinz et al. 2019). There has also been a decrease in the number of biological control agent releases that have had non-target effects in recent years due to the increasingly conservative approach taken by biological control practitioners and regulators (Hinz et al. 2020), so biological control is safer today than it has ever been in the past.

A global review of the success of biological control of invasive alien plants has recently been conducted (Schwarzländer et al. 2018). The study evaluated 1555 deliberate releases for 468 biological control agents targeting 175 invasive alien plants, which belonged to 48 different plant families (Schwarzländer et al. 2018). The analysis indicated a relatively high establishment rate with 332 (70.9%) of the 468 deliberately released biological control agents establishing populations where they were released (Schwarzländer et al. 2018). The deliberate release of biological control agents on 175 target invasive alien plants resulted in variable, medium, or complete control of 115 of these invasive alien plants (65.7%) (Schwarzländer et al. 2018). South Africa was ranked the second-highest country in its success rate (Schwarzländer et al. 2018).

Post-release evaluation studies are required to quantify the success of biological control programmes. These studies generally assess the impact and efficacy of biological control agents on targeted invasive alien plant populations. Recently, a new way to conceptualize success in biological control has been developed (Hoffmann et al. 2019). The level of success is based on the change in the trajectory of the invasion process due to biological control interventions (Hoffmann et al. 2019; Hill et al. 2020). Using this recently developed framework to estimate biological control success, if an invasive alien plant is reduced and maintained at a level below a 'tolerable threshold' where the plant is no longer considered a problem it is regarded as category A level of success, which is the equivalent of complete control (Fig. 1.1) (Hoffmann et al. 2019). Category B success is when the level of the invasion decreases to below a 'reversal threshold,' which means that the invasion has been reduced to below the point it had reach when the agent was released (Fig. 1.1) (Hoffmann et al. 2019). Category C is when invasive alien plant populations still increase after the release of the agent, but at a reduced rate and not to the same extent as if biological control had not been implemented (Fig. 1.1) (Hoffmann et al. 2019). The parameters used to evaluate which category a target weed should be assigned to are 1) weed density, 2) area occupied, 3) biomass of the weed, and 4) rate of spread (Hoffmann et al. 2019; Moran et al. 2021). In South Africa, thirty-nine of the 54 plants for which biological control has been implemented long enough to determine success have been reduced below the reversal threshold, and fifteen have been reduced below the tolerable threshold (Moran et al. 2021).

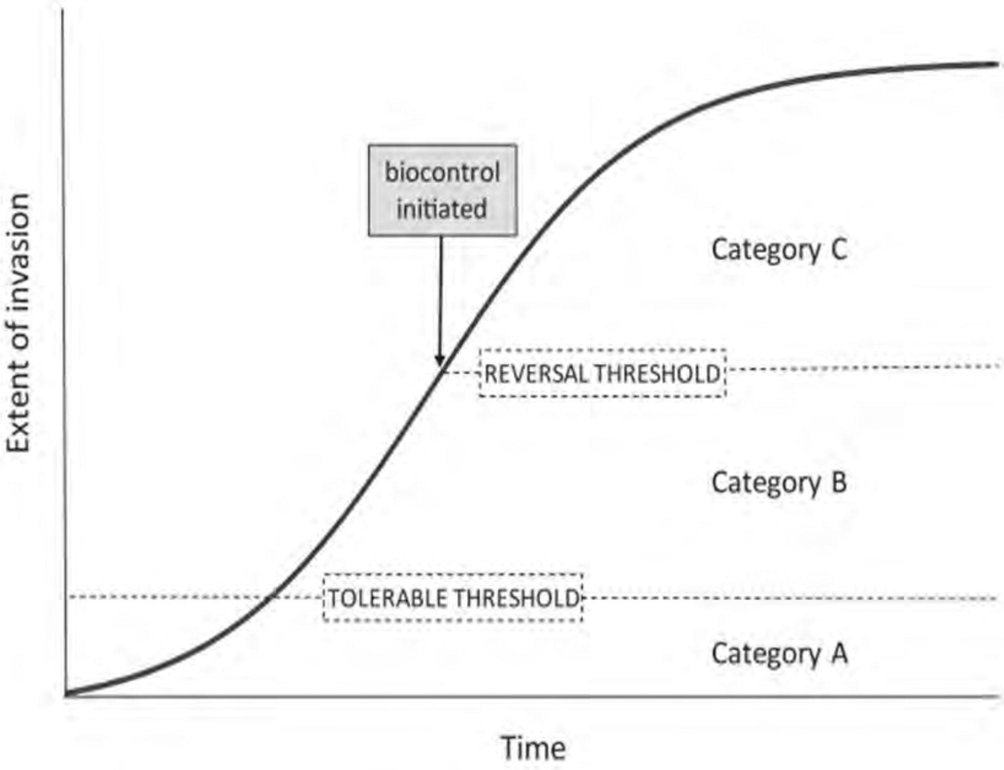

Figure 1.1 Diagrammatic representations of the outcomes of biological control categories at any given stage of an alien plant invasion. The sigmoid curve represents the hypothetical (albeit sometimes known or measured) extent of invasion (from Hoffmann et al. 2019).

1.3. Cactaceae species in South Africa

The Cactaceae family is one of the most diverse plant families, comprised of 130 genera and 1922 species, the vast majority of these species being indigenous to North, Central, and South America (Novoa et al. 2015a; 2019). Of the 1922 cactus species, 57 have become naturalized outside their native ranges and pose threats worldwide, particularly in arid and semi-arid rangelands (Novoa et al. 2015a; 2019). Cactus species are among the most dominant groups of invasive alien plants in South Africa (van Wilgen et al. 2012b; Novoa et al. 2015a). This is due to the country's arid climate favouring the establishment and growth of drought-tolerant plants, such as cactus species (van Wilgen et al. 2012b; Novoa et al. 2015a). Cactaceae were among the first alien plants introduced in South Africa (Walters et al. 2011) and since these first introductions, about 300 cactus species have been introduced to the country, primarily for ornamental purposes (Novoa et al. 2017a; 2019). Thirty-five cactus plant species are listed as Invasive Alien Species under the National Environmental Management: Biodiversity Act No. 10 of 2004 (NEM: BA) (DEA 2014; Kaplan et al. 2017).

These introduced cactus species have a significant negative impact on indigenous biodiversity (Novoa et al. 2015a). Invasive alien cacti trap and injure wildlife and livestock, reduce the carrying capacity of rangelands, reduce the value of land for agricultural and recreational activities, and restrict the access of animals to water sources and shade (Novoa et al. 2015a). Some cactus species have positive impacts as they generate revenue through the horticulture trade and provide fruits for human consumption and fodder for livestock (Novoa et al. 2016). For most individual cactus species and the plant family as a whole, the negative consequences outweigh the benefits, and thus the majority of cactus species should be controlled to reduce the associated negative impacts (Shackleton et al. 2017a).

1.3.1. Biological control of Cactaceae species

The biological control of cactus species in South Africa dates back to 1913 (Moran et al. 2013; Zimmermann et al. 2004). The first biological control programme against any plant in South Africa started with the release of the cochineal insect, *Dactylopius ceylonicus* (Green) (Dactylopiidae), to control the cactus weed *Opuntia monacantha* Haworth (Cactaceae)

(Zimmermann et al. 2004a). *Opuntia monacantha* was problematic to agriculture, particularly grazing, in the Eastern Cape Province (Zimmermann et al. 2004a). *Dactylopius ceylonicus* was very effective in reducing the density of *O. monacantha* and achieved a level of control where the weed is no longer a significant problem in just a few years after *D. ceylonicus* was released (Zimmermann et al. 2009; Paterson et al. 2011b). Since the successful biological control of *O. monacantha,* 14 other cochineals (species and lineages/biotypes) have been released in South Africa against 20 invasive alien cactus plants, all of which have been at least partially successful (Paterson et al. 2011b; Moran et al. 2021). Biological control of cactus species has resulted in complete control of 12 of these species under category A (A, A+, or A-) for all invasion-related parameters that were assessed. In contrast, the remaining cacti had lower levels of success (Moran et al. 2021). Biological control outcomes are categorized as A, B and C, most favourable outcomes are indicated by plus and the less favourable by minus symbol (Moran et al. 2021). This rate of success of control of Cactaceae is higher than that achieved for other plant families, not only in South Africa but also globally (Schwarzländer et al. 2018; Moran et al. 2021). One of the reasons contributing to this high level of success is that cactus species are only endemic to the Americas, except for one species *Rhipsalis baccifera* (J. Miller) Stern (Cactaceae), that is considered indigenous in Africa and Asia (Paterson et al. 2011b). This has permitted the use of oligophagous biological control agents, such as the cactus moth, *Cactoblastis cactorum* (Berg) (Pyralidae), and the mealybug *Hypogeococcus* sp. (Pseudococcidae), without posing a risk to non-target species (Paterson et al. 2011b). The close relationship between *Dactylopius* and cacti in the tribe Opuntioideae is also an important factor contributing to the high success rate. The cochineal insects in the genus *Dactylopius* are adapted to the unique architecture and growth form of the *Opuntia* cacti, and are usually host specific and damaging agents (Paterson et al. 2019).

1.3.2. Mass rearing

Many biological control agents released against invasive alien cacti in South Africa effectively reduce the density, area, biomass, and number of propagules produced by their target weeds. However, the agents for cacti are generally poor dispersers that do not spread easily to sites where they have not been released. This has resulted in some cactus weeds continuing to be problematic and even having increasing negative impacts, despite the existence of effective biological control agents (Henderson and Wilson 2017). Mass-rearing and releasing biological control agents can help increase the distribution of the agents and achieve widespread control on a national scale by ensuring that the agent is present wherever the target invasive alien plant is problematic. Mass-rearing and releasing not only increases the distribution of the agents but, in some cases, can augment agent populations in the field and therefore increase the damage inflicted and improve control levels (Zachariades et al. 2017). Mass-rearing is essential to produce enough agents to augment populations in the field to practice augmentative biological control, rather than relying on classical biological control only (Zachariades et al. 2017).

The mass-rearing of five biological control agents of cacti is conducted by the Centre for Biological Control (CBC) at a mass-rearing facility situated in Kariega, Eastern Cape Province. Each target invasive alien plant has a specific biological control agent, including species and lineages of cochineals (*Dactylopius* spp.) and the galling mealybug *Hypogeococcus* sp. (Table 1.1.). The CBC facility has been operational for five years and has produced and released large numbers of biological control agents for invasive alien cactus plants all over South

Africa. These biological control agents are available free of charge on request for land-users such as farmers, landowners, and conservationists. A team of eight people is responsible for mass-rearing, releasing, and monitoring the success of these biological control agents on target invasive alien cacti. The activities of the CBC Cactus Mass-Rearing Facility are primarily funded by WfW: NRM: DFFE.

Table 1.1 Biological control agents mass-reared, released and evaluated by the Centre for Biological Control Kariega Facility and their targeted invasive alien plants

Biological control agent	**Invasive alien plants (Target weed)**
Dactylopius ceylonicus (Green) (Dactylopiidae)	*Opuntia monacantha* Haworth
Dactylopius opuntiae "stricta" Cockerell (Dactylopiidae)	*Opuntia stricta* (Haworth) Haworth and *Opuntia cespitosa* Raf.
Dactylopius tomentosus "imbricata" (Lamark) (Dactylopiidae)	*Cylindropuntia imbricata* Haworth F.M. Knuth and *Cylindropuntia leptocaulis* (DC.) F.M. Knuth
Dactylopius austrinus De Lotto (Dactylopiidae)	*Opuntia aurantiaca* Lindley
Hypogeococcus sp. (Pseudococcidae)	*Harrisia martinii* (Labouret) Britton, *Harrisia pomanensis* (F.A.C. Weber ex K. Schum.) Britton & Rose, *Harrisia balansae* (K. Schum.) N.P. Taylor & Zappi and *Cereus jamacaru* De Candolle

1.3.3. *Dactylopius* (Dactylopiidae) biological control agents and their target cacti in South Africa

Dactylopius species are native to the Americas and have been introduced around the world for the production of dye (Lounsbury 1915), accidentally as agricultural pests of cactus crops (Moussa et al. 2017) and as biological control agents to control pest cactus species (Walton 2005). They are commonly known as cochineal insects and are sap-sacking insects that are host specific to plants belonging to the Cactaceae family (Klein 2002a). Eleven different cochineal species have been described, and each species is restricted in its host range to a single, or a few closely related, species of Cactaceae (Garcia Morales et al. 2016). The feeding damage of cochineal insects results in dry cactus cladodes (i.e. the pads of the cacti) that cannot root and form new plants (Klein 2002a) and results in a reduction in the quantity of fruit produced in some cactus species (Hoffmann et al. 2020). Vegetative reproduction from loose cladodes is the primary form of reproduction of many pest cactus species, so reducing the ability of cacti to spread through both a reduction in fruit and therefore seed, as well as a reduction in vegetative reproduction, can result in declines in cactus population densities (Hoffmann et al. 2020; Klein et al. 2020).

The host range of each cochineal species is restricted to a group of closely related cactus host plant species, and in some cases, intra-specific lineages of cochineal insects (often referred to as biotypes) are damaging to only a single or small group of closely related cactus species (Jones et al. 2015, 2016). Consequently, it is crucial to identify the correct cochineal lineage for each targeted weed (Jones et al., 2015, 2016). For example, the agent for *C. imbricata* and *C. fulgida* is the same species of cochineal, *Dactylopius tomentosus* Lamark (Dactylopiidae), but only the “imbricata” lineage of this cochineal species will provide complete control of *C. imbricata* and only the “cholla” lineage will provide complete control of *C. fulgida* (Paterson et al. 2011b; Klein et al. 2020). Another example is the inability of *Dactylopius opuntiae* “ficus” (Cockerell) (Dactylopiidae) to control *O. stricta* in Kruger National Park (KNP) (Lotter & Hoffmann, 1998; Volchansky et al. 1999). Only after the release of the correct lineage, *D. opuntiae* “*stricta*” for *O. stricta*, in the mid-1990s, was the invasive alien plant completely controlled (Hoffmann et al. 1999; 2020).

Cochineal insects are always gregarious and form clumps or clusters of individuals that live on the surface of the cactus plants (Klein 2002a). Adult females and adult males are morphologically different (Gunn 1979). Adult females are about the size of a match head and are not easily seen because they exude a white woolly wax that covers the organism's whole body (de Lotto 1974). The covering protects the female adults against predation and extreme weather conditions (Klein 2002a). Their bodies are filled with a dark red fluid that is high in carminic acid. This liquid has been used to produce red dye for many years (Eisner et al. 1980). The red dye is found in all cochineal insects, but the highest dye quantity is found in *Dactylopius coccus* Costa (Dactylopiidae), which is a domesticated cochineal species and has been used for dye production for thousands of years in Central America (Nejad and Nejad 2013). Female cochineal insects lay about a thousand eggs each, which hatch and emerge as tiny pink nymphs called crawlers. The crawlers are the most crucial stage for the dispersal of cochineal insects as they are mobile and carried by wind (Klein 2002a). On the other hand, adult males are not often encountered because they are short-lived and avoid attention or being notice (Klein and Zimmermann 2020). They are tiny pink, mobile insects with two semitransparent wings and long “tail” filaments (de Lotto 1974). Male cochineal insects can disperse as adults or crawlers, but they are very poor fliers as adults and will have very limited dispersal at this stage compared to the crawlers, usually remaining on the same individual plant (Klein 2002a). They have fewer and shorter bristles compared to female cochineal adults but can also be dispersed by wind (Klein and Zimmermann 2020).

1.3.4. *Opuntia monacantha*

Opuntia monacantha, commonly known as drooping prickly pear, is a shrub or a small tree that grows up to 2 m in height, occasionally with a short trunk (Fig. 1.2) (Klein and Zimmermann 2020). Its flattened cladodes are glossy green, narrowly obovate to oblong-lanceolate, and areoles (small raised or sunken cushions, often bearing a group of spines, glochids, flower, or shoots) have one to three grey or yellowish to reddish-brown spines with darker tips (Klein and Zimmermann 2020). The flowers have bright yellow petals and a light scent; flowering occurs during daylight and lasts for about 10 hours, and the fruits are fleshy and reddish-purple when ripe (Lenzi and Orth 2012; Klein and Zimmermann 2020). The fruit is large, pear-shaped, green with a red-purple shade, thick skin, and edible pulp (Klein and Zimmermann 2020). The plant is native to the east coast of Brazil and northern Argentina and had already been introduced into South Africa by 1722 (Klein and Zimmermann 2020). It was planted to support populations of *D. coccus* used for the production of dye in South Africa prior to the development of synthetic dyes (Lounsbury 1915).

Opuntia monacantha is a declared environmental and noxious weed in South Africa and Australia (Navie and Adkins 2008; Klein 2011). The plant creates dense impenetrable thickets, making it difficult for livestock to move around grazing lands, displaces native biodiversity, and blocks human and animals' movement in areas where it invades (Navie and Adkins 2008). The plant has spines and glochids (barbed hair or bristle produced in the areoles) that are harmful and cause skin irritation (Navie and Adkins 2008).

The first record of a cochineal insect controlling invasive alien plant populations took place in 1795 when *D. ceylonicus* was inadvertently released on *O. monacantha* in India (Lounsbury 1915). The cochineal insect was deliberately released in India in an attempt to start a dye industry for the British Empire and was therefore not intended for the control of *O. monacantha*, but the incorrect species of cochineal was released (Lounsbury 1915). Instead of releasing *D. coccus*, which produced large quantities of dye and is not very harmful to its host plant, *D. ceylonicus*, which produces far less dye and is very damaging to *O. monacantha*, was released. The damage caused by *D. ceylonicus* on *O. monacantha* in India attracted the attention of entomologists in Australia and South Africa, where *O. monacantha* was a severe agricultural pest, and the insect was imported into South Africa from India in 1913 (Lounsbury 1915). *Dactylopius ceylonicus* was then released in Australia in 1914, where it was also very effective at reducing *O. monacantha* populations (Lounsbury 1915; Walton 2005). Large and dense infestations of *O. monacantha* in South Africa were permanently reduced to just a few plants after the release of *D. ceylonicus* (Paterson et al. 2011b; Hill et al. 2020). *Opuntia monacantha* is a Category 1b invasive species, in terms of the National Environmental Management: Biodiversity Act No. 10 of 2004 (DEA 2014; Klein & Zimmermann, 2020). Category 1b are invasive species that are widely established and must be managed (Henderson 2001). *Opuntia monacantha* is considered under category A+ control in South Africa and is therefore controlled well below the 'tolerable threshold' (Moran et al. 2021) but at sites where the agent is not present, dense infestations still occur.

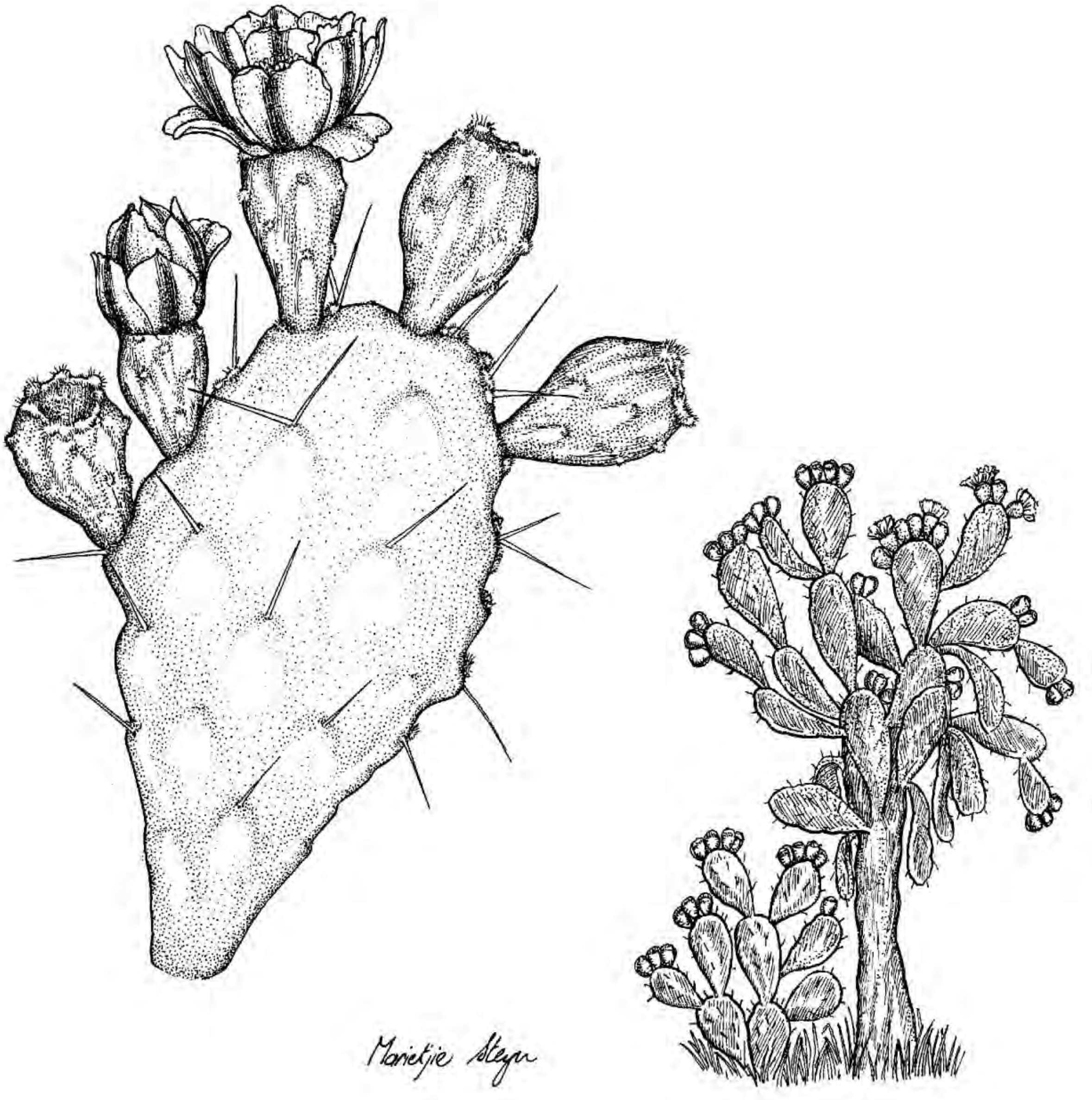

Figure 1.2 *Opuntia monacantha* (Drooping prickly pear) (Henderson L. 2001)

1.3.5. *Opuntia stricta*

Opuntia stricta, commonly known as Australian pest-pear, is native to the southeast USA, eastern Mexico, and some Caribbean Islands (Klein and Zimmermann 2020). Australian pest pear is a spiny, evergreen, succulent shrub with elongated blue-green cladodes and red-purple fruits (Fig. 1.3). This thicket-forming plant can grow up to a height of 2 m, cladodes are flattened and 10 – 20 cm long, and fruits are pear-shaped (Klein and Zimmermann

2020). The plant is found in all the provinces of South Africa, except the Western Cape and North West provinces, and it was primarily introduced to many areas as an ornamental plant or for hedging (Henderson 2001; Foxcroft et al. 2008). It was regarded as the most significant weed ever in Australia, but it is no longer a problem due to biological control, which achieved complete and permanent control (Raghu and Walton 2007). The negative impacts of *O. stricta* include reduced access by people and animals to conservation areas and rangelands, displacement of indigenous species, and injuries to people, livestock, and wild animals (Larsson 2004). It causes extreme discomfort to humans and livestock due to the plant's spines and glochids (Huxley 1992).

One of the most extensive infestations of *O. stricta* in South Africa was found in the Skukuza region of the KNP. It was first recorded in the 1950s and was estimated to occupy about 30 000 ha of the park (Hoffmann et al. 1998; Lotter et al. 1999). In the national park, the seeds were dispersed by elephants; this increased the seed germination rate and has made it difficult to control the plant using mechanical and chemical methods (Lotter et al. 1999). After years of limited success with mechanical and herbicidal control, biological control was considered (Hoffmann et al. 1998). *Cactoblastis cactorum*, the phycitid moth, was the first biological control agent released in 1998 against *O. stricta* in KNP. The moth established, and its populations increased; however, long-term monitoring studies of insect populations and plant density showed that the damage inflicted by the agent on *O. stricta* was limited, and other control methods were required (Hoffmann et al. 1998; Zimmermann et al. 2004b).

The cochineal insect, *D. opuntiae* was the second biological control agent released on *O. stricta* in KNP because it had already been released for the control of *Opuntia ficus-indica*

(L.) Miller (Cactaceae) in South Africa and because it had been so successful at controlling *O. stricta* in Australia, but unfortunately, *D. opuntiae* did not establish on *O. stricta* after it was released in the KNP (Lotter and Hoffmann 1998; Hoffmann et al. 1999). It was later discovered that *D. opuntiae* comprised of two lineages, one for *O. ficus-indica* (*D. opuntiae* "ficus") and another for *O. stricta* (*D. opuntiae* "stricta") (Volchansky et al. 1999). *Dactylopius opuntiae* released in the KNP was not the correct one for *O. stricta;* hence it did not establish (Volchansky et al. 1999). A different lineage of *D. opuntiae* "stricta" from *O. stricta* in Australia was then released in KNP (Volchansky et al. 1999). This lineage established immediately after the release and began to damage patches of *O. stricta* in areas where releases were done (Foxcroft and Hoffmann 2000). *Dactylopius opuntiae* "stricta" reduced the number of cladodes of *O. stricta* effectively from 30 cladodes/m^2 to less than five cladodes/$m^{2,}$ and chemical and mechanical methods were no longer required (Foxcroft and Hoffmann 2003; Paterson et al. 2011b; Hoffmann et al. 2020). *Opuntia stricta* is under complete control in KNP, but biological control is less effective in the cooler parts of the country (Moran et al. 2021; Hoffmann et al. 2020). The biological control outcomes of *O. stricta* are categorized as A+ for all parameters in sub-tropical habitats, such as KNP (Moran et al. 2021). In contrast, the agent is less effective in temperate habitats, with B- for density and area assessments and B+ for reducing biomass and rate of spread (Moran et al. 2021). The National Environmental Management: Biodiversity Act No. 10 of 2004 has declared *O.stricta* a Category 1b invasive species in South Africa (DEA 2014; Klein & Zimmermann 2020).

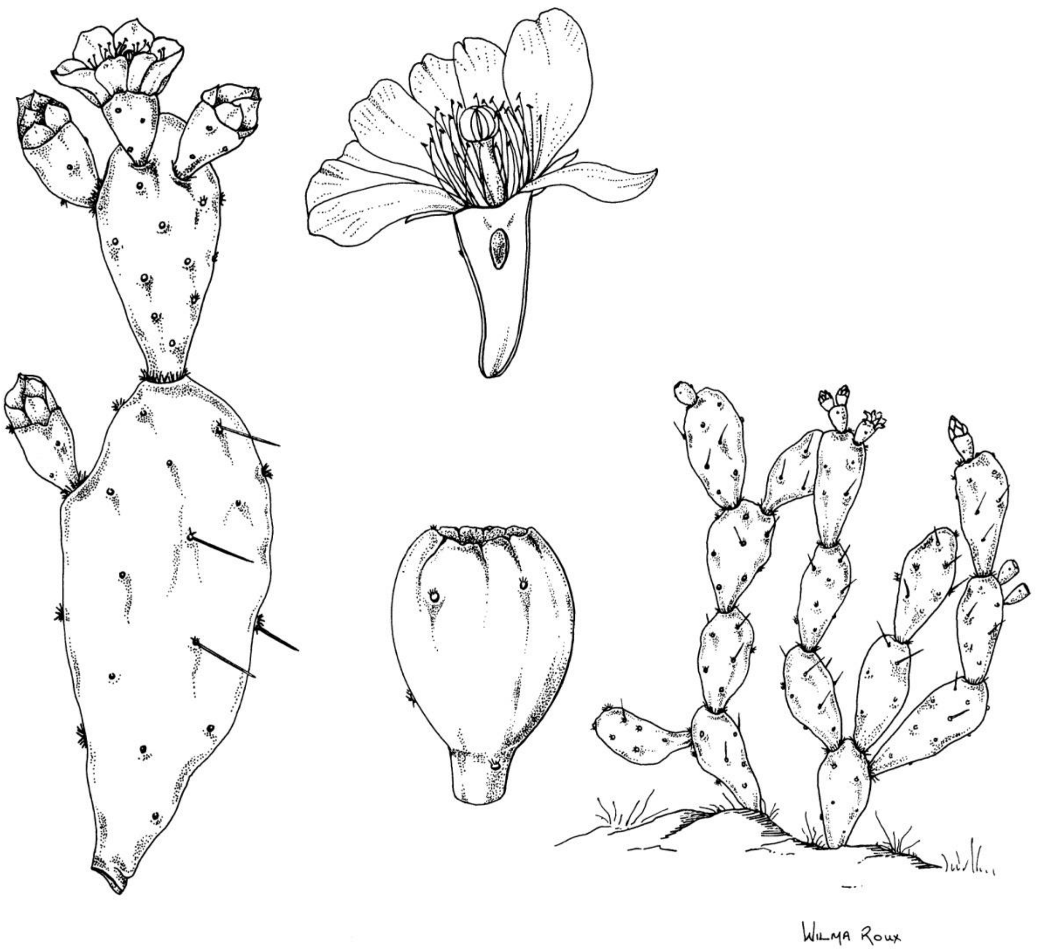

Figure 1.3 *Opuntia stricta* (Australian pest-pear) (Henderson L. 2001)

1.3.6. *Opuntia cespitosa*

Opuntia cespitosa is commonly known as creeping prickly pear and is indigenous to Central America and the southern-east parts of the USA (Klein and Zimmermann 2020). The plant is a prostrate prickly pear that does not grow taller than 30 cm from the ground (Fig. 1.4) (Klein and Zimmermann 2020). Its large yellow flowers appear from October to December; cladodes are pale green and flattened, fruits are sweet-tasting succulent red or purple berries (Henderson 2001; Klein and Zimmermann 2020). The plant was first recorded in South Africa near the border between Limpopo and Mpumalanga provinces (Henderson 1999). Originally introduced as an ornamental plant, it now occurs in all South African provinces and is problematic in dry grassland and savannah biomes (Henderson 2001; Rule and Hoffmann 2018). *Opuntia cespitosa* forms dense impenetrable monocultures that exclude all other plants.

No formal biological control programme was conducted against *O. cespitosa* in South Africa, but the history of the plant in Australia suggested that biological control could be effective (Rule and Hoffmann 2018). The biological control agent for *O. stricta*, *D. opuntiae* '*stricta'*, was known to be damaging to *O. cespitosa* in Australia and was therefore also utilised for control in South Africa (Rule and Hoffmann 2018). Although there are reports that *D. opuntiae* inflicts extensive damage on *O. cespitosa*, there are also suggestions that the agent damages *O. cespitosa* less than it does *O. stricta* (Rule and Hoffmann 2018). The findings of a laboratory study conducted to evaluate the development of *D. opuntiae* on *O. stricta* and *O. cespitosa* indicated that both plants are suitable hosts for *D. opuntiae,* and the agent damages *O. cespitosa* efficiently, but no post-release evaluations have been conducted in South Africa to confirm this in the field (Rule and Hoffmann 2018). The plant is officially listed as a Category 1b invasive plant species under the National Environmental Management Act, No. 10 of 2004 (DEA, 2014). *Opuntia cespitosa* is considered under category A+ control for all habitats and parameters in South Africa and is therefore controlled well below the 'tolerable threshold' (Moran et al. 2021) but there are dense infestations of the plant in areas without the biological control agent.

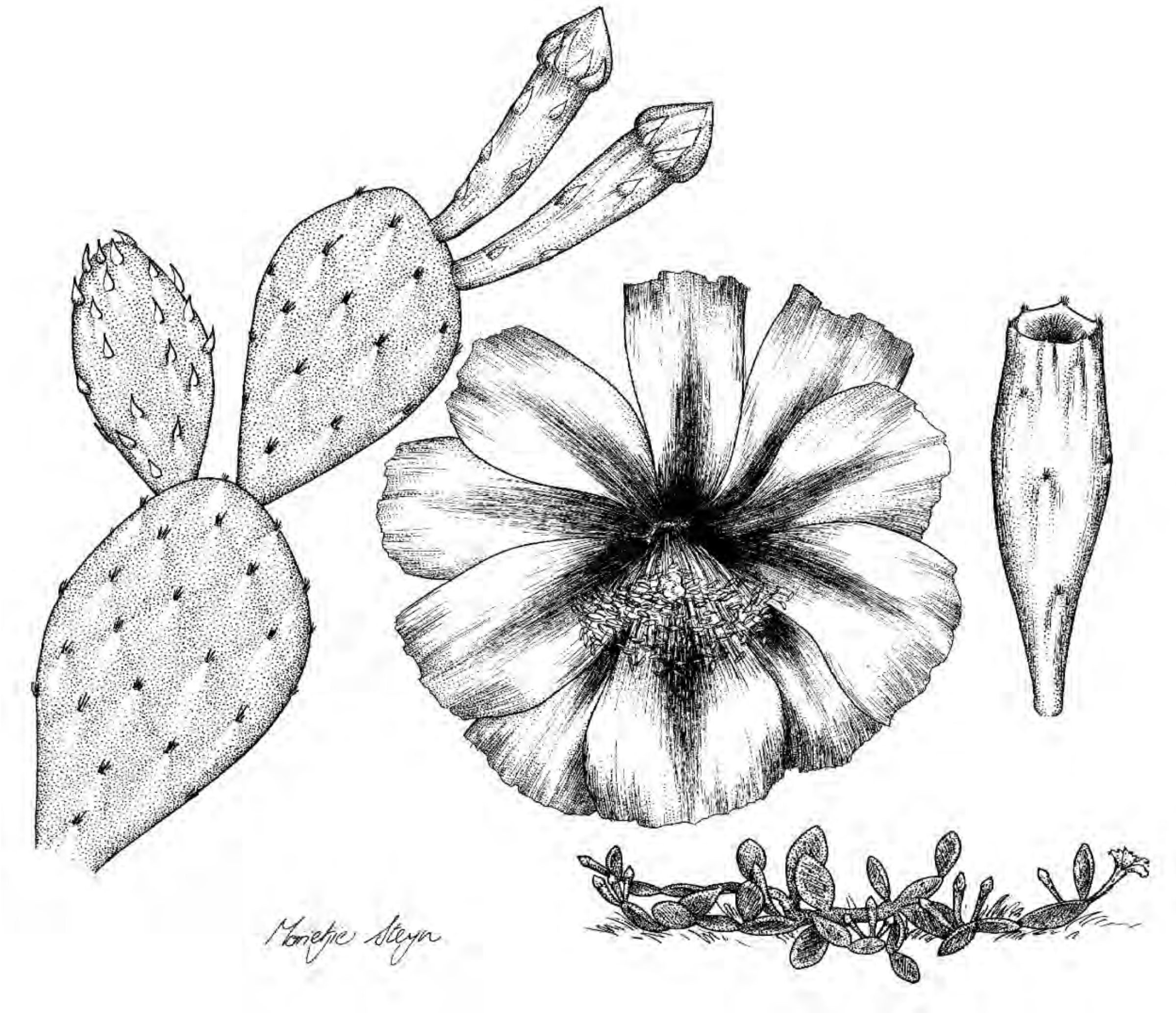

Figure 1.4 *Opuntia cespitosa* (Creeping prickly-pear) (Henderson L. 2001)

1.3.7. *Cylindropuntia imbricata* and *C. leptocaulis*

All *Cylindropuntia* spp. that are naturalised in Australia and South Africa, including *C. imbricata* and *C. leptocaulis*, cause environmental, agricultural, and recreational problems. The plants pose a threat to biodiversity by creating monospecific patches and displacing native plant species. The spines are injurious to native animals and prevent grazing activities in rangelands (Chuk and Waters 2010). The plants spread through vegetative reproduction as detached cladodes attached to animals, humans, and vehicles and are dispersed to other areas. When they contact the ground, they are likely to root and form new plants resulting in new infestations (Chuk and Waters 2010). Chemical and mechanical control methods have been implemented to control several *Cylindropuntia* sp. in Australia but have not been particularly effective (Jones et al. 2015). Chemical control is expensive over large areas and cannot be applied in remote and inaccessible locations. Mechanical control is not safe because the plant's spines are injurious when they come in contact with the skin (Jones et al. 2015). The biological control agent, *D. tomentosus* "imbricata," was released for the control of *C. imbricata* in Australia in 1925, and this was followed by South Africa releasing the agent in 1970 (Dodd, 1940; Moran and Zimmermann, 1991a). Several years after the agent was released on *C. imbricata* in South Africa, it was utilised for the control of the closely related *Cylindropuntia leptocaulis*, which was also found to be damaged by the same agent (Moran and Zimmermann 1991a).

Cylindropuntia imbricata, commonly known as imbricate cactus or Devil's rope cactus, is native to the south-western USA and northern Mexico (Henderson 2001; Klein and Zimmermann 2020). The plant was introduced as an ornamental plant in South Africa, and it now occurs in every province of the country (Henderson 2001). Imbricate cactus is an open, erect, branched succulent that grows up to 4 m (Fig. 1.5) (de Beer 1986). It has purple-red flowers that come into bloom from November to January, and the flowering season is followed by the appearance of yellow succulent egg-shaped fruits (de Beer 1986; Klein and Zimmermann 2020). In South Africa, the imbricated cactus has been declared a Category 1b invasive species by the National Environmental Management: Biodiversity Act No. 10 of 2004 (DEA, 2014; Klein & Zimmermann, 2020). It is considered to be under category B control for all habitats and parameters in South Africa and has therefore been reduced by biological control to below the ‘reversal threshold’ (Moran et al. 2021).

Cylindropuntia leptocaulis, commonly known as pencil cactus, native to Mexico and southern USA, now occurs in the Eastern Cape Province of South Africa (Moran and Zimmermann 1991a; Klein, 2011; Klein and Zimmermann 2020). Pencil cactus is an erect shrub that is usually not more than 1.5 m in height but it has the potential to grow up to 2.8 m in shaded areas under trees (Earle 1990; Klein and Zimmermann 2020). The plant has slender pencil-like spiny joints that are easily dislodged from the main plant resulting in vegetative reproduction and dispersal (Weniger 1991; Walker 2012; Klein and Zimmermann 2020). The flowers appear in May to July depending on climatic and environmental condictions and are greenish-yellow, occasionally with reddish tips (Vines 1960; Weniger 1991; Klein and Zimmermann 2020). The fruits are small, bright red, smooth and lack spines, contain brown glochids in the areoles and usually have less than 12 seeds per fruit (Earle 1990; Walker 2012; Klein and Zimmermann 2020). In South Africa, pencil cactus has been declared a Category 1b invasive species by the National Environmental Management: Biodiversity Act No. 10 of 2004 (DEA, 2014; Klein & Zimmermann, 2020). *Cylindropuntia leptocaulis* is regarded as being under complete control and is allocated to category A+ control for all habitats and parameters in South Africa, and is therefore controlled to below the ‘tolerable threshold’ (Moran et al. 2021).

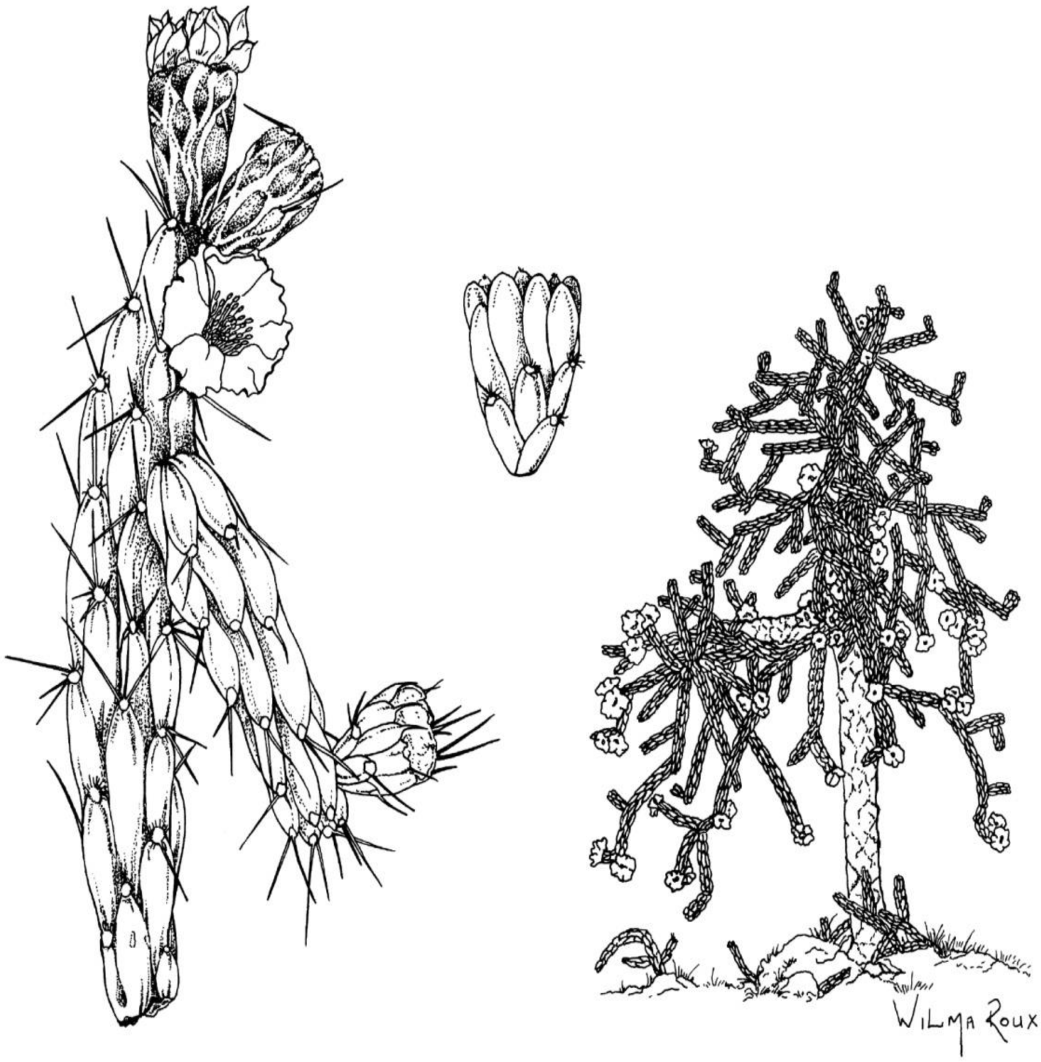

Figure 1.5 *Cylindropuntia imbricata* (Devil's rope cactus) (Henderson L. 2001)

1. 3.8. *Opuntia aurantiaca*

Opuntia aurantiaca is commonly known as jointed cactus and is a perennial, succulent, shrub-like plant (Figure 1.6). The plant usually grows no higher than 0.3 m but can reach a height of 2 m when supported by surrounding vegetation and growing under shady conditions (Figure 1.7) (Klein and Zimmermann 2020). A jointed cactus plant may be made up of over 100 cladodes covered by long barbed spines (Figure 1.6) (Hoffmann 1976; Zimmermann and van de Venter 1981). The plant has bright yellow flowers, not orange, as suggested by the specific epithet name ‘aurantiaca,’ and they emerge between November and January (Zimmermann and van de Venter 1981; Zimmermann 1981; Nieman 1983). Fruits develop into reddish club-shaped, unpalatable fruits that contain numerous seeds (Zimmermann and van de Venter 1981; Nieman 1983). The seeds of jointed cactus are infertile, so the plant reproduces only by vegetative means in South Africa (Gunn 1979; Zimmermann 1981). Every detached cladode can form roots under favourable conditions, resulting in new infestations (Hoffmann 1976; Annecke and Moran 1978; Zimmermann and van de Venter 1981; Robertson 1985). The detached cladodes are easily dispersed by floodwaters and attached to animals, car tyres, and footwear (Figure 1.7) (Hoffmann 1976; Zimmermann and van de Venter 1981). *Opuntia aurantiaca* is native to Paraguay, Uruguay, and Argentina in South America (Moran et al. 1976; Moran and Zimmermann 1991b). It is believed that jointed cactus was first brought to South Africa from England in 1843 as a collector’s item and was recorded by McGibbon at a botanical garden in Cape Town in 1858 (McGibbon 1858).

Jointed cactus has invaded approximately 1.9 million hectares of grazing lands in South Africa, reducing the carrying capacity of rangelands and having serious economic impacts (Moran and Zimmermann 1991b; Richardson and van Wilgen 2004). The plant is harmful to livestock; the sharp spines pierce the skin and cause sores. It decreases the quality of wool and mohair when it becomes attached to livestock (Hoffmann 1976; Hosking 1984a; Zimmermann 1981; Zimmermann and van de Venter 1981). The plant threatens native biodiversity because it competes with and replaces indigenous species (van Wilgen et al. 2004).

The cochineal insect, *D. austrinus*, is native to South America and is the most important and effective biological control agent released against jointed cactus in South Africa (Gunn 1979; Moran & Zimmermann 1991b; Zimmermann 1981). It is host-specific and feeds only on *O. aurantiaca* in South Africa, although it has other closely related host plants in the native distribution (Gunn 1979; Zimmermann 1979). The agent sucks the sap from the cladode, leaving it dry and unable to root and form new plants (Zimmermann 1981). When infected with the cochineal, plants fall apart into loose cladodes, but these cladodes cannot root and will slowly die, thus halting the plant's spread and reproduction because it reproduces only vegetatively (Zimmermann 1981).

Dactylopius austrinus was released against *O. aurantiaca* in South Africa in 1935 (Zimmermann 1981; Klein 2011). Dense infestations of jointed cactus over large areas were significantly reduced within 12 to 18 months, such that the use of chemical and mechanical control methods was stopped (Zimmermann 1981). The agent was actively redistributed all over the Eastern Cape Province and the Karoo region from 1938 to 1946 (Zimmermann 1981). After the dramatic decline following the release of the agent, there was a resurgence, probably due to the natural boom-bust cycle between the agent and the plant, which is mediated by changes in rainfall and temperatures (Zimmermann 1981). The regrowth caused Departmental of Agriculture officials to panic, and it was generally believed that the biological control had become ineffective. This resulted in the re-initiation of the use of mechanical control from 1947 to 1957 (Zimmermann 1981). Chemical control using herbicide was introduced in 1957 to improve the level of control of jointed cactus, which continued into the 1980s (Zimmermann 1981). Herbicide applied to *O. aurantiaca* does not kill below-ground tubers, making it challenging to control jointed cactus using chemical control (Whiting and Campbell 1984). In addition to this, both chemical and mechanical management are impractical because small detached joints are not easily detected in the field and are often overlooked (Moran and Zimmermann 1991b). Both mechanical and chemical control were ultimately ineffective against jointed cactus and biological control was made the only option for control, populations of the weed were maintained at low levels with fluctuations depending on the wet-dry cycles (Moran & Zimmermann, 1991b).

In terms of environmental legislation, jointed cactus is a declared Category 1b invasive species in South Africa by the National Environmental Management: Biodiversity Act 10 of 2004 (NEM: BA) (DEA 2014). The categorization of biological control outcomes classifies *O. aurantiaca* under category A+ for density and biomass and category A for area and rate of spread in dry, inland habitats (Moran et al. 2021). For coastal habitats, which are generally cooler and wetter, it is categorised for density as B+, for biomass as B, and as category C for the rate of spread and area (Moran et al. 2021).

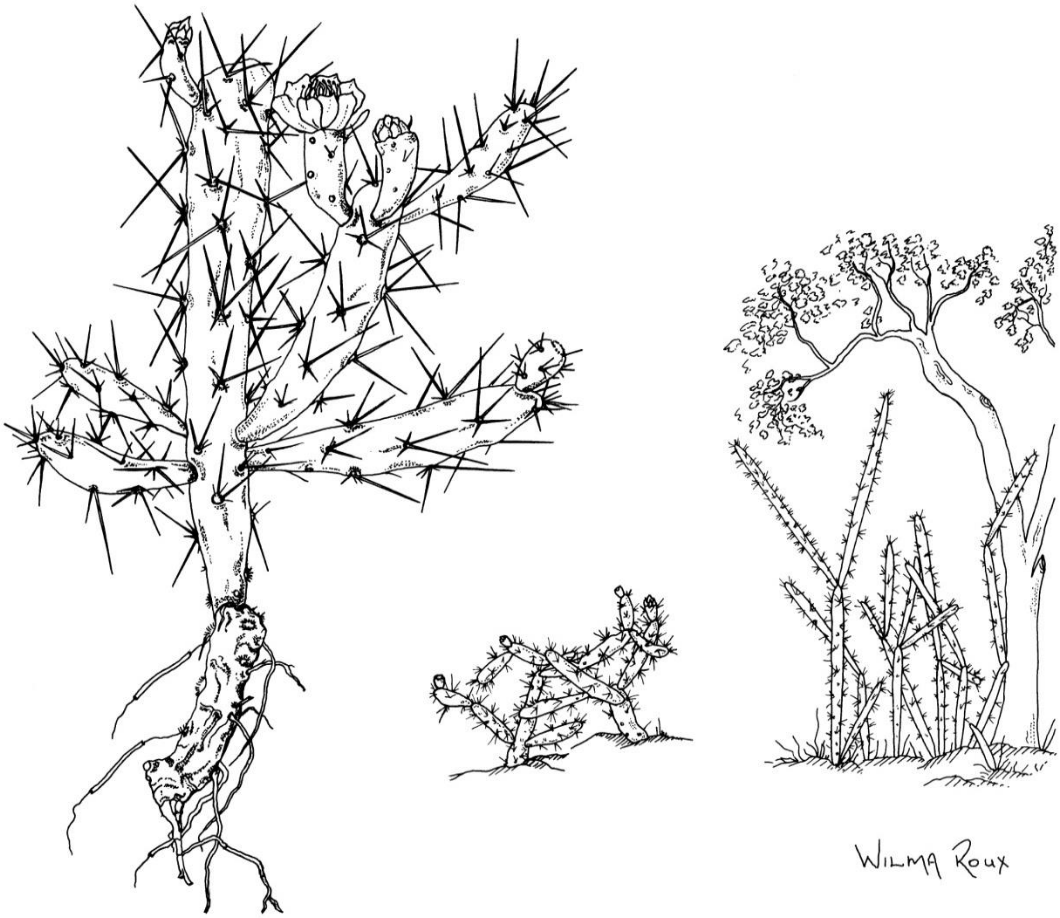

Figure 1.6 *Opuntia aurantiaca* (Jointed cactus) (Henderson L. 2001)

Figure 1.7 Detached cladode (circled) attached to an animal (Zimmermann and van Venter 1981)

1.3.9. *Hypogeococcus* sp. targets

Hypogeococcus sp., commonly known as the cactus mealybug, is a gall-forming mealybug initially released as a biological control agent for *H. martinii* but was later also found to be effective on *H. pomanensis*, *H. balansae,* and *C. jamacaru* in South Africa (Klein and Zimmermann 2020). Each of these plant species is briefly discussed below. *Hypogeococcus* sp. occurs naturally from Argentina to Paraguay and was first introduced in Australia from Argentina in 1975 for the control of *Harrisia* cactus (Tomley and McFadyen 1984). It was then released in South Africa on *Harrisia* cactus in 1983 by researchers from a culture that was received from Australia (Klein 2002b; Klein and Zimmermann 2020). The mealybug is a stem sucker and galler that targets the stem tips of the cactus in particular, and its feeding damage stunts growth and deforms and distorts the plant (Klein 1999; Klein and Zimmermann 2020). The agent has been established well at several South African sites and is a very effective biological control agent that has reduced its host plant densities (Klein 1999; Klein and Zimmermann 2020).

Hypogeococcus sp. spreads naturally by wind in a very similar way to cochineal insects (Klein 2002b; Klein and Zimmermann 2020). The newly emerged nymphs, known as crawlers because they have functional legs, will crawl up to a point where they can be blown away by the wind to other host plants (Klein 2002b; Klein and Zimmermann 2020). When they land on a host, they move to the stem tip or between the buds and feed by piercing the stem to suck the sap (Klein 2002b; Klein and Zimmermann 2020). Male nymphs move to a more exposed plant part before they undergo pupation, and when they emerge as winged adults, they fly away to find a female (Klein 2002b; Klein and Zimmermann 2020). Female nymphs stay on one plant part and wait to be fertilized by the male once they become apterous adults (Klein 2002b; Klein and Zimmermann 2020).

Harrisia martinii is commonly known as moon cactus (Klein and Zimmermann 2020). This plant's common name is derived from the white flowers that appear at night during summer from November to January (Henderson 2001). It is native to the Chaco Province of Argentina and Paraguay (South America) and was possibly introduced into South Africa and Australia as an ornamental plant (Tomley and McFadyen 1985; Henderson 2001; Klein and Zimmermann 2020). It is an unwanted plant in South Africa and Australia that is challenging to control using chemical and physical removal (Julien et al. 2012). It was regarded as of minor importance and believed to have a restricted distribution in South Africa (Moran & Zimmermann 1991a), but its distribution has expanded more recently. It is now an extensive problem, mainly in Savanna areas, and occurs in the Western Cape, Eastern Cape, Free State, KwaZulu-Natal, Limpopo, and Gauteng provinces. The plant is a spiny succulent shrub with climbing multi-branched stems, which grows to a height of 3 m when not supported by surrounding vegetation (Fig. 1.8) (Henderson 2001; Klein and Zimmermann 2020). Fruits are bright red, spherical with white spines, and contain black seeds that are the main means of reproduction (Klein and Zimmermann 2020). Stems usually arch downwards, take root, and form new plants when they contact the ground (Henderson 2001; Klein and Zimmermann 2020).

In Australia, *H. martinii* is under substantial control due to the efficacy of *Hypogeococcus* sp., which reduced dense, impenetrable infestations of the weed within four years of release (Houston and Elder 2019). The damage from the mealybug reduces the plant's ability to flower and fruit, and when in high enough densities, it causes plants to die (Houston and Elder 2019). In South Africa, *H. martinii* is also considered under complete control where the agent is present (Klein 2011). The biological control agent must however be released in areas with new infestations and where it has not been released in the past as the agent is a poor disperser. *Harrisia martinii* is a declared Category 1b invasive species in South Africa, according to the National Environmental Management: Biodiversity Act No. 10 of 2004 (DEA, 2014; Klein & Zimmermann, 2020). The plant is categorized under category A+ of biological control outcomes in South Africa for all habitats and parameters and is therefore below the 'tolerable threshold' (Moran et al. 2021); however, in areas without the agent, there may be dense infestations of *H. martinii*.

Figure 1.8 *Harrisia martinii* (Moon cactus) (Henderson L. 2001)

Harrisia pomanensis, commonly known as the Midnight lady, comes from Bolivia, Paraguay, and Argentina (Hart 2005; Franck 2016; Klein and Zimmermann 2020). It is an evergreen plant with spiny, fleshy-jointed stems (Hart 2005). The long, thin stems, with four to seven rounded ribs, support clusters of spines 1 to 2 cm long (Hart 2005). The flowering season occurs from November to February, when the plant produces white, funnel-form flowers that open during the night and are about 18 cm long and 10 cm in diameter (Hart 2005;

Klein and Zimmermann 2020). The fruits are spineless, spherical, red, and fleshy, containing numerous black seeds (Hart 2005; Franck 2016; Klein and Zimmermann 2020). *Harrisia pomanensis* is a declared Category 1a invasive species in South Africa under the National Environmental Management: Biodiversity Act No. 10 of 2004 (Wilson et al. 2013; Klein and Zimmermann 2020). The damage inflicted by *Hypogeococcus* sp. to *H. pomanensis* is moderate, and the control status is not determined as it has only recently been targeted for biological control (Klein 2011). Until very recently, this plant was considered a possible eradication target, but with the discovery of infestations in four provinces (Eastern Cape, Western Cape, Northern Cape and Limpopo), it is now considered too widespread for eradication and should be managed with other means as complete eradication is not feasible.

Harrisia balansae, commonly known as the strangler prickly apple, is native to Argentina and has been recorded in the North-west, Limpopo, and Eastern Cape provinces of South Africa (Klein 2011). The plant is a climbing succulent shrub with a conspicuously woody trunk and is about 1-6 m tall; it is comprised of spiny cylindrical stems, spines are clustered together with one spine longer than all other several spines (Oakley and Kiesling 2013; Walters et al. 2011). The flowers of the plant are nocturnal, large, tubular, and spineless, white to pale yellow (Klein and Zimmermann 2020). The fruits are bright red, covered with wart-like knobs, and contain viable seeds, which are the primary mode of dispersal (Walters et al. 2011; Klein and Zimmermann 2020). The plant is under Category 1a of invasive species in South Africa under the National Environmental Management: Biodiversity Act No. 10 of 2004 (Wilson et al. 2013; Klein and Zimmermann 2020). Category 1a invasive species are plants with limited distributions that pose significant threats and must be eradicated. It is still too early to evaluate the success of the biological control programme against *H. balansae* as its biological control agent, *Hypogeococcus* sp. was only released very recently.

Cereus jamacaru is a large, tree-like, cylindrical cactus of 3 m to 15 m in height (Fig. 1.9) (Anderson 2001; Klein and Zimmermann 2020). The plant has a short, woody trunk and many succulent stems (Britton and Rose 1919). The flowers are long, funnel-shaped, white in colour, and appear at night from November to January, giving the plant its common name, 'Queen of the Night' cactus (Anderson 2001; Klein and Zimmermann 2020). The fruit is large, red to pink, with edible white flesh, containing numerous small, black seeds (de Beer 1986; Klein and Zimmermann 2020). It is native to South America and was brought to South Africa by succulent collectors as an ornamental plant and for security hedges (de Beer 1986; Klein and Zimmermann 2020). Populations of *C. jamacaru* were primarily limited to the hot and drier areas of the country in the 1980s (de Beer 1986), but the plant has now been recorded in all nine provinces of South Africa, with large infestations found in Gauteng and Northern Cape provinces by the 1990 (Klein 1999), and large infestations in KwaZulu-Natal Province in the 2000s (Paterson et al. 2011b). One of the most extensive infestations recorded in South Africa occupied over 300 ha and about 40 000 plants per hectare on a farm in the Moloto-Witnek area (25.28 S; 28.37 E) about 100 km north of Pretoria (Taylor and Walker 1984). *Cereus jamacaru* is a Category 1b invasive species (National Environmental Management: Biodiversity Act No. 10 of 2004 (DEA 2014; Klein & Zimmermann 2020). The first quantitative post-release evaluation of this cactus weed was conducted in 2016 and showed that the release of *Hypogeococcus* sp. successfully controls *C. jamacaru* (Sutton et al. 2018). The agent results in a dramatic reduction of fruit production, mortality of small plants, and the complete control of infestations within eight to 15 years after release (Sutton et al. 2018). *Cereus jamacaru* is considered under category A- for density, biomass, and area, and A+ for the rate of spread for all habitats (Moran et al. 2021).

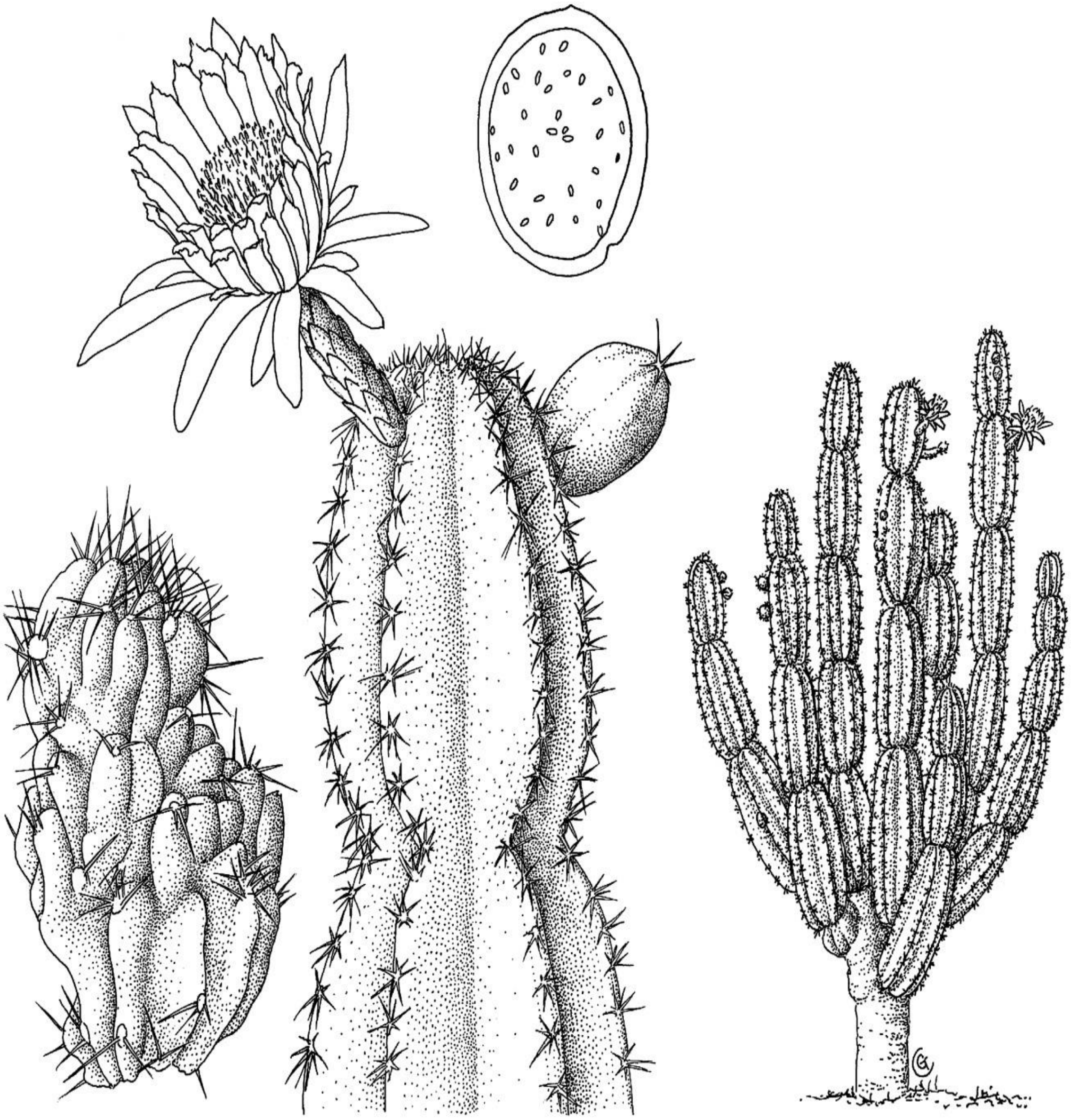

Figure 1.9 *Cereus jamacaru* (Queen of the night) (Henderson L. 2001

1.4. Study aims and objectives

The main objective of this study was to evaluate the impact of actively mass-rearing and releasing cactus biological control agents in South Africa. Biological control is widely regarded as an effective management tool to reduce densities and negative impacts of cactus invasive alien plants. However, there is no detailed evaluation of the success of cactus biocontrol on a national level. The study aims 1) to determine whether the current mass-rearing effort for biological control of invasive alien cacti is sufficient for the control of cacti on a national scale, 2) to determine whether releases of cactus agents are effective in reducing alien invasive cactus populations and to what extent, and 3) to determine whether land-users of South Africa benefit from these releases. This will be done by 1) assessing the release efforts conducted by the CBC and comparing them to the distributions of where the cactus weeds occur (Chapter 2), 2) by quantifying, before and after densities of the weeds and correlating this to the density of biological control agents (Chapter 3), and 3) by quantifying the opinions of the success of biological control from land-users such as landowners, managers, and farmers (Chapter 4). Moran et al. (2021) recently evaluated the success of biological control programmes in South Africa, and the data presented in this thesis were included as support for the decisions made as to how successful these cactus biological control programmes have been. The overall aim of the study was to quantify the success of the management of invasive alien cacti species in South Africa using biological control at a national level, and to use this information to determine how to improve levels of control.

CHAPTER 2: AN ASSESSMENT OF MASS-REARING AND RELEASE EFFORTS AGAINST INVASIVE ALIEN CACTUS IN SOUTH AFRICA

2. 1. Introduction

Biological control is considered one of the most important management interventions for invasive alien cactus weeds (Paterson et al. 2011a). Classical biological control is the most widely utilised approach to biological control of weeds, in which agents are released until established and become a part of the ecosystem, hopefully providing some level of control on the invasive plant population (Nafiu et al. 2014; van Driesche and Abell 2008). Another approach to implementing biological control is augmentative biological control, which involves mass-rearing natural enemies and periodically releasing them in large quantities to boost the field populations of natural enemies with the intention of achieving a greater level of control in a shorter period of time (van Lenteren and Bueno 2003; van Driesche and Abell 2008). Augmentative biological control requires more funding and capacity than classical control (Briese 2000). Whether classical or augmentative biological control will be the most effective approach depends on the target weed, the biological control agent in question, as well as the environment into which the biological control agent is introduced.

Biological control has been used to effectively control a number of cactus weeds globally, but there are still many cactus species that are problematic (Novoa et al. 2015b). Some of the cactus species that continue to be problematic do not have biological control agents, but others have effective biological control agents that require redistribution and augmentation in the field in order to control the weed (Zachariades et al. 2017; Kaplan et al.

2017). For example, *Hypogeococcus* sp. is an effective biological control agent for the invasive alien cactus *C. jamacaru* in South Africa (Sutton et al. 2018). However, the plant is still considered one of the top ten invasive alien plant species in the country, with an increasing distribution and abundance over the last ten years (Henderson and Wilson 2017). The most effective biological control agents for invasive alien cacti are cochineal insects (*Dactylopius* spp.) and the galling mealy-bug, *Hypogeococcus* sp. (Klein 2011; Paterson et al. 2011b). Both cochineal insects and the mealybug are poor dispersers, so active implementation is required to release them at sites where they are not present. In some cases, even if the agent is present at the site, augmentative releases may be required to achieve the most significant degree of control possible in the shortest possible period of time (Zachariades et al. 2017). Low population densities of biological control agents in the field could be caused by unfavourable climatic conditions, and for cactus agents, in particular, it could also be caused by having the incorrect biological control agent (Wilson et al. 2013; van Steenderen et al. 2020). Mass-rearing and releasing could therefore improve the efficacy of biological control agents and the levels of control for target weeds by ensuring that the agent is present wherever it is required, that population densities of the agent are as high as possible, and that the correct agent is present (Zachariades et al. 2017; van Steenderen et al. 2020).

Mass-rearing of biological control agents involves producing large quantities of the agent with the objective of maximizing establishment and population density in the field (Parra 2008; Leppla 2014). Mass-rearing in the biological control of agricultural pests utilises parasitoids of agriculturally important insect pests of crops that are produced in large numbers and repeatedly released to maintain pest populations below a damage threshold

(Bale et al. 2008; Sorensen et al. 2012). Mass-rearing of insects is also commonly used for sterile insect technique that requires very large quantities of sterilized insects to be produced in order to be effective (Orozco-Davilla et al. 2017). On the other hand, mass-rearing and augmentative releases are less commonly utilised in weed biological control, which has historically relied primarily on a classical biological control approach (McFadyen 1998).

In weed biological control, there are certain groups of target weeds, such as cactus weeds and aquatic weeds, for which control can be significantly improved through the use of an augmentative approach (Zachariades et al. 2017). Aquatic weeds are often found in isolated infestations separated by large land areas, so dispersal of agents between water bodies is limited, resulting in the need for releases to be conducted at each of the isolated sites (Goode et al. 2019). In addition, releases to augment populations of water weed biological control agents have been shown to improve levels of control if these releases are done at the appropriate time of year (Miller et al. 2021). Mass-rearing and releases have increased success in agent establishment for the five most problematic aquatic weeds in South Africa, *Pontederia crassipes* (Mart.) Solms (Pontederiaceae), *Pistia stratiotes* L. (Araceae), *Salvinia molesta* D.S. Mitchell (Salviniaceae), *Myriophyllum aquaticum* (Vell.) Verdc. (Haloragaceae) and *Azolla filiculoides* Lamarck (Azollaceae) (Hill and Coetzee 2017).

Mass-rearing and releases are considered important for cactus invasive alien plants (Zachariades et al. 2017), but this is primarily for the purpose of establishing the agents at all the sites where they cannot disperse because the agents are poor dispersers and require human intervention to reach distant and isolated targeted invasive alien plant infestations. It is possible that augmentation of agent populations that are already established may improve the level of control of cactus plants and shorten the time it takes for control to be achieved, but there are no studies that have investigated this directly, so little is known about the importance of augmenting agent populations for cactus plant biological control.

There are only three biological control mass-rearing facilities in South Africa for alien invasive cacti at present. These facilities are the Addo Elephant National Park mass-rearing facility, the Kruger National Park (KNP) mass-rearing facility, and the Centre for Biological Control mass-rearing facility. The two national park facilities only produce a single agent each (*D. austrinus* for *O. aurantiaca* in Addo National Park, and *D. opuntiae* "stricta" for the control of *O. stricta* in the KNP) and only conduct releases within the national parks and their buffer zones. The Centre for Biological Control (CBC) facility, located near Kariega in the Eastern Cape Province, is the only facility that serves the country as a whole and target invasive alien cacti. The CBC facility produces five biological control agents to control ten target cactus weeds and releases these agents at any site in South Africa where the target invasive alien plant occurs (Chapter 1; Table 1.1).

Establishing the correct biological control agent on every population of the target invasive alien cactus in South Africa is the ultimate goal of the cactus biological control implementation programme. This study aimed to describe and assess the outputs of the only national mass-rearing facility for cactus agents in the country, to determine whether mass-rearing efforts are sufficient to implement cactus biological control on a national scale in South Africa and to identify gaps where efforts need to be increased, such as target species or geographic areas that have been neglected.

2.2. Materials and Methods

2.2.1. Mass-rearing and releasing of *Dactylopius* species

There are several different *Dactylopius* lineages, each damaging to a particular invasive alien cactus species or group of closely related species. The correct *Dactylopius* species and lineage must be released on the correct cactus host to ensure that control is achieved (van Steenderen et al. 2020). In South Africa, *D. ceylonicus* is effective in controlling *O. monacantha* infestations, *D. austrinus* is effective for *O. aurantiaca*, *D. opuntiae* "stricta" is effective against the two closely related cactus plants, *O. stricta* and *O. humifusa,* and *D. tomentosus* "imbricata" is effective against *C. imbricata* and *C. leptocaulis* (Chapter 1; Table 1.1).

The mass-rearing technique for all the different cochineal species and lineages involves several steps, the first of which is collecting or growing cladodes of the target cactus weed that are not infected with the agent or any other cochineal of the incorrect lineage/species. Wooden crates measuring 53 cm X 50 cm X 32 cm are filled with uninfected cladodes of the relevant cactus species along with five cladodes that have been infested with the correct species and lineage of cochineal (Fig 2.1). Genetic techniques (nucleotide sequencing of three genes: 12S rRNA, 18S rRNA and CO1, and two inter-simple sequence repeats ISSR) are used to ensure that the correct cochineal is inoculated onto the clean cladodes (van Steenderen et al. 2020). The wooden crates are then placed on open shelves in a greenhouse (Fig 2.1). Greenhouses are polyurethane, which allows sunlight into the tunnel but excludes all precipitation, providing a hot and dry climate ideal for cochineal. The sides of the greenhouses are shade cloth rather than polyurethane to allow for ventilation so that humidity in the greenhouse is not significantly greater than ambient. After about a week, the cochineal insects on the cochineal-infested cladodes will start to infest all the cladodes in the wooden boxes. Since the temperature is not controlled within the greenhouses and the developmental time of cochineal is temperature dependent, the length of time that it takes for a box of cladodes to be infected with the cochineal is dependent on the temperature at the time, with much quicker developmental times in summer. When cochineal insects have spread and infested all the cladodes in the boxes, the biological control agents are ready for release in the field.

Figure 2.1 Mass-rearing wooden boxes filled with *O. stricta* cladodes infested by *D. opuntiae "*stricta"

The release of *Dactylopius* species on their host cactus in the field involves placing one to three cochineal-infested cladodes on selected host plants within an infestation. Dense stands of cactus are targeted because cochineal spreads more easily in dense stands than between isolated plants. A pair of tongs is used to release and position cochineal-infested cladodes between fresh cladodes in the cactus canopy, ideally well above the ground and partially shaded by the canopy. After a few weeks of conducting a release, the cochineal insect will spread and cover the cladodes of the inoculated plant and then spread to other cactus plants nearby. The cladodes in the boxes can be kept for a maximum of two weeks in a dry cool place before releasing them in the field.

2.2.2. Mass-rearing and releasing of *Hypogeococcus* species

Hypogeococcus sp. requires actively growing plant tissue to form galls. While the insect can survive on cut segments of the stem, they do not form galls unless the plant is growing. Potted plants are therefore used for mass-rearing of *Hypogeococcus* sp., unlike the cochineal insects that all thrive on dislodged cladodes. *Hypogeococcus* sp. feeds on a group of closely related cacti, including *Cereus* and *Harrisia* species. Of the plants present in South Africa, *H. martinii* is the most preferred host plant and is therefore used for mass-rearing (Klein 1999; McFadyen 1979). Galls of *Hypogeococcus* sp. are broken into $2cm^2$ segments and attached to the growing tips of potted *H. martinii* plants using wooden skewer sticks. The potted *H. martinii* plants are kept in a greenhouse with the same design as those used for cochineal species. After a plant has been inoculated with the agent, it will generally develop galls on all new growing tips that develop, and the plants can be kept alive by frequent watering, fertilizing, and harvesting the galls. The harvesting of galls results in new stems growing from below the damaged plant tissue, resulting in more shoot tips, which are the optimal part of the plant for the development of the agent. After successive harvests of the galls, old plants are eventually replaced with new ones, and if galls are not harvested often enough the plants will die.

To release *Hypogeococcus* sp. into the field, a gall or section of a gall of any size is skewered with a wooden skewer, and this is then stuck into the terminal growing tip of a cactus stem on a plant in the field. The gall should be in direct contact with the growing tip and be held in position indefinitely. The tissue of the growing tip of the cactus will then be galled as it grows, and the agent will then spread to other growth tips. The galls on the stem tips will inhibit the growth of the plant, and plants infested by the mealybug will develop twisted and deformed stems. Cactus plants affected by the mealybug produce very few flowers and almost no fruits. The decrease in the number of fruits will result in the reduction of dispersal rates of the plants as all plants targeted by *Hypogeococcus* rely heavily on fruit for dispersal and reproduction. Small plants die within one to two years after the release, whereas for large plants, it usually takes a greater number of years for the plant to die. The agent will therefore result in quicker control of smaller cactus species individuals than larger ones.

2.2.3. Assessing the release efforts of cactus agents

The distribution records of invasive alien cactus plants were extracted from the Southern African Plant Invader Atlas (SAPIA) (Henderson 1999). SAPIA was launched in January 1994 to record data on the distribution, abundance, and habitat types of invasive alien plants in Southern Africa, and the records are frequently revised (Henderson and Wilson 2017). The SAPIA database is a useful tool that gives baseline information for invasive alien plant control programmes on a national scale and helps with data required for categorizing invasive plants under the *Alien and Invasive Species Regulations of the National Environmental Management: Biodiversity Act*, Act 10 of 2004 (NEM: BA A & IS Regulations) (Department of Affairs, 2014; Henderson & Wilson, 2017). The SAPIA database has more than 87 000 locality records of ~ 773 invasive alien plants (Henderson and Wilson 2017). SAPIA comprises geo-referenced records at a quarter degree square (qds) resolution (Henderson 2007).

The distribution data of invasive alien cactus from SAPIA was compared with GPS co-ordinates from the CBC cactus biological control agent releases database from June 2015 to March 2021. The CBC records all the GPS co-ordinates where biological control agents on cactus plants have been conducted. An evaluation was then conducted to determine which qds where the target weed had been recorded have been targeted for biological control releases. ArcMap version 10.6.1 was used to create maps of SAPIA localities of cactus species and CBC release sites. All ten invasive alien cactus species for which agents are mass-reared and released by the CBC were included in the analysis (Table 2.1).

2.3. Results

2.3.1. Releases of biological control agents by the CBC

Three hundred and eighty-four releases of cactus biological control agents have been conducted, with a total of 385 184 infected cladodes and galls being released against ten invasive alien cacti (Table 2.1). *Opuntia aurantiaca* had the greatest number of cochineal-infested cladodes released by the CBC, and *C. leptocaulis* had the least number of cochineal-infested cladodes released by the CBC (Table 2.1). On the other hand, the CBC has conducted the greatest number of releases for *O. stricta* compared to all other releases, and only two releases have been carried out for *C. leptocaulis* (Table 2.1). A total of 5 141 *Hypogeococcus* galls have been released on the four target weeds that the agent is used for (Table 2.1).

Table 2.1 Cactus biological control agents released by the CBC Kariega Mass-Rearing Facility

WEED	AGENT	Total June 2015-March 2021	
		# releases	# cladodes/galls
O. aurantiaca	*D. austrinus*	64	332583
O. stricta	*D. opuntiae* "stricta"	81	6231
O. cespitosa	*D. opuntiae* "stricta"	40	5321
O. monacantha	*D. ceylonicus*	43	4863
C. imbricata	*D. tomentosus* "imbricata"	66	26758
C. leptocaulis	*D. tomentosus* "imbricata"	2	308
C. jamacaru	*Hypogeococcus* sp.	39	2554
H. martinii	*Hypogeococcus* sp.	14	650
H. pomanensis	*Hypogeococcus* sp.	3	1457
H. balansae	*Hypogeococcus* sp.	5	480
TOTAL		**357**	**381205**

2.3.2. Release efforts per quarter degree square (qds)

Opuntia monacantha

Opuntia monacantha was present in six provinces of South Africa and occupied a total of 51 quarter degree square (qds) according to SAPIA records (Fig. 2.2). The CBC releases of *D. ceylonicus* on *O. monacantha* have occupied 17 (33%) qds (Fig. 2.2). Therefore, there were 34 (67%) qds with *O. monacantha* where the CBC has not done releases (Fig. 2.2). The largest gaps in the CBCs release efforts appear to be in the northern parts of the Eastern Cape Province all the way to southern KwaZulu-Natal, as well as the Western Cape Province (Fig. 2.2).

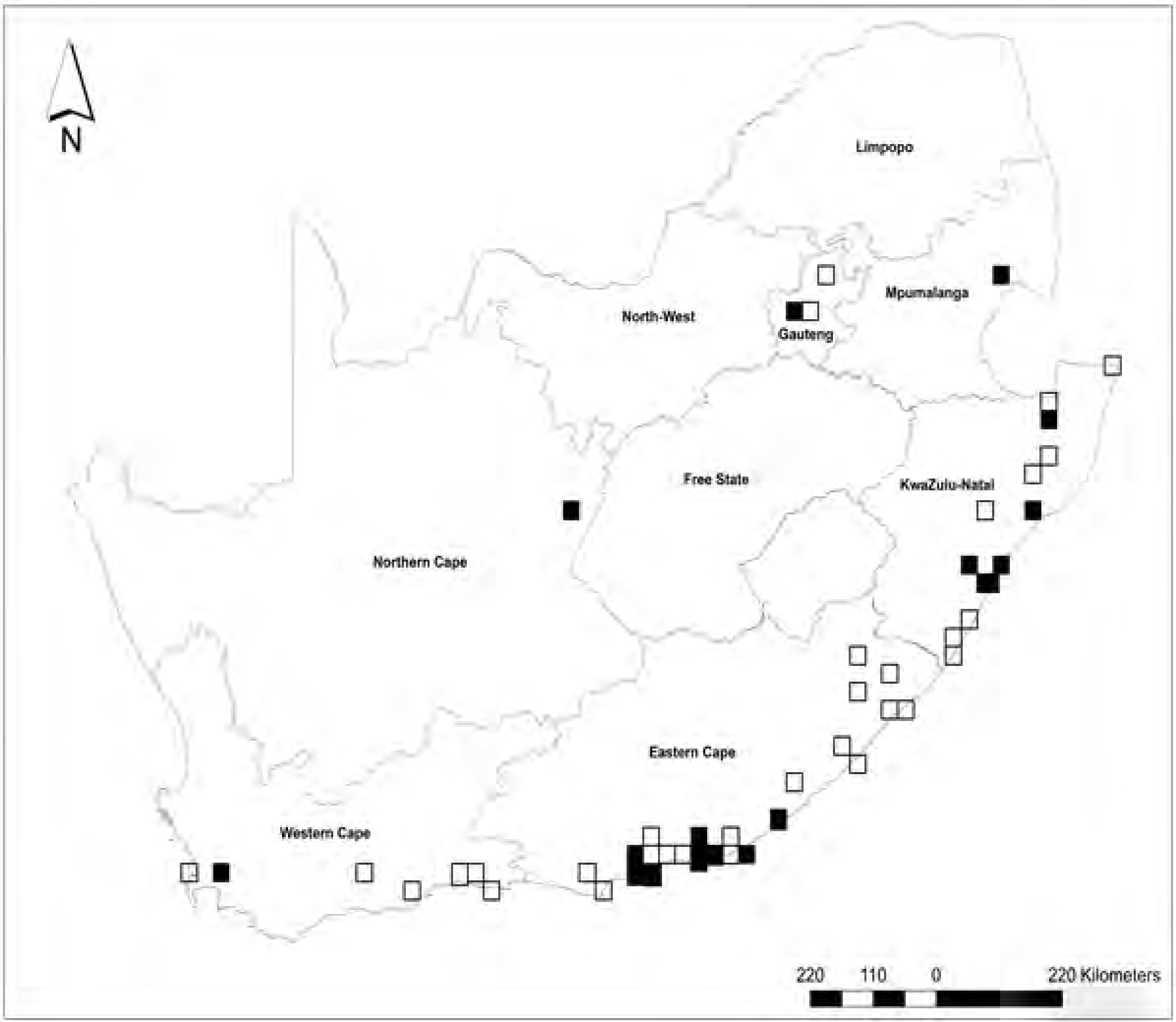

Figure 2.2 Map of South Africa showing quarter degree squares (qds) that are in the SAPIA and CBC databases for *Opuntia monacantha*. Black squares indicate qds where CBC has conducted releases. Open squares indicate qds where *O. monacantha* is recorded as present but no releases have been conducted.

Opuntia stricta

Opuntia stricta was recorded in 142 qds according to the SAPIA database; 109 (77%) qds had no biological control agent released in them (Fig. 2.3). Of the 33 qds where releases had been conducted, eleven were located in the Western Cape, seven in KwaZulu-Natal, and four or less in the other provinces of the country (Fig. 2.3). A high proportion of qds in the Free State, KwaZulu-Natal and Western Cape had releases conducted in them, while only

15% of the 33 qds in the Eastern Cape had been targeted (Fig. 2.3). Limpopo Province had the highest number of qds where the release of biological control agent for *O. stricta* had not been conducted compared to all other provinces, and both Limpopo and North-West are clearly areas where the target weed is widely distributed, but very few releases have been conducted (Fig. 2.3).

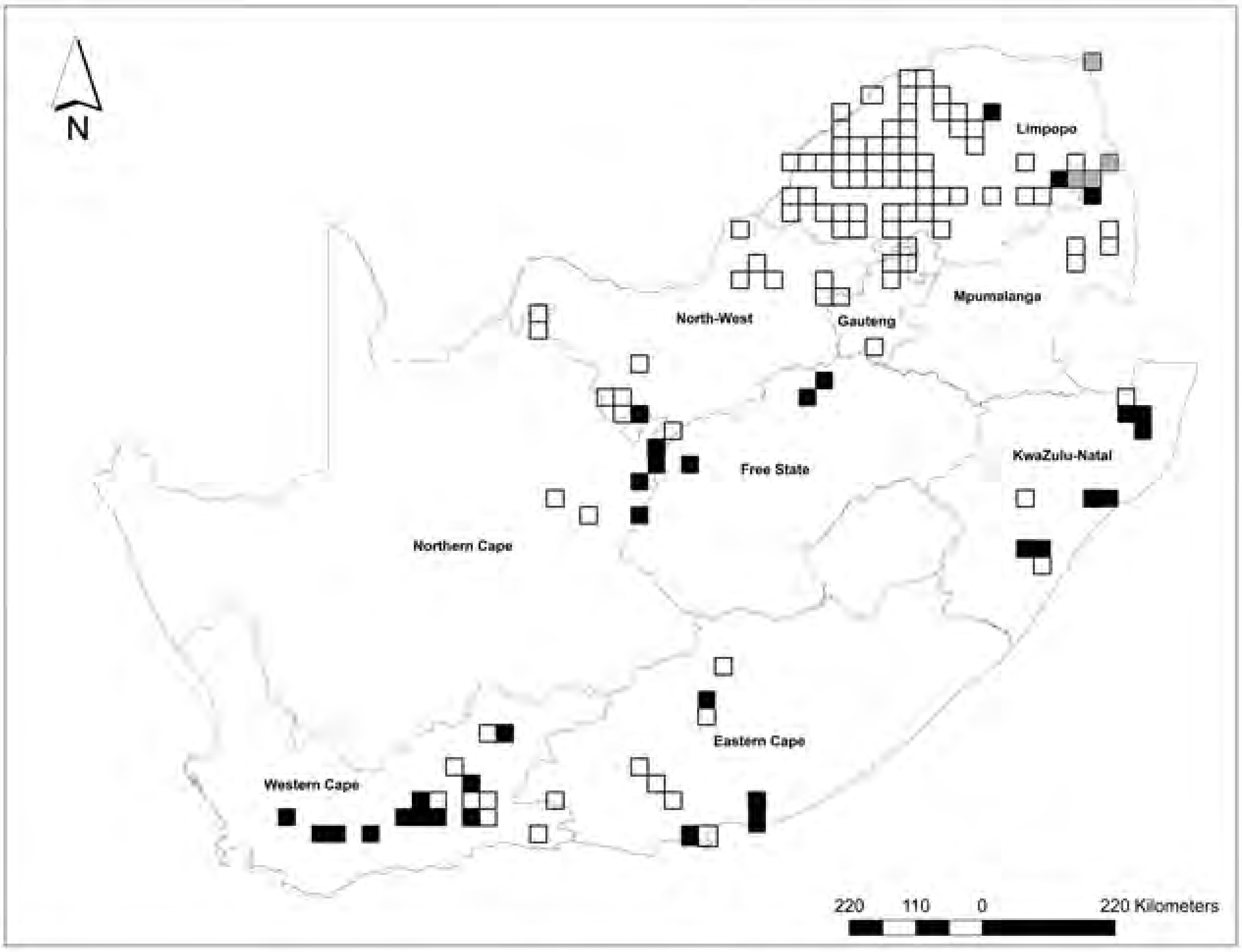

Figure 2.3 Map of South Africa showing quarter degree squares (qds) that are in the SAPIA and CBC databases for *Opuntia stricta*. Black squares indicate qds where CBC has conducted releases. Open squares indicate qds where *O. stricta* is recorded as present but no releases have been conducted. Grey squares indicate qds in the Kruger National Park where the park staff mass-rears and releases *D. opuntiae* “stricta” on *O. stricta*, it is not the CBC’s mandate to release in that area.

Opuntia cespitosa

SAPIA recorded 73 qds with *O. cespitosa* in all the provinces of South Africa. Of the 73 qds, the CBC has released in only 21 (29%) (Fig. 2.4). Despite releases only having been made in about one in five of the quarter degree squares country-wide, the spread of releases has been relatively even, with releases being conducted in six of the nine provinces of South Africa (Fig. 2.4). Most releases were conducted in the Eastern Cape and Northern Cape compared to all other provinces; in these two provinces, releases covered six qds (Fig. 2.4). There were three qds where releases had been conducted in the Western Cape and Free State, two in KwaZulu-Natal, and a single qds in Gauteng (Fig. 2.4). There are many qds that have not had releases in the Eastern Cape, Northern Cape, North-West, Gauteng and Limpopo (Fig. 2.4).

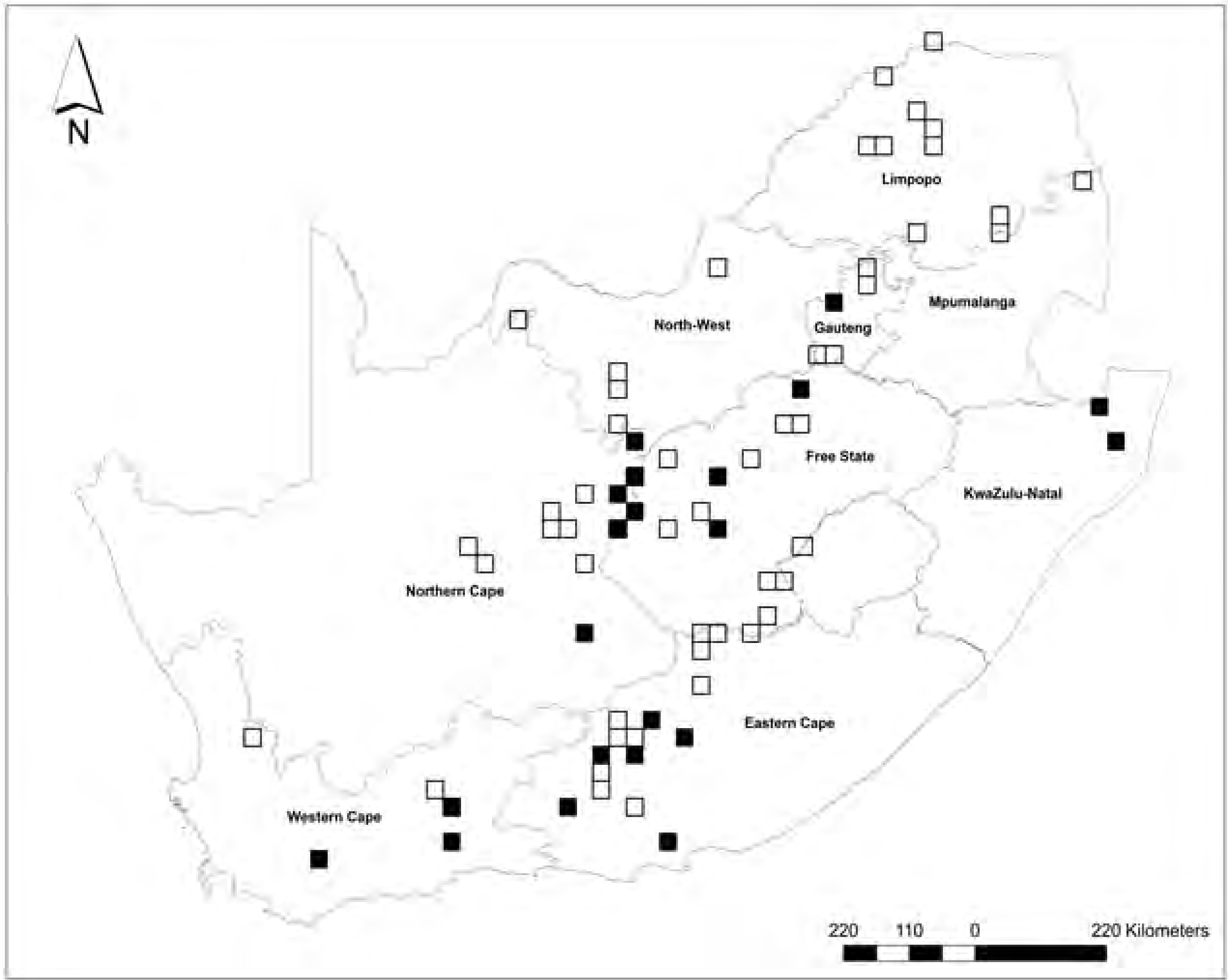

Figure 2.4 Map of South Africa showing quarter degree squares (qds) that are in the SAPIA and CBC databases for *Opuntia cespitosa*. Black squares indicate qds where CBC has conducted releases. Open squares indicate qds where *O. cespitosa* is recorded as present but no releases have been conducted.

Cylindropuntia imbricata and *Cylindropuntia leptocaulis*

Cylindropuntia imbricata was recorded in a total of 137 qds (Fig. 2.5). The biological control agent, *D. tomentosus* “imbricata,” has been released in thirty-seven (27%) of the 137 qds (Fig. 2.5). Of the 37 qds where the agent has been released, thirteen were in the Eastern Cape, eight were in the Western Cape, while the other provinces had four or less releases.

All the provinces except KwaZulu-Natal and Mpumalanga, where the plant is rare, have many qds where the agent has not been released (Fig. 2.5). There were four qds where *C. leptocaulis* is present in South Africa (Fig. 2.5). These qds are situated in the Eastern Cape, Western Cape and North-West (Fig. 2.5). The CBC has done releases on *C. leptocaulis* in two (50%) qds; both of which were located within the Eastern Cape (Fig. 2.5).

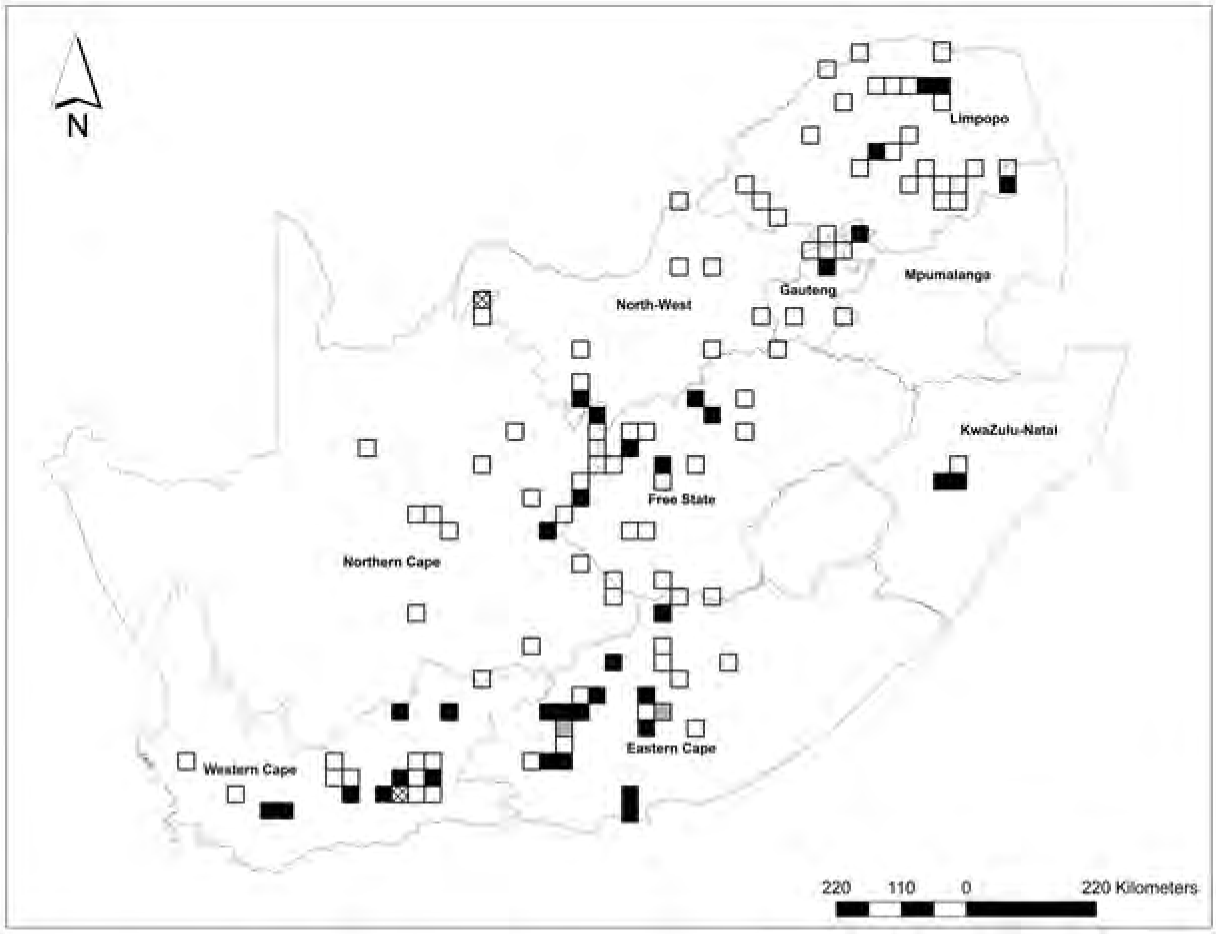

Figure 2.5 Map of South Africa showing quarter degree squares (qds) that are in the SAPIA and CBC databases for *Cylindropuntia imbricata* and *Cylindropuntia leptocaulis*. Black squares indicate qds where CBC has conducted releases on *C. imbricata*. Open squares indicate qds where *C. imbricata* is recorded as present but no releases have been conducted. Grey squares indicate qds where CBC has conducted releases on *C. leptocaulis,* and squares with lines indicate qds, where *C. leptocaulis* is recorded as present but no releases have been conducted.

Opuntia aurantiaca

SAPIA records indicated the presence of *O. aurantiaca* in 55 qds in all the provinces of South Africa except the Western Cape (Fig. 2.6). There is a clear cluster of qds where this weed occurs in the Eastern Cape Province, indicating an area where the plant is most widespread and abundant (Fig. 2.6). Releases have been conducted in 23 (42%) qds in four provinces; with the Eastern Cape having the highest number of releases compared to Free State, Kwa-Zulu Natal, and Limpopo which each only had one qds in which releases have occurred (Fig. 2.6). There were 32 (58%) qds where agents have not been released; the greatest number of these were located in the Eastern Cape, followed by KwaZulu-Natal (Fig. 2.6). There were 14 qds where releases had not been conducted in the Eastern Cape and 11 qds where the agent has not been released in KwaZulu-Natal (Fig. 2.6).

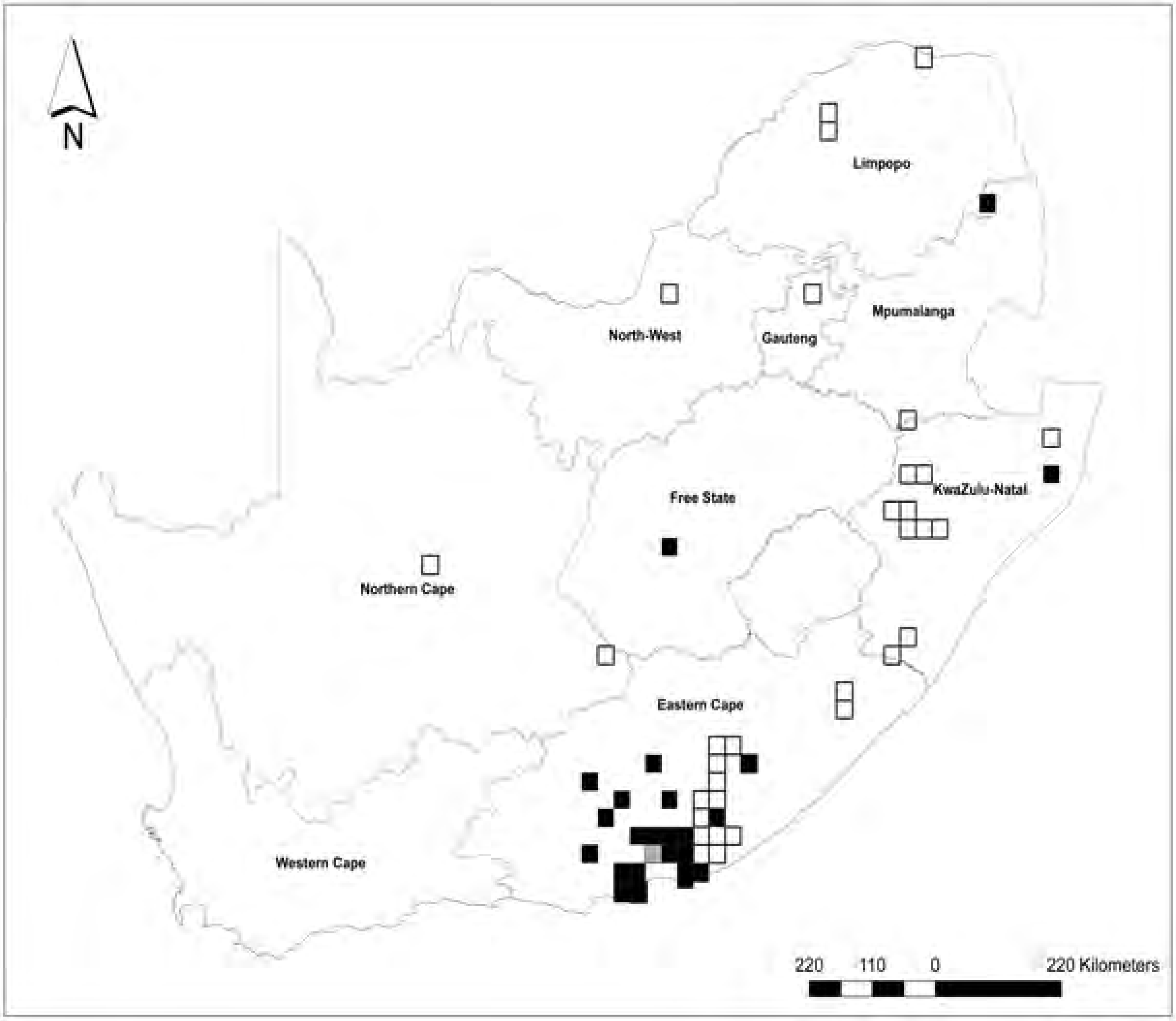

Figure 2.6 Map of South Africa showing quarter degree squares (qds) that are in the SAPIA and CBC databases for *Opuntia aurantiaca*. Black squares indicate qds where CBC has conducted releases. Open squares indicate qds where *O. aurantiaca* is recorded as present but no releases have been conducted. The grey square indicates Addo National Park, where the park staff mass-rears and releases *D. austrinus* on *O. aurantiaca* and is not the CBC's mandate to releases in that area.

Harrisia balansae, Harrisia martinii and *Harrisia pomanensis*

Harrisia balansae was present in five qds and four of these had biological control releases conducted in them (Fig. 2.7). There is a single locality in North-West Province where the CBC has not released the agent. For *H. pomanensis,* three of the six qds in the country have been targeted for releases by the CBC (Fig. 2.7).

There were 33 qds where *H. martinii* was recorded, and releases have been made in nine (27%) of these (Fig. 2.7). Eastern Cape, Western Cape, and Northern Cape had two qds each, and the other three provinces, KwaZulu-Natal, Free State, and Limpopo, had one qds each where the agent was released (Fig. 2.7). Limpopo had the highest number of qds with *H. martinii* localities with no biological control releases and all other provinces had between one and three qds occupied by *H. martinii* (Fig. 2.7).

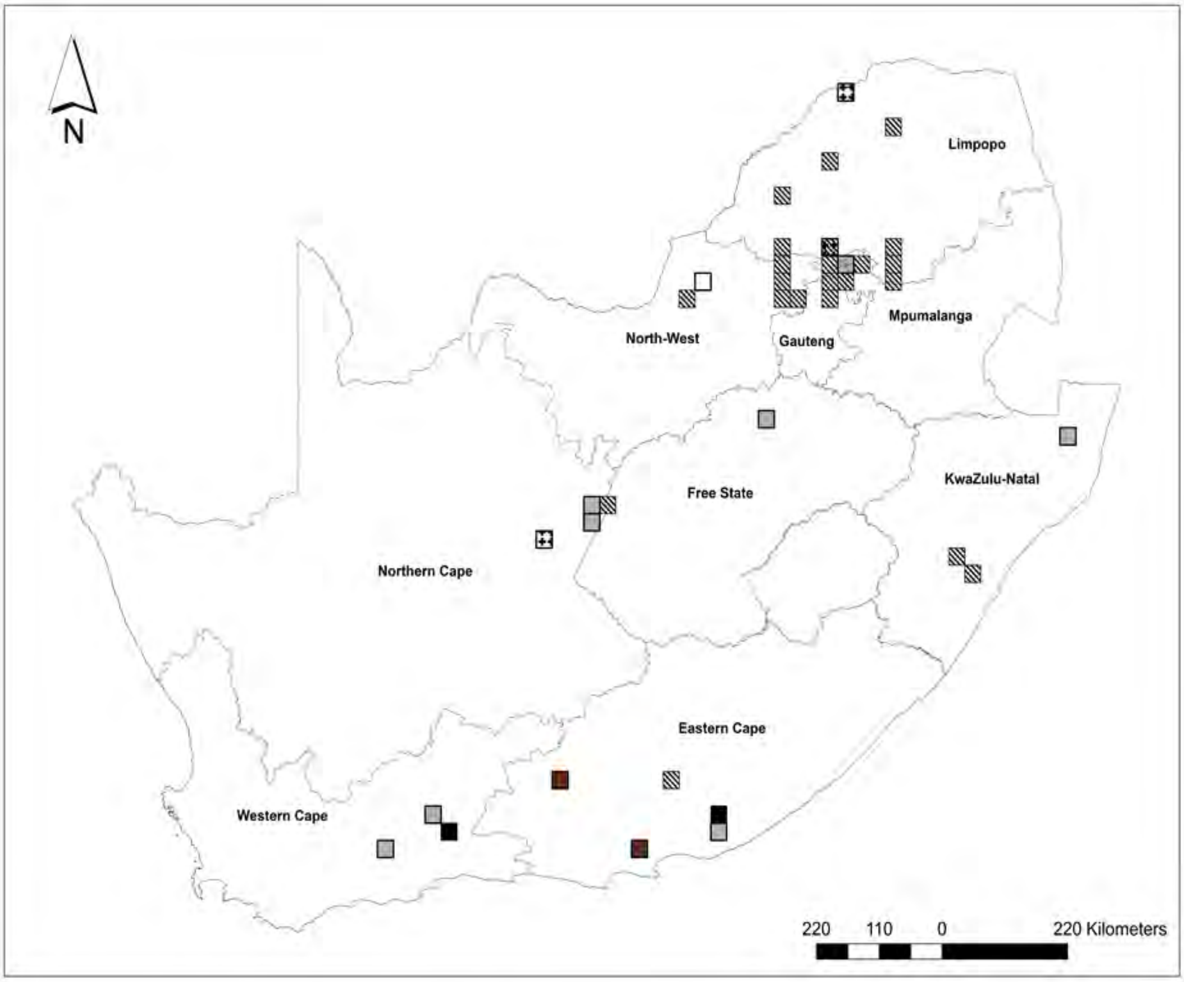

Figure 2.7 Map of South Africa showing quarter degree squares (qds) that are in the SAPIA and CBC databases for *Harrisia balansae, Harrisia martinii,* and *Harrisia pomanensis.* Black squares indicate qds where CBC has conducted releases on *Harrisia balansae*. Open squares indicate qds where *H. balansae* is recorded as present but no releases have been conducted. Grey squares indicate qds where CBC has conducted releases on *H. martinii*. Squares with lines indicate qds where *H. martinii* is recorded as present but no releases have been conducted. Brown squares indicate qds where CBC has conducted releases on *H. pomanensis*. Black squares with white circles indicate qds where *H. pomanensis* is recorded as present but releases have been conducted.

Cereus jamacaru

A total of 147 qds located in all the provinces of South Africa were occupied by *C. jamacaru* (Fig. 2.8). This makes *C. jamacaru* the most widespread cactus weeds targeted for biological control by the CBC mass-rearing facility. Out of 147 qds, 24 (16%) qds have had *Hypogeococcus* sp. released, and there were 123 (84%) qds where releases have not been recorded (Fig. 2.8). Most of the biological control agent releases conducted by the CBC were located in the KwaZulu-Natal with 11 qds, followed by Western Cape with four qds, Eastern Cape with three qds, Free State with two qds, and lastly Northern Cape and Gauteng with one each (Fig. 2.8). There were qds with no biological control agent released by the CBC in all the provinces, but Limpopo, and the northern parts of North-West and Mpumalanga, had the highest numbers of qds without releases by the CBC (Fig. 2.8).

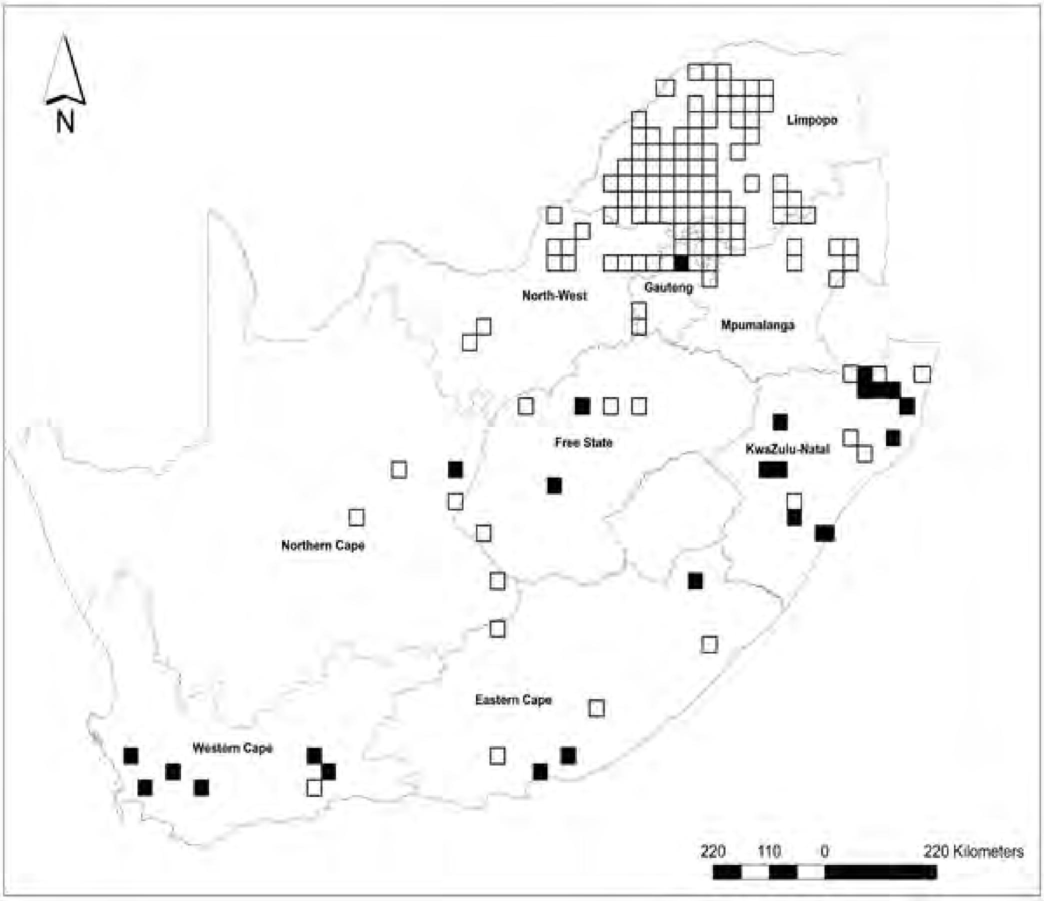

Figure 2.8 Map of South Africa showing quarter degree squares (qds) that are in the SAPIA and CBC databases for *Cereus jamacaru*. Black squares indicate qds where CBC has conducted releases. Open squares indicate qds where *C. jamacaru* is recorded as present but no releases have been conducted.

Overall, only a small proportion of the distributions of the invasive alien cacti in South Africa have been targeted with biological control releases by the CBC (Fig. 2.9). For example, releases by the CBC on *O. stricta*, *C. imbricata*, and *C. jamacaru* covered less than 30% of the distribution occupied by the plants (Fig. 2.9). The qds where the CBC has released agents occupied more than 30% of the distribution of *O. monacantha* and *O. aurantiaca* (Fig. 2.9). Limpopo and Eastern Cape had the highest number of qds that have not had biological control agents released in them compared to all other provinces, and these two provinces had the highest number of qds occupied by invasive cacti (Fig. 2.10). The number of qds occupied by invasive alien cacti was less than 60 qds in KwaZulu-Natal, Western Cape, and Northern Cape, resulting in a higher proportion of qds being targeted despite lower releases efforts compared with the Eastern Cape (Fig. 2.10). On the other hand, Limpopo Province has the highest number of qds occupied by invasive alien cacti and one of the lowest release efforts (Fig. 2.10). The number of qds occupied by invasive cacti in North-west, Free-State, Gauteng, and Mpumalanga were relatively low compared to other provinces (Fig. 2.10).

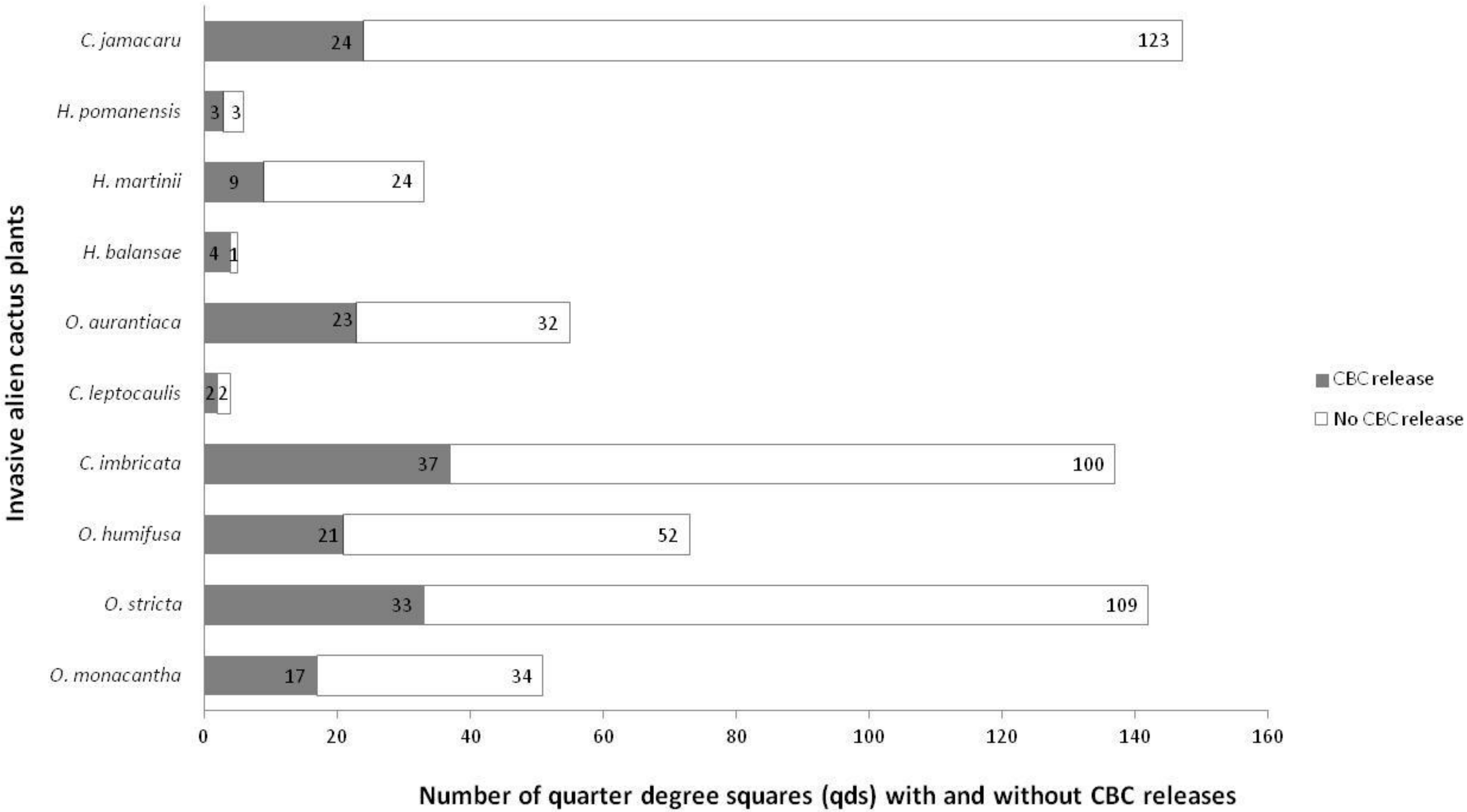

Figure 2.9 The number of quarter degree squares (qds) occupied by each invasive alien cactus species, and the proportion of these qds where the CBC has released biological control agents.

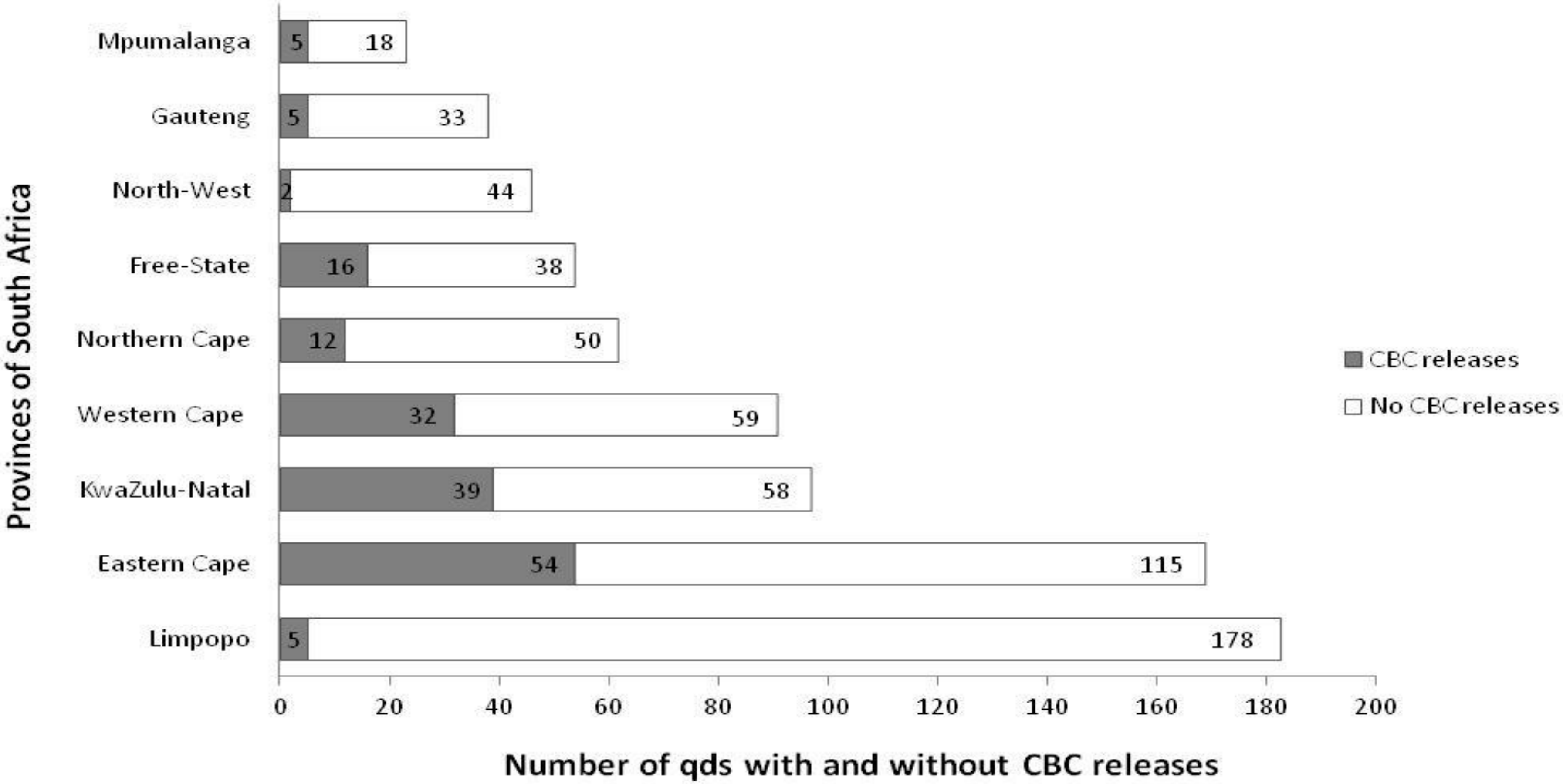

Figure 2.10 The number of quarter degree squares (qds) where cacti targeted for biological control by the CBC are present in each province of the country and the proportion of these qds where releases have been conducted.

2.4. Discussion

The CBC has conducted releases of biological control agents on invasive cacti in only 26% of the qds that have cacti recorded in them in South Africa. This clearly indicates that mass-rearing and releasing efforts should be increased substantially. The intention of the CBC mass-rearing facility is to release in every qds because the biological control agents are unlikely to be dispersed more than 27km based on previous studies (Gunn 1979). Given that a quarter degree squared is about 30 km^2 in South Africa (the area of a qds changes depending on latitude), aiming to release each biological control agent in at least one site within each quarter degree squared that the target weed occupies is a reasonable management goal.

Data on the current abundance of cactus species per qds (i.e. whether biological control is needed) and whether the biocontrol agent may already be present (i.e. whether releases are needed) is limited. There is a real need for on-the-ground surveys to be done at a national level to determine the abundance of the weed and presence of the agent (and also whether, if the biological control agent is present, it is the correct species and lineage, in the case of *Dactylopius* sp.).

The CBC needs to target and prioritize areas where there have not been releases conducted but the target cactus weed is present. This can be done by actively engaging farmer unions and conservation area managers in regions where few releases have been conducted, and the target cactus weeds are known to occur. By doing this, the CBC will get an opportunity to educate land-users about biological control as a control method. This is an important step towards releasing agents in all the qds in the country because, in some areas, land-users are not well-informed about weed biological control and are therefore sceptical about using it on their land. Engaging with land-users to help them understand how biological control works and providing them with the correct information may alleviate their concerns and result in an increase in requests for agents for their area (Novoa et al. 2017b; Shackleton et al. 2019a).

The distribution of *O. monacantha* covers 51 qds in South Africa, but the majority of the qds were recorded in the Eastern Cape. The CBC's releases have been primarily restricted to the coastal regions; however, there are still *O. monacantha* infestations recorded in the SAPIA database in both coastal and inland areas where the CBC has not released *D. ceylonicus*. It is not known whether the biological control agent is present at all or some of the sites where the CBC has not released, but it is likely that some of the sites do have the agent, as this agent has been present in the countries for over 100 years and is therefore widespread (Paterson et al. 2011b). Since biological control does not eradicate the target weed completely, records in the SAPPIA database may be of populations of plants that already have the agent. For sites that have large amounts of the agent already, there will be no need for additional releases; however, if the agent is only present at low densities or if the agent is absent, releases are required. Geographic gaps in the distribution of releases of the agent that should be the primary focus for new releases are in the northern parts of the Eastern Cape Province, southern KwaZulu-Natal, and the north-east of the Western Cape.

For *O. stricta*, the CBC has released *D. opuntiae* "stricta," but the releases only cover a small proportion of where the plant is catalogued as present in the SAPIA database. The main focus for the next releases of *D. opuntiae* "stricta" should be in the Limpopo and North-West provinces; these two provinces have a wide distribution of *O. stricta* and large areas where no releases have been conducted. Biological control of *O. stricta* in the KNP is well documented and has been extremely successful (Hoffmann et al. 2020). This suggests that the agent should be very effective in other parts of Limpopo and Mpumalanga province near the KNP, which have similar climates. The KNP mass-rears and conducts releases within the park, so the CBC should focus on releasing this agent in areas outside the park's boundaries and in the remaining infestations in Limpopo and Mpumalanga provinces.

Mass-rearing and release efforts for the control of *O. cespitosa* should be increased in most provinces, including the North-West, Northern Cape, Limpopo, and the south-east Free State and north-west of the Eastern Cape. It is recommended that the CBC should engage with communities in these areas to release *D. opuntiae* “stricta” at sites where there is no agent or the agent population is low.

Cylindropuntia imbricata has a very widespread distribution in South Africa, and it is challenging to ensure that the agent is distributed to all the areas where it is required. The CBC has released *D. tomentosus* “imbricata” in all the provinces of South Africa, but there are many qds where releases are still required. KwaZulu-Natal and Mpumalanga have few localities and releases have been conducted in some of these localities, so these provinces are not a high priority. The CBC should focus on conducting releases on *C. imbricata* in all other provinces. For *C. leptocaulis,* there are only four known sites in the country*,* and all have been reported to have been destroyed by *D. tomentosus* “imbricata.” The agent has been extensively damaging at the *C. leptocaulis* infestations at two of these sites where the agents were released by the CBC. At the two other sites, the agents were known to be present from releases conducted in the 1980’s and have significantly reduced the target weed densities at those sites (Moran and Zimmermann 1991a). This suggests that if any new

C. leptocaulis infestations are reported releases should be conducted, but no further release efforts are required at this stage.

Opuntia aurantiaca is most abundant in the Eastern Cape; this is due to climatic conditions that are favourable for its growth and not favourable to the biological control agent. *Dactylopius austrinus*, the biological control agent, was released many years ago before the SAPIA project was initiated, and the plants' distribution in South Africa has probably been completely changed due to the biological control agent, but this change is unrecorded (Moran and Zimmermann 1991b). The findings of this study have indicated that the CBC has conducted a high proportion of releases on the qds in which *O. aurantiaca* is present, mostly in the southern parts of the Eastern Cape. Eastern Cape and KwaZulu-Natal provinces should be given more attention because, according to SAPIA records, *O. aurantiaca* is still present in a large number of qds compared to other provinces, and in the majority of these qds, the agent has not been released by the CBC. *Opuntia aurantiaca* is still a significant problem in the Eastern Cape, but the releases of *D. austrinus* have reduced the density of *O. aurantiaca* and improved the level of control compared to how it was prior to biological control (Moran and Zimmermann 1991b; Hill et al. 2020). Climatic conditions influence the survival and performance of *D. austrinus*; the agent works effectively under hot and warm temperatures ranging from 25°C to 30° (Zimmermann 1981; Hosking 1984b). Reports from the few releases conducted outside of the cooler, wetter south-central Eastern Cape Province suggest that the agent has extensively damaged the plant and resulted in the drastic reduction in plant populations. This trend is confirmed by historical observations that there were large and dense infestations of *O. aurantiaca* in KwaZulu-Natal Province, where temperatures are warm, prior to the release of the agent in the 1930s (Moran &

Zimmermann 1991b). This indicates that releases of cochineal should be conducted everywhere in the KwaZulu-Natal Province using small releases because there is a high likelihood of achieving good control. Within the south-central Eastern Cape, there is a need to augment populations with large releases to ensure that the agent is present at all sites.

Few releases have been conducted on *H. balansae* and *H. pomanensis,* and while these species occupy small numbers of qds in some provinces, it is still important that releases are conducted to prevent further dispersal. These species are emerging weeds that should be targeted with biological control in the early stages of the invasion process to stop them from ever becoming widespread and problematic (Olckers 2004).

Cereus jamacaru had a distribution restricted to Limpopo, Gauteng, and North-West provinces up to 1987, but the plant had spread to all the provinces of South Africa by 1999 (Klein, 1999). Recently, there has been an increase in density and the number of sites infested with *C. jamacaru* in the Eastern Cape and KwaZulu-Natal (Paterson et al. 2011b; Henderson & Wilson, 2017). The first releases of *Hypogeococcus* sp. on *C. jamacaru* were conducted in the northern provinces of South Africa in 1990 over a fairly wide area (Moran & Zimmermann, 1991a). By 1999, the population of the agent had noticeably increased on the plant populations on which it had been released (Klein, 1999). In 2011, the *C. jamacaru* infestations on which releases had been made were heavily colonized with *Hypogeococcus* sp., where the release was done (Paterson et al. 2011b). Despite no recent releases being conducted in the area, the biological control agent is actually very widely distributed in North-West, Limpopo, and Gauteng (Sutton et al. 2018). It will be worth surveying these areas and localities in the northern provinces to ensure that the agent is established at every site. The CBC releases should therefore focus on the EC and KZN where the plant is considered a problem that is getting worse, and the agent is not present at many sites.

For all cactus weeds, correct biological control agents may be present in some of the qds where the CBC has not released. There are few published or available records of biological control implementation from the past (prior to 2015). It is also important to note that biological control does not eradicate the weed, so the weed can be present in the SAPIA database and already under complete control from biological control interventions. A survey to the SAPIA localities to evaluate if the biological control agents (the correct agents) are present is warranted for all targeted species. Although quarter degree squares have been used to visualize the spread of releases across the country, the SAPIA and CBC databases have the GPS localities for the majority of localities, so these could be visited in order to assess the status of the agent at these sites and the maps could be updated to give presence and absence of agents at sites where releases have not been made.

The results of this study have shown that significant progress has been made towards the goal of releasing agents wherever biological of cactus weeds is required; but an increase in mass-rearing and releasing efforts is required. There are still gaps in most provinces of South Africa for *O. monacantha*, *O. stricta*, *O. humifusa*, *C. imbricata*, *O. aurantiaca*, *C. jamacaru* and *H. martinii*. *Opuntia stricta, C. imbricata,* and *C. jamacaru* are the species with the most localities where releases have not been conducted and should therefore be prioritized. The two provinces that have the most qds where releases have not been conducted are the Eastern Cape and Limpopo. Release efforts should be increased in both these provinces. This could be achieved by increasing capacity, particularly in the production of the agent for *O.*

aurantiaca at the CBC mass-rearing facility in the Eastern Cape, and by setting up a new mass-rearing facility in Limpopo Province to serve that part of the country.

CHAPTER 3: MONITORING THE IMPACT OF BIOLOGICAL CONTROL AGENTS RELEASED ON INVASIVE ALIEN CACTACEAE IN SOUTH AFRICA

3. Introduction

3.1. Post-release evaluation

Post-release evaluation is an important phase in the biological control process that should receive the same emphasis and effort as other phases, such as exploration and host specificity testing (McEvoy and Coombs 1999; van Klinken and Raghu 2006; Morin et al. 2009). Quantitative post-release data can be used to optimise release strategies of existing agents and, therefore, the level of control achieved for the target weeds. For example, post-release data can inform biological control practitioners whether further releases of agents are required to augment agent populations and when and where releases will be most effective (Reid et al. 2008; Morin et al. 2009). Furthermore, assessments of successful biological control programmes justify continued and additional funding towards conducting biological control research and implementation (Schwarzländer et al. 2018; Ivey et al. 2018).

The success of a biological control programme should ultimately be determined by quantifying the decrease in negative impacts posed by the invasive alien plant due to biological control (Barton et al. 2007; Paterson et al. 2011a). The negative impacts of the target weeds are usually proportional to the density and distribution of the weed population, so in most cases, the damage from the agent must result in a decrease in the density and distribution of the target weed populations to reduce these negative impacts.

Biological control is a long-term control method. It can take many years after the release of the agent before any population-level impacts to the target weed are evident. It is therefore recommended that a period of 10-20 years after the agent is first released should be allowed before post-release evaluations are conducted (McFadyen 1998), but in at least some cases, the impacts of biological control have been much more rapid (McConnachie et al. 2004). Biological control is also intended to control widespread populations of target weeds across a large geographic area, and the level of control may vary due to differences in climatic and environmental conditions (McClay 1995; Müller-Schärer and Schaffner 2008). Therefore, it is suggested that post-release evaluations should be conducted over many years and in different regions to lessen confounding factors such as climatic differences that vary in space and time (Denoth and Myers 2005).

3.1.1. Parameters measured in biological control post-release evaluations

Post-release evaluations can include measuring changes in agent-related, weed-related, and ecosystem-related parameters before and after release (Morin et al. 2009). The agent and plant-related parameters are relatively easy to measure, whereas changes in ecosystem-related parameters are more complicated (Morin et al. 2009). Agent-related measurements are taken to quantify the population of the agent by counting the number of each life-stage of the agent and access agent establishment, phenology, and dispersal patterns (Morin et al. 2009). Other agent-related parameters include damage to the target weed, such as the number of damaged leaves, the number of damaged shoots or stems, the number of galls formed, and the number of damaged flowers, fruits, or seeds (Dhileepan and McFadyen 2001; Paynter et al. 2006). Agent-related measurements are appropriate for biological control programmes in the early stages after the release of the agent when reductions to target weed population densities are unlikely, and the negative impacts of the target weed are unlikely to have been reduced (Reid et al. 2008; Morin et al. 2009). It is, however, also important to include agent-related assessments in post-release evaluations for agents that have been established for more extended periods of time because correlating agent density and damage with changes in weed-related parameters, such as weed density, can serve as important evidence that the agent is the causal factor (Reid et al. 2008; Morin et al. 2009). For example, if the agent population and damage increase while weed density decreases, this serves as evidence that the agent is likely to have caused the change in weed density. If the agent density is not assessed, then the change in weed density could be attributed to another factor.

Weed-related measurements are recorded at the individual and population levels of the target plant. Individual-level parameters to be measured may include the size and number of flowers, fruits, and seeds, and the population-level may involve recording plant density, cover, and spread (Dhileepan and McFadyen 2001; Morin et al. 2009). Demographic studies that measure reproductive output and age structure of target weed populations and the response of the population to the agent are helpful in post-release evaluations and have been used for a number of biological control programmes in the past (Briese et al. 2004; Paynter 2005; Sutton et al. 2018). These studies can provide data on the life stage of the plant that should be targeted for the maximum impact on plant densities and could therefore help determine specific times when augmenting agent populations would maximize success (Raghu et al. 2006).

Ecosystem-related measurements are taken to evaluate changes to the state and functioning of an ecosystem due to the release of a biological control agent (Morin et al. 2009). These measurements include, but are not limited to, the diversity and abundance of native and invasive alien plants, productivity in agricultural or forestry systems, as well as ecosystem services such as water quality, and the rate of ecosystem processes such as nutrient cycling and decomposition (Schaffner et al. 2020). The change in ecosystem functioning after the decrease in weed density is ultimately the most important measure of biological control success because it is the primary reason why there is a need to control invasive alien plants in the first place (Schaffner et al. 2020). Measuring changes in ecosystem functioning is challenging, but there have been some recent studies that have done this (e.g., Motitsoe et al. 2020), and a greater emphasis on this level of post-release evaluation would be beneficial to the science and practice of weed biological control (Schaffner et al. 2020).

3.1.2. Post-release evaluation methods

There are several different experimental methods utilized to evaluate changes to agent, plant, and ecosystem-related parameters in post-release evaluations of biological control programmes (Morin et al. 2009). Some of these methods work better than others, some are only appropriate in certain situations depending on the agent-target weed system, and each design has its advantages and disadvantages (Reid et al. 2008; Schaffner et al. 2020). These methods include comparing sites (or plots) with and without agents, manipulative experiments, comparing before and after release data, and stakeholder surveys (Morin et al. 2009).

Comparing sites (or plots) with and without agents

Comparing sites or plots with and without agents is feasible when the agent is not widely dispersed and is present at some sites and not others (Dhileepan 2003; Morin et al. 2009;

Schaffner et al. 2020). These sites can be paired and replicated to measure agent, plant, and ecosystem parameters of interest (Carson et al. 2008). This method does not involve insecticides to exclude the biological control agents, and it allows the researcher to collect data easily and within a short space of time (Adair and Groves 1998). The results of this method can be affected by site features such as biotic or abiotic conditions that may differ between sites, so the impact of the agent could be under or overestimated due to these confounding factors (Adair and Groves 1998). Studies using this method are recommended to be undertaken on many sites over several years to minimize this possibility (Adair and Groves 1998). This method has been employed in the post-release evaluation of a stem-mining moth, *Carmenta mimosa* Eichlin and Passoa (Sesiidae), a biological control agent released against an invasive alien tree, *Mimosa pigra* L. (Mimosaceae) in Australia (Paynter 2005). The results of this comparative study indicated that there was a reduction greater than 90% of seed rain sites that had high population densities of *C. mimosa* compared to sites where *C. mimosa* was absent (Paynter 2005).

Manipulative experiments

Inclusion and exclusion experiments are two types of manipulative experiments used to evaluate the impact of biological control agents (Morin et al. 2009; Jones et al. 2018). An inclusion experiment involves releasing the agent into a closed caged area in the field. Inclusion experiment results may be misleading because the agent population densities could be greater in the cages than under normal conditions in the field (Morin et al. 2009; Jones et al. 2018). Exclusion experiments use insecticides, or fungicides if the agent is a plant pathogen, to exclude the agent from some plots, and compare these plots to those where the agent density is left unchanged (McClay 1995; Dhileepan 2003; Jones et al. 2018).

Field exclusion experiments provide more consistent and realistic results than other experiments (Morin et al. 2009). The use of insecticides may not be an appropriate method in areas with high rainfall or at remote sites because frequent re-applications of the insecticide will be needed to effectively exclude the agent (Paynter et al. 2006; Reid et al. 2008). The need to repeatedly apply insecticides makes insect exclusion experiments over long time periods, large areas, and multiple sites unfeasible and manipulative post-release evaluations more generally are time-consuming and expensive and therefore usually only conducted over small areas and over short periods of time (Morin et al. 2009; Jones et al. 2018).

Comparing before and after release data

Comparing before and after release data to assess the efficacy of biological control agents can be undertaken by comparing pre- and post-release measures of agent-, weed- and ecosystem-level parameters (Morin et al. 2009). Historic comparisons involve comparing historical and contemporary data of plant densities and distributions. These comparisons are most valuable if data were collected over multiple years, both before and after the release of the agent. In some cases, data may be intentionally collected to make before and after comparisons (e.g., Paterson et al. 2011a), but in other cases, there may be data collected for other reasons that may fortuitously be useful. For example, comparisons of historical maps of invasive alien plant distributions before release compared with maps created in the years after releases of biological control agents have been conducted for many invasive alien plants targeted for biological control in South Africa (Henderson and Wilson 2017). Another example is the post-release assessments for the biological control programme against diffuse (*Centaurea diffusa* Lam.) and spotted knapweed (*Centaurea* *stoebe* L.) (Asteraceae), which was conducted by comparing historical and contemporary vegetation cover data (Gayton and Miller 2012).

Stakeholder surveys

Stakeholder surveys evaluate the perceptions of the land-users or other stakeholders about the success of the biological control programme (Andreu et al. 2009; Morin et al. 2009). These surveys are usually completed before the initial release of biological control agents and then repeated years after the release to assess the social, environmental, and economic benefits of implementing biological control (Andreu et al. 2009; Morin et al. 2009). For example, surveys were used to evaluate the impacts and the decline in the problem status of ragwort, *Jacobaea vulgaris* L. (Asteraceae) after the release of the flea beetle *Longitarsus flavicornis* Stephens (Chrysomelidae) in Tasmania (Ireson et al. 2006). The participants of the survey perceived the weed as having an impact on agricultural productivity, and natural ecosystems, and the participants believed that the infestations of ragwort have been reduced after the release of *L. flavicornis* (Ireson et al. 2006). Surveys are a relatively easy and cheap way to evaluate the efficacy of biological control programmes compared to other methods, and are especially useful in assessing ecosystem-level impacts of agents, which are very difficult to measure directly. The results obtained from this method can however be misleading because it is based on people's perceptions and is therefore subjective and can be influenced by a variety of confounding factors.

The before-and-after method is particularly suited to cactus biological control evaluations because biological control agents for cactus weeds often result in control much quicker than agents for other weeds. Additionally, the biological control agents have limited dispersal capability, so there are many sites where the release of the agent can be made at sites that have never had the agent in the past. While comparisons of sites with and without the agent would be possible for assessing these biological control programmes, the before and after approach avoids the confounding influence of spatial variability in climatic conditions, which has a large impact on the biological control agents for cactus weeds as well as cactus populations themselves. Insect exclusion experiments are not feasible at multiple sites and over a reasonable period of time and can therefore not provide an assessment over as broad a geographic area or over as long a time period as before-and-after assessments. The influence of climatic variability over time is also incorporated into this study as assessments are continued for years after the release, so any variability of control over time should be recorded. Ideally, pre-release assessment would be made over several years to compare to post-release assessments, but this was not feasible for this study.

This study utilized the before and after release method to compare agent-related and plant-related parameters for biological control programmes against four of the invasive alien cactus species that were assessed in Chapter 2. Of the species covered in Chapter 2, *C. leptocaulis*, *H. balansae* and *H. pomanensis* have very few localities in the country that could be monitored and were therefore excluded from this assessment, while for *H. martinii* monitoring could not be conducted at enough sites for statistical analysis. *Cereus jamacaru* was excluded because there has been a recent post release evaluation conducted for this species (Sutton et al. 2018). As only agent and plant related parameters were included in this the study, the exclusion of ecosystem-level parameters in our assessment was dealt with through a stakeholder survey that was conducted concurrently and included an assessment of ecosystem-related parameters (Chapter 4). The aim of this study was to conduct post-release evaluations to quantify the success of biological control agents against specific cactus weeds in South Africa. These post-release evaluations justify the implementation efforts associated with the biological control of these cactus weeds and could be used to improve how biological control is implemented to maximise the levels of control that are achieved.

3.2. Materials and Methods

The CBC team conducts mass-rearing and releases of biological control agents for invasive alien cacti across South Africa. This study focuses on the post-release evaluation of four of the agents' mass-reared and released by the CBC on four invasive alien cactus species (Table 3.1). A pre-release assessment of weed populations was conducted at the time of the release of the agent at each site, followed by long-term monitoring to evaluate the success of the agent at each site. The first follow-up sampling events were at least 6 months after the release and sampling events were pooled within 6-month intervals (i.e. 0-6 months, 6-12 months, 12-18 months and 18-24 months) for *O. stricta* and *O. aurantiaca*, and year-long intervals for *C. imbricata* and *O. monacantha*. The difference in the time intervals was based on the frequency with which sites could be visited. All sites included in this study were situated in the south-west of the Eastern Cape Province near Rhodes University and the Kariega mass-rearing facility for logistical reasons (Fig 3.1).

Table 3.1 The four biological control agents and their target invasive alien plants that were mass-reared, released, and evaluated in this study.

Biological control agent	**Invasive alien cacti species**
Dactylopius austrinus	*Opuntia aurantiaca*
*Dactylopius opuntiae "*stricta"	*Opuntia stricta*
Dactylopius ceylonicus	*Opuntia monacantha*
*Dactylopius tomentosus "*imbricata*"*	*Cylindropuntia imbricata,*

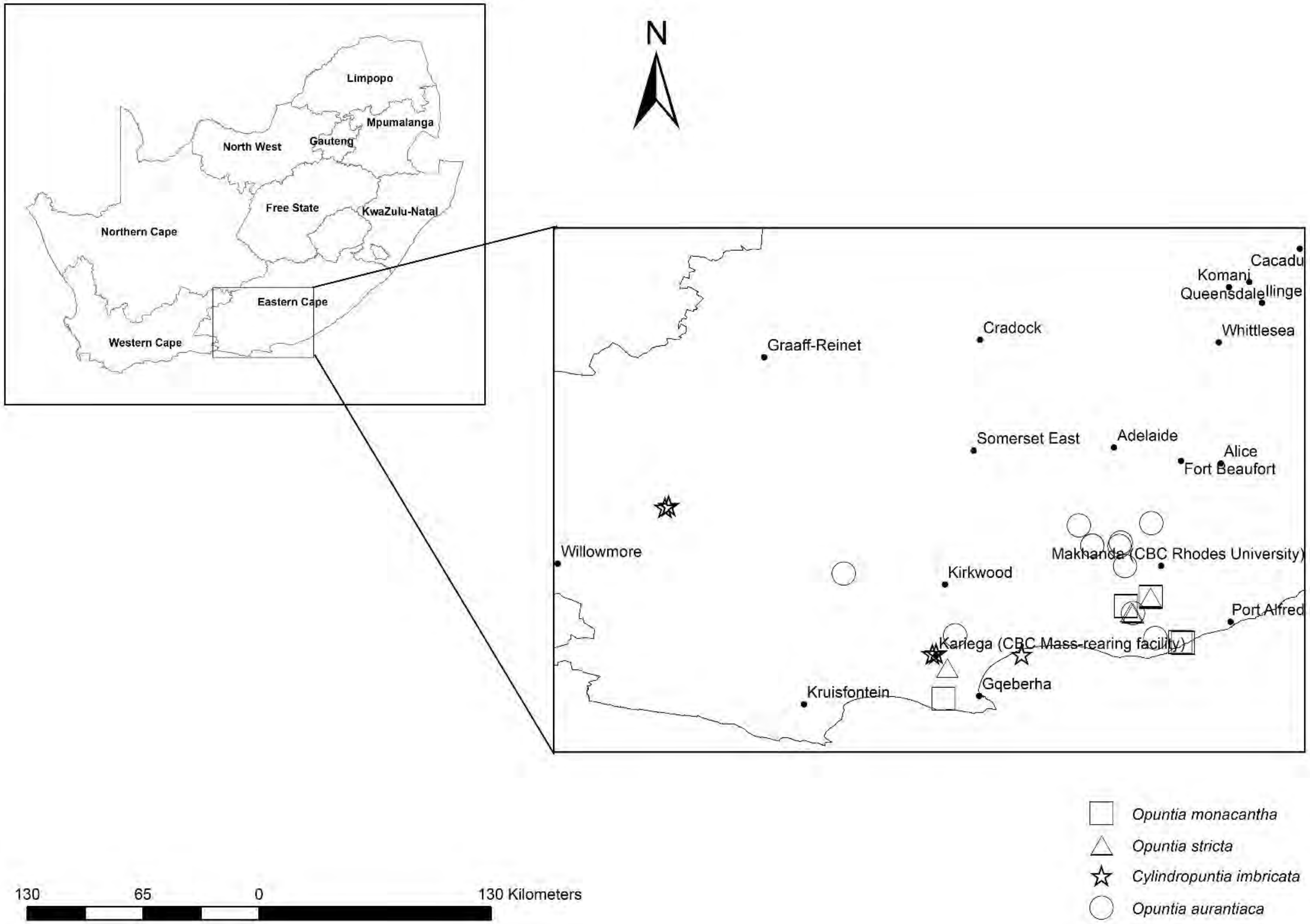

Figure 3.1 Map of South Africa showing the location of monitoring sites, CBC Rhodes University and the CBC Mass-rearing facility in the Eastern Cape

Slightly different monitoring protocols were adapted for each target weed. This was necessary because the agent and plant parameters measured for one cactus species are not always appropriate for others due to the mode of reproduction of the cactus species (i.e., whether seeds, or dislodged cladodes, or both, are important for reproduction), the plant architecture (i.e., whether it is possible or feasible to count the number of cladodes on a given plant or in a given area), and how the agent damages the plant (i.e., does it result in changes to plant size, reduce fruiting, kill small plants, or other means of damage).

Opuntia monacantha, Opuntia stricta* and *Cylindropuntia imbricata

Monitoring of all the above cactus weeds involved recording GPS co-ordinates of each tree selected for monitoring. Five trees were randomly selected across a wide area of the infestation and were at least 100 m apart from each other. The selected trees were permanently marked with labeled wooden poles hammered into the ground next to the base of the tree. In the event that a plant died completely, the pole would be used as the point where the plant was so that plant density could be recorded by measuring distances to the nearest plant. Parameters measured were the total number of cladodes, the number of fruits, and the number of cochineal-infested cladodes. The number of fruits was not recorded for *C. imbricata* because the fruits of *C. imbricata* do not contain viable seeds and are not important for reproduction or spread of the plant, but the height and width of the *C. imbricata* trees were recorded in addition to the number of cladodes and number of cochineal-infested cladodes. Tree average density (no. of individuals per area) was estimated by using the nearest/closest individual distance method, which is a plot-less technique (Cottam and Curtis 1956; Zhu and Zhang 2009). This method involves measuring the distances from each of the five marked trees to the ten individual trees nearest to each of them (Cottam et al. 1953). The mean distance was then calculated by dividing the sum of the distances by 50 (the number of trees multiplied by five, which is the number of permanently marked trees). A correction factor formula: MA = $2d^2$ was used to obtain the mean area (Cottam and Curtis 1956). Density is equal to the inverse of the mean area, and the output was converted to the number of plants per hectare (Elzinga et al. 1998). This was repeated for each monitoring event over time.

Opuntia aurantiaca

Two wooden poles were used to mark the beginning and end of three 20 m transects that were erected at each site. Each transect was separated by a distance of at least 100 m. A measuring tape was then placed between the two poles. A quadrat of 1 m^2 was placed at each meter distance along with the tape measure with 0.5 m^2 on each side of the tape, and the number of cladodes and cochineal-infested cladodes were counted within each quadrat. The number of cladodes and the number of cochineal-infested cladodes in each quadrat were allocated to a density category because it was not practical to count the number of cladodes in very densely infested sites and quadrats (Table 3.2). *Opuntia aurantiaca* densities were measured in cladodes per meter square rather than number of plants because, in dense infestations, it is not possible to see individual plants. The numbers of cladodes and cochineal-infested cladodes were counted up to 50 cladodes per m^2, and any density greater than this was allocated to category 4 (Table 3.2). This was repeated for each monitoring event over time.

Table 3.2 Categories of cladodes density and cochineal-infested cladode density used for post-release evaluations of *O. aurantiaca*

Categories	Number of cladodes per m^2
1	1-5 cladodes
2	5-20 cladodes
3	20-50 cladodes
4	50 plus cladodes

3.2.1. Data analysis

Data were analysed using the statistical programme STATISTICA version 13.5.0.17 (©TIBCO Software, Inc., USA, 2018). Repeated measures ANOVA were performed by general linear modelling (GLM) followed by Tukey HSD post-hoc tests to identify homogenous groups. Data are presented as comparisons between sampling events to facilitate statistical analysis.

3.4. Results

3.4.1. Post-release evaluations

A total of 26 sites were used for long-term monitoring. Initial measurements were taken at the time of release at all of these sites (first monitoring event). Second monitoring was conducted at 20 sites, a third at 15, a fourth at 17, a fifth at 13, and a sixth at three sites. Each of the monitoring events has a minimum interval of five months to one year after the previous monitoring event, but the length of time between each sampling event varies. This is because it was logistically possible to visit the sites during these times. *Opuntia monacantha* and *C. imbricata* had an interval a year for monitoring events; on the other hand, six months to a year were an interval for monitoring events *O. stricta* and *O. aurantiaca*.

3.4.2. Monitoring the impact of *Dactylopius ceylonicus* on *Opuntia monacantha*

Change in plant parameters over time after the releases

Monitoring was conducted at the time of release at five sites. Four sites have been monitored after six, 12 and 18 months from the time of release. Three sites were visited for monitoring after 24 months of releasing. Of the five sites where the CBC conducted releases and monitoring, two sites were near Kenton-on-sea, one site was situated in Gqeberha, and the last two sites were within the Salem area near Makhanda (Fig. 3.1). There were overall significant differences in the number of cladodes per plant between the sampling events ($F_{4.9} = 2.87$; $p = 0.027$; Fig. 3.2). There was a decline in the average number of cladodes after the release from 89 (± 12.7) to 32 (± 5.64) cladodes recorded at the last monitoring event (Fig. 3.2). The numbers of cladodes at the first monitoring event were significantly greater than all other monitoring events except for the second monitoring event (Fig. 3.2).

There were also overall significant differences in the number of fruits per plant between times of monitoring ($F_{4.9} = 7.84$, $p < 0.001$; Fig. 3.2). The number of fruits decreased from 135 (± 15.6) at the first monitoring event to 56 (± 15.1) after one year, this decrease continued to the next monitoring events and resulted in an average of 17 (± 7.4) fruits at the last monitoring event (Fig. 3.2). The average number of fruits per plant at the first monitoring was significantly greater than subsequent monitoring events (Fig. 3.2).

There were significant differences in the percentage of cochineal-infested cladodes per plant between monitoring events ($F_{4.9} = 5.85$, $p < 0.001$; Fig. 3.2). Four sites had cochineal insects on all of the trees, and only one site had no cochineal insects measured on any of the trees before the initial release. The percentage of cochineal-infested cladodes per plant was at the lowest percentage of 9% (± 2.5) at the beginning of monitoring before the releases (Fig. 3.2). This was significantly different from the second, third and fifth monitoring events, while cochineal densities were slightly lower in the fourth monitoring and this was significantly lower than the third monitoring event (Fig. 3.2). There was a significant increase in the percentages of cochineal-infested cladodes per plant in the second, third and fifth monitoring events. The percentage of cochineal infected cladodes was significantly higher in the final monitoring event compared with that measured at the time of release (Fig. 3.2).

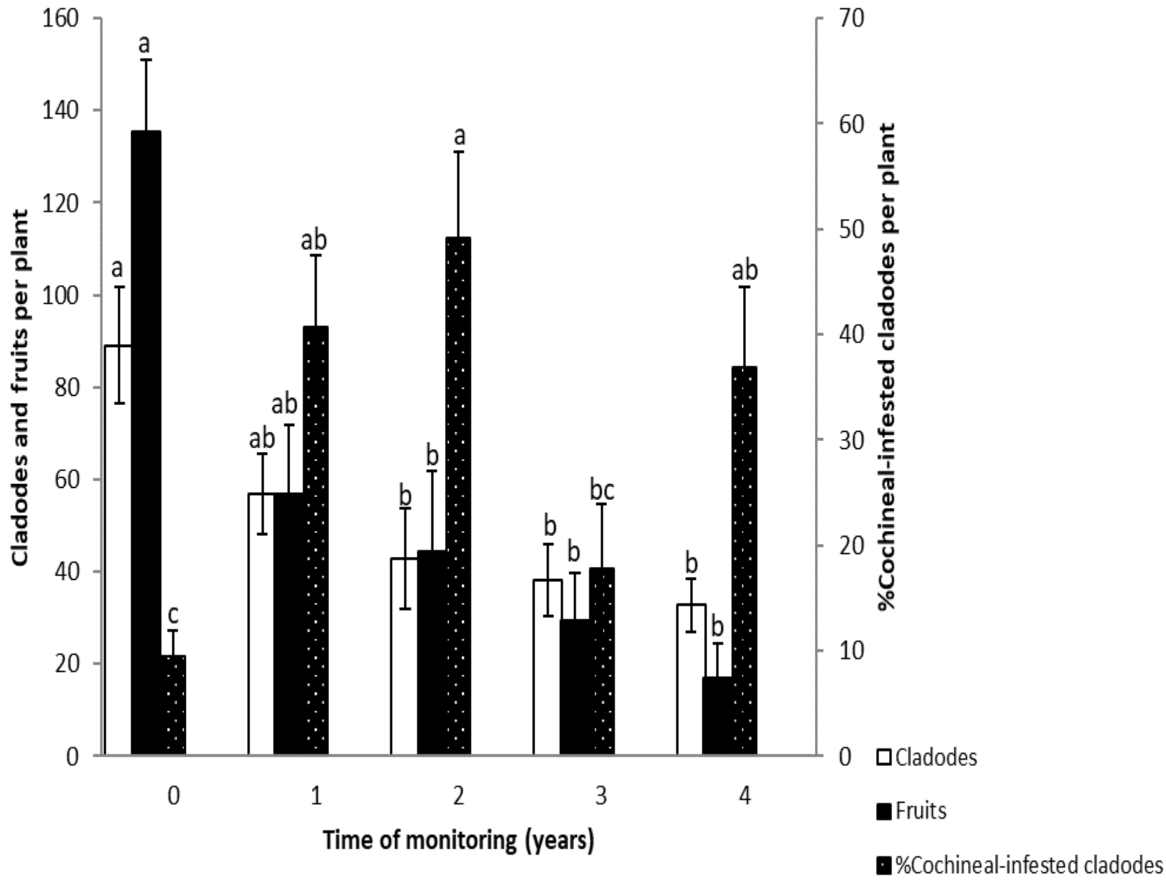

Figure 3.2 Mean number of cladodes, fruits, and percentages of cochineal-infested cladodes per plant between different monitoring events of *O. monacantha*. Letters indicate significant differences ($p < 0.05$) according to Tukey HSD post-hoc test. Error bars indicate the standard error around each mean.

Change in plant population over time after the release

Lindale had the highest average plant density of 5080 trees per ha compared to all other sites, while the lowest density of 595 trees per ha was recorded at Otter's Vlei (Table 3.3). Two of the five marked plants at Klipfontein and Otter's Vlei sites, and one at Lindale, died completely during the duration of the study (Table 3.3). It is important to note that other plants that were not marked were also killed by the cochineal and that this would impact the change in plant density. No measurable change in plant density was recorded at three of the sites, but a reduction in plant density was evident at Klipfontein, Lindale and Otters Vlei (Table 3.3).

Table 3.3 Actual change in plant/tree density per ha recorded during monitoring events for *O. monacantha* sites after the release of *D. ceylonicus*

Sites	***D. ceylonicus* present (1) /absent (0) during the initial release**	**Plant/tree density per ha during the third year of monitoring**	**Plant/tree density per ha during fourth year of monitoring**	**Actual change in plant/tree density recorded between monitoring events**	**Number of dead plants**
Klipfontein	1	3966	3785	181	2
Kragga-kamma	1	1647	1647	0	0
Lindale	1	5080	3950	1130	2
Otters vlei	0	595	560	35	1
Spring grove	1	2192	2192	0	0

3.4.3. Monitoring the impact of *Dactylopius opuntiae* "stricta" on *Opuntia stricta*

Change in plant parameters over time after the releases

Monitoring was conducted at the time of release at four sites. Three sites were visited between 6-12 months and 13-18 months after the release. Four sites were monitored after two and three years of releasing the biological control agent. Of the four sites, three sites were situated in the Salem area outside Makhanda, and one site was located outside Kariega (Fig. 3.1). There were overall significant differences in the number of cladodes per plant between monitoring events ($F_{4.8} = 3.03$, $p = 0.022$; Fig. 3.3). The average number of cladodes per plant was significantly reduced from 141 (± 19.3) per plant at the beginning of monitoring to an average number of cladodes of 35 (± 13.2) per plant during the final monitoring event (Fig. 3.3).

There were overall significant differences in the number of fruits per plant between monitoring events ($F_{4.8} = 4.01$, $p = 0.005$; Fig. 3.3). The first monitoring event had the greatest average number of 126 (± 20.1) fruits per plant, and this was significantly higher compared to the number of fruits per plant for all the subsequent monitoring events (Fig. 3.3). The average number of fruits per plant reduced drastically to 19 (± 6.8) fruits per plant after 6-12 months and remained low with no significant differences in subsequent sampling events (Fig. 3.3).

There were overall significant differences in the change of the percentages of cochineal-infested cladodes per plant between the different monitoring events ($F_{4.8} = 7.27$, $p < 0.001$; Fig. 3.3). Three of the sites had no cochineal on any of the plants prior to the releases, but only one of the sites had cochineal present. The percentage of cochineal-infested cladodes per plant increased from 13% (± 7.3) for the first monitoring to 53% (± 9.9) after 6-12 months but then reduced to levels not significantly different to those prior to release except for 18-24 months (Fig. 3.3).

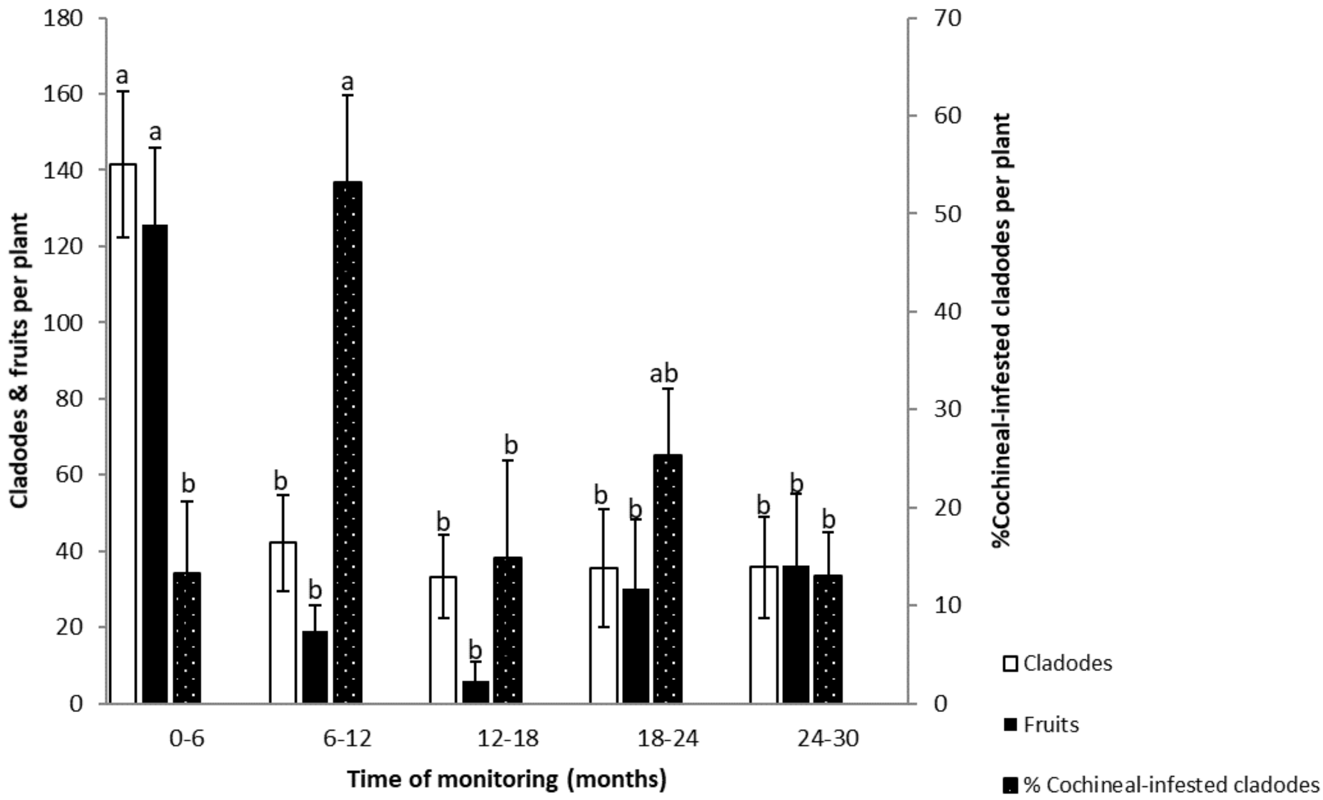

Figure 3.3 Mean number of cladodes, fruits, and percentages of cochineal-infested cladodes per plant between different monitoring events of *O. stricta*. Letters indicate significant differences ($p < 0.05$) according to Tukey HSD post-hoc test. Error bars indicate the standard error around each mean.

Change in plant population over time after the release

The greatest average plant density was recorded at Longfoot, and the lowest density was at Lindale (Table 3.4). There was a reduction in the average plant density only for the Khayamnandi site, where two marked plants were completely dead due to the damage by the biological control agent released (Table 3.4). There were no reductions in the average plant density at other sites that were monitored (Table 3.4).

Table 3.4 Actual change in plant tree density per ha recorded during monitoring events for *O. stricta* sites after the release of *D. opuntiae* “stricta”

Sites	***D. opuntiae* "stricta" present (1) / absent (0) during the initial release**	**Plant /tree density per ha during the period of 18-24 months of monitoring**	**Plant /tree density per ha during the period of 24-30 monitoring events**	**Actual change in plant/ tree density recorded between monitoring events**	**Number of dead plants**
Khayamnandi	0	3817	3331	486	2
Kikuyu Lodge	0	5887	5887	0	0
Lindale	0	243	243	0	0
Longfoot	1	13025	13025	0	0

3.4.4. Monitoring the impact of *Dactylopius tomentosus* “imbricata” on *Cylindropuntia imbricata*

Change in plant parameters over time after the releases

Monitoring was conducted at the time of release at six sites; three sites were visited in the first year after release, four sites in the second and third years, and two sites in the final year of the study. Four of these sites were situated in Kariega, and two sites were located near Willowmore (Fig. 3.1). There were no overall significant differences in the average change of plant height ($F_{4.9} = 1.61$, $p = 0.179$; Fig. 3.4). The average plant height per plant was reduced from 176 (± 7.3) per plant at the beginning of monitoring to 151 (± 13.4) per plant at the last monitoring event, but this was not statistically significant (Fig. 3.4).

There were overall significant differences in average plant width over time ($F_{4.9} = 7.04$, $p = 0.178$; Fig. 3.4). The average plant width per plant of 158 (± 8.6) recorded for the first monitoring event was significantly greater than the average plant width per plant of 97 (± 23.8) in the fourth year of monitoring (Fig. 3.4). Overall, plant width reduced slightly during the years of monitoring after the biological control agent release compared to the first monitoring event at the time of release (Fig. 3.4).

There were no significant differences in the number of cladodes during the years of monitoring events, but there were reductions in the average number of cladodes after the release of biological control agents ($F_{4.9} = 1.61$, $p > 0.001$; Fig. 3.4).

The percentage of cochineal-infested cladodes had overall significant differences between mean percentages recorded during different years of monitoring ($F_{3.6} = 16.9$, $p < 0.001$; Fig. 3.4). Three of the sites had no cochineal on any of the plants prior to the releases conducted by the CBC, and three sites already had cochineal present. There was a significant increase in the percentage of cochineal-infested cladode from less than 5% (± 0.4) at the beginning of monitoring to 34% (± 7.6) recorded two years after release (Fig. 3.4). There were no significant changes in the percentage of cochineal-infested cladodes in subsequent years of monitoring, but the percentage of cochineal-infested cladodes remained above an average of 30% (Fig. 3.4).

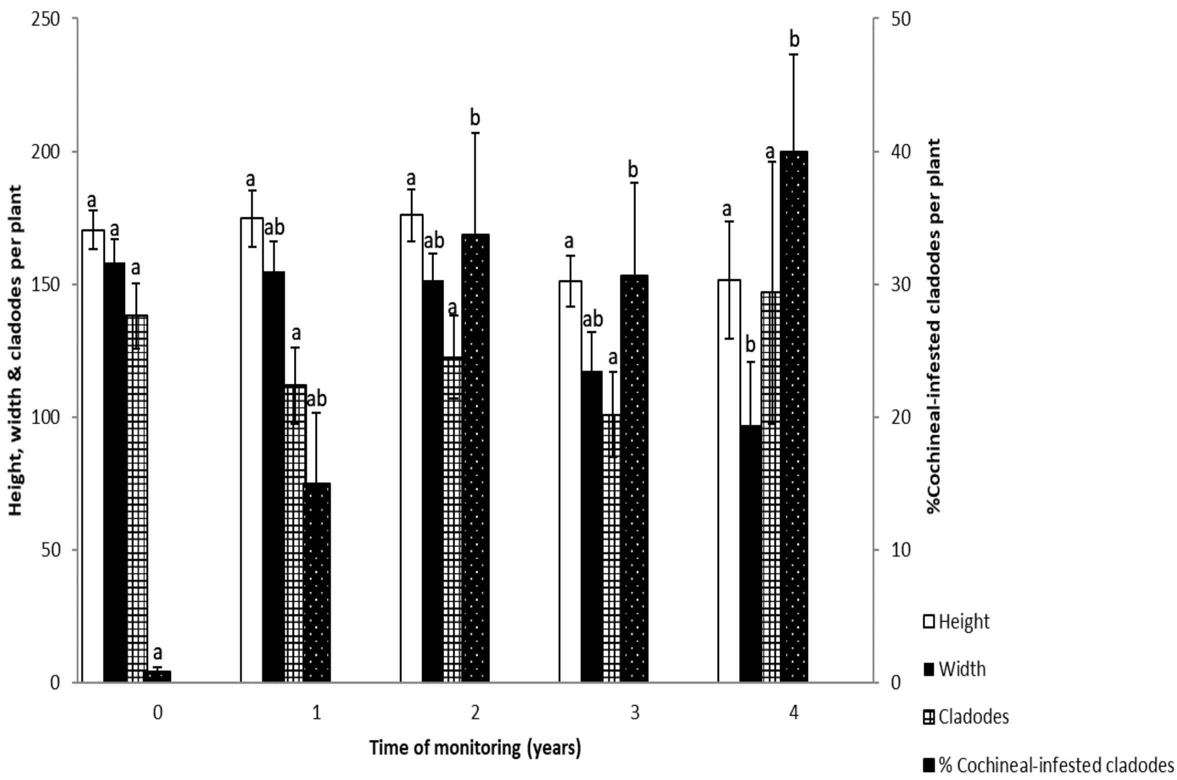

Figure 3.4 Mean measurements of plant height, plant width, number of cladodes, and percentage of cochineal-infested cladodes per plant between different monitoring events of *C. imbricata*. Letters indicate significant differences ($p < 0.05$) according to Tukey HSD post-hoc test. Error bars indicate the standard error around each mean.

Change in plant population over time after the release

Plant densities for *C. imbricata* ranged from 61 to 9542 with an average density of 2337 (SE ± 1487) across all sites. No changes in plant density that were recorded over the period of this study and none of the marked plants died during the study.

3.4.5. Monitoring the impact of *Dactylopius austrinus* on *Opuntia aurantiaca*

Monitoring was conducted at the time of release at eleven sites; ten sites were visited after 6-12 months, four sites were monitored two years after the release, five sites after 30 months, and four sites after three years, and there were three sites monitored in the final monitoring event. Five of these sites were located in the Riebeek East area; three sites were around the Salem region (both Riebeek East and Salem areas are outside Makhanda), two of the sites were outside Kariega, and the last site was situated south of Makhanda (Fig. 3.1). There were overall significant differences in the change in the number of cladodes per m^2 between the monitoring events ($F_{5.2} = 31.9$, $p < 0.001$; Fig. 3.5). The number of cladodes per m^2 was reduced significantly between monitoring events with an overall change from an average number of cladodes of 31 (± 0.78) per m^2 to 18 (± 1.5) per m^2 (Fig. 3.5). The first monitoring event, when the agents were released, had the highest average number of cladodes and it was significantly higher compared to all other monitoring events (Fig. 3.5). There was a significant reduction in the number of cladodes per m^2 from 6-12 months to 18-24 months monitoring events (Fig. 3.5). In the last two monitoring events, there was no significant reduction in the number of cladodes per m^2, but the average remained low compared to that of pre-release densities (Fig. 3.5).

There were overall significant differences in the percentages of cochineal-infested cladodes per m^2 between the different monitoring events ($F_{4.3} = 63.4$, $p < 0.001$; Fig. 3.5). All sites had some cochineal presence at the time of the release with an average percentage of 10% (± 0.85) cochineal-infested cladodes per m^2; this percentage was significantly lower than monitoring at 6-12 months and 30-36 months after the release (Fig. 3.5). There was a significant increase in the percentage of cochineal-infested cladodes during 6-12month monitoring event, with an average of 30% (± 1.26) cochineal-infested cladodes per m^2 (Fig. 3.5). This increase was followed by a drastic decrease of almost half in the percentage of cochineal-infested cladodes per m^2 for the two subsequent monitoring events to 10% (± 1.42) for 18-24 months (Fig. 3.5). At the three-year monitoring period, there was an increase in the percentage of cochineal-infested cladodes per m^2 that was statistically different from other monitoring events except for the 12-18 months and 36-42 months monitoring events (Fig. 3.5). The last monitoring event was significantly lower in the number of cladodes and percentage of cochineal-infested cladodes per m²than the second monitoring event at 6-12 months but similar to all other monitoring events, including the first monitoring event prior to the release of the biological control agent (Fig. 3.5).

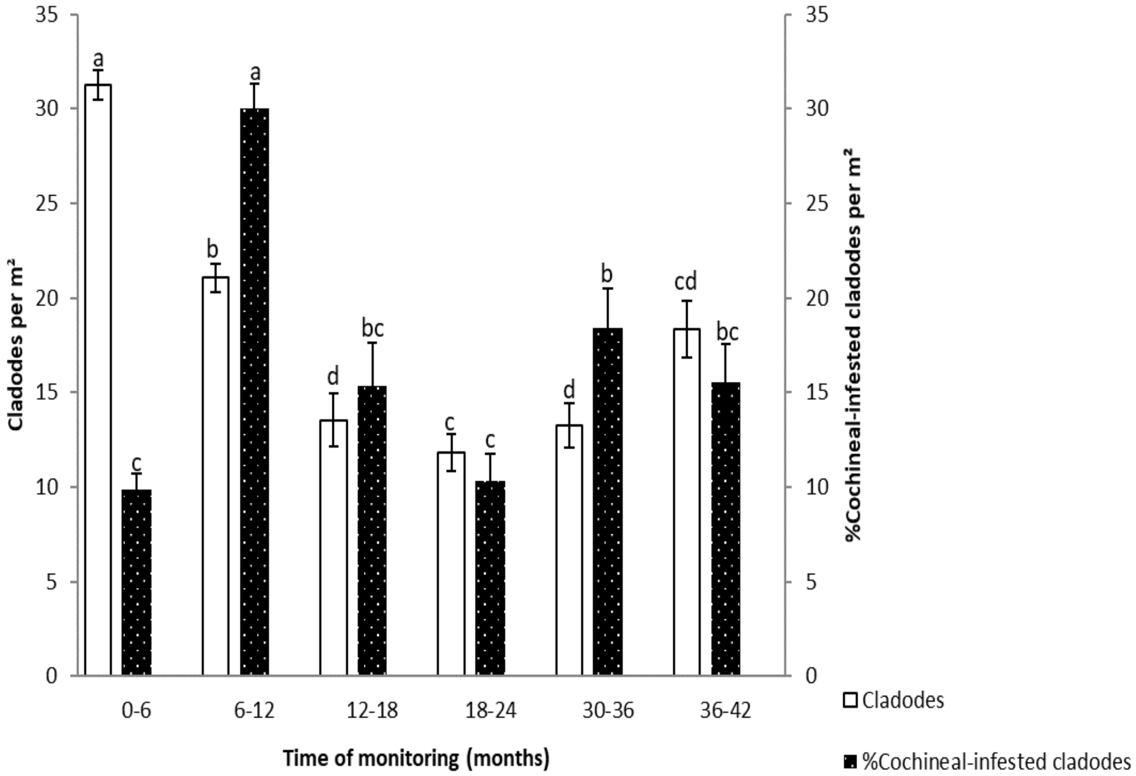

Figure 3.5 Mean number of cladodes and percentages of cochineal-infested cladodes per m^2 between different monitoring events of *O. aurantiaca*. Letters indicate significant differences ($p < 0.05$) according to Tukey HSD post-hoc test. Error bars indicate the standard error around each mean.

3.5. Discussion

In the majority of cases, there was an increase in the amount of cochineal at the site after the release, which was associated with a decrease in plant parameters such as the number of cladodes and fruits per plant. The fact that in most cases, the change in plant parameters followed an increase in cochineal densities of the four cactus weeds is evidence that the changes in plant parameters are due to the impact of the agent and not another confounding factor (Morin et al. 2009). *Cylindropuntia imbricata* was the least impacted species, with only a small reduction in width and no statistically significant reduction in other parameters, while all other species had significant reductions in biomass and reproductive output measured as the number of cladodes and fruits. These data, therefore, generally support the conclusion that biological control is effective at reducing cacti populations to some extent.

The biological control programme against *O. monacantha* using *D. ceylonicus* was the first successful biological control programme against an invasive alien plant in South Africa (Moran et al. 2013; Lounsbury 1915). This programme is well-known and still remembered in South Africa, even though *O. monacantha* is no longer a prominent problem in most parts of the country (Hill et al. 2020). The results of this post-release evaluation confirm that biological control of *O. monacantha* using *D. ceylonicus* is very successful and damages *O. monacantha* extensively within a short period of time and that the control is permanent. It also confirms that releases of the agent improve control. There was a small reduction in the density of *O. monacantha* plants over time, and this is expected to decrease further in the future as more plants die completely. It is important to take note that the density of *O. monacantha* at these sites was likely to have increased over this time period without the biological control agent, so the agent is not only reducing the density from the baseline that was measured during the study but stopping the weed from increasing in density (Hoffmann et al. 2019; Moran et al. 2021). The reduction in the number of fruits produced also reduces the chances of the plant spreading to new sites.

Dactylopius opuntiae “stricta” contributed extensively to reducing the biomass and reproductive output of *O. stricta* in the Eastern Cape Province at the sites selected for this study. The number of cladodes was reduced after the releases and remained low until the study was terminated. The slight increases in the number of fruits at the third and fourth monitoring events were not significant and were not greater than the number of fruits recorded before the release of *D. opuntiae* “stricta” at the first monitoring event. The slight increase in fruits is probably because of climate at the time of releasing *D. opuntiae* “stricta” on *O. stricta*. There is no set fruiting season, but the plants respond to heat and rain, so the number of fruits will vary between sampling events but at a much lower average than prior to the introduction of biological control agents.

The results of this study are comparable to the long-term monitoring of *D. opuntiae* “stricta” against *O. stricta* in the KNP (Hoffmann et al. 1999, 2020). The total area infested by *O. stricta* was approximately 30 000 ha, and it was spreading quickly before the release of *D. opuntiae* “stricta” (Foxcroft and Hoffmann 2000; Foxcroft and Hoffmann 2003). Overall the average number of cladodes was reduced by about 90% in the KNP (Lotter and Hoffmann 1998; Foxcroft and Hoffmann 2003; Hoffmann et al. 2020). There have been no fruits produced in the monitoring plots in the KNP since 2005 due to the impact of the agent (Lotter and Hoffmann 1998; Foxcroft and Hoffmann 2003; Hoffmann et al. 2020). In comparison to the KNP, in the Eastern Cape, the number of cladodes has reduced by about 25% and the number of fruits by 33%. Biological control is therefore considerable less damaging to *O. stricta* in the Eastern Cape than in the KNP.

In the past, biological control of *O. stricta* was generally considered to be less effective in the Eastern Cape than in KNP due to climatic conditions (Paterson et al. 2011b), but this study has shown that the difference in impact is considerable. Comparatively low temperatures in the Eastern Cape may have resulted in either lower levels of control or control being achieved over a longer period of time. A study of the thermal physiology of the *D. opuntiae* “stricta” cochineal is recommended to evaluate the effect of temperature in the biological control of *O. stricta*. Although there is some evidence that control in the KNP was slightly better than in the Eastern Cape, the agent was still effective, reducing biomass and reproductive output, and there is evidence that plant densities have started to reduce and will hopefully continue to reduce in the future.

The efficacy of *D. opuntiae* “stricta” could also have been influenced by the high densities of *O. ficus-indica* with *D. opuntiae* “ficus” cochineal found at the sites selected for this study (Hoffmann et al. 2002; Hoffmann 2004). The results of this study indicated that there was limited biological control success for *O. stricta* at Longfoot, where the “ficus” cochineal was abundant. Hybridization between the two cochineals is likely to result in reduced efficacy if the incorrect cochineal is more abundant. It is recommended that releases of *D. opuntiae* “stricta” should only be done where the *D. opuntiae* “ficus” is not present, thus directing release efforts to areas where there will be the greatest impact (Hoffmann et al. 2002). Alternatively, augmentative releases could be implemented at *O. stricta* in areas where both cochineals occur to increase the populations of *D. opuntiae* “stricta” and improve the level of control. Genetic techniques are required to determine whether the correct cochineal is present at these sites, as the cochineal insects are morphologically identical (van Steenderen et al. 2020).

The impact of *D. tomentosus* "imbricata" on *C. imbricata* was less evident over the time of this study than for the other cactus weeds. This may be due to the structure of the plant, which differs from the other targets in that it consists of a large woody trunk up to 25 cm in diameter (Mokni et al. 2020; Maroyi 2017). The trunk of the plant is a source of energy to regenerate from after cochineal damage, and *D. tomentosus* "imbricata" does not feed on the woody part of the plant, so after plants have been defoliated by the cochineal, the cochineal population declines, and the plants can regenerate new cladodes from the unharmed woody stem. This case is comparable to the post-release data collected for *Dactylopius tomentosus* 'cholla' released against *Cylindropuntia fulgida* var. *fulgida* (Cactaceae) at a site located in Douglas, Northern Cape (Klein et al. 2020). The agent was effective in damaging detached cladodes and small plants, but an additional mechanical intervention was required for large, old, and woody plants (Klein et al. 2020). Ten plants were felled as an integrated approach; seven of these plants were completely dead after three years, and the three remaining plants only had an average of eight cladodes each, all of which were expected to die in time due to cochineal damage (Klein et al. 2020). The plants that were not felled, lost cladodes but there were new cladodes on the woody trunk of the plant during the wet season, and the plants did not die (Klein et al. 2020).

A similar, integrated control strategy is suggested for *C. imbricata*, where the larger trees are felled once they are covered in high densities of cochineal (Moran and Zimmermann 1991a). This integrated control method is expected to result in complete control, but long-term monitoring at these sites should continue after large *C. imbricata* trees are felled in order to evaluate the efficacy of this integrated method. The results of the study showed that *D. tomentosus* "imbricata" populations increased, then stabilised, and did not seem to be increasing more from when 25% of all the cladodes were infected with cochineal. This indicates that cochineal-infested trees should be felled between 18 and 24 months after the release of the agent as the agent population is unlikely to increase further from this point. It is important to note that the study marked large trees for measuring parameters and did not include the thousands of small plants which often grow directly under the large trees. Many of these small plants were killed during this study, but this parameter was not measured. The long-term monitoring protocol used in this study could be improved by including these plants in the evaluation of the impact of biological control in the density of *C. imbricata*.

Augmentation of *D. austrinus* populations appears to be useful for the control of *O. aurantiaca* based on the results of this study. Releases resulted in a decreased average density of *O. aurantiaca* in subsequent monitoring events. However, the results also indicated that the increase in cochineal densities the following release was temporary. The length of time that high population densities of the agent are present, as well as the impact that this has on the target weed populations, is most likely impacted by climatic conditions, but the population densities of cochineal did eventually reduce after an initial increase following release for all sites. This suggests that further augmentative releases may be useful in further reducing *O. aurantiaca* populations, especially if these releases are done during times of favourable climatic conditions (i.e., hot and dry periods).

There was also significant variability between sites in the level of control of *O. aurantiaca.* This was most likely due to release site conditions such as temperature, precipitation (including mist), and importantly, the amount of shade (Shea and Kelly 1998). These factors are important, and it is recommended that these should be looked into further because this study did not investigate them directly. Continuous assessments of agent and weed densities may provide important information about when to conduct augmentative releases for *O. aurantiaca* control. Sometimes releases fail due to poor climatic conditions just after the release, such as cold temperatures and heavy rainfall. In these cases, additional releases should be conducted to improve establishment success. The impact of *D. austrinus* is dependent on climatic conditions; this agent finds hot, dry conditions and temperatures between 25°C to 30°C favourable for its growth, performance, and reproduction (Hosking 1984b; Zimmermann 1981). If multiple releases do not result in an increase in cochineal after six months on multiple occasions, then releases should be stopped at that site. It is then recommended that field studies should be conducted to understand the driving factors to the failure at those sites so that releases techniques can be improved in the future. It is, however, evident from these data that *D. austrinus* is extremely advantageous in reducing *O. aurantiaca* densities and augmentative releases of *D. austrinus* contribute to achieving the desired control of *O. aurantiaca*.

The results of this study were from data collected by measuring agent-related parameters and weed-related parameters before and after release. These were the two parameters feasible to be measured and recorded during the timeframe of the study. It was not possible to directly measure ecosystem-related parameters over this short time period. Measuring changes in ecosystem-related parameters such as the recovery of agro-ecosystems and indigenous biodiversity years after releasing biological control agents would however be valuable data. The questionnaire study (Chapter 4) was designed to provide information on ecosystem-related parameters, but direct measurement of these parameters would be a beneficial long-term study.

The releases of biological control agents are effective in reducing the infestations of invasive alien cacti in the Eastern Cape. Although there is significant variability between sites, there is evidence that, in general, the release of the agents has been beneficial to some extent for all the target weed species and indications that the level of control will improve further in the future for some species. This study also provided evidence that for some agents, it is worth conducting additional releases even if the biological control agent is already present in the field in order to augment the agent population. This is moving away from classical biological control to augmentative biological control strategy and treating the agents more as green herbicides than classical biological control agents. Post-release evaluations are important for understanding the factors that contribute to the failures and successes of biological control programmes. The success for biological control of invasive alien cacti is optimised when mass-releasing, releasing large quantities of biological control agents, and post-release monitoring are involved in the management strategy. Long-term monitoring should continue to collect more data and quantify the benefits and the impact of biological control.

CHAPTER 4: EVALUATING LAND-USERS PERCEPTIONS ABOUT BIOLOGICAL CONTROL OF INVASIVE ALIEN CACTUS WEEDS IN SOUTH AFRICA

4. Introduction

4.1. The negative impacts of invasive alien plants on human communities

Most ecologists regard invasive alien plants as a threat to agricultural productivity, water security, and indigenous biodiversity (Wilcove et al. 1998; Perrings et al. 2002). Invasive alien plants constraint agriculture by reducing the carrying capacity of rangelands for livestock, outcompeting grasslands that are productive for grazing, blocking access to grazing and water points, and reducing the availability of water (Pimentel et al. 2005; Vilà et al. 2011; Le Maitre et al. 2020). The opinion of the general public is often different to scientists, but in the case of invasive alien plants' negative impacts, there is good evidence that the public agrees that invasive alien plants have negative impacts (Witt et al. 2019). Public opinion surveys have indicated that there is a perception that invasive alien plants have negative impacts, which include: threatening native biodiversity, reducing livestock and crop production, reducing the mobility of people, reducing water quality and quantity, and increasing the risk of fire (Potgieter et al. 2019; Shackleton et al. 2019b). In Kenya, agricultural land has been heavily infested by the invasive alien plant *Prosopis juliflora* (Sw.) DC. (Fabaceae) which creates impenetrable thickets to the extent that the infested area cannot be used for crop cultivation (Shackleton et al. 2014). The majority of local people from three villages perceived *P. juliflora* as a bad invasive plant and that their livelihoods would be better without the presence of the plant (Maundu et al. 2011). The community members in some areas of Sri Lanka considered *Prosopis* sp. infestations to have a negative impact on livelihoods through reduced areas for grazing lands and the thorny branches being harmful to humans and animals (Perera et al. 2005). There are also negative implications of alien invasive plants directly to society. A survey study conducted in the Eastern Cape, South Africa, reports that 41% of local people noted that invasion by Australian *Acacia* species provides hiding spots for criminals, and 52% of the community members stated that they feared walking in the forests of invasive alien plants, especially females who are often responsible for firewood collection (de Neergaard et al. 2005).

Invasive alien cacti in South Africa threaten indigenous flora and fauna, are harmful to humans and animals because of the sharp spines and are particularly problematic as contaminants of wool (Kaplan et al. 2017). Land-users who suffer from these negative impacts indicated that they would like the weeds to be controlled because the weeds do not provide any benefits and are harmful (Kaplan et al. 2017; Shackleton et al. 2017b; Novoa et al. 2019). For example, all villagers from Tidbury in the Eastern Cape of South Africa who participated in a study identified *O. aurantiaca* as a threat to livestock and human beings (Shackleton et al. 2007). They reported that children are vulnerable to being harmed, particularly when they are playing barefoot; and sometimes livestock suffered injuries that resulted in mortality due to *O. aurantiaca* (Shackleton et al. 2007). The villagers have collected, dried, and burnt the plant in an attempt to control it, but this was not successful because the plant grew back in heavy infestations, so they gave up on their efforts (Shackleton et al. 2007).

4.1.2. Control of invasive alien plants

Biological control, chemical control, and mechanical control are different measures used either individually or in combination to manage invasive alien plants and reduce their negative impact (Adriaens et al. 2018; Wittenberg and Cook 2005). Most land-users utilise chemical and mechanical control methods compared to those who have used biological control for the control of invasive alien plants (Aigbedion-Atalor 2020). The majority of land-users have limited information about biological control and do not fully understand how it works (Groote et al. 2003). For example, a questionnaire study was carried out in Southern Benin to evaluate the impact of the biological control agents, *Neochetina eichhorniae* Warner *and Neochetina bruchi* Hustache (Curculionidae), which are used for the control of water hyacinth, *P. crassipes* reported that out of 362 participants in the study, only 86 participants were aware of the agents, 68 understood the role of the agents in control of the invasive alien plants, and only 54 were able to recognize the damage caused by the agents (Groote et al. 2003). Some land-users who utilize mechanical control and apply herbicides to contain invasive alien plants on their land could be using biological control, which in some cases is known to be much less expensive and more effective (Day and Witt 2019). The reason why these land-users have not utilised biological control is most likely due to a lack of awareness.

One of the most important misconceptions and misunderstandings of biological control, and one of the reasons why the public is sceptical of the use of biological control, is a fear of non-target effects (Downey and Paterson 2016; Hinz et al. 2020). Despite the excellent track record and evidence of efficacy and safety of weed biological control (Barratt et al. 2018; Hinz et al. 2020; Paynter et al. 2020), there are still people who are reluctant to use biological control because they have concerns about risks and safety of non-target plants (Havens et al. 2019). Public support is important so that biological control implementation can continue because public opposition sometimes results in delays or cancellations of biological control releases (Warner et al. 2008; Bean and Dudley 2018). It is crucial to educate about biological control because the method has often been misunderstood and criticised by land-users (Barratt et al. 2018; Messing and Brodeur 2018). Although some scepticism around the release of new biological control agents is understandable, agents which have been tested and have proven to be host-specific and beneficial for many years after release, pose absolutely no risk to land-users who may benefit from utilizing them.

4.1.3. Public knowledge and perception of weed biological control

Public awareness is recommended as an essential tool to increase general knowledge about available control methods, like biological control, which some land-users are not familiar with (Bremner and Park 2007; Marchante et al. 2010). Biological control is more complicated than other control methods in the sense that it works over a long period of time, does not get rid of the weed completely, and some people do not understand how it works; hence some land-users believe that it is not effective in controlling invasive alien plants (Barratt et al. 2018). A knowledgeable public is a very strong vector in helping to avoid further introductions, minimize the dispersal, and support management actions of weeds (Bremner and Park 2007; Garcia-Llorente et al. 2008; Reis et al. 2013). A number of tools have been recommended to raise awareness, for example, developing websites or printed documents such as handouts, giving school talks or holding public events, educational workshops, screening short educational films, and publishing popular science articles (Novoa et al. 2017b).

Public perception of invasive alien species has been neglected compared to the attention given to the ecological and economic impacts of invasive alien species (Garcia-Llorente et al. 2008; Schüttler et al. 2011). Out of 9192 interdisciplinary studies on invasion science conducted between 1950 and 2014, only 3% of the studies connect social and ecological science (Vaz et al. 2017). Public opinions about invasive alien species can differ due to a number of factors, such as socio-demographic features, conflicts of interests, as well as background and prior knowledge about the problem (Bremner and Park 2007). These factors can either have negative or positive implications on the control and management of invasive alien species (Woodford et al. 2016). Understanding different public opinions is an important component of successful management (Andreu et al. 2009; van Wilgen 2012c; Potgieter et al. 2019). It is important to understand these opinions for decision-making and formulating management strategies because it is likely to reveal conflicts of interests between stakeholders, as well as areas and species that require urgent control (Bennett and van Sittert 2019; Potgieter et al. 2019).

4.1.4. Questionnaire surveys

One of the techniques used to understand social components of invasive alien species and their management is through conducting questionnaires-based surveys (Andreu et al. 2009). Questionnaires have been successfully used to evaluate public perceptions about the perceived impact of invasive alien plants and control (Bremner and Park 2007; Garcia-Llorente et al. 2008). While there have been many studies that investigate whether invasive alien plants are considered problematic or beneficial (e.g., Ngorima & Shackleton, 2019) and others access the need for control (Ireland et al. 2019), there are relatively few questionnaire studies that have been used to assess the success of management interventions, or on biological control in particular (McNeil et al. 2010; Hart and Larson 2014; Voukeng et al. 2019). Questionnaires can, however, be used to quantify the impact of the targeted invasive alien plant before and after the release of biological control agents; they are an easy and inexpensive way to collect qualitative data to evaluate the efficacy of biological control agents on target weeds (Morin et al. 2009). For example, in Tasmania (Australia), respondents who participated in a questionnaire study indicated that there was a decline in the threat of European blackberry, *Rubus fruticosus* L. (Rosaceae), and common ragwort, *Jacobaea vulgaris* Gaertn. (Asteraceae) after the release of biological control agents (Ireson et al. 2006). Questionnaires have been utilized to evaluate biological control success from the perceptions of participants. These surveys serve to gather information received from participants before and after the release of biological control agents (White et al. 2005; Bardsley and Edwards-Jones 2006; Morin et al. 2009).

However, the data collected from surveys can be misleading, as it is ultimately the opinion of the land-user that is providing evidence for the level and benefits of control, and this opinion can be affected by factors other than the success of the agent. For example, land-users have expectations from biological control; they want it to show quick results and get rid of the target weed within a few months after the release. Land-users get disappointed when desired and expected results are not achieved because biological control generally takes a long time before clear impacts are noticeable and because it does not result in the complete eradication of the target weed, even when complete control is achieved. This does not mean that the biological control agent has failed. In contrast, land-users who do not expect biological control to be effective may be very positive about even very small impacts from biological control agents. So, what is expected from biological control can have a large influence on how land-users perceive successful biological control, and this can be influenced by their knowledge of how biological control works and the length of time it takes for control to be achieved. Additionally, land-users' opinions may change over time, and the perceived density of the target weed may change due to changes in perception rather than the damage inflicted by biological control agents (Bardsley & Edwards-Jones 2006, 2007). For example, land-users often forget the successes of biological control (Zimmermann and Moran 1991) but will always remember the failure if the agent does not achieve the control of the target invasive alien plant on their land (Delfosse 2005). It is, therefore important that questionnaire survey studies should be conducted in conjunction with field-based quantitative studies to support and compare results (Bardsley and Edwards-Jones 2006, 2007).

When conducting questionnaires, it is important to identify the targeted participants in order to know the type of questionnaires that should be used and how to evaluate them (Desai and Potter 2017). The selected participants should be composed of groups of people to obtain different perceptions based on their own experiences and context (Desai and Potter 2017). Surveys can be done through **1) Face-to-face surveys,** which involve an in-person meeting with the investigator and the participants in a public space, the home of the participant, or at a community meeting, such as farmer and land-user meetings (Kelley et al. 2003; Doyle 2005). Face-to-face surveys make it possible for the investigator to explain the questions in detail and provide examples so that the participant understands the question, and this influences the responses provided by the participants (Fowler 2014). This method has a high response rate compared to other survey methods, but it is the most costly and time-consuming (Kelley et al. 2003; Doyle 2005). **2) Postal surveys** involve sending surveys to a large sample of participants located in different geographical areas (Kelley et al. 2003).

This method is cheaper than telephone surveys, but it has low response rates in comparison to other methods, and questions cannot be asked by the participants and answered by the investigator, but this also means that the investigator has less influence on what responses are given, which could be beneficial (Kelley et al. 2003). **3) Telephone surveys** provide the investigator access to the targeted population easily (Groves 1989). This approach yields a higher response rate than postal surveys but has a higher number of people who do decline to partake in the survey compared to face-to-face surveys (Kelley et al. 2003; de Rada 2011). **4) Online or email surveys** are similar to postal surveys and do not require the investigator to be present to conduct the questionnaire survey (Kellner, 2004). It allows participants to complete the survey in their own time and at a venue convenient for them (Kellner 2004).

This study aims to evaluate the efficacy of cactus weed biological control as perceived by land-users in South Africa through questionnaire surveys. This was conducted through a mixture of face-to-face, telephone, and email surveys. This study will be used as a post-release evaluation tool to assess the efficacy of the biological control programme against cactus weeds as perceived by land-users, as a way to i) quantify benefits of biological control to the community, ii) investigate if there is a need for educational awareness in biological control and, iii) assess whether communities of land-users who utilise biological control in South Africa perceive it as a safe control practice.

4.2. Materials and Methods

4.2.1 Study area

Biological control agents released for the management of invasive alien cactus species were provided by the CBC to the participants of this study. The sites where the participants were land-users, and where the agents were released cover all nine of the provinces of South

Africa (Fig. 4.1). The biological control agents were mass-reared at the national mass-rearing facility located in Kariega in the Eastern Cape Province. The CBC provided biological control agents for cactus weeds free of charge to the land-users because the government sponsored the mass-rearing and releases of agents as a public good through the Department of Forestry, Fisheries and the Environment (DFFE): Natural Resource Management (NRM) programmes.

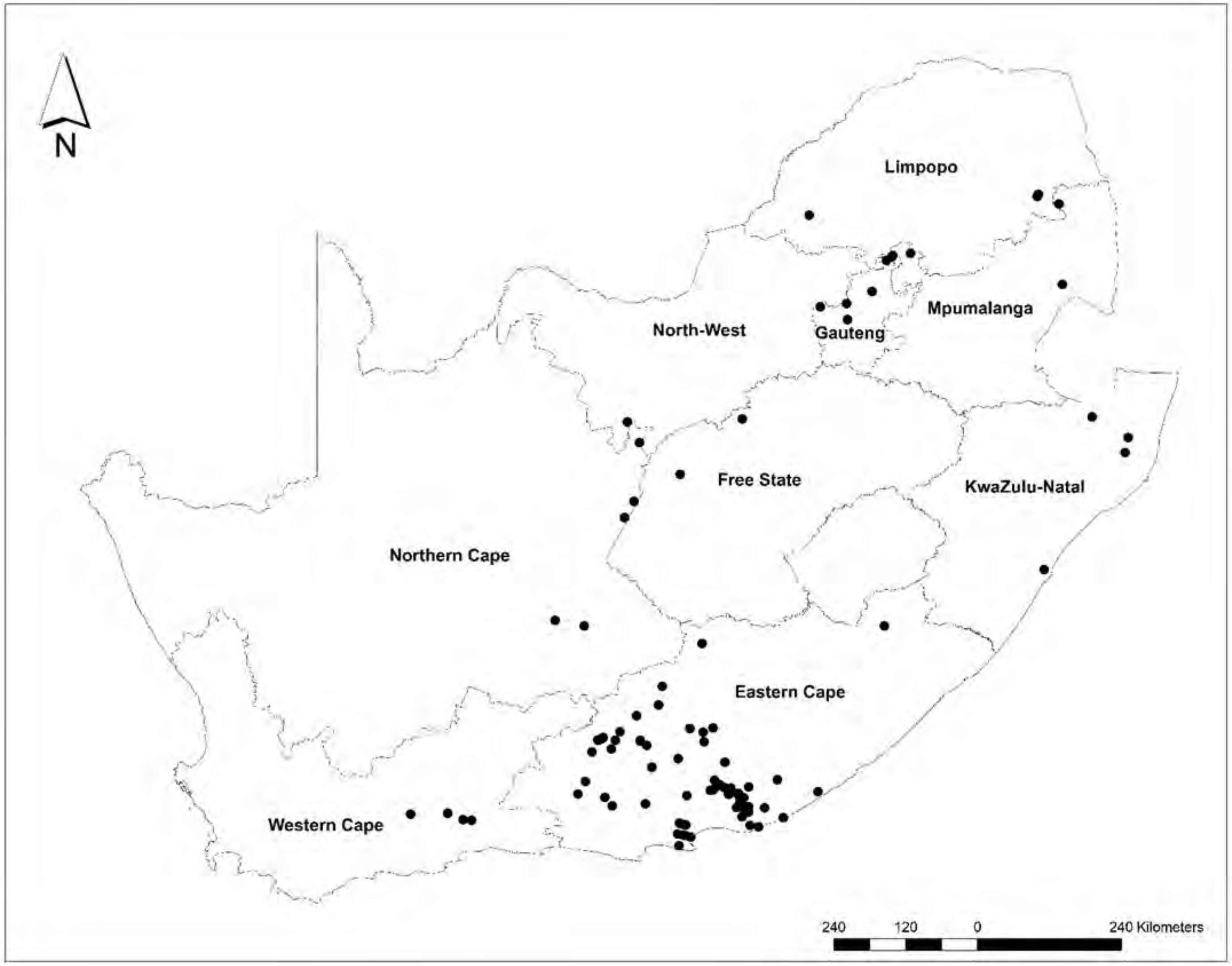

Figure 4.1 The distribution of sites where land-users who participated in this study have released agents provided by the Centre for Biological Control (CBC).

4.2.2. Ethics consideration

As this study included human participants, a separate ethical clearance was required for other studies in this thesis. The study proposal was submitted for review and approval to the Research Ethics Committee of Rhodes University before the initiation of this study, as suggested by Brockington and Sullivan (2003). The ethics application was approved on 14 September 2018, and the ethical clearance number is SCI2018/042 (Appendix D).

4.2.3. Participants

Land-users who have problems with invasive alien cacti on their land and have utilised biological control for the control of these cacti were the participants in this survey. Some of the participants contacted the Centre for Biological Control (CBC) to request biological control agents after they heard about the availability of agents through public talks and exhibitions such as farmers meetings, game farming and hunting association meetings, agricultural shows and science festivals, the CBC website, articles in magazines and via word of mouth from other land-users. Other land-users were approached by members of the CBC who were aware of cactus infestations on the land-users' properties. The study targeted these land-users to evaluate their opinions about the safety and efficacy of biological control against invasive alien cacti. Two relatively short questionnaires and one equally short follow-up questionnaire were developed to assess these perceptions (Appendix A, B, and C).

4.2.4. Questionnaire design

Structure of questionnaires

The first step taken to design the questionnaires was to choose which type of questionnaire to make use of (Desai and Potter 2017). There are three types of questionnaires: 1) structured questionnaire which consist of short and specific answers which are provided and are to be ticked by the participant; 2) unstructured questionnaires, which do not have specific answers and allows the participant to give answers freely with no limitations; and 3) semi-structured questionnaires which are composed of both open- and closed-ended questions. A semi-structured questionnaire was chosen for this study to allow explanations from the participants and not limit the participants by asking them to choose from the answers provided. Semi-structured questionnaires are the most commonly used type of questionnaire in similar studies (Desai and Potter 2017).

Three questionnaires were developed; Questionnaire 1 was intended for land-users that had already had the biological control agents released on their land between 2016 and 2018 and had had sufficient time to see whether there had been some impact from the agent. Questionnaire 2 was aimed at land-users who had had releases conducted very recently on their land and could not had seen any impacts (pre-release), and then Questionnaire 3, a follow-up questionnaire was done six months or more after these releases when an impact may have been evident (post-release). There were three sections to all questionnaires: general, background information, and participant's opinions. The general section included the name of the investigator and the geographic area of the farm. The second section was about participants' background information, including age, gender, and highest level of education, race, and home language. The third section focused on the participants' opinion about biological control of cactus weeds, including whether they thought that the agent population increased after the release and if there had been a decrease in the target weed, and if this had benefited them. Other important questions included were with regards to awareness, such as asking how the participant had become aware that the CBC had biological control agents for cactus weeds available. The farmers were also asked if the agents had fed on any other plants that they did not expect it to feed on. Although these agents are known to be host-specific, this question provides further evidence of the safety of biological control and the public's perception of biological control safety.

4.2.5. Logic and explanation of questions

Questionnaire 1

Questionnaire 1 was completed by participants who had cactus biological control agents released on their land at least six months prior to the time the questionnaire was conducted. Participants were asked where they first heard about biological control of cactus weeds (Appendix A, Q3.1) and how they got information that biological control agents for cactus weeds could be sourced from the CBC (Appendix A, Q3.2). There is a view that the general public is not aware of biological control as a method to control invasive alien weeds (Barratt et al. 2018). These questions were asked to get an idea about what people know about the biological control of weeds and to gain an understanding of where they heard it. The responses from land-users will indicate what forms of awareness have been successful.

Participants were then asked whether the biological control agent population had increased after the release of the agent on their land (Appendix A, Q3.3). The first measure of success after releasing a biological control agent is the establishment of the agent (McFadyen 1998; 2000). In some cases, the agent was already established at the sites where releases were conducted, and the intention of the release was to augment the population. In such cases, the release of the agent should result in an increase in the abundance of agents at the site. This question is therefore an agent-related parameter and is important as evidence that any changes in target weed density are due to the agent and no other confounding factors (Morin et al. 2009).

The next question asked if the biological control agents had reduced the amount of cactus weed on the participants' land (Appendix A, Q3.4). The aim of this question was to obtain perceptions of participants about any changes in the density of cacti species after the release of biological control agents. This question is therefore a weed-related parameter (Morin et al. 2009).

The land-users were asked to provide reasons as to why they wanted to control the invasive alien cacti and what impact these plants have on their land (Appendix A, Q3.5). This question was asked to assess the negative impacts of cactus weed on the land-users so that the benefits of biological control to the land-users could be understood. These questions led to asking participants if the negative impact of the weed had reduced after the release of biological control agents to determine if the releases had benefited them and how their land had improved due to the release of biological control agents (Appendix A, Q3.6). This question is, therefore, an ecosystem-related parameter assessment.

The perception of the safety of biological control among land-users was evaluated by asking participants if the biological control agents had any non-target effects on any plants besides the target weed (Appendix A, Q3.7). This was included to verify the specificity of these biological control agents post-release and to assess the perception of the safety of biological control among land-users.

Participants were asked if they would recommend biological control to other land-users. This question was included to gauge whether land-users who were not satisfied with the level of control would still suggest that it was worth trying because it is a safe method and because agents are provided for free, so there are no costs to the land-user in trying biological control, even if it is ineffective (Appendix A, Q3.8).

Questionnaire 2 and Questionnaire 3 (follow-up questionnaire)

Questionnaire 2 was completed by participants at the time of the first release of cactus biological control agents on their land and then followed up with another questionnaire six months later. Similar questions as those asked in Questionnaire 1 were given, but in this case, the change in individuals' perceptions could be tracked over time. In the initial questionnaire, the participants were asked about the density of the target plant and agent on their land, and this could be compared with the answers given six months after the release of the agents. These questionnaires essentially covered the same questions as those asked in Questionnaire 1, but before and after, such that an impact from the agent could have been evident. The amount of the agent that was present before release and after release was assessed, followed by the amount of the target weed and the change in the negative impacts of the weed (Appendix B). The safety of the agents was assessed by asking if the land-user expected the agent to feed on other plants and then asking whether it had fed on any non-target plants in the follow-up questionnaire (Appendix B).

A subject related to management options was included by asking the participants if they have ever used any other management options against invasive alien cacti and were asked to state them if they have (Appendix B, Q3.4). There are different weed control techniques such as mechanical and chemical used as management options for cactus weeds; some work effectively when applied individually or together, and some are effective for short periods of time (van Wilgen and Lange 2011; van Wilgen et al. 2013). This question was asked to evaluate the participants' practice and perceptions about the effectiveness of other control measures that they have used against invasive alien cacti. This question would also help determine if some participants had implemented biological control in the past and it was not effective, maybe due to the inadequate number of biological control agents released, incorrect biological control agents being released, or agents being released at inappropriate times such as in winter. This question would also give an idea about how badly the land-user wants to get rid of the invasive alien cactus plant on his land and whether they are willing to actually invest in removing it.

The opinions of participants about knowledge and understanding of biological control, and interactions and engagements with the CBC, were assessed by asking the question in Appendix B, Q3.10 similar to the first questions of the first questionnaire in (Appendix A, Q3.1). Participants were asked if the CBC provided them with all the required information to understand biological control and if they would have preferred more information. This will indicate if the CBC should provide land-users with educational material such as pamphlets or leaflets to broaden their knowledge about biological control. These questions were essentially about education on biological control of cactus weeds and interactions with the CBC, and if the land-users feel that they had been properly informed about the safety and efficacy of biological control and the way it works. A well-informed community is able to make wise decisions, such as choosing to use biological control on their land after they have seen how effectively it works (Schüttler et al. 2011).

4.2.6. Completion of the questionnaires

The study commenced in September 2018 and was completed in April 2021. Participants were contacted via email, telephone, and in-person to conduct the questionnaires. The questionnaires were conducted in a language best understood by the participants, and the questionnaires were translated into Afrikaans and IsiXhosa. For both telephone and face-to-face questionnaires, a translator was used in cases where participants did not understand English or participants who preferred the questionnaire to be conducted in their own mother tongue language. Participation was voluntary; informed consent was signed by both participant and investigator; for participants who were interviewed over the telephone, the consent form was emailed, and confidentiality assurances were given to all participants. One hundred and sixty-seven questionnaires were sent out to land-users, and 149 questionnaires were completed and returned by land-users across all the sites where releases of biological control agents have been conducted against invasive alien cacti of South Africa (Fig. 4.1). Of the 149 questionnaires that were completed, 47 questionnaires were completed for questionnaire 1, followed by 64 questionnaires for questionnaire 2 and 38 questionnaires for the follow-up questionnaire. Eighty-eight questionnaires were completed via email, 37 questionnaires were completed over phone calls, and 24 questionnaires were conducted face-to-face.

4.2.7. Data analysis

All questionnaire data collected were translated into English before conducting the analysis. Data obtained were organized and captured in Microsoft Excel before being imported and analysed using the Statistical Package for Social Science (SPSS) software (IBM SPSS, version 26) and STATISTICA version 13.5.0.17 (©TIBCO Software, Inc., USA, 2018). Descriptive statistics such as percentage, mean, measures of frequency, and Chi-squared test χ^2 used for data analysis were performed on SPSS software. Responses from open-ended questions where participants have provided more than one answer were categorized using content analysis (Erlingsson and Brysiewicz 2017). These responses were analysed by identifying similar phrases and were ranked in order of uniformity (Erlingsson and Brysiewicz 2017).

4.3. Results

4.3.1. Profile of participants

Participants were from all the nine provinces of South Africa, but the Eastern Cape was heavily biased, with 78 participants (Table 4.1). A greater number of participants were White, with 89 (80%) participants compared to 19 (17%) Black participants and three (3%) Coloured participants ($\chi^2 = 113.1$, $p < 0.05$) (Table 4.1). Afrikaans and English were spoken by most participants compared to all other languages ($\chi^2 = 196.2$, $p < 0.05$) (Table 4.1). A significantly higher number of the 81 participants were males (73%) rather than 30 females (27%) ($\chi^2 = 23.4$, $p < 0.05$) (Table 4.1). The average age of participants was 48.56 (SE ± 1.28), and ranged between 24 and 81 years of age, with most participants between the age of 55-65. Eighty-six (78%) of participants were highly educated with tertiary education, 23 (21%) of participants had completed high-school as the highest level of education, and only one (1%) participant had primary education as their highest level of education ($\chi^2 = 244.4$, $p < 0.05$) ($n = 111$) (Table 4.1).

Table 4.1 Profiles of land-users who participated in the study (n= 111)

Variable		Participants (%)	Chi-square
Gender	Male	73	$\chi 2= 23.4$, $p < 0.05$
	Female	27	
Race	White	80	$\chi 2= 113.1$, $p < 0.05$
	Black	17	
	Coloured	3	
Education level	Tertiary	78	$\chi 2= 244.4$, $p < 0.05$
	High School	21	
	Primary School	1	
Home language	Afrikaans	43	$\chi 2= 196.2$, $p < 0.05$
	English	34	
	IsiXhosa	8	
	English & Afrikaans	4	
	IsiZulu	4	
	Tshivenda	2	
	Tswana	2	
	Sepedi	2	
	Italian	1	
Province	Eastern Cape	70	$\chi 2= 395.4$, $p < 0.05$
	Western Cape	6	
	Gauteng	6	
	KwaZulu-Natal	4	
	Free State	4	
	Limpopo	3	
	Northern Cape	3	
	Mpumalanga	2	
	North-West	2	

4.3.2. Participants' opinion about biological control of invasive alien cacti

Extent and negative impact of invasive alien cacti

Participants were first asked about their opinion on the level of infestations of invasive alien cacti on their land prior to the introduction of a biological control agent (n = 64) (Appendix

B, Q3.1). Twenty-seven participants (43%) have expressed that their land was heavily infested by invasive alien cacti, while 17 participants (27%) viewed cactus infestations on their land as moderate, and 19 participants (30%) felt that the extent of the infestation on their land was small ($\chi^2 = 22.3$; $p < 0.05$) (n = 64) (Fig. 4.2). Participants who said that they have heavy infestation described the extent of infestations as being “very bad, everywhere”, “big problem”, “heavy infestation,” and “very bad, dense infestation”. For moderate infestations, participants said “the infestation is a mild infestation” and “moderate coverage, growing population”. Participants who wrote that they had small infestations on their land explained that it “occurs in isolated spots”, “not a big problem at the moment but has a potential to be a problem if not controlled,” and “very small” and “minimal impact”.

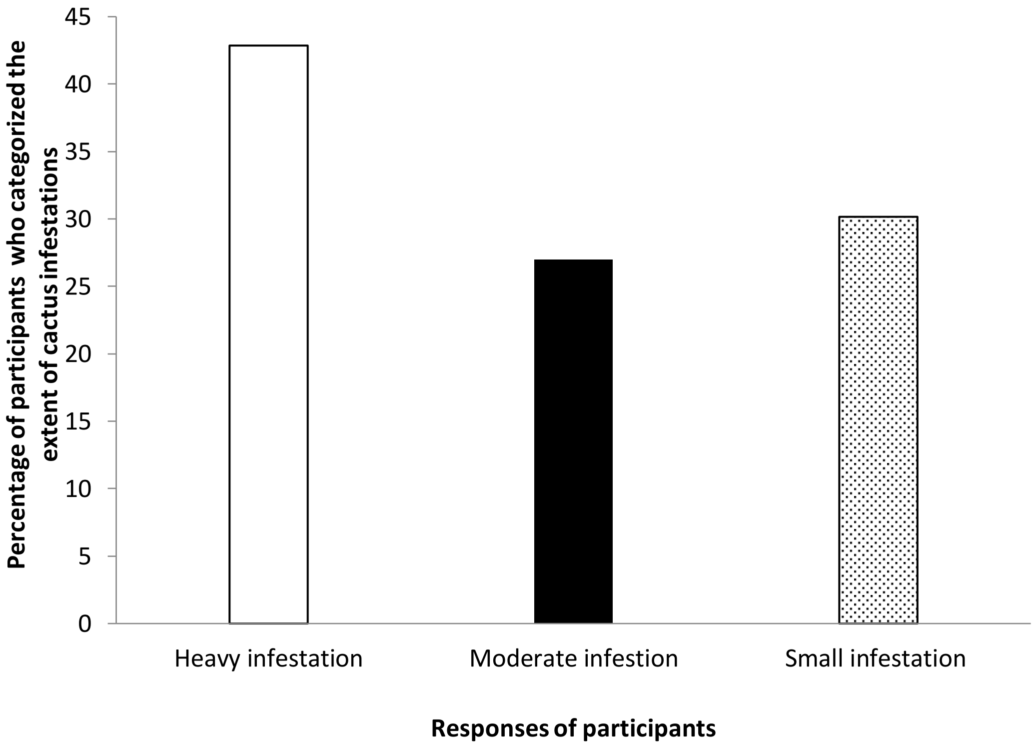

Figure 4.2 Percentage of participants who perceived the cactus infestations on their land as heavy, moderate, or small infestations (n = 64) (Chi-squared test, p > 0.05)

The question on the extent of invasive alien cacti infestations was followed by a question on the perceptions of participants on the negative impacts of invasive alien cacti and the reasons why they wanted to control them on their land (n = 111) (Appendix A, Q3.5 and Appendix B, Q3.2). Participants were not provided with a list of negative impacts; there was no limit to a number of negative impacts to specify, and as a result, some of the participants identified more than one negative impact of invasive alien cacti. The responses were all captured and ranked from the highest to the lowest frequencies according to how many times they have been mentioned by participants (Table 4.2).

All participants considered invasive alien cacti as having a significant negative impact on their lands, and a total of nine negative impacts were identified by participants (Table 4.2). Participants mentioned negative impacts caused by invasive alien cacti, which included impact on animals and human health, indigenous biodiversity, land-use, wool quality, agricultural economy, water quantities, soil quality, and risk to aircraft (Table 4.2). Impact on animals and human health was the most common negative impact of invasive alien cacti specified by 50 participants; one participant explained the negative impact cactus weed causes and said that “It attaches to and interferes with game (i.e. wildlife) & even people”. Forty-three participants perceived cactus weeds as having an impact on indigenous biodiversity, and one participant wrote that “It is an alien invader species and has a negative environmental impact over indigenous plants”. There were 40 participants who specified that invasive alien cacti plants caused land-use change because they posed a threat to grazing lands on their farms and they could not perform some of the activities they used to do prior to the invasion by invasive alien cactus plants, one of the participants stated: “(The cactus) takes away grazing land, blocks access to grazing land, sticks to animals and people”.

Nine participants mentioned that invasive alien cacti were a significant problem to wool production, and one participant describes how this harm occurs: “that the most important is that the thorns pollute wool”. Three negative impacts: impact on the agricultural economy, water quantities, and soil quality, were mentioned by each of three participants (Table 4.2). One of the participants wrote that an invasive alien cactus “uses large water quantities”. Another participant cited that “the cactus has affected the soil quality of the area”. The two final negative impacts of invasive alien plants specified by two participants each were a reduction in meat quality and risk to aircraft (spines of cactus weeds have a negative effect on the aircraft tyres) (Table 4.2).

Table 4.2 Different negative impacts caused by invasive alien cacti as cited by participants (n = 111). Some land-users indicated more than one negative impact.

Type of impact caused by invasive alien cacti	**Negative impact response frequency**
Animals and humans	50
Native biodiversity	43
Land-use change	40
Wool quality	9
Agricultural economy	3
Water quantities	3
Tourism	3
Soil quality	1
Meat quality	1
Risk to aircraft	1

The negative impact of invasive alien cacti led to a question about primary land-use before infestation by invasive alien cacti and about land-use change due to the infestation by invasive alien cacti (n = 64) (Appendix B, Q3.3). The majority of participants (48 participants, 75%) used the land as grazing lands before the infestation by invasive alien cacti, six participants (9%) stated that the land is used for nature conservation, three (6%) participants used the land for other agricultural activities, and three participants (6%) were not sure of the primary use of the land before it was infested by invasive alien cacti. Two participants (3%) wrote that the land was used to perform recreational activities such as recreational hunting and hiking, 2 participants stated that the land was not used before the infestation by cacti and 1 participant (2%) used the land as a homestead. Participants were then asked to specify if there was a change in the land-use after the infestation by cactus weeds (n = 64) (Appendix B, Q3.3). The majority of 32 participants (50%) indicated that there had been no change in land-use after the infestation by invasive alien cacti, and this number was significantly higher than 22 participants (34%) who specified that there had been a change in the land-use due to the presence of invasive alien cacti and to 10 participants (16%) who did not specify either way ($\chi^2 = 10.3$; $p < 0.05$). One participant explained why he no longer uses the land and said that "livestock avoids areas with the weed which makes livestock grazing difficult".

Control of invasive alien cacti

Participants were asked about any control interventions that have been implemented on their land to manage cactus weeds. The second part of this question requested the participants to state any methods they have used (n = 64) (Appendix B, Q3.4). There were 48 participants (75%) who stated that they have tried to control the invasive alien cacti and 16 participants (25%) said that they have never attempted control (χ^2 = 18.1; p < 0.05).

Of the 48 participants who had implemented some sort of control intervention, one participant did not specify the control that had been used on the land. Twenty-one participants (45%) had implemented chemical control, ten (21%) used a combination of chemical and mechanical control, seven participants (15%) used mechanical control, six participants (13%) have had biological control agents released on their land, two participants (4%) used biological and chemical control; and one participant (2%) had implemented all the control methods (biological, chemical and mechanical) (χ^2 = 23.7; p < 0.05) (Fig. 4.3). One of the participants who used chemical control said it “helped for a while but seemed like they have planted it ‘(the cactus weed)’ because the plant came back and infested stronger than it was before herbicide application”. Another participant explained that “tried biological control and after the release, there was rainfall, so cochineal was washed off by the rain”. Another participant wrote that “my dad nearly 30+ years ago fought the cacti with poison and chopped it out. He also put cochineal on the cacti. I have also sprayed the cacti since 1999 and also cut and burn them. I also transferred the cochineal to other cacti”.

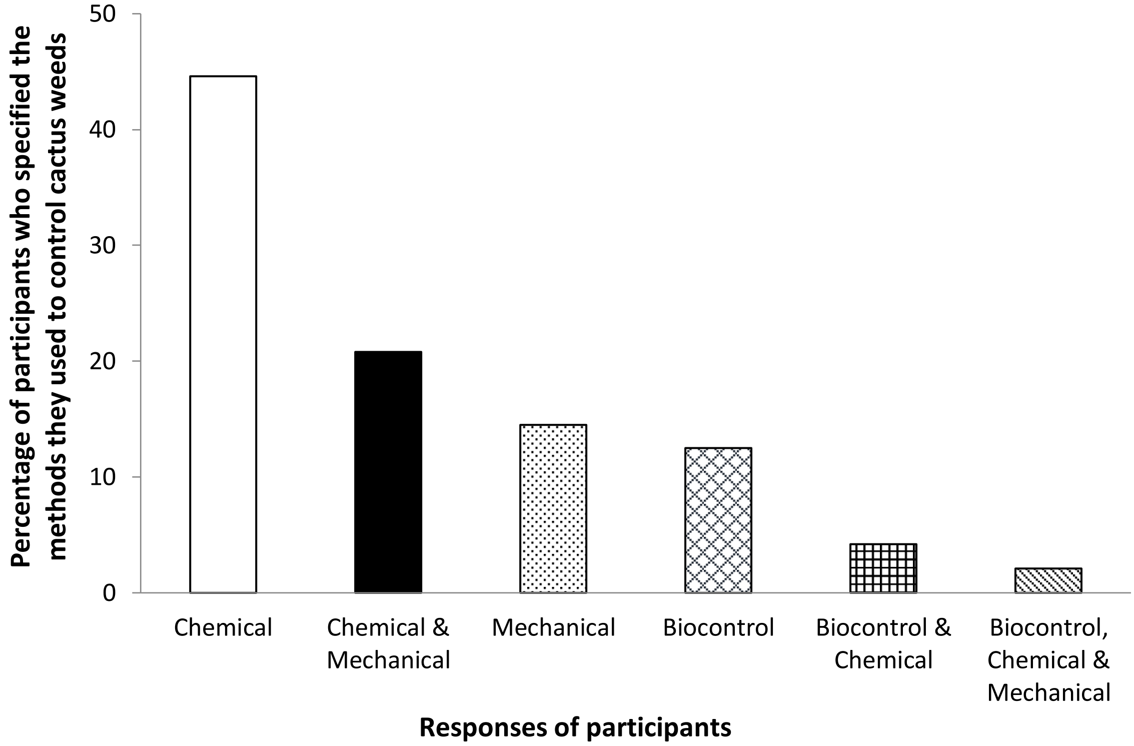

Figure 4.3 Percentage of participants who have tried different control methods to reduce invasive alien cacti on their land before the CBC intervention (n= 47) (Chi-squared test, $p < 0.05$)

Knowledge about biological control and the CBC

Participants were asked about where they first learnt about the biological control of invasive alien cacti (Appendix A, Q3.1, Appendix B, Q3.5 & Q3.6). Forty-seven participants (73%) expressed that they had heard about the biological control of weeds before the releases by the CBC, and 17 participants (27%) said they had not (n= 64) ($\chi^2 = 14.1$, $p < 0.05$) (Appendix B, Q3.5). In some cases, more details about where or how they learnt about biological control was given (n = 95). Twenty-nine participants expressed that they learnt about biological control during their tertiary studies as nature conservation, biology, or game ranch management students (Table 4.3). Twenty-three participants learnt for the first time about biological control from the CBC, with seven of the 23 having had discussions with the CBC researchers and students about biological control before contacting the CBC to request biological control agents for cactus weeds on their land. The CBC reached out to six participants by asking if they would be interested in using biological control to manage invasive alien cactus on their land. Six participants wrote that they learnt about biological control from the community engagement events in which the CBC participates by having an exhibition or giving presentations, such as agricultural shows, workshops, and farmer's association meetings. One participant mentioned that he learnt about biological control from the weed biological control short course run by the CBC. Of the 95 participants, 20 heard via word of mouth from their fellow colleagues, neighbours, land-users and farmers who shared their knowledge and information about biological control (Table 4.3). More than fifteen participants heard from biodiversity conservation organisations such as the Department of Forestry, Fisheries and the Environment (DFFE), Cape Nature, South African National Biodiversity Institute (SANBI), Agricultural Research Council (ARC), and Mountain Zebra Camdeboo Protected Environment (MZCPE) who work together with the CBC in controlling invasive alien cacti (Table 4.3). Ten participants stated that they learnt about biological control by means of media, literature, and research (Table 4.3). Another ten participants wrote that they first learnt about biological control of invasive alien plants in their workplaces (Table 4.3). Nine participants first learnt about biological control through visual observations of biological control agents damaging other cactus species such as *O. ficus-indica* on their land (Table 4.3). Only one participant did not know where he learnt about biological control (Table 4.3).

Table 4.3 Responses of participants about where they learnt about biological control (n = 95). Some land-users indicated more than one response.

Responses provided by participants	Number of participants who provided the responses
Tertiary studies	29
Centre for Biological Control	23
Word of mouth	20
Biodiversity Conservation Organisation	19
Media, Research and Literature	10
Workplace	10
Visual observations	9
Do not know	1

Additionally, participants were asked about how they heard that they could obtain biological control agents from the CBC's Kariega Facility (Appendix A, Q3.2 and Appendix B, Q3.8). Similar to the previous questions, the participants were not given a limit to the answers to provide for each question, so some participants gave more than one response.

Forty-four participants were contacted by the CBC to ask if they were interested in using biological control or heard about the CBC from the CBC's community efforts, such as agricultural shows, farmer's association meetings, and game ranger's association meetings (Table 4.4). Other participants heard about getting biological control agents for cactus weeds from the CBC through a variety of different ways (Table 4.4). These included forty-four participants via communicating with biodiversity conservation organisations, twenty-nine participants via word of mouth from land-users who had previously utilised the CBC, nine participants read or heard from media platforms such as magazines, newspaper articles, Facebook posts and Twitter, and lastly one participant received information that the CBC provides biological control agents from an academic conference (Table 4.4).

Table 4.4 Responses of participants about knowledge of obtaining cactus weed biological control agents from the CBC (n = 111). Some land-users indicated more than one response.

Responses provided by participants	**Frequency of responses from participants**
Centre for Biological Control	44
Biodiversity Conservation Organisation	44
Word of mouth	29
Media platforms	9
Biological control conferences	1

Change in the biological control agent population

Participants were asked if the biological control agent populations increased after the releases were conducted (Appendix A, Q3.3 and Appendix C, Q1.1). Fifty-nine participants (69%) observed that the biological control agent had established and increased after the releases, which was significantly greater than the 18 participants (21%) who believed that the agents did not increase, and the eight participants (9%) who were unsure whether the agents increased or decreased after the initial release (χ^2 = 91.7; $p < 0.05$) (Fig. 4.5). The participants who said that the agent density had increased since the releases stated that “YES, observations of the plants on which the agent was applied suggested that the agent has proliferated and spread across the plants” and “YES, there's more biological control agent since the release and are observable in all cactus that was released on”. One participant wrote that he was uncertain whether there was an increase or not because “It is difficult to say, as there were already agents present”.

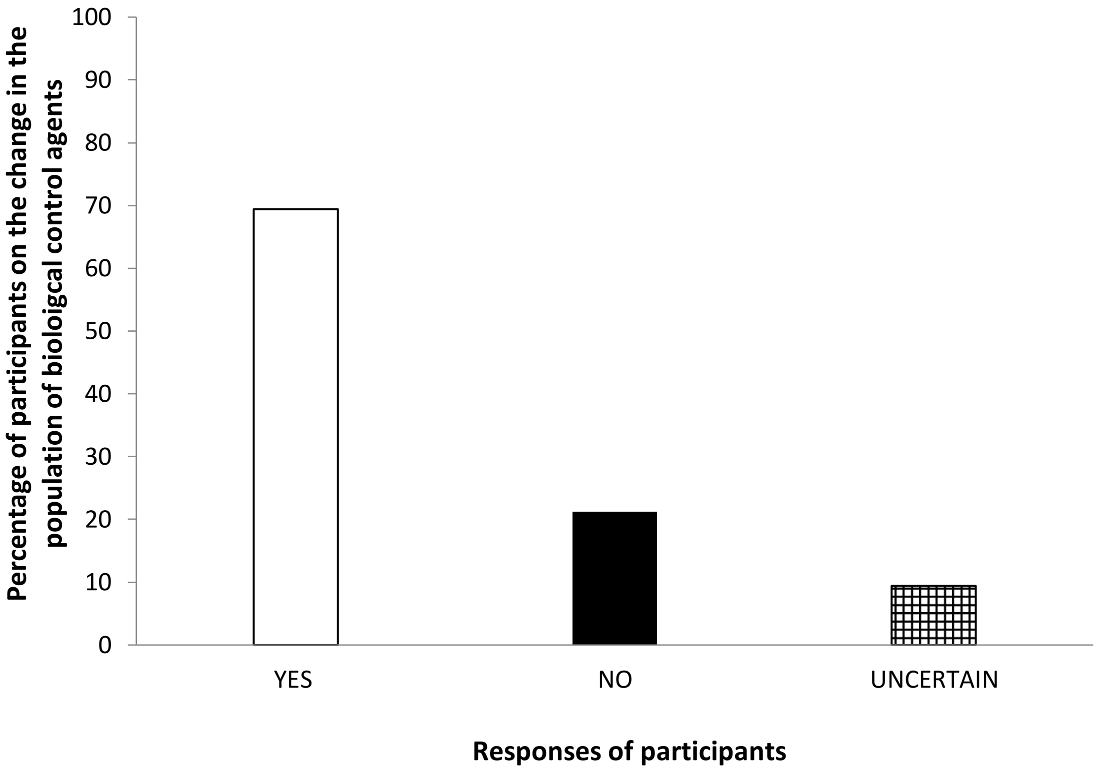

Figure 4.5 Response of participants when asked if the agent population increased after biological control agent releases (n= 85) (Chi-squared test, $p < 0.05$)

Impact of biological control agents on the invasive alien cacti

Participants were asked if the cactus was less harmful or caused fewer negative impacts since the release of biological control agents on their land (Appendix A, Q3.6 and Appendix C, Q1.4). Sixty-nine participants (81%) perceived that there were less invasive alien cacti due to the damage of the agent; this was significantly more than 15 participants (18%) who stated that the amount of the targeted plant was still the same as before the release of the agent, and only one participant (1%) who wrote that he was uncertain ($\chi^2 = 50.4$; $p < 0.05$). Two participants who believed that the amount of cacti had reduced said that "YES to a lower degree" and "YES definitely, see some places the katyi [a local name for *O. aurantiaca*] can't survive because it is dead". One participant said that there was no change in the amount of invasive alien cacti, citing the phrase “more weed,” and another participant said that it “has not really reduced”.

Change in the negative impact of invasive alien cacti after the release

Participants were asked if there were any changes in the negative impact caused by invasive alien cacti after the release of biological control agents (n = 85) (Appendix A, Q3.6 and Appendix C, Q1.4). Significantly more participants (49%) believed that the negative impact caused by the invasive alien cacti had reduced after the release of the agent compared to 38% who believed that there was no change. Six participants (7%) said that it was too early to tell whether the harm caused by the cacti had decreased after the release and 5 participants (6%) were uncertain ($\chi^2 = 51.7$; $p < 0.05$) (Fig. 4.6). Two participants explained that there was a change in the impact of invasive alien cactus after the release of agents using phrases such as “YES, positive impact,” and another participant said that “YES, satisfied”. One participant who said the impact has not yet been reduced said “NO not yet decreased”. For the uncertain response, one participant said, “Not sure yet”. One participant felt that it was too early to assess the impact of cactus weeds said that “It is too early to access, but I have no doubt that the negative impact will decrease as time passes”.

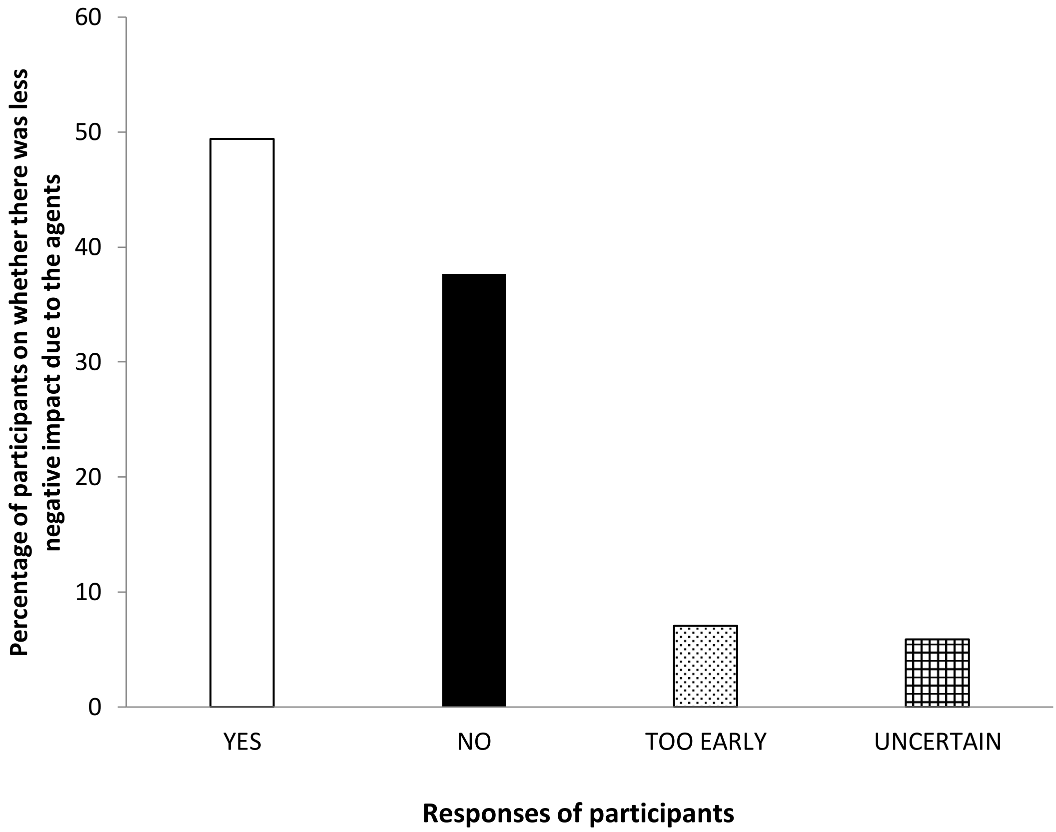

Figure 4.6 Response of participants when asked if the negative impact caused by cacti decreased after biological control agent releases (n= 85) (Chi-squared test, $p < 0.05$)

Of the 42 participants who mentioned that cactus weeds had a negative impact on animals and humans, 19 said that the invasive alien cacti no longer pose a threat to animals and human health. For the impact on indigenous biodiversity, out of 39 participants who wrote that the cactus weeds threaten indigenous biodiversity, 15 perceived that there was a recovery in the indigenous biodiversity after the damage caused by biological control agents. Thirty-three participants specified that cactus weeds had negatively impacted on land-use change; nine of these participants said that they could now use all the land to perform activities they used to do in the past before invasive alien cactus plants infested the land. Seven participants had indicated that wool production was negatively impacted and four of these mentioned that fewer spines were found as wool contaminants after biological control.

Biological control safety

Participants were also asked whether the biological control agents that had been released fed on any other plants besides the target weeds (n = 146) (Appendix A, Q3.7, Appendix B, Q3.7, and Appendix C, Q1.5). One hundred and forty-two participants (97%) said that there were no other plants on their land that had been damaged by the agents. This was significantly different from the three participants (2%) who were uncertain whether the agents damaged other plants, and the single participant (1%) who said that the agents did feed on other non-targeted plants ($\chi^2 = 268.6$; $p < 0.05$) (Fig. 4.7). One participant who believed that biological control agents did not harm other plants said, “NO, and that's what I like about the agent; kills specifically the cactus”.

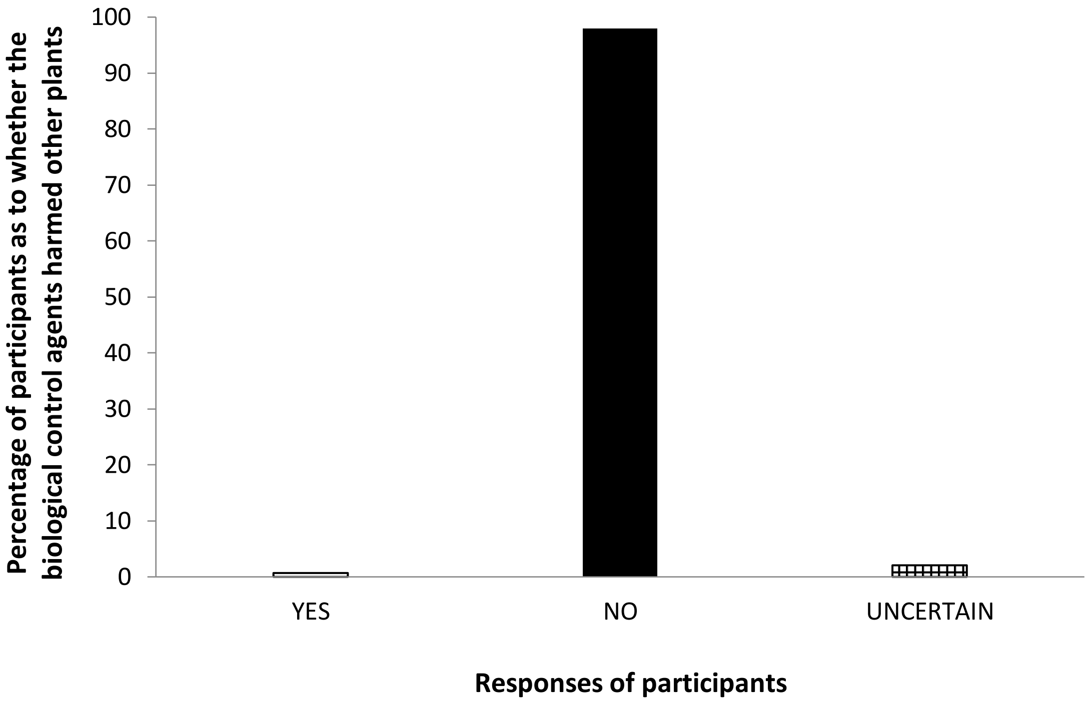

Figure 4.7 Response of participants as to whether the biological control agents harmed other plants (n = 146) (Chi-squared test, $p < 0.05$)

Recommending biological control to fellow land-users

Participants were asked if they would recommend biological control as a method used to control invasive alien plants to their fellow land-users (n = 85) (Appendix A, Q3.8 and Appendix C, Q1.8). Eighty participants (94%) would recommend biological control to their fellow colleagues, and this was significantly more than the three participants (3%) who said that they would not, and two participants (3%) were not sure ($\chi^2 = 211.3$; $p < 0.05$).

Biological control information and engagements with the CBC

Participants were asked to specify if they had been given enough relevant information about biological control or whether they would have liked to get more information from the CBC (n = 149) (Appendix A, Q3.9, Appendix B, Q3.10, and Appendix C, Q1.6). One hundred and thirty-five (91%) participants felt that the information about biological control that they received from the CBC was sufficient, and 14 participants (9%) were not satisfied with the amount of information provided ($\chi^2 = 98.3$; $p < 0.05$). Several participants said that they would like to receive more information about biological control to further their knowledge and share it with other fellow land-users. One participant said, “Any interesting and relevant information, brochures, publications (popular and scientific) would be great! I use it to learn more about it and for awareness amongst land owners”. Another participant wrote that he understands how biological control works, but he needs more information to raise awareness to his colleagues and neighbours: “Yes, I do [want more information] it [biological control] takes time, but it is effective, would like to get presentations for my neighbouring farmers and landowners”.

4.4. Discussion

The vast majority of the participants in this survey were white men who spoke English or/and Afrikaans and were well-educated, usually having completed a degree in a tertiary education institute, such as a university. This is not a proper reflection of the demographics of South Africa, where 81% of people are not white, and 94% have no tertiary education (Stats SA 2019). This is partly due to the legacy of apartheid in South Africa, which discriminated against non-white people, and has resulted in most farmers and landowners in South Africa being white, educated men (Daniels 1987; Rehbein 2018). The higher levels of education of white people have given them an opportunity to connect with the CBC and receive biological control agents. Poor rural communities are possibly the people that are most negatively impacted by invasive cacti in South Africa (de Neergaard et al. 2005), and are also the least likely to be aware of the benefits and availability of biological control agents. This indicates that the strategy used for raising awareness of weed biological control needs to change and be directed at all sectors of communities in South Africa, such as other races, less educated communities, and people speaking other languages. Most questionnaires were completed via email compared to face-to-face and over the telephone. So, the participants reached by the CBC were engaged via a medium that is not necessarily available to poor and marginalised people. It is recommended that the CBC should reach out to poor rural communities that need to benefit from biological control. This may be achieved by scheduling to meet in person with poor rural communities and by partnering with land reform agencies such as the Department of Rural Development and Land Reform to reach the targeted people through that agency (DLA 1997).

The land-users included in this study were generally well informed about biological control, with many of them having learnt about biological control as part of their formal education, and the vast majority of land-users saying that they had a good understanding of biological control and did not feel that they required additional information. This may be due to the sector of society that has received biological control agents from the CBC, who on average have a higher level of education of seventy-eight percent than six percent for the general public of South Africa (DHET 2019), and also because many of the land-users actively approached the CBC for agents, and therefore must have been aware of biological control and its potential benefits before the release. Furthermore, the level of knowledge that land-users have regarding biological control, and their perceptions of it, both of which influence the likelihood that they will adopt biological control practices, are in turn sometimes positively influenced by education level (Goldberger and Lehrer 2016; Abdollahzadeh et al. 2017). A survey study conducted in Canada to determine the perceptions of 1000 people on the use of biological control for pest management showed that the majority of the respondents who perceived the use of organic methods or biological control safer for food production compared to food with pesticides was associated with a higher level of education (McNeil et al. 2010). Educating the public specifically on biological control is important to build well-informed societies which are able to make the best and most well-informed decisions (Warner et al. 2008). This has also been shown in the Indian Western Ghats for the release of the rust fungus and biological control agent, *Puccinia spegazzinnii* de Toni (Pucciniaceae) against *Mikania micrantha* Knuth (Asteraceae). All the farmers with the problem of *M. micrantha* infestations on their land were informed about the biological control benefits before the releases were conducted, and this resulted in more than 90% of farmers who were eager to attempt implementing biological control against *M. micrantha* infestations on their land (Sankaran et al. 2008).

The results of this study suggest that South African land-users are generally well informed about the use of biological control prior to coming into contact with members of the CBC. There are many well-known, successful weed biological control programmes for aquatic weeds, such as *Salvinia molesta* D.S Mitch. (Salviniaceae) and terrestrial weeds, *Acacia* spp. (Mimosaceae) and *Opuntia* spp. (Cactaceae) in South Africa (Zachariades et al. 2017). These successes have resulted in positive public perceptions about biological control in the country, but another important factor contributing to such perceptions is the community engagement activities of the biological control community in South Africa (Martin et al. 2018). The school curriculum includes information on biological control, and some schools are involved in mass-rearing, releasing, and monitoring biological control agents (Martin et al. 2018). This affords learners an opportunity to gain knowledge about biological control and assist in the release efforts of the biological control agents (Martin et al. 2018). The use of a variety of outreach and awareness programmes helps biological control to be perceived positively in South Africa (Martin et al. 2018).

The results showed that the majority of participants perceived invasive alien cacti as a significant problem on their land. The data pool is, however, biased because the only people interviewed were those who requested or accepted agents for cactus weeds. These data should therefore not be considered a representation of the wider population of South Africa, but provide evidence that there are land-users who are negatively impacted by invasive alien cactus weeds. This limitation of biased data is well known in other studies that have utilised questionnaires to assess public opinion, and if data are interpreted with this limitation in mind, the results are still of value (Cardell et al. 2016). All livestock farmers who participated believed that cactus infestations impacted livestock production. The most common invasive alien cacti species mentioned by livestock farmers as major problems were *O. aurantiaca*, *C. imbricata,* and *O. humifusa*. The negative impacts included harm to animals and humans, reduction of indigenous biodiversity, and land carrying capacity; all of these are considered by questionnaire participants as the main reason why cactus weeds are problematic.

This study has highlighted that the participants perceive biological control as an effective method to control invasive alien plants even before they observed its damage on the targeted cactus invasive alien plants on their land (Ellison et al. 2014). High number of participants believed that biological control would solve the problem of invasive alien cacti on their land prior to the release of the agent. This study had a significantly higher percentage of people who believed that biological control would help alleviate the problem with the target weed than previous similar studies (Ireland et al. 2019). This is probably due to the fact that the participants had contacted the CBC and therefore actually already believed that biological control intervention would help them solve the problem of invasive alien species on their land. Additionally, this is influenced by the fact that cactus biological control has been used for many years in South Africa and is extremely successful within a short period of time (Moran and Zimmermann 1991b; Paterson et al. 2011b).

Nearly all of the participants reported that there was no non-target feeding by biological control agents on any other plants. Only one participant stated that the biological control agent had non-target effects. This is a testimony to both the specificity of the agent and the educational information that is provided by the CBC to land-users, as well as previous education about biological control of weeds received by the land-users in the past. The great support and trust for biological control from the participants may be because they were well-informed about host-specificity of biological control agents prior to the releases being conducted and had benefited directly from the releases of biological control agents. This is similar to a results of the study conducted in Canada to evaluate the use of biological control as a management tool for pests (McNeil et al. 2010). The findings of this study indicated that the interviewed people perceived biological control as a safe method for crop production compared to the use of pesticides because they had concerns about the health risks posed by pesticides, so they benefitted from and supported biological control (McNeil et al. 2010). This positive perception from this study is in contrast to some countries, such as European countries and the USA, where there is substantial negative public opinion on the safety of biological control. For example, in Europe, there are biological control agents that have naturally spread in the continent and are effectively reducing weed densities; however, there is still a need to educate decision makers about the safety of biological control, and very little public support for the science and practice (Shaw et al. 2018).

The majority of participants believed that the biological control agent had decreased the amount of cactus on their land. This is a very positive result because it shows that participants understand that biological control is a long-term solution which takes some time before successes are evident. It has only been a maximum of four years since biological control has been implemented by the CBC on the participants' land, so although there has been a reduction in the weed-related measurements recorded in the post-release evaluation, there has been little change in the density of the weed (Chapter 3). Many participants who responded to the questionnaires had only witnessed the initial stages of biological control. If the same question was asked in five years' time, it is possible that a higher percentage of participants would indicate that the agent had reduced invasive alien cacti on their land.

Importantly, land-users perceived to have gained benefits after the release of biological control agents. The benefits they gained included reduced threat posed by invasive alien cactus plants to animals and humans, recovery of indigenous biodiversity, decreased cladodes and thorns polluting wool, water security benefits, improved agricultural economy, and soil quality. Directly quantifying these benefits may be challenging, but they are the ultimate measure of success in weed biological control, and therefore, an attempt to directly quantify them should be made (Morin et al. 2009; Schaffner et al. 2020). The use of land-user surveys has allowed for some insights into the types of benefits that weed biological control has provided to land-users, but direct quantification of these benefits would be very valuable.

Over 90% of participants indicated that they would recommend biological control to other land-users. This positive perception is assumed to have been influenced by a number of factors. Firstly, the biological control agents worked effectively in reducing cactus infestations in the majority of cases, and secondly, the land-users are well-informed that biological control agents are host-specific. Another factor that could have influenced the responses to this question is the fact that the CBC provides biological control agents to land-users free of charge, with no cost to the land-users, so as long as the land-users perceive the agents as safe, they may not need to be convinced of the efficacy of the agent to recommend their use. The perception would have been different if there were funds paid by the land-users in order to receive the biological control agents from the CBC.

Most participants had positive perceptions about the effectiveness of biological control agents. They have expressed that biological control is a useful invasive alien cacti management option and has made a positive contribution to reducing the negative impacts of cactus weeds on their land. For this reason, they will recommend it to their fellow colleagues who are also impacted by cactus weeds. There is still a need for land-users to work with each other in the re-distribution of biological control agents. The CBC should also consider offering the biological control agents at a cost to those who can afford to pay for this service, this could assist in getting more biological control agents to the Black and poor communities who really need them. This study highlighted the importance and the need to integrate the public into the planning, decision-making, and management of invasive alien cacti in South Africa. This study also presented a foundation for further investigation that incorporates an economic analysis to evaluate the cost and benefits of biological control. Such a study could improve allocation of resources in future. Data gathered from this study will help the CBC to understand the perceptions and expectations of land-users about biological control in order to improve the way they engage and interact with the public. It also provides some important evidence that biological control effectively reduces invasive alien cactus populations and that this is beneficial to land-users in South Africa.

CHAPTER 5: GENERAL DISCUSSION

The research conducted for this study was performed to assess the impact of cactus biological control agents released in South Africa. This was done by 1) comparing where agents have been released with where the invasive alien plants have been recorded (Chapter 2), 2) performing post-release monitoring to evaluate the efficacy of biological control agents on cactus invasive alien plants at release sites (Chapter 3) and 3) using questionnaires to evaluate the opinions of land-users about the success of biological control on their land (Chapter 4). The main objective of this study was to quantify the success of biological control programmes and to identify gaps where improvements could be made in South Africa.

The results of this study indicate that mass-rearing and release efforts are currently insufficient to occupy every quarter degree square (qds) where invasive alien cacti are known to occur in South Africa. This suggests that there are many cactus infestations in the country where biological control releases would be beneficial. All the cactus biological control agents are poor dispersers that are unlikely to disperse to areas where they have not been released. They therefore require human intervention to reach distant and isolated infestations. Based on the results of this study it is evident that there is a need for other cactus mass-rearing facilities to enable mass-releases and long-term monitoring to be feasible in other provinces of the country that are situated far from the CBC cactus mass-rearing facility in the Eastern Cape. Currently, the CBC packs biological control agents (cochineal-infested cladodes and galls) into cardboard boxes and couriered to where they are required for the release (Klein and Zimmermann 2020). Packing them should be done in a way that they do not move around the box in order to avoid the agents being damaged.

The biological control agents are kept in place with the crumpled tissue or newspaper (Klein and Zimmermann 2020). It is important to ensure that the box is kept dry to prevent moisture which increases mortality significantly (Klein and Zimmermann 2020). Despite these efforts to keep the agents healthy in transit, the closer the mass-rearing facility is to the release site, the healthier the insects will be when they are released. Mass-rearing facilities in key parts of the country would help mitigate this problem.

Additionally, areas with established populations of cactus biological control agents can be used as nursery sites in order to make it possible for collections and redistribution of agents to be conducted. There is also a need to undertake a nationwide survey to assess the distribution of agents, as in most cases, the presence of the cactus is just recorded, but it is not known whether the agent is present. A database of the distribution of the cactus biological control agents would be very valuable. If nation-wide surveys of cactus and agent distributions could be repeated periodically then sites where agents need to be released could be identified and changes in cactus distributions over time in relation to the presence of the agents could be tracked.

Mass-rearing facilities located in other provinces of South Africa would not only be beneficial for the control of cactus weeds, but would also provide employment opportunities. This is aligned with the dual mandate of WfW programme managed by the DFFE: NRM established in 1995 to manage invasive alien plants and provide employment focusing mainly on women (60%), youth (20%), and disabled people (5%) (van Wilgen et al. 1998). The WfW programme implements chemical, physical and biological control methods to control invasive alien plants (Macdonald 2004). This programme has employed more20 000 people and provided them with training to gain skills annually (DEA 2016).

Therefore, if mass-rearing and releasing continue and is expanded to other provinces this will increase the number of job opportunities created as well as control problematic invasive alien cactus plants in South Africa. Mass-rearing facilities also play a vital role in promoting biological control through hands-on experience and teaching in schools. Small-scale mass-rearing facilities have been set up in schools in South Africa and biological control has been incorporated in the school curriculums (Weaver et al. 2017). These facilities play a role in increasing the numbers of biological control agents mass-reared as well as developing interest in weed biological control, invasive species control and biology more generally, at a grass-roots level (Weaver et al. 2017).

The recently implemented system to access biological control success on a broad scale in South Africa relies on either expert opinion, or quantified post release data, for each of the target weeds that were assessed (Moran et al. 2021). Each target weed species is given a category related to the level of control and highlighted (in bold) if the biological control outcome was assessed based on post-release data, while is left in standard text if the assessment was made based on expert opinion, without post-release data (Moran et al. 2021). Out of 54 invasive alien plants that have been included in the categorizations of outcomes, 20 (37%) have post-release data, while 34 (63%) assessments were based on expert opinion (Moran et al. 2021). Of the 20 species with post-release data, seven (13%) were invasive alien cactus plants (Moran et al. 2021). The results of this thesis were included in that assessment and have therefore played an important role in understanding the levels of biological control success on a broad scale in South Africa.

The new system is an important tool for quantifying the success of biological control of weeds and is a significant improvement on previous systems. Incorporating more quantified data to support the decisions made in the allocation of each weed to each category will be an important focus for the weed biological control research community of South Africa in the future. The parameters used in categorising success of biological control are biomass, area and rate of spread for different habitats (Moran et al. 2021). These are all plant or population-level parameters and not ecosystem-level parameters (Moran et al. 2021). The new system does not have any direct quantified benefits of biological control (i.e., ecosystem-level assessments). Chapter 4 of this thesis was a quick and easy way of assessing ecosystem-level benefits, and although this is not as good as directly measuring these benefits, it could be used as a relatively easy way of quantifying the benefits for other weeds.

5.1. Assessing the success of biological control of invasive alien cactus species targeted by the CBC

Four species of invasive alien cactus species were the main focus of this study, and with evidence from the distribution of release efforts (Chapter 2), quantification of damage and impacts to plants and plant populations (Chapter 3), and perceptions of the benefits of biological control from land-users (Chapter 4), an overall assessment of the status of biological control of these species can be made. The success outcomes of each cactus invasive alien plant are discussed individually below.

Opuntia monacantha

Although the biological control agent for *O. monacantha* was released in South Africa over 100 years ago (Moran et al. 2013), this study is the first to quantify the impact of the biological control agent on *O. monacantha* and formally evaluate the success of the programme. Previous assessments have been based on the opinions of experts, but now there is evidence to support these opinions (Moran et al. 2021). The results of this study support the assessment that *O. monacantha* has been reduced to a density below a 'tolerable threshold' after the release of *D. ceylonicus* (Moran et al. 2021). The agent has been released or is already present in most areas where the plant occurs; the presence of the agent reduced fruit and biomass dramatically; very few land-users, therefore, require the agent, and all those that have used it perceive it as providing a high level of control. *Opuntia monacantha* is no longer a problem, but there may still be areas where there are infestations without *D. ceylonicus*, or new infestations in areas where the agent is not present may appear in the future.

Opuntia stricta

The biological control success of *O. stricta* in subtropical habitats is considered under category A+ for all parameters, and these categorizations were all confirmed by the results of quantified data (Moran et al. 2021). The data from subtropical habitats was largely taken from the intensive post-release evaluations conducted in the KNP (Paterson et al. 2011b; Hoffmann et al. 2020). In contrast, *D. opuntiae* "stricta" was less effective in temperate habitat and achieved category B (Moran et al. 2021). The success outcomes B- for density and area are categorizations based on expert opinion while the categorizations B+ for biomass and rate of spread are confirmed by the post-release evaluation studies that have been conducted during this study (Moran et al. 2021). The level of biological control achieved by the agent for *O. stricta* according to this study validates the assessment that this weed has been controlled to below the 'reversal threshold' in temperate areas such as the Eastern Cape (Moran et al. 2021). *Dactylopius opuntiae* "stricta" resulted in fewer fruit and reduced biomass, and the land-users have perceived that the releases have reduced the density and negative impact of *O. stricta*.

Cylindropuntia imbricata

Cylindropuntia imbricata falls into category B according to the outcomes used to assess the success of its biological control programme for all habitats and parameters (Moran et al. 2021). Although biological control has not reduced *C. imbricata* populations to levels below the reversal threshold, there is a possibility that the overall level of control of this weed could be improved through the integration of biological control and mechanical control in the correct manner. Moran & Zimmermann (1991a) first recommended the integration of these two methods, and Klein et al. (2020) showed the value of integrating biological control with mechanical control for the very similar and closely related *C. fulgida*, for which another lineage of the same species of cochineal is used for control (i.e., *Dactylopius tomentosus* 'cholla'). The fact that integrated control is needed has been highlighted by the findings of this study that after two years of monitoring, there were no further increases in the percentage of cochineal-infested cladodes at the sites. Felling the large trees of C. *imbricata* should be done when all the cladodes are covered by *D. tomentosus* “imbricata”. It is anticipated that this method will improve the control of *C. imbricata* to achieve category A over a five-year period, but a quantitative post-release evaluation will be required to assess this.

Opuntia aurantiaca

The success of biological control of *O. aurantiaca* is variable depending on the climate, and this is reflected in the assessment provided by Moran *et al.* (2021). *Opuntia aurantiaca* is considered under category A+ for density and biomass and A for area and rate of spread in dry, inland habitats (Moran et al. 2021). For coastal habitats, which are generally wetter and milder in temperature, it is assessed as B+ for density, B for biomass, C for distribution and rate of spread (Moran et al. 2021). The findings of this study confirm that *O. aurantiaca* is correctly placed in category B for biomass and density in temperate areas because although there was an overall reduction in these parameters measured at the sites, there was often resurgence in these parameters over time and at some sites there was no evidence of improved control. It is also clear from the post-release evaluation data collected in this study that control is variable in both space and time, and the level of control is largely influenced by climatic conditions. These fluctuations have been reported in previous studies, including a post-release evaluation conducted over a 12 year period (Fig. 5.1) (Moran and Zimmermann 1991b). Similarly, to Moran and Zimmermann (1991b), the results of this study also indicated an overall trend of reduced density of the target weed over time (Fig. 5.1). It is essential that land-users dealing with *O. aurantiaca* infestations are educated and informed about population dynamics and expectations of biological control of *O. aurantiaca* after the release of *D. austrinus* on their land. Land-users should be aware that better control will be achieved during hot, dry times and that although a resurgence of *O. aurantiaca* is expected during wetter periods, the growth of the population will not continue to increase indefinitely (Moran and Zimmermann 1991b).

Biological control of *O. aurantiaca* does not result in control below the tolerable threshold in many parts of South Africa, but the release of the agent is beneficial and if the agent were not present in the country, it would be a much more serious weed. There is also evidence from previous studies indicating that chemical and mechanical control is not effective (Moran and Zimmermann 1991b). Therefore, it is important to make sure that *D. austrinus* is present wherever the weed is present and that population densities of the agent are as high as possible in the field. Mass-rearing and releasing efforts should be increased to achieve this.

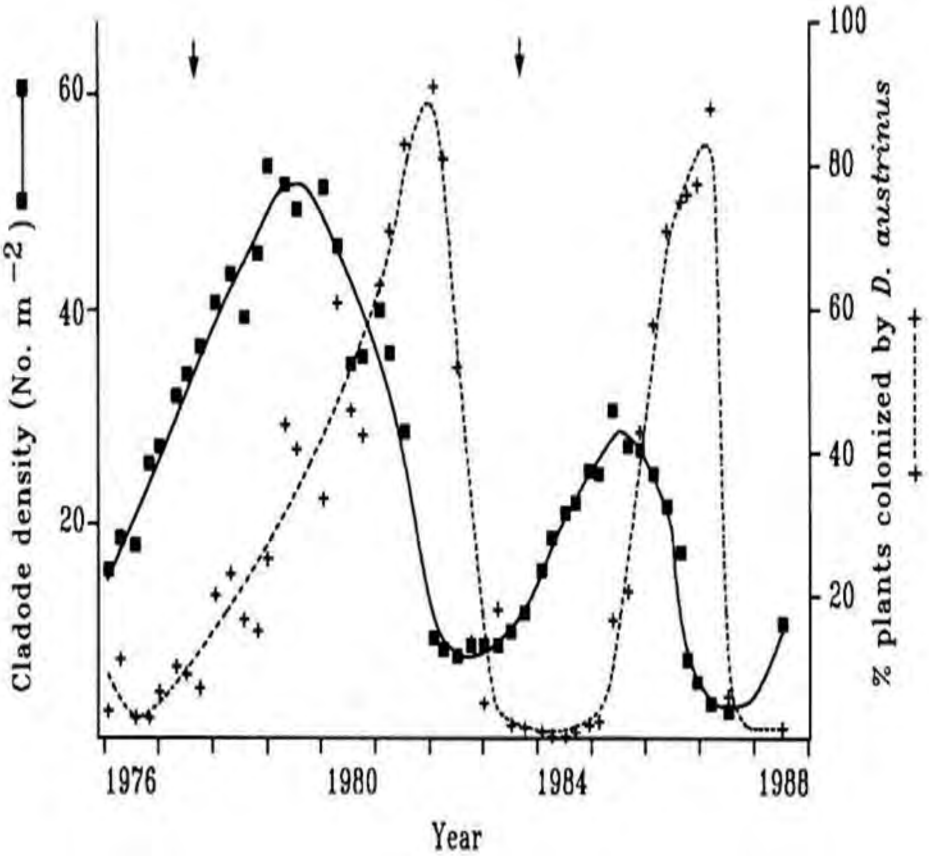

Figure 5.1 Average population variations of *O. aurantiaca* and *D. austrinus* from 1976 to 1988 on twenty 50 m^2 permanent plots (from Zimmermann and Malan 1989)

5.2. CONCLUSION

The benefits of biological control of invasive alien plants at the ecosystem-level are important and should be included as a part of categorizing levels of success (van Driesche et al. 2010). Biological control programmes have made a major contribution to the protection and restoration of natural systems worldwide (Causton 2001), and the benefits of biological control programmes have improved the functioning of natural ecosystem services which include air quality, flood control, fire regulation, and maintenance of healthy soils (van Driesche et al. 2010). The questionnaire survey reported on in this thesis was a relatively quick and simple way of quantifying the changes in ecosystem-level benefits as opposed to directly measuring these benefits, which is usually difficult to undertake, and similar studies could be conducted for other groups of target weeds. In combination with directly measuring impacts of biological control agents at some field sites, and assessing where agents have been released compared with the known distributions of the target weed, a supported assessment of the overall success of cactus biological control in South Africa can be made.

It is important to assess the success of biological control to validate the continuation of its use as a tool to manage invasive alien plants (Morin et al. 2009). This study was valuable to evaluate mass-rearing and releasing efforts and to quantify the impact and safety of biological control through long-term monitoring and land-users' perceptions. This was a short-term study, and continued monitoring over a longer period of time would be beneficial to more accurately quantify the benefits of cactus weed biological control. Even within the short period of this study undertaken, the benefits from cactus biological control are evident and there are indications that the level of control, as well as the benefits of control, will increase in years to come, especially if mass-rearing and release efforts continue. These data can be used to justify continued and increased investment in biological control of invasive alien cacti in South Africa.

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APPENDICES

APPENDIX A: QUESTIONNAIRE 1

RHODES UNIVERSITY
Where leaders learn

Centre for Biological Control

PROJECT TITLE: BIOLOGICAL CONTROL OF CACTUS WEEDS

CONSENT FORM

Dear respondents,

I am conducting a survey on the benefits of using biological control as a method against cactus weeds on your land. Data from this questionnaire will be used as part of my PhD research and your assistance is highly appreciated for my successful completion of this research project. Please try to answer all questions. Thank you very much for your time.

I,…………………………………………voluntarily agree to take part in the following questionnaire for research purposes.

I understand that even though I agree to participate now, I can pull out of the research project at any time or refuse to answer any questions.

I understand that I have the right to request that data from the questionnaire should not be used within two weeks after the interview; in this case, the material will be deleted.

I understand that the information I provide in the questionnaire will strictly be used for research purposes.

I have heard the purpose and nature of the study explained to me in writing. I have been given an opportunity to ask questions about the study.

I understand that all the information I provide for this study will be treated confidentially and my identity will remain anonymous.

I understand that some of the data collected from this questionnaire may be quoted in a, for example, thesis, oral or poster presentations, published papers, and manuals or protocols.

Signature of the respondent……………………………………………………………

I,…………………………………………………………………………have witnessed that the respondent is giving consent to part take in this research study.

Signature of the investigator: Date:

Venue:

QUESTIONNAIRE 1

Questionnaire No…..…………..

Date:

1. GENERAL

1.1. Name of the investigator:

1.2. Name of the area:

2. RESPONDENTS' BACKGROUND INFORMATION

2.1. Age ……………………………………………………………….

2.2. Gender……………………………………………………………….

2.3. Marital status ………………………………………………….

2.4. Highest level of education ……………………………….

2.5. Race …………………………………………………………………

2.6. Home language …………………………………………….

3. RESPONDENTS' OPINION ABOUT BIOLOGICAL CONTROL OF CACTUS WEEDS

3.1. Where did you first learn about biological control?

……
……

3.2. How did you hear that you can get biological control agents from the Centre for Biological Control's Uitenhage Facility?

……
……

3.3. Has the biological control agent increased since the release?
……
……
……

3.4. Has the biological control agent reduced the amount of cactus weed?
……
……
……

3.5. Why do you want to control the cactus weed? What harm does the cactus weed do on your land?
……
……
……

3.6. Does the cactus weed do less harm since the release of biological control agents?
……
……
……

3.7. Have the biological control agents harmed any other plants besides the problem cactus?
……
……
……

3.8. Would you recommend biological control to your fellow farmers/land managers?
……
……
……

3.9. Were you given enough information regarding biological control when it was first released? Or would you have liked more information?

………
………
………..

3.10. Have you been happy with your interactions with the Centre for Biological Control from Uitenhage? If no, how can we improve our engagements with you?

……….
……….
……….
……….

APPENDIX B: QUESTIONNAIRE 2

RHODES UNIVERSITY
Where leaders learn

Centre for Biological Control

PROJECT TITLE: BIOLOGICAL CONTROL OF CACTUS WEEDS

CONSENT FORM

Dear respondents,

I am conducting a survey on the benefits of using biological control as a method against cactus weeds on your land. Data from this questionnaire will be used as part of my PhD research and your assistance is highly appreciated for my successful completion of this research project. Please try to answer all questions. Thank you very much for your time.

I,……………………………………………….voluntarily agree to take part in the following questionnaire for research purposes.

I understand that even though I agree to participate now, I can pull out of the research project at any time or refuse to answer any questions.

I understand that I have the right to request that data from the questionnaire should not be used within two weeks after the interview; in this case, the material will be deleted.

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I understand that some of the data collected from this questionnaire may be quoted in a, for example, thesis, oral or poster presentations, published papers, and manuals or protocols.

Signature of the respondent………………………………………………………………

I,…………………………………………………………………have witnessed that the respondent is giving consent to part take in this research study.

Signature of the investigator: Date:

Venue:

QUESTIONNAIRE

Questionnaire No…………..

Date:

1. GENERAL

1.1. Name of investigator:

1.2. Name of the area:

2. RESPONDENTS' BACKGROUND INFORMATION

2.1. Age…………………………………………………………………..

2.2. Gender…………………………………………………………….

2.3. Marital status…………………………………………………….

2.4. Highest level of education……………………………….

2.5. Race……………………………………………………………….

2.6. Home language……………………………………………..

3. RESPONDENTS' OPINION ABOUT THE IMPACT OF CACTUS WEEDS

3.1. What is the extent of cactus weeds on your land?

………

………..

3.2. What is the negative impact of cactus weeds on your land?

……

………..

3.3. What was the land primarily used for before infestation by cactus weeds? And has the use changed since the infestation?

……

……

……

3.4. Are there any measures that have been implemented to control cactus weeds? If yes, may you please state them below?

……

……

……

3.5. Was it your first time to hear about biological control as a weed control method?

Yes ☐ No ☐

3.6. If no, where did you learn about biological control method before?

………

……

………..

3.7. Is biological control safe, or do the agents consume other valuable/beneficial plants?

……

……

……

3.8. How did you hear that you can get biological control agents from the Centre for Biological Control's Uitenhage Facility?

………

………..

3.9. Is there cochineal or any other biological control agent on your farm? If yes, does the cochineal or other biological control agent result in less of the cactus?

……

……

……

3.10. Do you understand how biological control works; do you need any more information?

……

……....

3.11. Do you think that the CBC releasing biological control agents will help solve your problem?

……

……

……

APPENDIX C: FOLLOW-UP QUESTIONNAIRE

PROJECT TITLE: BIOLOGICAL CONTROL OF CACTUS WEEDS

CONSENT FORM

Dear respondents,

I am conducting a survey on the benefits of using biological control as a method against cactus weeds on your land. Data from this questionnaire will be used as part of my PhD research and your assistance is highly appreciated for my successful completion of this research project. Please try to answer all questions. Thank you very much for your time.

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I understand that all the information I provide for this study will be treated confidentially and my identity will remain anonymous.

I understand that some of the data collected from this questionnaire may be quoted in a, for example, thesis, oral or poster presentations, published papers, and manuals or protocols.

Signature of the respondent……………………………………………………………

I,………………………………………………………………….. have witnessed that the respondent is giving consent to part take in this research study.

Signature of the investigator: Date:

Venue:

FOLLOW-UP QUESTIONNAIRE

1. RESPONDENTS' OPINION ABOUT BIOLOGICAL CONTROL OF CACTUS

The Centre for Biological Control (CBC) released a biological control agent on your farm. We would like to ask you a few questions about the process and the impact of the agent in order to improve our service.

1.1. Is there more of the biological control agent since the release?

……

……

1.2. Has the biological control agent damaged the cactus since the release?

……

……

1.3. Is there less cactus weed because of the biological control agent?

……

……

1.4. Has the negative impact of the cactus weeds decreased on your land since the release of the biological control agent?

……

……

1.5. Have any other plants been affected by the biological control agent?

……

……

1.6. Did the CBC give you all the necessary information required in order to understand what the biological control agent was expected to do, what it is and how to use it?

……

……

……

1.7. Would you have liked more information?

……

……

……

1.8. Would you be able to raise awareness about biological control method to other people?

Yes ☐ No ☐

1.9. If yes, may you please tell us who you would share it with?

……

…….

1.10. Will you be using biological control as a control method on your land?

……

……

…….

APPENDIX D: ETHICAL CLEARANCE

RHODES UNIVERSITY
Where leaders learn

Science Faculty Ethics Sub-committee
Biological Science Building, Grahamstown, 6139, South Africa
PO Box 94, Grahamstown, 6140, South Africa
t: +27 (0) 46 603 8443
f: +27 (0) 46 603 7576
e: j.dames@ru.ac.za

www.ru.ac.za

14 August 2018

Dear Dr Iain Paterson and Zezethu Mnqeta

Tracking Number SCI2018/042
Date Submitted 21 August 2018
Proposal title Evaluating the perception of biological control of cactus weeds in South Africa

This letter serves to notify you of the outcome of your ethics application submitted to the Science Faculty Ethics Sub-committee.

	Outcome	**Comments**
Approved	X	Approved no ethical concerns
Provisional approval with stipulations		
Refer to RUESC-HE/RUESC-AE		
Does not require ethics approval:		
Referred back to applicant		
Rejected		

Please ensure that the Science Faculty Ethics Committee is notified should any substantive changes be made, for whatever reason, during the research process. An annual progress report is required in order to renew approval for the following year. The purpose of this report is to indicate whether or not the research was conducted successfully, if any aspects could not be completed, or if any problems arose that the ethics committee should be aware of. If a thesis or dissertation arising from this research is submitted to the library's electronic theses and dissertations (ETD) repository, please notify the committee of the date of submission and/or any reference or cataloguing number allocated.

Prof Joanna Dames (Chair)