Augmentative releases of *Dactylopius austrinus* De Lotto (Dactylopiidae; Hemiptera) for biological control of *Opuntia aurantiaca* Lindley (Cactaceae), in South Africa

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Abstract

Opuntia aurantiaca Lindely (Cactaceae) is an invasive alien cactus which has detrimental effects on agroecosystems and indigenous biodiversity in South Africa. Dense infestations over large areas reduce grazing capacity and indigenous biodiversity. Despite the release of a biological control agent, the cochineal insect *Dactylopius austrinus* De Lotto (Dactylopiidae), the weed is still considered a major problem in many parts of the country. Biological control has relied heavily on classical biological control, with little augmentative biological control implemented. This study investigated the outcome of mass-rearing and augmentative releases of *D. austrinus* for the control of *O. aurantiaca*. Augmentative releases are thought to improve the level of control by increasing agent densities in the field and thus increasing the level of damage inflicted to the plants. All data were collected with the intention to optimize release strategies so that the maximum benefit from the biological control agent could be achieved.

An impact study was conducted using potted plants in a greenhouse to quantify the efficacy of multiple releases of the agent on the target weed. All three of the release treatments showed consistently higher proportion of cochineal than the controls, as well as the insect exclusion treatments, and these differences were statistically significant. The number of cladodes per plant increased significantly for the insect exclusion and control treatment over the period of the study, whilst all three release treatments decreased steadily over the same period. This study indicated that the agent is damaging to *O. aurantiaca* and that a single release event was beneficial but that multiple releases did not result in greater levels of control.

A post-release evaluation was carried out to quantify the impact of releases of *D. austrinus* on O. *aurantiaca* in the field. Plots where the agent was excluded were compared with those where the agent was left at natural field densities and three treatments where agent populations were augmented to varying degrees through releases. The percentage of cochineal infested cladodes for all treatments decreased over time from the initiation of the experiment in October 2017 until the end of the experiment in October 2018. *Opuntia aurantiaca* densities also decreased over time for all treatments. The insect exclusion treatment had the greatest number of plants for the duration of the study, but this was not significantly different from other treatments.

Dactylopius austrinus was damaging to *O. aurantiaca*, but climatic conditions in the field limited the efficacy of releases. Although *O. aurantiaca* density decreased during the experiment, it was evident that the reduced number of plants was not due to augmentation of the cochineal populations from the releases that were conducted. The experiment was conducted over a very dry period, when cochineal was particularly effective, so although augmentative releases did not improve the level of control, the natural population of cochineal was high and very damaging to *O. aurantiaca* over the course of the experiment. Releasing during wet periods, when the agent is less effective, could augment agent populations at a time when natural populations would be low, and hence improve levels of control further.

Although this study was limited to a short period of two years, the results of this study suggest that the number of releases is less important than the timing of releases. Releasing immediately after periods of high rainfall is likely to be beneficial, while releasing during dry periods, or during winter when temperatures are low, is less effective. *Dactylopius austrinus* populations should be constantly monitored so that releases can be conducted when cochineal populations are low and the climatic conditions are correct. If the timing of release events is appropriate, then the over level of control of *O. aurantiaca* using *D. austrinus* could be improved.

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Chapter 1: General Introduction

1.1 Introduction

Biological control of the invasive alien plant, *Opuntiae aurantiaca* Lindley (Cactaceae) or jointed cactus, has been implemented in South Africa since the release of the biological control agent, *Dactylopius austrinus* De Lotto (Dactylopiidae) in 1935 (Klein 2011). The biological control programme has significantly reduced the negative impacts of this damaging environmental and agricultural weed, but there are still many parts of South Africa where the plant is problematic, including parts of the Eastern Cape Province such as Grahamstown (Moran & Zimmermann 1991a). This study investigates the possibility of improving the level of biological control of jointed cactus through the development of a release strategy that aims to increase biological control agent populations in the field through mass-rearing and augmentative releases. In this chapter, the background to invasive alien plants, biological control of weeds and the agent-weed systems is provided.

1.2 Invasive Alien Plants

Invasive alien plants are an increasing threat to all ecosystems in the world (Richardson *et al*. 1997; Mack *et al*. 2001; Van Wilgen *et al*. 2004; Brooks *et al*. 2004; Le Maitre *et al*. 2004). These plants (often referred to as 'weeds') are a major threat to economic productivity, and they impact indigenous plants by altering the composition and community structure of invaded areas (Vitousek 1990; Pimentel *et al*. 2001; Brooks *et al*. 2004; Zimmermann *et al*. 2004a). The negative impacts associated with invasive alien plants have resulted in landowners increasingly looking for cost-effective control methods to reduce these negative impacts (Van Wilgen *et al*. 2004). Mechanical clearing, chemical control by application of herbicides, biological control and a combination of these methods (integrated control), are ways to reduce the population of invasive alien plants, thereby reducing their impacts (Van Wilgen & De Lange 2011).

Biological control of weeds is the introduction of non-native natural enemies (plant-feeding insects and mites, or plant pathogen) with the intention of using them to reduce the density and invasiveness of invasive alien plant populations (Briese 2000; Van Wilgen & De Lange 2011).

There are two different approaches to biological control referred to as classical biological control and augmentative biological control. Classical biological control is the mainstay of biological control of weeds and entails the use of introduced natural enemies against invasive alien plants that do not require further releases once they have established in the field (McFadyen 1998; Briese 2000). Similarly, augmentative biological control is the use of natural enemies, but it involves the mass production of natural enemies and large releases at regular intervals to reduce the population of the target weed (McFadyen 1998; Briese 2000; Van Lenteren 2012). Augmentative biological control is often conducted using generalist predators or insectary-reared parasitoids to control pest insects in agricultural ecosystems, but has more recently also been used for control of invasive alien plants, though on a much smaller scale (Van Lenteren & Bueno 2003: Dudley *et al*. 2006; Zachariades *et al*. 2017). One of the main reasons for the recent growth in both biological control approaches is the current international attitude regarding pesticides, which has helped make biological control a widely recognised control method used by farmers, conservationists and land managers for the control of pest organisms, including weeds (Van Driesche *et al*. 2010).

Augmentative biological control for weeds has been used in cases where biological control agents have poor dispersal capacity and/or where the weed occurs in fragmented habitats (McFadyen 1998). Augmentative biological control is therefore used when densities of biological control agents are low in space and time. This is done to increase the impact of biological agents by distributing them more widely and therefore providing a more extensive suppression of the weed over a greater area (McFadyen 1998; Dudley *et al*. 2016). Examples of augmentative biological control of weeds include the regular redistribution of mealybugs and cochineal insects in Australia (Hosking *et al*. 1988) and South Africa (Moran & Zimmermann 1991a); and agents for the control of various water weed species that are all restricted to waterbodies making it difficult for agents to disperse to new sites (McFadyen 1998; Hill & Coetzee 2017). One of the most well-known and successful biological control programmes, the control of *Opuntia stricta* Haworth (Cactaceae) using the agent *Cactoblastis cactorum* (Berg) (Pyralidae) in Australia, relied on an extensive mass-rearing programme which was key to the success of the biological control programme as a whole (Raghu & Walton 2007). Mass-rearing and redistribution of agents is considered an essential component of the overall biological control of weeds effort in South Africa and significant resources are allocated towards it (Zachariades *et al*. 2017).

1.3 Biological control of weeds

Evidently the abundance of invasive alien plant can be rapidly reduced by deliberately introducing specialist natural enemies from the native range of that plant (Keane and Crawley 2002). Therefore biological control of weeds is regarded as the most effective method for managing problematic weeds over large areas and over long periods of time (McFadyen 1998). It is environmentally safe because in most cases the target invasive alien plant is the only organism that is negatively affected (Briese 2000). The agents released are self-sustaining in that, once established, they can distribute themselves throughout the target weed distribution, which includes areas that are often difficult or impossible to access by human (Briese 2000; Culliney 2007). In examples where successful control is achieved, the densities of the agent population are able to adjust themselves in response to the target weed's population, so with an effective agent that reduces weed populations there will be a corresponding reduction in the density of the agent (Culliney 2007). In some cases insect herbivores are regulated by restricted access to high-quality food, and the reduction in host plant densities also reduces insects densities due to starvation caused by limited food (Crawley, 1989). No harmful chemicals are used in biological control, and the agents are host specific, making it the most appropriate method to control invasive alien plants in ecologically sensitive conservation areas and aquatic habitats (Culliney 2007).

Chemical and mechanical control of environmental weeds can often be expensive and unsustainable in the long term as they require repeated intervention over a prolonged period (Clewley *et al*. 2012). They are sometimes difficult to apply in areas that are inaccessible, and are not economically feasible where the weed is widespread (Culliney 2007). Chemical control is not appropriate in environmentally sensitive habitats because of the high costs relative to the economic value of land, especially when management over a large area is required. In a similar way, mechanical control involves hand-pulling, hoeing, tillage, bulldozing or harvesting; it disrupts habitats and disturbs wildlife, and contributes to soil compaction and erosion (DiTomaso 1997). In addition, both herbicides and mechanical control can lead to extensive disturbances that create niches for colonisation by other invasive species. Biological control is therefore widely considered as the most effective weed control method for invasive alien plant species in the long-term (Zachariades *et al*. 2017).

The biological control of weeds is not always successful, but the success rates are high enough to warrant an investment. Some of the failures in biological control programmes may be caused by factors such as ecological attributes of target plants versus agents, climate and suitability of agent. Globally, the high level of safety (Suckling & Sforza 2014) in combination with the relatively high success rate of about 65% (Schwarzlander *et al*. 2018) has resulted in the widespread use of biological control (Winston *et al*. 2014). South Africa is one of the countries which has had a high level of success and an excellent safety record in biological control of weeds (Zachariades *et al*. 2017) and this has resulted in biological control becoming a crucial component in the management of problematic weeds over the last 100 years and has increasingly received support from the government in South Africa (Moran *et al*. 2013).

There are unique challenges in every biological control programme, but all follow the same framework. Biological control entails a series of stages: from pre-introductory surveys where the agent is intended for release, to surveys to source potential candidate agents in the native distribution, testing the agents for possible non-target impacts on other plant species (host specificity testing), pre-release studies to quantify possible efficacy, mass-rearing and finally the release of the agent followed by monitoring the agent's success in the field (Briese 2000; Van Klinken & Raghu 2006).

Pre-introductory surveys in the introduced range

Pre-introductory studies provide information on the invasive alien plant in its introduced range prior to the development of biological control agents (Dudley *et al*. 2006). This usually involves a detailed study of the weed's biology and ecology; as well as genetic and climatic matching (Wapshere *et al*. 1989; Briese 2000; Sheppard 2003). Pre-introductory studies provide evidence on the invasiveness of the weed, such as the extent of invasiveness, and traits that enables it to thrive in the introduced range. The information obtained from the pre-introductory study can help identify factors that determine agent performance and ultimately efficacy p, and/or stages of the weed's lifecycle that are likely to be good targets for control. Moreover, pre-introductory studies provide evidence of whether or not enemy release is a factor promoting a plant's invasiveness, and determines if there are any biological control agents that are already established in the introduced range (Hoffmann 1988; Keane & Crawley 2002; Dudley *et al*. 2006). For example, in South Africa, Canavan *et al*. (2014) conducted a pre-introductory study on *Arundo donax* L. (Poaceae) in the Free State Province, prior to

development of biological control programme. The pre-introductory investigation was able to determine that the potential biological control agent, *Tetramesa romana* Walker, had already established in South Africa (Canavan *et al*. 2014).

Selecting natural enemies in the native range

After pre-introductory surveys have been completed, promising potential biological control agents must be sourced from the native distribution. It is believed that most effective natural enemies will be from areas into which the eco-climatic conditions are broadly similar to those where the weed is problematic (Briese 2000; Goolsby *et al*. 2006; Robertson *et al*. 2008; Paterson *et al*. 2014). As a result, organisms selected where the climatic conditions match that of the introduced region (Robertson *et al*. 2008). In the same way, genotype matching is considered useful when searching for natural enemies, because natural enemies feeding on native plants with a similar genotype to the plants in introduced range have increased chances of local adaptions for feeding on the invasive plants genotypes (Goolsby *et al*. 2006; Paterson *et al*. 2014). The chances of finding such natural enemies are high at the "centre of diversification" of weed tribes, genera and sub-genera (Wapshere *et al.* 1989, Briese 2000). Such organisms can then be prioritised and studied further to determine their potential as biological control agents. Prioritisation of potential natural enemies based on the likely efficacy of the agent is also considered a vital step in biological control programmes (Van Klinken & Raghu 2006; Paterson *et al*. 2014). Prioritising potential biological control agents increases the likelihood of successful biological control and reduces costs (Klinken & Raghu 2006). Once a promising agent is selected, it must be imported into quarantine in the introduced distribution for further testing.

Host-specificity testing

Safety of candidate natural enemies for biological control of weeds is one of the primary concerns in biological control programmes. It is requisite for all selected candidate natural enemies to undergo host-specificity testing before they are released in the field (Van Klinken 2000; Sands & Van Driesche 2000; Heard 2000; Briese 2000; Klein *et al.* 2011). This is regulated by the Department of Agriculture, Forestry and Fisheries (DAFF) as when as the Department of Environmental Affairs (DEA) in South Africa. Host-specific testing is designed to determine whether the release of the natural enemy into a new region will result in an

unacceptable damage to non-target plants (Marohasy 1998; Sands & Van Driesche 2000; Van Klinken 2000; Sheppard *et al*. 2005; Fowler *et al*. 2012; Paynter *et al*. 2015). It can take up to three to five years to perform host-specificity testing on selected natural enemies and this often requires considerable financial support (Fowler *et al*. 2000).

The testing method has evolved from testing only economical valuable plants to using the "centrifugal phylogenetic" testing procedure developed by Wapshere (1974). In this procedure, plants that are close relatives to the target weed form part of the testing list, for example, congeneric plants, then plants within the tribe or subfamily, then within the family and then other close relatives and/or distant relatives of the target weed are tested (Wapshere *et al.* 1989; Pemberton 2000; Briese 2000). A greater proportion of species of close relatives to the target weed are tested with fewer and fewer species of more distantly related taxa. It has been argued, that this method focuses more on taxonomic circumscriptions and instead tests should focus more on phylogenetic relatedness, biogeography overlap and ecological similarities (Briese 2005; Briese & Walker 2002). Modernizing the centrifugal phylogenetic method will ensure that test plants include the close phylogenetic relatives, as these are the plants that are most likely to be suitable non-target hosts (Briese 2005; Briese & Walker 2002). This is due to the fact that plants that belong to the same genus as the target weed are more susceptible to non-target damage than more distant-relatives (Pemberton 2000).

The sum of plant species that a natural enemy is able to utilize is defined as the natural enemies' host range (Van Klinken 2000). Host range can be divided into the fundamental host range and the realised host range. The fundamental host range is defined as all the plants that a natural enemy is able to accept and/or utilize when no alternative is offered (Van Klinken 2000; Sheppard *et al*. 2005). The realized host range or field host range is what a natural enemy is able to accept and/or utilize under field conditions, such as the conditions in the introduced range after introduction (Van Klinken 2000). There are a number of different methods that are employed to determine host ranges of a candidate natural enemy (Withers *et al.* 2000; Sutton *et al*. 2017). In traditional no-choice tests, the candidate natural enemy is confined to a cage in the presence of non-target plant species one at a time under standard conditions (Marohasy 1998; Withers *et al*. 2000; Sheppard *et al*. 2005). In choice tests, a candidate natural enemy is presented with several test plant species including the target plant species for varying periods of time (Sand & Van Driesche 2000; Withers *et al*. 2000; Marohasy 1998). The most reliable

form of determining the realised host range of candidate natural enemies is open field trials because these trials are considered the best representative of what is likely to occur in the introduced range (Sheppard *et al*. 2005; Paynter *et al*. 2015). However, open field trials are not always possible, because natural enemies have to be kept under quarantine conditions in the introduced range, and due to the fact that test plant species that are not found in the native range of the target plant may not be able to be imported as they may be at risk of becoming invasive (Paynter *et al*. 2015; Sutton *et al*. 2017).

The results of host specificity testing may overestimate (false positive) and underestimate (false negative) the fundamental host range of a candidate agent (Sheppard *et al*. 2005; Marohasy 1998). According to Marohasy (1998), a "false positive" is when the test indicates that there will be an attack to non-target plants whereas there will be no potential attack under field conditions. A "false negative" is when the test indicates that there is no attack to a non-target whereas there is a potential for attack under field conditions (Marohasy 1998). False negative and false positive results may be caused by behavioural mechanisms such as effects of experience and learning. For example, the natural enemy may be stimulated to oviposit on a test plant that it wouldn't use under field conditions if plant volatile cues from the target weed have accumulated around the non-target test plant (Sutton *et al*. 2017). Such mechanisms used by the natural enemy for host-plant selection should therefore be taken into consideration when interpreting results (Sutton *et al*. 2017). False negatives occur when the natural enemy is not responsive on lower-ranked taxa of test plant species while in the presence of the primary host plant, however, may feed on it when released in the field (Paynter *et al*. 2015). False negatives could therefore lead to the release of the insect that might feed on non-targets. Choice-minus-target tests in which the natural enemy is presented with a number of non-target plants in the absence of target plant have since been developed to succeed in dealing with some of these problems (Shepard *et al*. 2005).

It is no longer acceptable to just declare a natural enemy 'safe' for release. There is a need to clarify all possible impacts however minimal, such as localized infrequent adult feeding with short-term effects, and/or whether there is a chance of plants being colonized by reproducing populations with long-term ecological and evolutionary consequences (Briese 2005). Risk assessors in biological control programmes need to be able to interpret and communicate host specificity testing data in a way that is understandable to regulators (Briese 2005). However,

regulators still need to make a decision in every case about whether the likelihoods of success are worth the risks, without absolute certainty of what might happen if a release goes ahead (Briese 2005; Downey & Paterson, 2016).

The possibility of biological control agents feeding on non-target plant is one of the main concerns in the safety of biological control (Pemberton 2000). This has resulted in biological control practitioners focusing primarily on host specificity testing and only releasing agents that are very clearly host specific to the target weed. Despite a highly risk-averse approach to the release of biological control agents, some agents are paradoxically released even though host-specificity tests show the potential for non-target effects (i.e. a predicted non-target risk) (Downey & Paterson 2016). An example is one of the few cited cases of non-target impacts given by Louda *et al*. (1997). The introduced flowerhead weevil, *Rhynocyllus conicus* Froeh., was released in North America in the 1960s for biological control of Eurasian thistles of the genus *Carduus L*. (Pemberton 2000; Louda *et al*. 1997). Although an attack on non-target species was anticipated from the results of host specificity testing (Zwölfer & Harris 1984), the flowerhead weevil was released and did feed on native thistles of the genus *Cirsium,* and had a direct effect on the seed production of native thistles which as a result threatened the existence of these species (Louda *et al*. 1997). This example is widely regarded as a biological control programme with unintended consequences, but these consequences were accurately predicted in host specificity testing and were regarded as less important than the damage done by the weed (Downey & Paterson 2016).

Although biological control has been criticised due to non-target impacts, there are a few recorded cases of non-target impacts compared to the number of released agents (Suckling & Sforza 2014). Of 512 examples, there are only two significant non-target effects from biological control agents and both of these were predicted from host specificity testing (Suckling & Sforza 2014). South Africa has not had a case of any agent causing substantial damage to non-target plants (Moran *et al*. 2013), and globally the safety record of biological control is greater than 99% (Suckling & Sforza 2014). Host specificity testing is therefore a reliable way to predict whether biological control agents are safe for release, making biological control an environmentally safe management practice as long as host specificity testing procedures are followed correctly.

Determine the potential impact of the agent

If a potential agent proves to be suitably host specific, the next step is to conduct pre-release studies to evaluate the efficacy of the agent. Releases of agents that are not suitably damaging should be avoided, and although it is very difficult to determine how damaging an agent will be prior to release, it is important that research is done to show that the agent can reduce the invasiveness of the target weed (McClay & Balcianus 2005). These studies are either conducted in the field in the native range or under a controlled environment in quarantine in the introduced range. The efficacy of the agent is sometimes determined by using the formula Impact= Range × Abundance × Per capita effect (Goolsby *et al*. 2009). In this formula 'range' and 'abundance' are measured in the native distribution during surveys and 'per capita effect' is calculated in quarantine. In this way, effects of the agent on relevant parameters to the plant's performance in the adventive range; such as biomass, seed production and its ability to proliferate and out-compete indigenous plants, are measured and quantified (McClay & Bacillus 2005; Goolsby *et al*. 2009). The results obtained in these studies are therefore of importance and are one of the tools that are considered useful when assessing the effectiveness of candidate biological control agents.

Post-release evaluation of agents

Once the host-specificity and impact testing of candidate agents is complete, appropriate release sites are selected throughout the invaded distribution where detailed establishment and population increase of the agent can be studied without disturbance (Wapshere *et al*. 1989). This is followed by monitoring short-term effects on the weed population by comparing sites with and without the agent (Morin *et al*. 2009). Short-term monitoring focuses on the population increase of released agents and their impacts on target weeds (Briese 2000). Long-term monitoring on the other hand focuses on the efficacy of the agents, and understanding factors influencing their effectiveness (Morin *et al*. 2009). Data collected from long-term monitoring studies can then be used in cost-benefit economic analyses to justify the investment in biological control and to make a case for continued investment in the science and its application (Morin *et al*. 2009). Long-term monitoring studies can also allow us to quantify reductions in the weeds density as well as the negative impacts caused by the weed (e.g., an increase in indigenous biodiversity or increase in water quality/quantity) (Paterson *et al*. 2011b;

Zachariades *et al*. 2017). Studies like these are however, rarely conducted due to funding, time and availability of resources (Morin *et al.* 2009).

1.4 Improving control through mass-rearing and releases

Release strategies in biological control are generally not very well studied. Some work has been done on testing different release strategies to increase establishment success (Grevstad 1999a,b), but there are no published studies that test different biological control release strategies and then quantify the relative impact of each release strategies on populations of the target weed in the field. With the increased use of augmentative biological control in South Africa (Zachariades *et al*. 2017) there is a need to better understand how this method should be implemented in order to maximise the benefits of biological control. How many releases, what quantity of insects in the releases, and when releases should be made, are just some of the questions that need to be answered for each weed species that is targeted for augmentative biological control. The ultimate goal is to reduce weed densities, so an understanding of how the different release strategies influence agent populations in the field and how this in turn impacts the density of the target plant population is needed.

1.5 Efficacy of biological control

Four categories are recognized in evaluating weed biological control programmes in South Africa (Hoffmann 1995). The categories are defined based on the assessment of how the use of alternative control methods, such as chemical and mechanical control, have been reduced since the introduction of the biological control program (Hoffmann 1995; Klein 2011).

Complete: alternative control methods are not required to decrease the weed population to adequate levels, at least in areas where the agents have established.

Substantial: alternative control methods (either chemical or mechanical method) are still needed but to a lesser extent than if agents had not been released (e.g. a reduced amount of herbicide or a reduced number of regular herbicidal applications).

Negligible: control of weeds is still dependent on the application of alternative control measures, in spite of damage inflicted by biological control agents.

Not determined: the agent has been released too recently to evaluate or the program has not undergone an evaluation for another reason.

These categories are useful when estimating the success of biological control programmes, many of which lack quantitative data on post-release evaluations (Zachariades *et al*. 2017). Since many programmes do not have formal post-release evaluations, the system proposed by Hoffmann (1995) allows for an estimation of biological control success on a broad scale.

South Africa has well-documented cases of the successes of the release of biological control agents in managing alien invasive species (Zachariades *et al*. 2017). In South Africa, of the 106 natural enemies released, seventy-five (71%) have become established on 48 invasive alien plant species in 14 plant families (Klein 2011). Biological control has led to the complete control of 10 (21%) of the 48 plant species on which the agents have become established, and substantial levels of control in 18 (38%) (Klein 2011). Fourteen (29%) of the target weed species are considered under a negligible degree of control and the status of five (10%) of them is as yet, unknown (Klein 2011).

A recent study by Schwarzländer *et al*. (2018), reported on biological control introductions of all intentional releases made until 2017 worldwide. The report includes data on 1555 releases of 468 biological control agent species released against 175 target weeds which belong to 48 plant families in 90 countries. Of the 468 biological control agents released, 332 (70.9%) established in at least one instance. Of the 313 agent, for which impact could be categorized, 172 (55.0%) caused medium, variable or heavy levels of damage (impacts). From all the releases made world-wide, the overall control rate stood at 65.7% (Schwarzländer *et al*. 2018).

1.6 Biological control of Cactaceous weeds in South Africa

In South Africa, biological control is considered the most effective and economically viable method of managing alien invasive cactus species (Moran & Zimmermann 1991b; Zimmerman & Moran 1991; Klein 1999; Paterson *et al*. 2011a). Biological control against invasive Cactaceae has had a long history and has included the use of a wide range of insects including various cochineal species (Zimmermann *et al*. 2009). The first ever use of cochineal insects for biological control of invasive cactus species was recorded in India. Initially referred to as "Indian cochineal", the cochineal insect, *Dactylopius ceylonicus* (Green) (Dactylopiidae), was

the first ever biological control agent deliberately released for biological control of an invasive alien plant anywhere in the world (Lounsbury 1915). In India, *D. ceylonicus* successfully controlled large infestations of the prickly pear *Opuntia monacantha* Haw., although it was not intentionally released for control, but rather for the dye that is produced by the cochineal insect (Lounsbury 1915). Destruction of *O. monacantha* by the cochineal insects in India drew the attention of entomologists who wished to control cactus weeds in other countries and the agent was then released in South Africa in 1913 (Lounsbury 1915) and Australia in 1914 (Walton 2005). In South Africa, *O. monocantha*, commonly referred to as drooping prickly pear, was recognized as an important pest plant along the eastern coastal region of the country. Within a few years, the cochineal insects had reduced these infestations to just a few plants, resulting in complete and permanent control (Zimmermann *et al*. 2004a; Zimmermann *et al.* 2009; Moran *et al*. 2013).

The successful biological control of *O. monocantha* led to the further release of 14 cochineal entities (species and biotypes) for biological control of 15 cactaceous weed species in South Africa (Paterson *et al*. 2011a). Of the 15 cactaceous species, four are considered to be under complete control, eight under substantial levels of control, one under negligible control, while the level of control for the two remaining cactus species is yet to be determined (Klein 2011). The biological control agents were initially released against eight target species (including *O. monocantha*), however, they eventually became established on seven additional cactus species (Paterson *et al*. 2011a).

Biological control of cactus weeds has been more successful than for most other weed taxa. This is partly because, with the exception of the cosmopolitan cactus species, *Rhipsalis baccifera* (J. Miller) Stern., there are no cactus species that are native outside of the Americas (Zimmermann *et al*. 2009; Paterson *et al.* 2011a). This phenomenon has allowed the use of agents that are less host specific (i.e. oligophagous species that feed on many cactus species) than is usual in other biological control programmes (Zimmermann & Granata 2002). Oligophagous cactophages can be used for biological control without risk to non-target native or commercial plants, thereby broadening the pool of suitable candidate agents that are available (Paterson *et al*. 2011a). The successful biological control of Cactaceae in South Africa has also been influenced by the unusual aspects of the biology of the plants and of cactophagous insects (Paterson *et a*l. 2011a). The taxonomic isolation and unique morphology

and anatomy of the family Cactaceae are the main attributes that contribute to adaptations in their insect herbivores which has resulted in very few cactophage insects being able to feed on any plant species outside the family (Mann 1969; Moran 1980). Cochineal insects and *Hypogeococcus* species, both cactus specialists, have contributed to the successful biological control of many cactus species in South Africa. For example, the biological control of various cacti species such as *Cereus jamacaru* De Candolle and several *Harrisia* species have been controlled by *Hypogeococcus* sp. (Moran and Zimmerman 1991b; Klein, 1999; Paterson *et al*. 2011a; Sutton *et al.* 2018); while *Opuntia stricta* (Haw.) Haw. (Moran & Zimmermann 1991b; Hoffmann *et al*.1999) *Opuntia. ficus-indica* (L.) Mill.) (Zimmermann & Moran 1991),and *Cylindropuntia fulgida* (Engelm.) F.M.Knuth var. *fulgida* (Paterson *et al*. 2011a) have all been controlled using various cochineal insects.

1.7 Jointed cactus, *Opuntia aurantiaca* Lindley

Morphology and biology

Jointed cactus, *Opuntia aurantiaca* Lindley, the focal organism of this thesis, is a low-growing cactus, which consists of one or many jointed stems which grow from tuberous roots (Fig. 1.1) (Gunn 1979). Jointed cactus plants seldom grow to more than 0.5m in height, however, they can grow up to 2 m height in dense bushes as a result of stems getting support by surrounding vegetation (Zimmermann & Van De Venter 1981). Each cladode is 5- 20cm long with numerous barbed spines of 1-3cm in length (Zimmermann 1981). Young cladodes are slightly flattened and are bright green in colour, whereas older cladodes become cylindrical with a corky surface (Zimmermann 1981). Climatic conditions and habitat play a role in the morphology of jointed cactus plants, with plants growing under bushes and in high rainfall areas characterized by slender, long cladodes and shorter, thinner spines (Zimmermann & Van De Venter 1981). Jointed cactus plants in South Africa do not produce viable seeds and therefore reproduce by vegetative means only (Gunn 1979; Zimmermann 1981). Cladodes become detached from the plants easily and adhere to livestock, wild animals and vehicles, until they fall to the ground and root (Zimmermann, 1981).

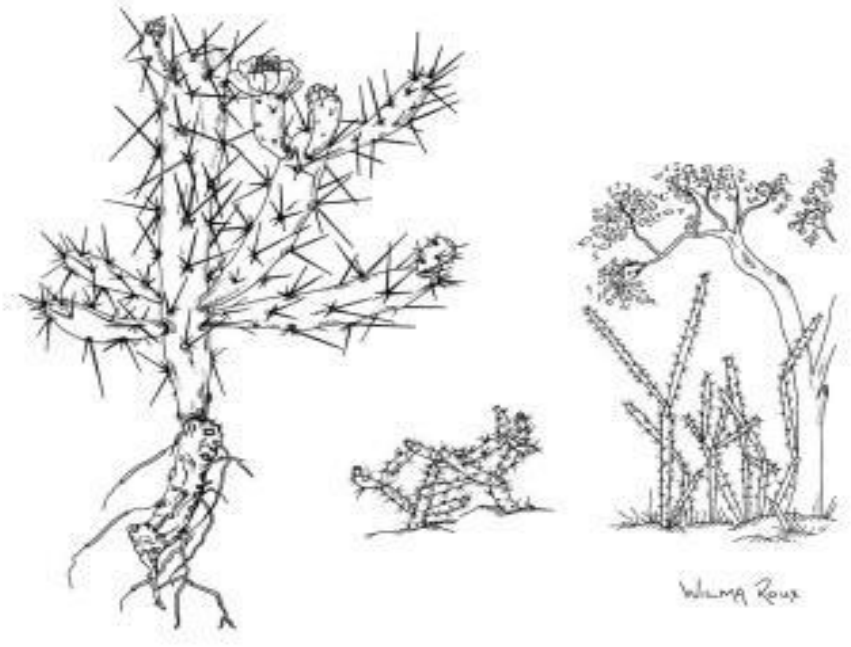

Figure 1.1. *Opuntia aurantiaca*. (From Henderson 1995).

Distribution

Opuntia aurantiaca is native to east Argentina and Uruguay (Gunn 1979; Zimmermann 1981). At first, there was confusion with regards to the identity and native range of the species. It was suggested that the "centre of evolution" of jointed cactus was the Caribbean and that it was introduced into Argentina and Uruguay (Moran *et al*. 1976). However, based on seed sterility, pollen morphology, cytology, insect associations, morphology and distribution, it was later suggested that jointed cactus is a hybrid taxon with *Opuntia salmiana* Parm. ex Pfeiffer and *Opuntia discolor* Britton & Rose, as putative parents (Arnold 1977; Gunn 1979; Zimmermann 1981). It is now generally accepted that jointed cactus is native to east Argentina and Uruguay where it is distributed along the riverine bush of the Parana and Uruguay rivers (Gunn 1979; Zimmermann 1981).

Jointed cactus is known to have been brought deliberately into South Africa as a collector's item in the 1840s and was first recorded by McGibbon in the Ludwigsberg Gardens in Cape Town (McGibbon 1858). Jointed cactus then naturalised and spread, with dense infestation noted in the Eastern Cape in the Gamtoos River region, further north in the country on the bank of the Vaal River between the towns of Christiana and Douglas, and isolated infestations in southern KwaZulu-Natal (Gunn 1979; Zimmermann 1981). To date, jointed cactus is

widespread in the Eastern Cape and is present, but less abundant, in other provinces of South Africa (Fig. 1.2).

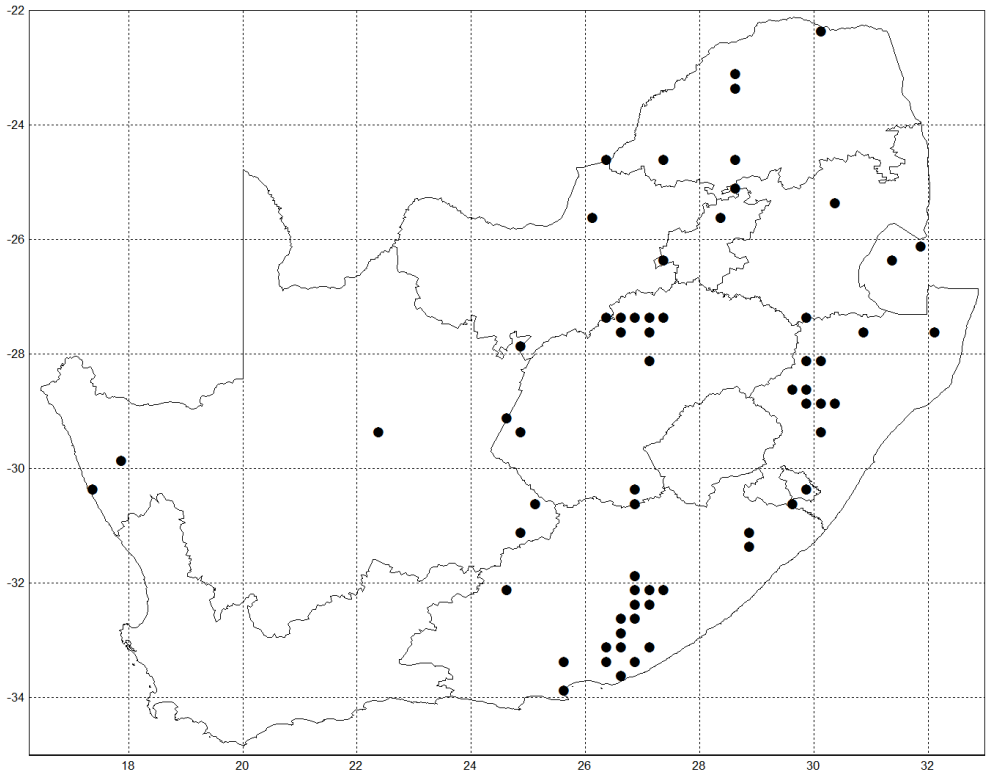

Figure 1.2. Distribution of *Opuntia aurantiaca* in South Africa (Drawn by L. Henderson, 2016; South African Plant Invaders Database [SAPIA], ARC-Plant Protection Research Institute, Pretoria, South Africa). Filled black circles indicate the presence of *O. aurantiaca* in a quarter-degree grid cell.

Negative impacts

In South Africa, dense infestations of jointed cactus over large areas reduce grazing capacity and replace nutritive plants (Zimmermann & Van De Venter 1981). Dense infestations of large plants, or highly aggregated population of jointed cactus, make it difficult for grazing and browsing animals to move through the veld for grazing and accessing water sources. Sharp spines on the easily detached cladodes penetrate the skin causing sores, abscesses and often lameness in smaller stock (Hosking & Deighton 1980; Moran & Zimmermann 1991a). As a result, the value of wool is adversely affected with significant negative economic consequences (Gunn 1979). Jointed cactus is not only a problematic weed in South Africa, but it is also a

weed of importance in Queensland and New South Wales, Australia, where it is commonly known as Tiger pear (Zimmermann 1981).

1.8 Chemical control of jointed cactus

Chemical control of jointed cactus encompasses spot-spraying plants with herbicides (Zimmermann 1981). This control method together with mechanical methods, were the first considered for control of invasive jointed cactus before biological control was introduced in the 1920s. Chemical control is expensive and time-consuming, and requires labour to spot-spray the plants (Zimmermann 1981). Chemical and mechanical control did not successfully reduce jointed cactus infestations and was withdrawn when biological control was introduced (see section 1.5) (Zimmerman, 1981). For example, a study on the chemical control of jointed cactus by spot-spraying plants with the herbicide Tordon (Iso-octyl ester picloram) diluted with illuminating paraffin (kerosene) was conducted on the farm Maastricht located near Grahamstown in the late 1970s (Zimmermann & Malan 1980). The herbicide had a positive effect on large plants (i.e., killed all large plants); but also had a negative effect on the cochineal insects that were already present on the plants (Zimmermann & Malan 1980). Chemical control as a whole proved to be less effective than biological control because spot-spraying failed to locate small plants and some medium-sized plants as a result of plants being hidden under grass and low bushes (Zimmermann & Malan 1980). Later, the use of water-based MSMA was recommended because Tordon had a greater negative insecticidal effect on the cochineal (Zimmermann 1981).

1.9 Biological control of jointed cactus

Native to Argentina where its host plant is *Opuntia delaetiana* Weber, the cactus moth, *C. cactorum* is a natural enemy that was released for biological control of prickly pear (*O. ficus-indica*) in South Africa in 1933 (Moran & Zimmermann 1991a; Klein 2002). *Cactoblastis cactorum* not only became established on *O. ficus-indica* but also colonized many other cactus species, including jointed cactus (Moran & Zimmermann 1991a). The cactus moth is a generalist cactophage that specialises on feeding on most *Opuntia* species and the close relative of the genus, so the utilisation of other cacti in South Africa was expected (Paterson *et al.* 2011). *Cactoblastis cactorum* is an internal feeding lepidopteran and therefore prefers large cladodes (such as those on prickly pear) rather than the small jointed cactus cladodes. Although

jointed cactus plants are damaged by *C. cactorum*, its contribution to the biological control of jointed cactus is considered insignificant (Klein 2002).

The phycitid moth *Tucumania tapiacola* Dyar is an oligophagous moth that is widely distributed in central Bolivia as well as central and north Argentina, and was first introduced in Australia in 1935 for biological control of *O. aurantiaca*. Although *T. tapiacola* became established causing minor damage to joined cactus in Australia, two consignments of *T. tapiacola* that were introduced into South Africa failed to establish (Moran & Zimmermann 1991a). Another insect that was attempted for biological control of jointed cactus was the pyraustid moth, *Mimorista pulchellalis* (Dyar), which was released between 1979 and 1982 in three sites around Grahamstown but also failed to establish (Moran & Zimmermann 1991a).

1.10 Cochineal insects

Native to South America and North America, cochineal insects (family Dactylopiidae, genus *Dactylopius*) are sap-sucking insects that only feed on cactus species (De Lotto 1974; Moran & Cobby 1979). There are only nine cochineal species that have been described, and each species is known to utilize and live in colonies on the surface of only a few closely related cactus species (Klein 2002). Adult females and adult males are morphologically distinct from each other but males rare (Gunn, 1979). Females are covered with a coat of white, woolly wax and are composed of body fluids which are dark red in colour as a result of the high content of carminic acid, which is the source of red cochineal dye (De Lotto 1974). Adult males, on the other hand, having no resemblance to females, are tiny, pink, mobile insects with two semi-transparent wings and long "tail" filaments (De Lotto 1974). Females lay up to a thousand eggs which hatch within minutes of oviposition and give rise to mobile pink nymphs, referred to as crawlers, which are less than 1mm in length (Klein 2002). Crawlers are easily dispersed by wind because they are covered with elongate, stiff bristle that acts as parachute for wind dispersal (Klein, 2002). During warm conditions, crawlers move upwards to a high point of the host plant or other vegetation from where they are dispersed by the wind (Klein 2002).

Although cochineal insects are very successful biological control agents, there are some important limitations that are worth noting. Females are sessile so dispersal is limited to the crawler stage and crawlers depend on the wind for dispersal because they are unable to fly (Klein 2002). Cochineal insects are therefore more effective in dense infestations of cactus

plants than in sparse infestation. High rainfall regions are less suitable for cochineal insects because rain erodes and washes away the protective wooly wax that is excreted buy the cochineal, exposing the insects to predators and sometimes dislodging the sessile insects from the plant.

1.11 The cochineal insect, *Dactylopius austrinus* De Lotto

The cochineal insect, *Dactylopius austrinus* De Lotto (Dactylopiidae) has also been referred to as D. sp. *confusus* and species 'J' (Zimmermann 1981). *Dactylopius austrinus* is indigenous to dry areas of central, north and western Argentina (Gunn 1979; Zimmermann 1981). In its indigenous country, it is found on various low-growing cactus species namely *O. retrorsa, O. discolor, O. canina, O. kiska-loro, O. palmadora, O. sulphurea, O. brunnescens,* and *Opuntia (Austrocylindropuntia) salmiana* (Gunn 1979; Zimmermann 1981). *Dactylopius austrinus* was introduced into South Africa in 1938 (Klein 2011). The original host plant of the consignment that was released in south Africa was thought to be an unidentified prickly pear, possibly *O. sulphurea*, found in the province Catarmaca in Argentina. The cochineal from this plant was transferred to *O. aurantiaca* and then shipped to South Africa and Australia (Gunn 1979). *Opuntia aurantiaca* is however the only plant that host *D. austrinus* in South Africa (Klein 2011).

Females *D. austrinus* oviposit eggs, which are 0.5 m long, in a single or chain manner depending on the rate of production (Gunn 1979). Hatching of first instar crawler can occur a minute or a few hours after oviposition (Gunn 1979; Zimmermann,1981). According to Moran and Cobby (1979), within 24 hours after hatching, crawlers can be easily sexed. Male develops long glassy filaments on the abdomen posteriorly only, whereas the female has more conspicuous filaments on the dorsal surface of the body anteriorly and posteriorly.

The female crawlers move away from the parent after hatching, colonize a new host-plant or new feeding site (this usually takes up to ten days), while males settle near the protective wax-covering of the parent female where they feed intermittently (Moran & Cobby 1979; Zimmermann 1981). After colonizing a new feeding site, the female crawlers insert their mouthparts, start to feed, remain sessile and continue to feed for the rest of their lives (Moran & Cobby 1979; Gunn 1979). Shortly after feeding, first instar males secrete a woolly wax which is shed together with the old skin at the first moult (Moran & Cobby 1979; Klein 2002).

Second-instar males spend about five days feeding and wandering away from the females and secrete more wax of which a cocoon is formed (Moran & Cobby 1979). Whilst inside the cocoon, the males undergo three further moults, and finally become winged adults (Klein 2002). The adult males do not feed and die after inseminating a single female (Moran & Cobby 1979). *Dactylopius austrinus*, just like any other cochineal insect, have a poor mode of dispersal that is limited to first instar crawlers which are dispersed by wind, as the males are very week fliers (Gunn 1979). No records exist about discrete generations in the field, and all instars of *D. austrinus* are believed to be found on host plants throughout the year (Gunn 1979).

Jointed cactus plants damaged by *D. austrinus* are characterized by yellow, rotten and abscised cladodes. It takes about a year and six months for nymphs and adult females to successfully feed and destroy jointed cactus plants. For example, most of the outer parts will die and fall to the ground, leaving only hard, woody main stems which may later die depending on the weather conditions (Klein 2002). *Dactylopius austrinus* is more effective at damaging high densities of jointed cactus plants growing in dry conditions than wet conditions because; rainfall tends to erode and wash away woolly wax exposing cochineal insects, washing insects off the plant and making them susceptible to predators (Hosking & Deighton 1980; Klein 2002). Water stressed *O. aurantiaca* also have a reduced ability to survive feeding by *D. austrinus* (Hosking & Deighton 1980).

1.12 Biological control of *Opuntia aurantiaca* in South Africa

Dactylopius austrinus was first released in South Africa in June 1935 (Moran & Zimmerman 1991a). Infestations over large areas were dramatically reduced 12 to 18 months after release (Zimmermann 1981). Consequently, mechanical and chemical control programmes against jointed cactus were discontinued and biological control became the only means of control from 1938 to 1946 (Zimmermann 1981). Although biological control using cochineal was initially considered successful, survival and extensive regrowth of plants alarmed Department of Agriculture officials and landowners and, as a result biological control of jointed cactus was discontinued in 1946 (Gunn 1979; Zimmermann 1981). The reduction in the impact of the cochineal is believed to have been from a combination of factors, including high rainfall and that the cochineal was so effective that the crash in the cochineal population following the decline in jointed cactus resulted in such low cochineal densities that the plant populations had time to recover (Zimmermann 1981). A mechanical clearing programme was introduced to

replace the biological control programme from 1947 to 1957 with the justification that the biological control agent had 'lost efficacy' and efforts were increased by reintroducing chemical control method in conjunction with the mechanical clearing of plants (Zimmermann 1981; Moran & Zimmermann 1991a). The herbicide was issued free from the government and land owners were then responsible for applying the herbicide (Zimmermann 1981). Labour to spray the herbicide was at the expense of the land-owner, so land-owners were left with no choice but to participate in a costly chemical control programme (Moran & Zimmermann 1991a). 2, 4, 5-T in paraffin was initially used for chemical control of jointed cactus, but was replaced by Iso-octyl ester picloram, and later by MSMA (Zimmermann 1979; Zimmermann 1981).

Success in biological control of jointed cactus using *D. austrinus* can be enhanced or reduced by climatic conditions (Hosking 1984). Temperature plays a vital role in the development, survival, and reproduction of *D. austrinus*. Temperatures ranging from 25°C to 30°C favour the development of *D. austrinus* but, during winter in the Eastern Cape, when mean daily temperature range from 17°C to 19°C, conditions are suboptimal (Hosking 1984; Zimmermann 1981). Precipitation also plays an important role. For example, at moist coastal sites, jointed cactus plants are healthier and more vigorous, while populations of *D. austrinus* are low (Moran & Zimmermann 1991a). The climatic conditions therefore are important factors affecting the biological control programme.

In the absence of persistent control methods jointed cactus has the potential to spread and expand its range and density in South Africa (Moran & Zimmermann 1991a). Integrated control, by selectively applying herbicide in those areas where prospects for successful biological control are less favourable was considered the most appropriate management strategy (Zimmermann 1981). Different management strategies are necessary for each of the many vegetation types and climatic regions where dense infestation of jointed cactus occur (Moran & Zimmermann 1991a). For example, the strategy should differ in higher rainfall regions where cochineal insects are less effective in reducing weed populations, compared to those in dry regions (Moran & Zimmermann 1991a). Despite control efforts, jointed cactus is still perceived as a weed of importance and there is a lack of evidence that the spread and distribution in South Africa are decreasing (Moran & Zimmermann 1991a).

Opuntia aurantiaca is therefore still a major problem in many parts of South Africa despite the fact that biological control has significantly reduced infestations across the whole distribution of the plant. The biological control programme has however relied heavily on classical biological control, with little augmentative biological control being implemented. Mass-rearing and releasing *D. austrinus* for the purpose of increasing the agent population in the field is encouraged and is believed to improve the level of control but little work has been conducted to determine what densities of the agent are required in the field, whether releases of the agent increase field populations and how often releases should be made.

1.13 Aims and Rationale

This study aims to investigating the outcome of mass-rearing and releasing *D. austrinus* for the control of jointed cactus in South Africa. Increasing agent populations through augmentative biological control may improve the level of control provided by the agent by increasing field populations of the agent and hence decreasing jointed cactus populations. The outcome of this investigation will optimize release strategies so that the maximum benefit from the biological control agents can be achieved.

Chapter 2: The impact of varying release sizes of *Dactylopius austrinus* on the proportion of cochineal and number of cladodes of jointed cactus under laboratory conditions.

2.1 Introduction

The level of damage inflicted by different densities of biological control agents to a target weed can be used to predict what densities of the agent are required in the field for adequate control (McClay & Balciunas 2005). This principle is based on the 'damage curve' developed for quantifying the impact of insect pests to agriculture (Peterson & Higley 2001), but is equally applicable to weed biological control (McClay & Balciunas 2005). While classical control relies on natural population increase, augmentative biological control is intended to improve control by increasing population densities of the agent in the field (Zachariades *et al.* 2017). Calculating a density of the agent that results in a suitable level of control gives biological control practitioners a target density of the agent that must be achieved in the field through mass-rearing and releases.

Opuntia aurantiaca is considered under substantial control in South Africa (Klein 2011) but it is still considered a problematic invasive alien plant in some parts of the country (Moran and Zimmermann 1991a). Mechanical and chemical control have been shown to be ineffective against *O. aurantiaca*, and new biological control agents are unlikely to be sourced from the native distribution, so the current biological control agents need to be used as best as possible in order to maximise levels of control (Moran and Zimmermann 1991a). Augmentation of the biological control agent *D. austrinus*, through mass-rearing and multiple releases, is thought to improve levels of control by increasing the populations of the agent in the field and therefore reducing *O. aurantiaca* populations. Whether the agent populations are increased after mass-rearing and releases, and whether this has an impact on weed populations has however not been formally assessed.

The impact of *D. austrinus* is known to be very variable depending on climatic conditions and the success of the biological control programme against jointed cactus will therefore vary in time and space (Moran and Zimmermann 1991a). While field based studies would give a more realistic representation of what will happen when different release strategies are implemented, the variability in time and space is problematic as field based studies are limited to a certain site, or at least a limited number of sites, and must be done during a limited time period.

Laboratory based studies allow for controlling the environment, thus partially mitigating some of the problems with field based studies, such as variable climatic conditions. A combination of both field based experiments and laboratory based experiments is the most appropriate way to determine how *O. aurantiaca* will respond to different release strategies.

Most laboratory based assessments of the impacts of biological control agents are those that are conducted in quarantine prior to the release of the agent (Goolsby *et al.* 2009; Paterson *et al.* 2014). These studies are restricted to a single agent density, or different combinations of agent species at a single density, and so cannot be used to calculate a density threshold at which the agent becomes damaging to the target weed. By exposing the target weed to different densities of the agent it is possible to quantify levels of damage expected in the field. Although these laboratory based studies are valuable and informative, they are however difficult to interpret and extrapolation of the results to field conditions is problematic (Morin *et al.* 2006; 2009).

In this study, the impact of releasing *D. austrinus* on *O. aurantiaca* is quantified in a laboratory based study. The densities of the agent were manipulated by performing different numbers of release events on plants and compared to control plants where the agent was not present. The results are intended to inform biological control practitioners as to whether mass-rearing and releasing *D. austrinus* increases the agent population and reduces jointed cactus populations. It was also intended to determine how many release events would result in the optimal level of control of *O. aurantiaca* using *D. austrinus*.

2.2 Materials and methods

Opuntia aurantiaca plants were collected from Table Farm outside Grahamstown (33º15’12” S; 26º26’54”E) in the Eastern Cape Province, South Africa in November 2017, and were grown in a greenhouse at the Waainek Research Facility at Rhodes University in Grahamstown, Eastern Cape, South Africa. Plants were dug up with the roots and transplanted to 23cm plastic pots, and allowed to grow for a period of four months before the experiment was started. A 50:50 ratio of sandy soil and potting soil was used. Plants were watered and fertilised when required and all plants were subjected to exactly the same watering and fertilising regime.

2.2.1 Experimental design

Five treatments, each with ten replicates, were included in the experiment (Table 2.1). The control treatment were plants which had natural levels of cochineal (as collected from the field). The insect excluded treatment were plants where the cochineal was removed by application of insecticide. The 1-release treatment were plants where cochineal was released once on the plant, the 2-release treatment was exposed to two cochineal release events, and the 3-release treatment was exposed to three events.

Table 2.1. The details of the five treatments used in the laboratory based impact study.

Treatments	Description
Control (C)	Natural levels of cochineal (as collected from the field)
Insect Excluded (IE)	Insect excluded by application of insecticide
1-Release (1)	Single release of cochineal insect in April
2- Release (2)	Release of cochineal insects in April and a second release in June.
3-Release (3)	Releases of cochineal in April, June and August

The first release event was conducted in April 2018 when three cladodes infected with cochineal were placed in the canopy of all the plants in the release treatments (1-release; 2-release and 3-release treatments). All infected cladodes had similar levels of cochineal with about 50% of the cladode covered. Infected cladodes were sourced from the Centre for Biological Control Cactus Mass-Rearing Facility and the method of exposing the infected cladodes to the plants, as well as the intensity of the release (i.e. the number of infected cladodes released per plant), simulate release events conducted by the mass-rearing team when field releases are made. The second release event was conducted in the same way in June 2018, but infected cladodes were only placed on the 2-release and 3-release treatment. Finally, the third release event was conducted in August 2018 when releases were made on only the 3-release treatment plants. This method simulated different release intensities that could be conducted by the cactus mass-rearing team by conducting a single release at a site in a year, two releases in a year, and three releases in a year.

The plant parameters that were measured in this study included total number of cladodes per plant and the number of cladodes infested with cochineal per plant. These parameters were measured on a monthly basis from a month before the first release event in March 2018, until two months after the final release event, ending in October 2018.

2.2.2. Details of insecticide

There are no insecticides registered specifically for *D. austrinus*. Makhro Cyper Cypermethrin pyrethroid 200g/l (Makhro-Agro SA (pty) Ltd is an insecticide which is used for control of various pests in gardens. It is registered for ornamentals and flowers pests such as: aphids, mealy bugs and Australian bug, flower beetles, lily borer, pine emperor moth and caterpillars, shield bug and twig wilters, and white fly. This insecticide was applied following instructions provided by the manufacturer.

2.2.3. Statistical analysis

All statistical analyses were performed using the statistical software STATISTICA ver. 13.0 (©1984-2017 TIBCO software inc.). The means for proportion of cladodes infested with cochineal and the total number of cladodes were calculated for each sampling event. Repeated measures ANOVAs were performed by General Linear Model (GLM) to investigate the variation in means of cochineal proportions, and means of total cladodes for each treatment, for each monthly sampling event.

2.3 Results

2.3.1 Impact of augmentative releases on cochineal population

Measurements recorded throughout this study, showed a slight decrease in the number of cladodes infested with cochineal overtime. The statistical results indicated a significant difference between treatments but not between months or a combination of the two factors (Table. 2.2). The significant difference between treatments was due to the insect exclusion treatment where no cochineal was present after it was sprayed with insecticide (Fig. 2.1). Increases in the proportion of cochineal were shown in some treatments after release events, with a particularly pronounced increase in the 3-release treatment after August, but the majority of the increases following releases were subtle and were not statistically significant (Fig. 2.1).

All three of the release treatments had consistently higher numbers of cochineal than the control as well as the insect exclusion treatments and in most months this was significantly different (Fig. 2.1). This result is however, complicated by the fact that the plants randomly chosen for the release treatments also had higher proportions of cochineal at the start of the experiment. In future studies a stratified method of random selection should be used so that the size of plants is more evenly distributed among treatments.

Jointed cactus plants in the control treatment had a reduced proportion of cladodes infested with cochineal by the end of the experiment compared with the starting point (Fig. 2.1). Similarly, the plants in the insect exclusion treatment had reduced cochineal infestation proportions that quickly reduced to zero after insecticide was applied in April and remained at zero for the rest of the study (Fig. 2.1). The first release of cochineal in April did not have any impact on cochineal infestation in 1-release treatment plants 12.9 ± 2.73; mean ± se (April) and 11.7±2.57; mean ± se (May), with the proportion of infested cladodes dropping slightly after the release (Fig. 2.1). A slight increase in the proportion of cladodes infested with cochineal was recorded in the 2-release treatment after the first release event with an increasing proportion of infected cladodes from April, when the release was conducted, until May, but this was followed by a decrease to slightly below initial proportions of infected cladodes by June (Fig. 2.1). A similar trend was recorded over the same time period for the 3-release treatment which also increased slightly after the first release event but decreased again a month later (Fig. 2.1). The second release event, which was conducted in June in the 2-release and 3-release treatments, did not result in any increase in cochineal in either treatment (Fig. 2.1). The third release event in August, which was only conducted on plants in the 3-release treatment, did result in a substantial increase in the proportion of infected cladodes in that treatment, but again, this only lasted for a brief period in September and cochineal levels dropped significantly by the end of the experiment in October (Fig. 2.1). There was also considerable variation between replicates for the increase in September (Fig. 2.1).

Table 2.2 Results of repeated measures ANOVA for the effects of augmentative releases of cochineal insects to the proportion of cochineal infected cladodes.

Effect	**Df**	**F**	**P-value**
Treatment	4	7.8367	0.0000
Month	7	1.2328	0.2841
month*treatment	28	26.100	0.0000

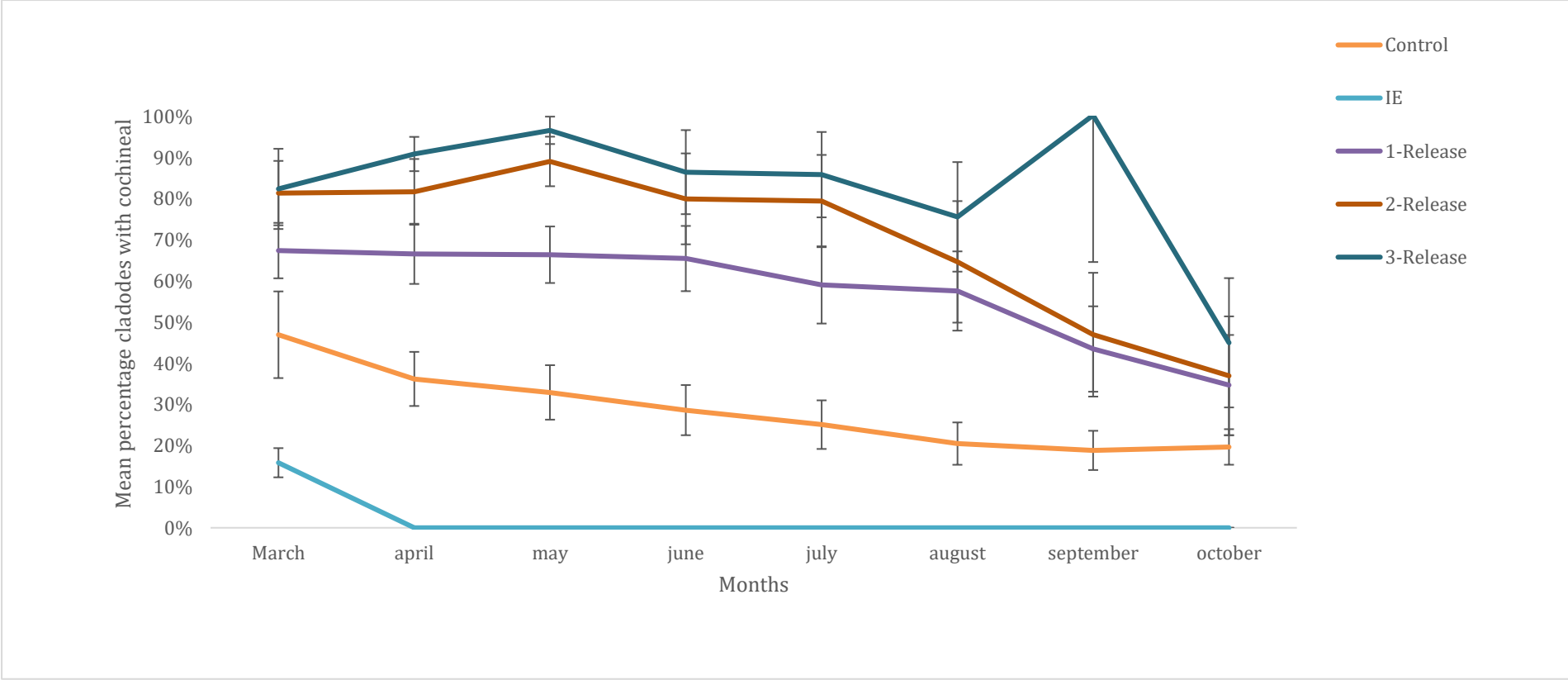

Figure 2.1 Mean proportion of cochineal infected cladodes (%) for each treatment. Treatments include C=control treatment, 1=1-Release treatment, 2=2-Release treatment, and 3=3-Release treatment. Error bars delineate standard errors. The IE treatment was significantly different from other treatment in all monthly sampling events (Table 2.2).

2.3.2 Impact of augmentative release of cochineal on total number of *O. aurantiaca* cladodes

The number of cladodes per plant increased over the period of the study for the insect exclusion treatment, as well as the control treatment, while all three treatments in which releases were made, decreased steadily over the same period (Fig. 2.2). There was a significant difference in terms of both treatment and month of sampling but the combination of the two effects, although close to the 0.05 threshold, was not statistically significant (Table 2.3). There were no significant differences between treatments at the initiation of the experiment, but after five months the control and insect exclusion treatment had significantly more cladodes than the release treatments (Fig. 2.2). At the end of the experiment in October these significant differences remained, and the plants that had been subjected to release events had decreased in the average number of cladodes per plant, while those that had not had releases, or had the insect excluded using an insecticide, had increased in the average number of cladodes per plant (Fig. 2.2).

There were no significant differences between the 1-release, 2-release and 3-release treatments, but the 1-release treatment consistently had more cladodes than the other two treatments for the entire length of the experiment. This was followed by the 2-release treatment which was intermediate, and then the 3-release treatment which was consistently the treatment with the lowest number of cladodes (Fig. 2.2). There was no indication that release events resulted in an immediate decrease in the number of cladodes followed by an increase one month later as could have been expected given the response of the cochineal population to release events (Section 2.3.1).

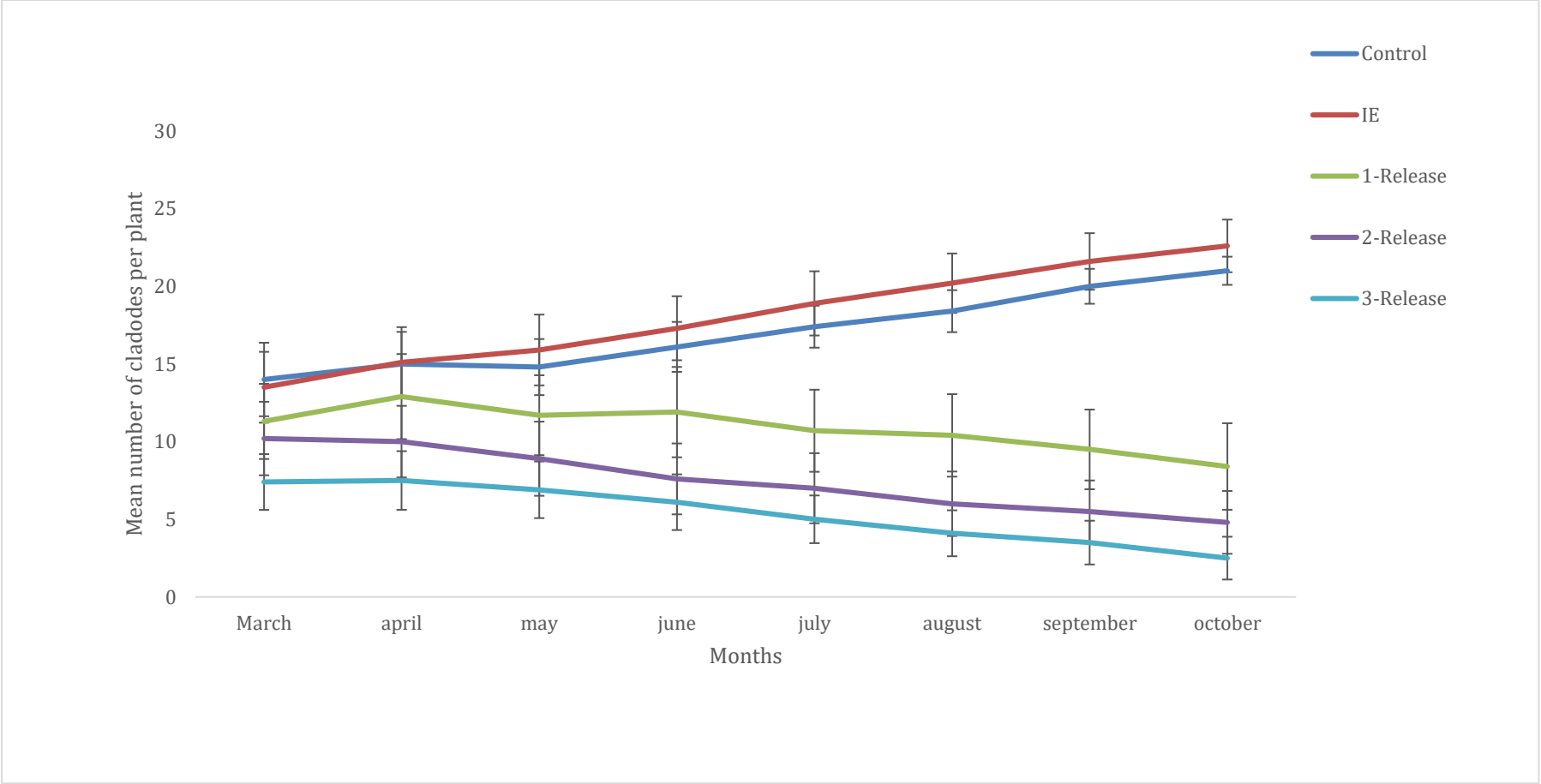

Figure 2.2. The mean number of cladodes per plant over time. Treatments include C=control treatment, 1=1-Release treatment, 2=2-Release treatment, and 3=3-Release treatment. Error bars delineate standard errors. Treatments C and IE were significantly different from other treatments in the last three months of sampling (Table 2.3).

Table 2.3. Results of repeated measures ANOVA for the effects of augmentative releases of cochineal insect on total number of cladodes.

Effect	Df	F	P-value
Treatment	4	33.948	0.0000
Month	7	12.781	0.0000
month*treatment	28	1.4070	0.0871

2.4 Discussion

This study aimed to determine the impact of augmentative releases of the cochineal insect *D. austrinus* on jointed cactus in a controlled laboratory experiment. The results indicate that the cochineal insect was damaging to jointed cactus plants, with all treated plants having reduced numbers of cladodes while plants left at the field density of the agent, and plants where the agent was excluded, had increased numbers of cladodes at the end of the experiment. No correlation between an increase in the number of infected cladodes was evident in this study, as can be seen by the fact that the treated plants with the lowest number of cladodes at the end of the experiment were not the plants with highest proportion of infected cladodes. This indicates that percentage of infested cladodes is an inappropriate way in which to quantify the abundance and/or damage of cochineal. Plants in release treatments were clearly impacted by the releases, so more cochineal must have been feeding on these plants, although the number of cladodes infected with cochineal did not increase significantly. It is likely that the number of cochineal insects per cladode increased and this had an impact on overall damage to the plant. The total amount of cochineal on each plant, rather than the number of infected cladodes, is therefore likely to be a better parameter to measure in order to assess cochineal densities in future.

Observations from this study concur with the theory of augmentative biological control for weeds which suggested that periodic or frequent releases of insects can increase levels of control (Zachariades *et al.* 2017). It also confirms that *D. austrinus* is a damaging natural enemy that has an impact on jointed cactus, and can have a significant impact if the agent is present in high enough densities (Moran & Zimmermann 1991a). Although there were no significant differences between the three release treatments in terms of the number of cladodes per plant,

the 3-release treatment did consistently have the lowest number of cladodes and 1-release treatment had the highest. This may suggest that the more releases that are conducted the greater the level of control, but the results should be interpreted with caution. There was no statistical difference at the end of the experiment and the size of the plants at the end of the experiment also mirrored that of the starting size, with the plants with the least cladodes on average all randomly being allocated to the 3-release treatment at the start of the experiment. Further studies are required to determine whether increasing the number of release events will result in a decrease to the average number of cladodes per plant, but it is clear that releasing the agent at least once is beneficial.

The total number of cladodes per plant in the control treatment and the insect exclusion treatment increased in contrast to all release treatments. This indicates that under natural levels of cochineal or in the absence of cochineal insect, jointed cactus has the potential to grow new cladodes and increase in size. Also, plants that have no cochineal insect at all had higher plant growth rate than plants that have natural levels of cochineal, although this was not statistically significant. Hosking and Deighton (1980) also found that plants without cochineal increased in the number of cladodes per plant, while those with cochineal did not. The increase in the number of cladodes for plants with natural field densities of the cochineal insect suggest that the site where the plants were collected did not have sufficient amounts of cochineal to significantly damage jointed cactus and that further releases are therefore warranted.

Augmenting insect populations might or might not increase field insect population. The efficacy of any biological control agent against a weed depends on the interaction between the weed, the agent and the environment (Groves *et al*. 1979). Factors such as climate , nutritional status, and dispersion of cochineal insect as well as predation rates by generalists in the introduced ranges can affect the efficacy of the agent (see Chapter 3). Studies have shown that various herbivorous insects remain at low densities because of limited access to high-quality food (Crawley 1989) and there may therefore be a density threshold of cochineal in the field that cannot be exceeded. If herbivorous insects are regulated by limited access to high-quality food or the nutritional status of the host plant, it is not likely that they will be abundant enough to have significant impact on plant performance (Crawley 1989). Thus, whether the insect populations have been augmented or not, the nutritional status of the host plant might reduce the insect's effectiveness as a biological control agent (Crawley 1989). In some cases, insect

populations are reduced by the amount of host plant available, which results in insect having little or no impact on host plants population density and may be because growing season of host plant exceeds the feeding period of the insect, so that post-defoliation regrowth is possible (Crawley 1989). There are therefore many other factors that will be important under field conditions making it difficult to extrapolate the results of this laboratory based experiment to the field. Difficulties in extrapolating impact assessments done under laboratory conditions into the field is a common problem in biological control (Morin *et al*. 2006; 2009) but laboratory studies of the density thresholds of Biocontrol agents are still valuable as they confirm what the greatest possible level of damage could be under field conditions (McClay & Balcianus 2005).

This study has confirmed that *D. austrinus* can be damaging to *O. aurantiaca* and that augmenting the populations of *D. austrinus* can increase the level of control that is provided, at least under the conditions under which this experiment was conducted. There is strong evidence from this study that mass-rearing and releasing should be continued, and there is some evidence to suggest that more than one release of *D. austrinus* should be conducted per year, though this evidence is statistically supported. Under the conditions in this experiment, a single release in a year had a similar impact to three releases in a year, so the evidence suggest that an optimal release strategy would be to augment field populations once every year. This single release is however essential because if field densities of cochineal were not augmented, there was very little or no impact to plant growth and the plants increased in size throughout the study period, while with a single release the plants decreased in size steadily. Field densities of cochineal will differ in time and space depending on climatic conditions (Moran & Zimmermann 1991a), so the impact of further releases is also likely to vary. Sites with very high levels of cochineal may not benefit from further releases, while those with low cochineal densities may.

Under controlled conditions in a laboratory study releases of *D. austrinus* improved the level of control of jointed cactus. These conditions exclude abiotic variables such as temperature fluctuations, precipitation, wind, nutrients and sunlight; as well as biotic variables, such as plant stress and predation of the cochineal insects. Field based studies are therefore required in order to determine whether releases of *D. austrinus* will result in the augmentation of

population under the conditions in the field where the plant is problematic, and whether this will result in increased levels of control.

Chapter 3: Augmentative releases of *Dactylopius austrinus* on jointed cactus under field conditions

3.1. Introduction

3.1.1 Post- release evaluations

Post-release evaluations are important in biological control programmes because they determine the extent to which released agents are reducing the ecological, social and economic impacts of weeds (Morin *et al*. 2009; Meyer *et al*. 2012). Post-release evaluations should demonstrate how effective the agent is in the introduced range over time by quantifying parameters such as the level of damage inflicted by the agent, the impact of the agent on individual plants, the change in plant population densities and ultimately the reduction in the negative impacts of the weed (Morin *et al*. 2009). However, despite successes in many biological control programmes, it is rarely evaluated objectively and quantitatively (Thomas & Reid 2007). Most post-release evaluations are subjective assessments of the impact of an agent or are limited to measuring damage to the plant or changes in plant density at very few sites (Morin *et al*. 2009). Few studies have quantified the reduction in weed populations caused by released agents and even fewer have attempted to quantitatively measure changes to the negative impacts from the weed (Barton *et al*. 2007; Paterson *et al*. 2011). It is important to understand the level of success of biological control programmes in order to learn from past successes and failures, as well as to justify further biological control programmes and the resources required to run them (Müller-Schärer & Schaffner 2008; Morin *et al*. 2009). Thus, the evaluation of weed biological control programmes is important to advance the ability to predict the effect of future introductions and, therefore, underpins the continued use of biological control as an effective way of managing invasive alien plants (Paynter *et al*. 2006). Post-release evaluations have also been used to compare different management practices (such as release techniques) in order to learn how best to implement biological control. This allows for post-release evaluations to inform management on how best to implement biological control so that the maximum level of control that is possible can be achieved.

In cases where more than one biological control agents exists, post-release evaluations can improve the implementation of biological control by prioritising the redistribution of the most effective agents (Paynter 2005). For example, Paynter (2005) did a study on the impact of a biological control agent *Carmenta mimosa* Eichlin & Passoa (Sesiidae) on the woody wetland weed *Mimosa pigra* L. (Mimosaceae) in Australia. The study showed that out of the six biological control agents that were established on mimosa, *C. mimosa* alone supressed the weed's populations (Paynter 2005). *Carmeta mimosa* is a slow dispersing agent and therefore, to increase the benefits, redistribution of the agent throughout the mimosa infestations where the agent is absent should be carried out (Paynter 2005). Evaluating the agents' effectiveness may also indicate whether altering other management practices would increase the effects of agents (Morin *et al*. 2009). Monitoring success of different management strategies, such as augmentation of agent populations, could therefore improve the implementation of biological control programme.

In the past, evaluation studies have focused mainly on the establishment of the agent and the response of target plants at the individual plant level (Morin *et al*. 2009). Thus, many estimates of the efficacy are qualitative and lack details about the extent and level of biological control and little information on the factors that influence its success or failures (Van Klinken & Raghu 2006; Thomas & Reid 2007). There are many different ways that post-release evaluations can be done, and in most cases, methods must be adapted for each different weed species or agent, but the different approaches to post-release evaluations can be broadly divided into the following groups: agent-related studies, weed-related studies, studies that compare before and after release data, and manipulative experiments.

Agent-related studies

Agent-related studies involve quantifying the impact/damage that an agent has had on the target weed. There are various measurements that are taken to quantify the damage inflicted by agents at different infestations on individual plants. This can be achieved by measuring the number of individuals of each life-stage and feeding damage measurements which include portions of leaf area removed, proportion of leaves exhibiting agent damage, severity of the damage per unit size/biomass, and number of stem tips damaged (Morin *et al*. 2009). This data is valuable and helps to understand trends in the agent populations, phenology and dispersal patterns (Morin *et al*. 2009). However, these measurements are not always feasible due to the nature of insects

feeding mode (endophagous), and some difficulties in sampling (such as large trees). Despite some challenges, these studies are reliable post-release studies which quantify the agent's effectiveness but are most appropriate at the early stages of a post-release evaluation soon after the release of the agent because they do not measure changes to populations of the target weed or changes to the negative impacts that are associated with the weed. Having agent-related parameters in a post release-evaluation is however important because correlating agent damage to changes in weed densities ensures that the agent is responsible for these changes rather than any other factor, such as a changing climate.

Weed-related measurements

Weed measurements in post-release evaluations are usually taken at the individual or population level (Morin *et al*. 2009). For individual plants, growth parameters (number, size and biomass) and reproductive parameters (number and biomass of flowers, fruits or seeds) are measured. At the population level, parameters include density, cover, stand size and age structure, seedling recruitment and survival; and viable seed bank density (Morin *et al*. 2009). The parameters, however, vary depending on the target weed and the agent. For example, for seed attacking agents of long-live plants, the seed set and seed bank may be most important, but for other weeds, such as vines, the biomass, density and percentage cover at a site may be more important. In most cases, population level parameters such as weed density and biomass do not change until many years after biological control has been implemented, so quantifying changes to these parameters over time is challenging. For some weeds, an assessment of population structure can predict what the impact of the agent will be in future, mitigating this problem to some extent (Sutton *et al*. 2018).

Comparison between sites with and without agents

Comparisons of sites with and without agents can provide details of how successful an agent has been at reducing the weed population (Carson *et al*. 2008). Comparison studies are usually possible in cases where the agent is not widely distributed by natural means or can be excluded from certain sites in some way. In this type of study, the agent is released in both randomly selected sites (release sites) paired with non-release sites (control sites) in a replicated manner, stratified across relevant temporal and spatial biotic and abiotic gradients (Carson *et al*. 2008). Agent and weed related parameters are then quantified in both sites prior to any releases and

periodically thereafter. There are advantages to this approach, because there is a control, so the changes to the weed population can be confidently attributed to the action of the agent, but there are disadvantages to having controls at different sites, because plant parameters may differ between sites for other reasons that have little to do with the agent's impact (Adair & Groves 1998).

Manipulative experiments

Manipulative experiments are another way that can be used to determine the effectiveness of biological control agents. This is done by performing manipulative experiments such as exclusion and inclusion, and augmentative releases experiments. The exclusion and inclusion experiments determine the influence that the agents have in regulating the target weed population. Exclusion experiments can be done using cages to restrict the presence of the agent, or insecticides and/or fungicides to exclude them (Morin *et al*. 2009). Insect exclusion has been very effective in some systems, such as on the environmental weed *Chrysanthemoides monilifera* (L.) Norlindh (Asteraceae) in Australia (Adair & Holtkamp 1999). This study indicated that the use of pesticides for evaluating the effect of biological control agents on *C. monilifera* is a valid and readily applied method. Manipulating insect density is not only to quantify if weed densities have been reduced or not, but can also determine what densities of agent are required to reduce weed densities to an acceptable level. This can in turn inform management of what densities will need be achieved in the field for biological control to be effective. Mass-rearing and releases may be able to increase field populations for some agent-weed systems.

Post-release evaluation studies are therefore an integral part of biological control programmes. Although there is often a lack of post-release evaluation studies due to limited of funding, it is important to note that these studies provide valuable information and therefore are worth investing in because they will improve management and implementation techniques, and hence improve levels of control.

3.1.2. Augmentative biological control of jointed cactus

Classical biological control of jointed cactus in South Africa has resulted in substantial levels of control of this weed (Klein 2011). Mass-rearing and augmenting insect population in the field might increases agent populations and hence the level of control, but this has not been sufficiently studied. The release of *C. cactorum* on *O. stricta* in Australia is one of the few studies in which a biological control agent was mass-reared for field augmentation on a large scale. The mass-rearing programme resulted in the release of almost two billion eggs sticks in a four-year period across the entire range of *O. stricta* in the Australia (Raghu & Walton 2007). In South Africa, an intense mass-rearing programme released about 580 million egg sticks between 1933 and 1941 (Zimmermann *et al*. 2004b; Raghu & Walton 2007). The mass-rearing programme in Australia was extremely successful and is possibly the most well-known weed biological control success (Raghu & Walton 2007). Unfortunately, in South Africa, the mass-rearing programme did not successfully increase the densities of *C. cactorum*, possibly due to predation of the larvae or climatic factors (Paterson *et al*. 2011a). This indicates that in some cases mass-rearing can successfully increase agent populations and improve control, while in others the agent population cannot be increased, even with a substantial release effort. The number of releases per unit area of *C. cactorum* in South Africa were even higher than those in Australia, and no long-term increase in the agent population, or any associated decrease to weed densities was recorded. Regardless of the limited success of mass-rearing *C. cactorum*, South Africa also has an active mass-rearing programme of cochineal insects. Large numbers of cochineal are being mass-reared and released for the control of invasive cacti species by the Rhodes University Centre for Biological Control. *Dactylopius austrinus* for the control of *O. aurantiaca* is one of the most commonly requested agents from the facility because it is still a very problematic plant in the Eastern Cape Province. Most requests for this agent come from farms where the agent is already established, so farmers require augmentation of the agent populations on their farms, rather than a small release to get the agent established.

In this chapter, a post-release evaluation was carried out in the form of a manipulative experiment to quantify the impact of *D. austrinus* on *O. aurantiaca*, and to determine whether augmenting agent populations through multiple releases was possible, and if this augmentation would improve levels of control. In the previous chapter (Chapter 2), a similar experiment was conducted under laboratory conditions, while in this chapter, the same methods are used but

under field conditions at a site in the Eastern Cape where *O. aurantiaca* is problematic. The main aim of this chapter was to determine whether additional releases of *D. austrinus* would result in increased agent populations and whether this would result in greater levels of control under field conditions. These data will be useful in determining the most effective way to reduce *O. aurantiaca* populations and how to optimise mass-rearing and release efforts.

3.2. Methods and materials

3.2.1 Field site

The field study was conducted between October 2017 to October 2018 on Table Farm (33º15'12" S; 26º26'54" E), outside of Grahamstown, Eastern Cape Province, South Africa. Throughout the Eastern Cape Province, the cochineal insect is abundant and widespread at most sites and Table Farm is no exception, but the density of jointed cactus is of concern to the farmer who believes that the productivity of his land is reduced by the high density of jointed cactus (Pers. Comm. Andrew White; Table Farm). The study site is a pasture for grazing sheep. It is characterised by shrub vegetation, grasses, and a few acacia trees and prickly pear plants (*O. ficus-indica*). The area receives a mean annual rainfall of 683mm and average monthly temperatures between 6.6°C to 20.5°C during winter season, with July being the coldest month (5.4°C), and 15.4°C to 25.0°C during summer, with February being the warmest (26.8°C) (www.enclimate-data.org).

3.2.2 Experimental design and data collection

Twenty-five experimental plots of $5m^2$ were permanently marked where there are infestations of jointed cactus at Table Farm. The distance between the plots was a minimum of three to a maximum of five meters. The plots were divided into five treatments each with five replicates: control, insect excluded, 1-release, 2-release, 3-release (the same treatments as the laboratory study (Chapter 2)). The control treatment (C) is the treatment in which there were natural levels of cochineal that were already present at the site. The cochineal insect exclusion treatment (IE) is a treatment in which the insect was excluded by frequent application of insecticide (Cyper) by spraying every second day. This was the same insecticide used in the laboratory based experiment (Chapter 2). The 1-release treatment had a single release conducted in November

2017; the 2-release had releases in November 2017 and February 2018; while the 3-release treatment had releases in November 2017, February 2018 and May 2018.

The cochineal insect was released by placing *D. austrinus* infested cladodes in the canopy of the plants in the same way as described in Chapter 2. For each treated plot, cladodes were released according to the density of jointed cactus, (i.e., the higher the jointed cactus density the higher the number of cladodes released) with three cladodes released per large plants and less on smaller ones.

The density of *O. aurantiaca* and *D. austrinus* was recorded within each plot once a month. Every jointed cactus plant inside each plot was counted and assigned a plant size category.

Three categories of jointed cactus were used:

Category 1- all single jointed plants that are rooted on the ground

Category 2- all individual plants that had cladodes between 2-20

Category 3- all individual plants that had more than 21 cladodes

A single plant was also selected near the centre of each plot and labelled with a plant tag. The plants selected for each plot were always category 3 plants. These plants were examined in detail during each sampling event (once a month). The number of cladodes per plant and the number of cochineal infested cladodes per plant were recorded. These plants will be referred to as ‘subject plants’ and are directly comparable, in terms of the parameters that were measure, with plants in the laboratory experiment in Chapter 2.

Table. 3.1. The details of the five treatments used in the laboratory based impact study.

Treatment	Description of the event
Control (C)	The treatment plots had natural levels of cochineal that were already present at the site
Insect Exclusion(IE)	The cochineal was excluded by application of insecticide
1- Release (1)	Cochineal released in November 2017
2-Release (2)	Cochineal released in November 2017 and February 2018
3-Release (3)	Cochineal released in November 2017, February 2018 and May 2018

3.2.4 Statistical analyses

All statistical analyses were performed using the statistical software STATISTICA ver. 13.0 (©1984-2017 TIBCO software inc.). For each treatment, means for the number of jointed cactus plants were calculated for each sampling event. The proportion of cladodes infested with cochineal was then calculated for each treatment and sampling event. The same calculations were then conducted for each size category. Repeated measures ANOVAs were performed by General Linear Model (GLM) to investigate the variation in means of total number of jointed cactus cladodes, proportion of cochineal infested cladodes, total number of cladodes of subject plants, proportion of cochineal infested cladodes in subject plants, for each treatment and each sampling event.

3.3 Results

3.3.1 Cochineal density

There was a statistically significant effect for treatment, sampling month, and for the treatment-month interaction, according to the GLM (Table 3.2). Significant differences between release treatments, the control treatment, and the insect exclusion treatment, were measured at some monthly sampling events according to the post-hoc test, but these significant differences did not persist for any particular treatment over an extended period (Fig. 3.2).

All treatments had similar percentages of cochineal infested cladodes at the start of the experiment (Fig. 3.2). The percentage of cochineal infested cladodes for all treatments then decreased over time, with the control treatment (unmanipulated field densities of cochineal) decreasing from January 2018, and all three release treatments (1-release, 2-release and 3-release) also slowly reducing from February 2018 until the end of the experiment, with a small but temporary increase in June (Fig. 3.2). In the insect exclusion treatment, the percentage of infected cladodes reduced dramatically after initiation of the study due to the application of insecticide, but by the end of the experiment all treatments had reduced to the same low level of cochineal (Fig. 3.2). At the start of the experiment, the proportion of cochineal in all treatments was very high, even before the first release event, and the first releases therefore had little effect on cochineal densities. The release of cochineal for the second time, in May, had very little or no effect on the treated plots (2-release and 3-release treatments) in terms of the percentage of cladodes with cochineal, but the 3-release in August, which was only conducted for the 3-release treatment plots, resulted in a small increase in cochineal infestations and was significantly different from control and insect excluded treatments for that month (Fig. 3.2). All treatments had very low cochineal infestation in the last sampling month and there were no significant differences between treatments (Fig. 3.3).

Table 3.2. Results of a repeated measures ANOVA on the effect of augmentative releases of cochineal insect on the proportion of cochineal infected cladodes at Table Farm.

Effect	**Df**	**F**	**P-value**
Treatment	4	52.688	0.0000
Month	12	92.375	0.0000
month*treatment	48	7.151	0.0000

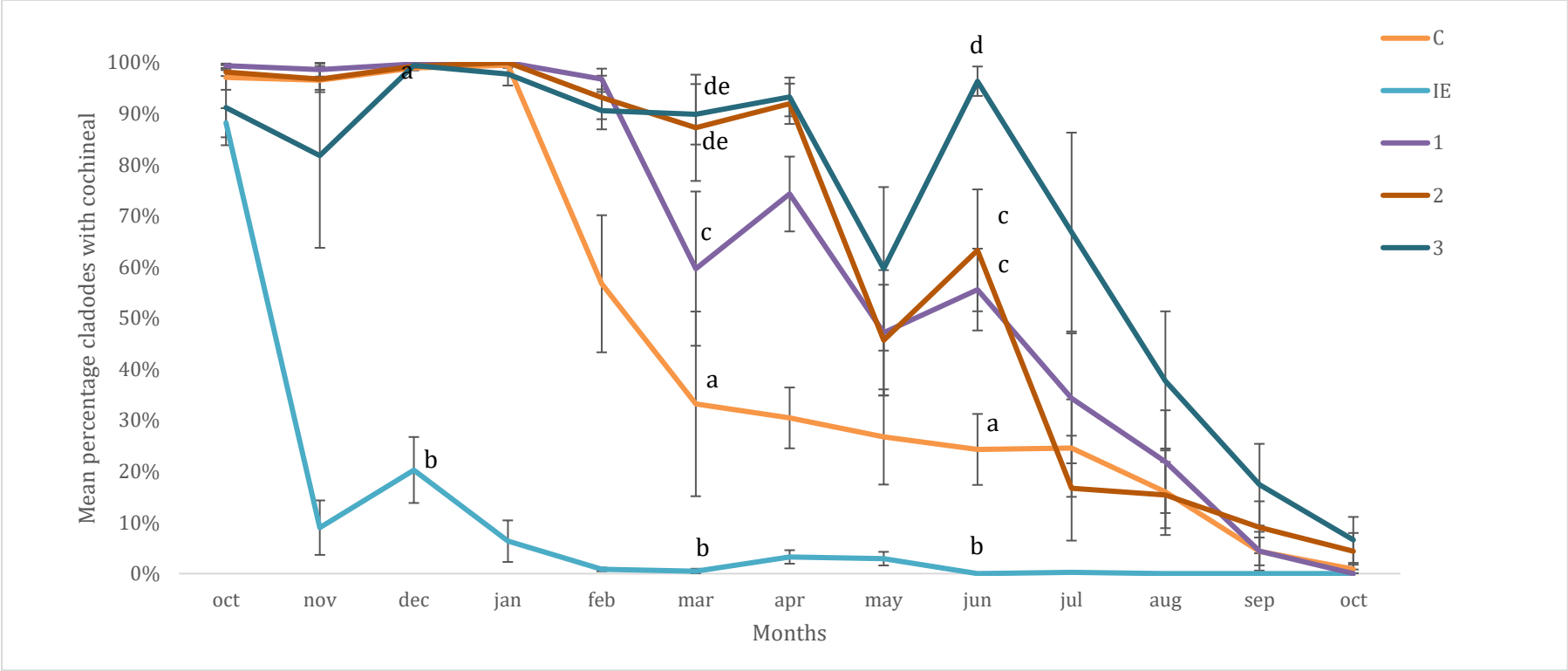

Figure 3.2 The mean percentage of cladodes infected with *Dactylopius austrinus* over the experimental period from October 2017 to October 2018. Treatments included are C=Control treatment, IE=Insect excluded treatment, 1=1-Release treatment, 2=2-Release treatment, and 3=3-Release treatment. Lines with different letters are significantly different from each other within the sampling event (ie: in that month) ($p<0.05$). Error bars delineate standard errors.

3.3.2 Jointed cactus density

Total number of plants

There was a significant effect for the total number of plants over the different sampling months, but no significant difference between treatments or the interaction between the two factors (Table 3.3). The post-hoc test indicated that the only significant differences between treatments were recorded during December 2017 (Fig. 3.3).

The density of jointed cactus decreased in all treatments over time (Fig. 3.3). By the end of the experiment, the insect exclusion treatments had the greatest number of plants, but this was not significantly different from other treatments (Fig. 3.3). Releases of cochineal did not appear to influence jointed cactus densities either immediately after the release or in the months following the release (Fig. 3.3). All the treatments showed an increase in jointed cactus density in the December sampling event, followed by a decline to levels below that of the starting density, and this density then remained relatively stable for the duration of the study (Fig. 3.3).

Table 3.3. Results of repeated measures ANOVA for the effects of augmentative releases of cochineal insect on jointed cactus densities at Table Farm.

Effect	**Df**	**F**	**P-value**
Treatment	4	0.4748	0.7537
Month	12	21.0250	0.0000
month*treatment	48	1.3099	0.0984

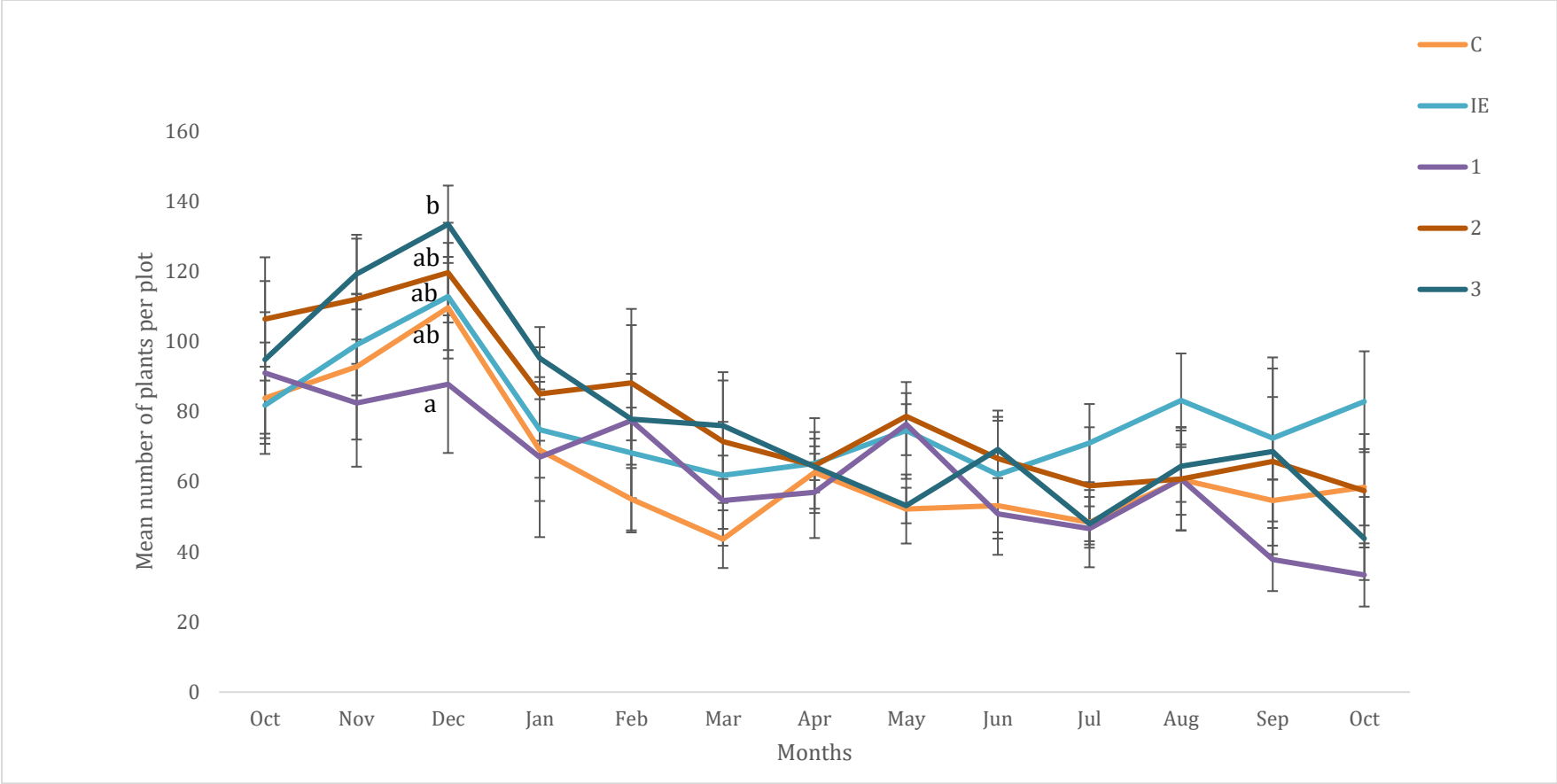

Figure 3.3 Mean number of jointed cactus plants per $5m^2$ plot over the period of the experiment from October 2017 until October 2018. Treatments included are C=control treatment, IE-Insect exclude treatment, 1=1-Release treatment, 2=2-Release treatment, and 3=3-Release treatment. Lines with different letters are significantly different from each other ($p<0.05$). Error bars delineate standard errors. Significant differences are indicated by different letters.

Category 1 plants

Category 1 plants were defined as plants that had single cladode that had rooted, loose cladodes that have fallen to the ground were not counted. Few numbers of category 1 plants were recorded during the first three months of sampling, however, the number decreased to zero over time. Therefore, there was little variation for Category 1 plant densities. (Fig. 3.5).

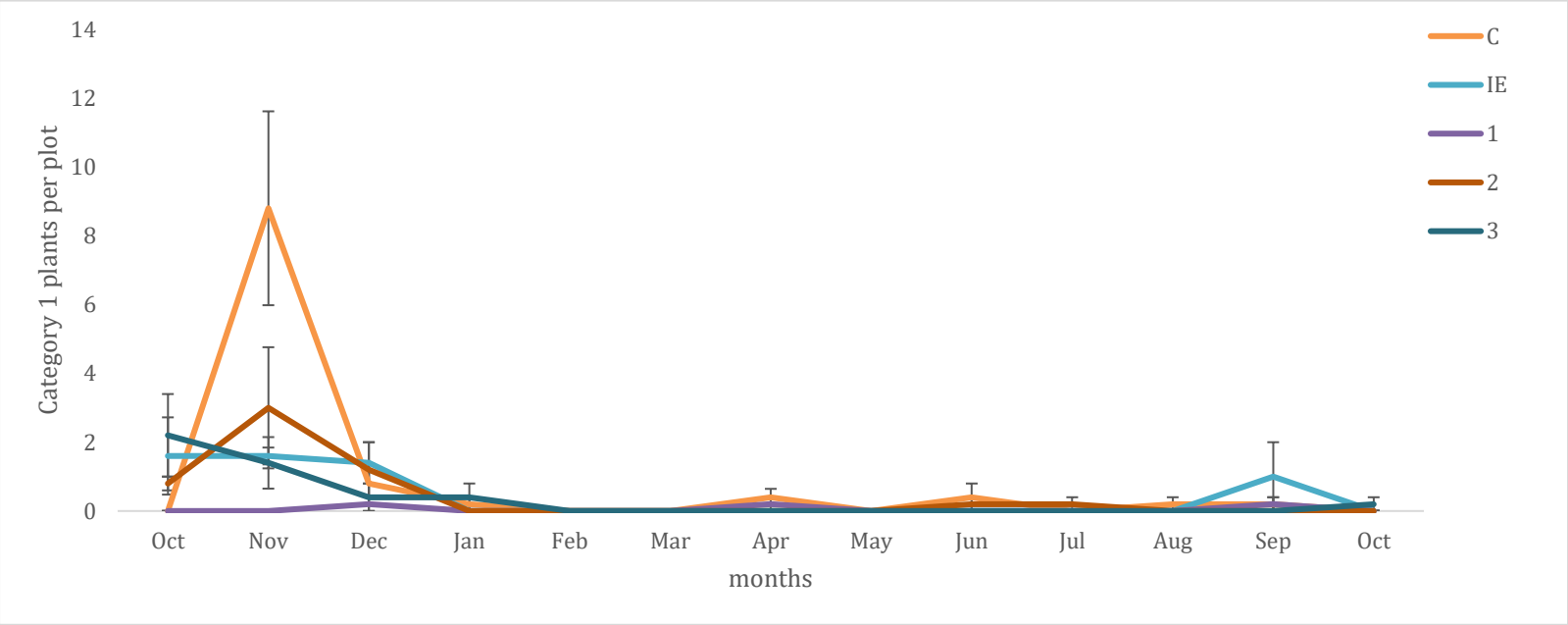

Figure 3.4 The mean number of Category 1 plants per $5m^2$ plot over the experimental period from October 2017 until October 2018. Treatments included are C=Control treatment, IE=Insect excluded treatment, 1=1-Release treatment, 2=2-Release treatment, and 3=3-Release treatment. Error bars delineate standard errors.

Category 2 plants

There was no significant difference between treatments, but there were significant differences between months, and between months and treatment interaction (Table. 3.4). There were less Category 2 plants at the end of the experiment than at the start of the experiment for all treatments except the insect exclusion treatment that had slight more Category 2 plants on average, though this was not statistically significant (Fig. 3.4). There was a slight increase in most treatments from November to December 2017 followed by a decreased until March 2018 and from this sampling event until the end of the experiment the number of Category 2 plants remained relatively stable.

All treated plots had less Category 2 plants two months before the cochineal was released for the second time in February 2018. 1-Release treatment plots had Category 2 plants densities which were significantly different from 3-release treatment in December sampling event (81.2 $\pm$ 18.24 and 127 $\pm$ 10.13; mean $\pm$ se; $p<0.0183$). 2-Release treatment had a notable reduction in Category 2 plant densities and was significantly different from 3-release treated plots in March shortly after the cochineal was released for the second time (26.8 $\pm$ 9.22 and 76 $\pm$ 18.3; mean $\pm$ se, $p<0.018$). Category 2 plants densities decreased in the 3-release treatment plots in June and July after the last release of cochineal which was conducted in May, and these were significantly different from other treatments.

Table 3.4. Results of repeated measures ANOVA for the effects of augmentative releases of cochineal insect on Category 2 plant densities at Table Farm.

Effect	**Df**	**F**	**P-value**
Treatment	4	0.4598	0.7642
Month	12	18.662	0.0000
Month*treatment	48	1.7729	0.0028

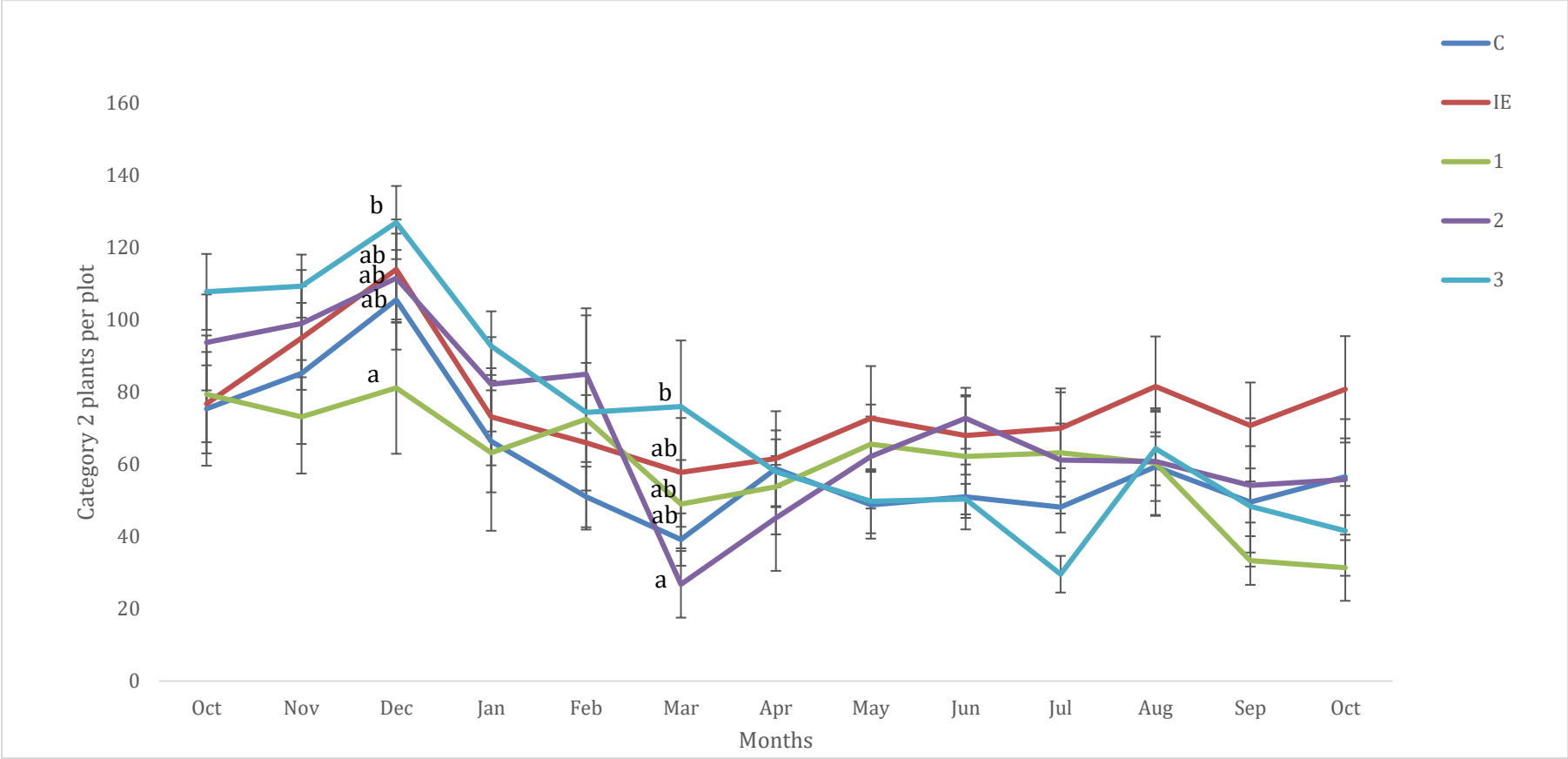

Fig 3.5 The mean number of Category 2 plants per $5m^2$ plot over the experimental period from October 2017 until October 2018. Treatments included are C= control treatment, IE=Insect excluded treatment, 1=1-Release treatment, 2-2-Release treatment, and 3-3-Release treatment. Lines with different letters are significantly different from each other ($p<0.05$). Error bars delineate standard errors and letters indicate significant differences.

Category 3 plants

There were significant difference between months, but no significant difference between treatments and between the months and treatments interaction (Table 3.5). There were significant differences between treatments at the first sampling event in October 2017, but by January 2018 all treatments had low numbers of Category 3 plants (Fig. 3.6). After January 2016, the average number of Category 3 plants per plot did not increase above 6 plants per plot, and by the end of the experiment in October 2018, there was an average of three plants in all treatments (Fig. 3.6). The first release event, which was conducted in November, may have resulted in the decline of Category 3 plants after that sampling event, but plants in the control treatment also declined and most treatments had already declined from October 2017, before the release was conducted (Fig. 3.6).

Table 3.5. Results of repeated measures ANOVA for the effects of augmentative releases of cochineal insect on Category 3 plant densities at Table Farm.

Effect	**Df**	**F**	**P-value**
Treatment	4	0.5604	0.6939
Month	12	22.500	0.0000
Month*treatment	48	1.3715	0.0656

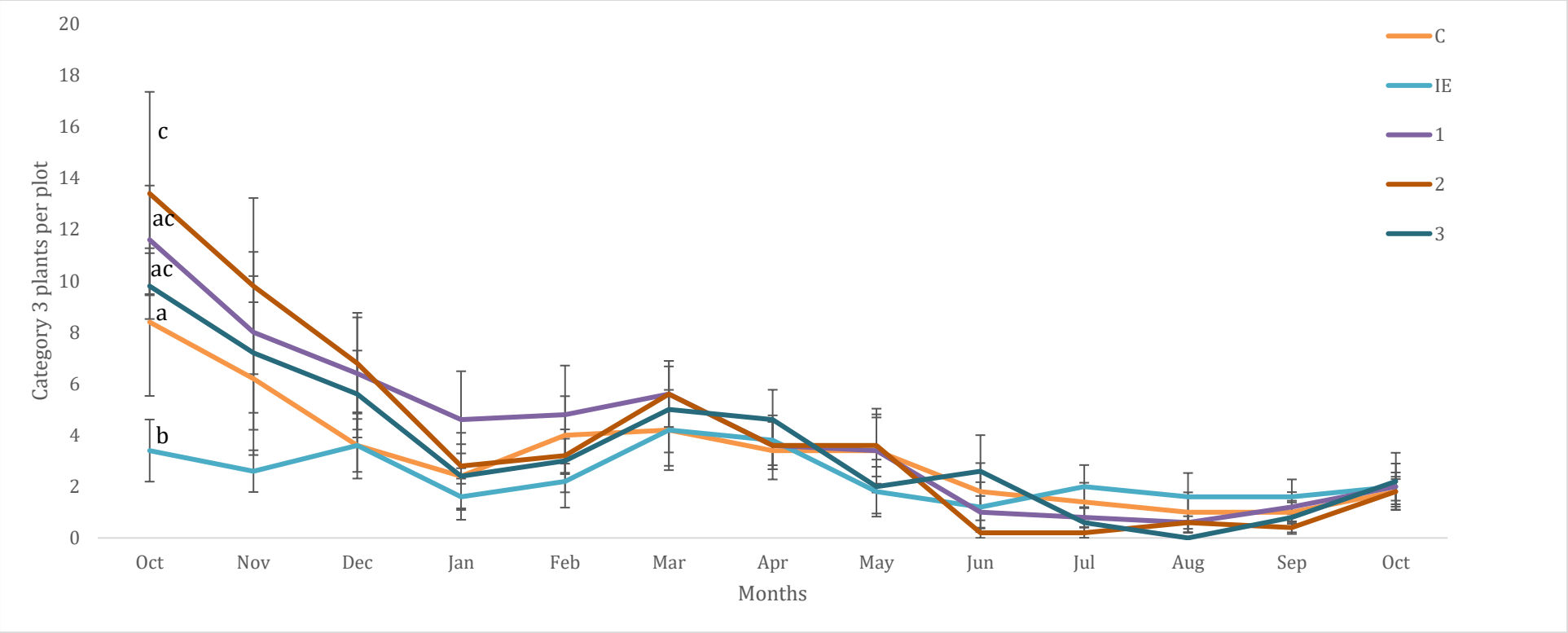

Fig 3.6. The mean number of Category 3 plants per 5m^2 plot over the experimental period from October 2017 until October 2018. Treatments included are C= control treatment, IE=Insect excluded treatment, 1=1-Release treatment, 2-2-Release treatment, and 3-3-Release treatment. Lines with different letters are significantly different from each other ($p<0.05$). Error bars delineate standard errors and letters indicate significant differences.

Subject plants

Subject plants had similar cladode densities in the October sampling event for all treatments and there were no significantly differences (Fig. 3.7). Total cladodes in the 1-release treatment plots increased in December after the cochineal had been released, but the 2-release treatment and 3-release treatment had fewer cladodes after the first release of cochineal, although none of these changes were significantly different (Fig. 3.7). Before the cochineal was released for the second time; almost two months after the first release, all subject plants in all treatments had notably reduced total cladodes, with the exception of the insect exclusion treatment (Fig. 3.7). However, this reduction only lasted for two months and therefore the subject plants had increased numbers of total cladodes in March, after the cochineal was released for the second time in February 2018 (Fig. 3.7). There was no statistical difference between the treatments, and between month and treatment interaction in all sampling events, but there were significant differences between months (Table. 3.6).

Table 3.6 Results of repeated measures ANOVA for the effects of augmentative releases of cochineal insect on the proportion of cochineal infected cladodes of subject plants at Table Farm.

Effect	**Df**	**F**	**P-value**
Treatment	4	0.7149	0.5914
Month	12	10.555	0.0000
month*treatment	48	0.9896	0.4985

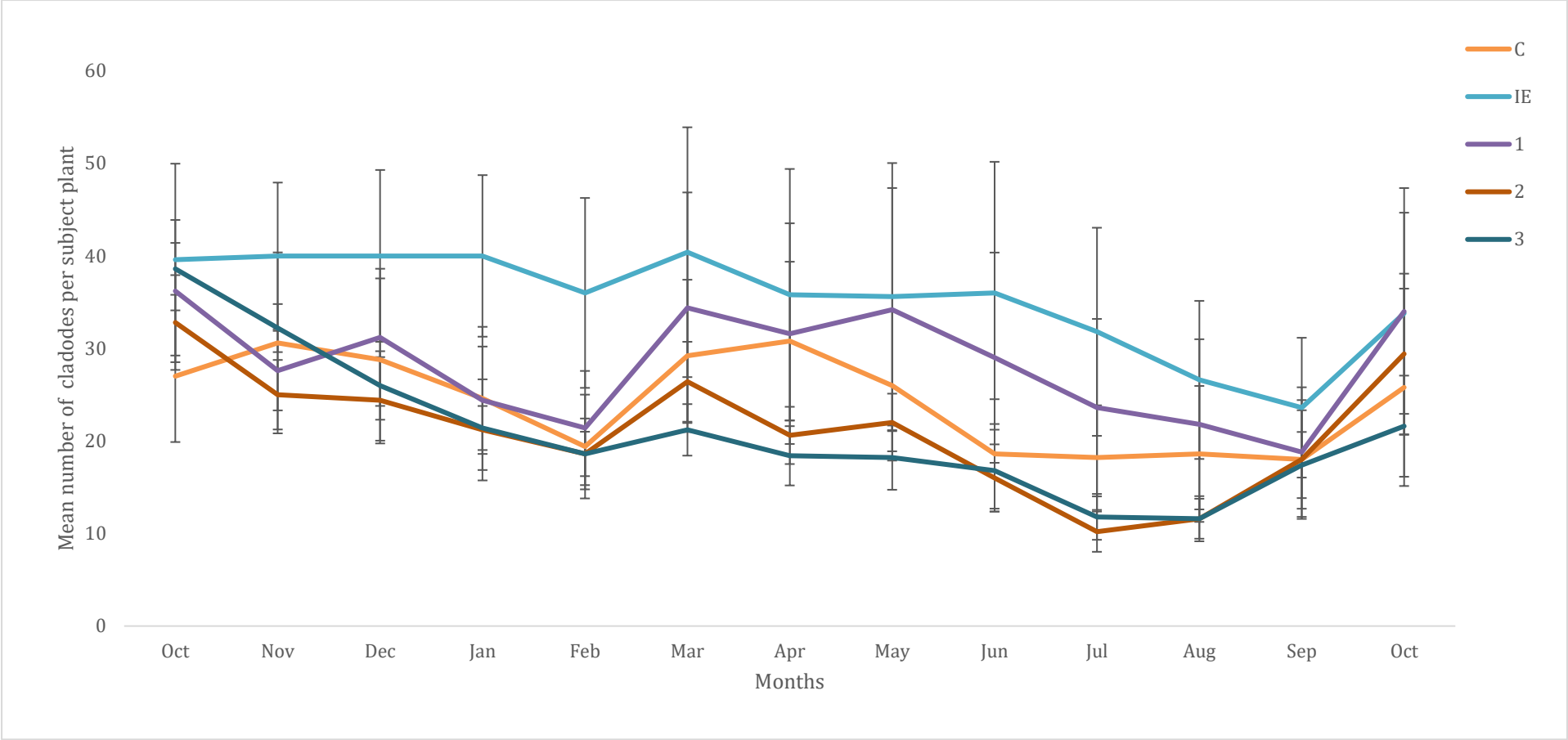

Figure 3.7. The mean number of cladodes per subject plant over the period of the experiment from October 2017 until October 2018. Treatments included are C= control treatment, IE=Insect excluded treatment, 1=1-Release treatment, 2-2-Release treatment, and 3-3-Release treatment. Error bars delineate standard errors.

3.4 Discussion

Findings from this study indicate that the cochineal insect, *D. austrinus*, had a subtle impact on *O. aurantiaca* at Table Farm over the period of this study. The plots where the biological control was excluded had the highest number of plants, and the highest number of category 2 plants (those between 2 and 20 cladodes in size), at the end of the study period. The subject plants without cochineal were also consistent in having the highest number of cladodes per plant. There is therefore some evidence that having cochineal is beneficial, but the differences between the plots with cochineal and those where cochineal was excluded were expected to be much greater based on previous studies. Potted *O. aurantiaca* plants have been shown to be severely damaged by *D. austrinus* under laboratory conditions (Chapter 2) and the damage recorded in previous studies under field conditions have been much greater (Zimmermann 1981).

Zimmermann (1981) conducted similar field based experiments to quantify the efficacy of *D. austrinus* on *O. aurantiaca* in areas with very similar climatic conditions and vegetational compositions as those found on Table Farm. All of the sites from Zimmermann (1981) were also in the same geographic area as Table Farm, between Grahamstown, Bedford, Adelaide and Fort Beaufort in the Eastern Cape Province. Over a four-year study period, jointed cactus plants without cochineal had a 100% survival rate, while those with natural levels of cochineal had only about a 23% survival rate (Zimmermann 1981). The number of cladodes produced in plots where the agent was excluded was also almost five times greater than those in plots with natural levels of cochineal (Zimmermann 1981). The effect of excluding the cochineal at Table Farm in this study was therefore much less pronounced than was expected. This difference in the results may be due to the length of time over which the studies were conducted (four years for Zimmermann (1981) and a single year in this study), or the climatic conditions at the time of the study. After excluding cochineal from plots in the insect exclusion treatment the plants would still require suitable climatic conditions to grow. Under very dry or cold conditions jointed cactus plants appear to be dormant, growing very little, or not at all.

Findings from this study indicate that augmentation of the cochineal insect had very little or no long-term implications for the density of the plant in the field. This is contradictory to laboratory based studies which indicated that releasing the agent did have an impact on plant size although there was no difference between a single release event or multiple release events

(Chapter 2). The difference between laboratory and field results in this case may be due to higher cochineal density on control plots under field conditions than those maintained in the laboratory. In the field, the insecticide exclusion plots had higher total number of plants, while there was no consistent difference between plots left at the natural level of cochineal and any of the release treatments. Similarly to what was found in laboratory studies, there was some evidence that cochineal densities increased after releases, however the increased cochineal infestation was only sustained for a short period of time. The decrease in cochineal infestation in control treatment shows that cochineal insect population would not build up naturally under the conditions in the field for the period of this study, which could have resulted in augmentative releases having very little effect, or that the effect from releases was temporary at best.

The first and second release of *D. austrinus* increased the density of cochineal on the treated plots for a short period of time. There was a slight increasing in cochineal populations, and decreasing of at least some of the plant parameters evident. But winter came shortly after the second release, which could explain why cochineal populations were unresponsive. Cold temperatures have a significant influence on the efficacy of biological control agents (Byrne *et al*. 2003), and *D. austrinus* is no exception, with much lower growth rates being expected under cold conditions. *Dactylopius austrinus* is also heavily impacted by rain (Hosking & Deighton 1980) while the plant thrives after rainfall events (Zimmermann 1981). Other cochineal insects, such as *Dactylopius tomentosus* (Lamark) for the control of *Cylindropuntia imbricata* (Haworth) Knuth., have also been shown to most effective under hot and dry conditions in South Africa (Moran & Zimmermann 1991b). Conditions were very dry at the initiation of this study, but there were higher amounts of precipitation after the winter period. This explains the very high densities of cochineal at the start of the experiment from October 2017 to February 2018, followed by a decline in cochineal due to cold winter conditions, and then a further decline at the beginning of the next summer due to high rainfall. Timing of releases is therefore likely to be critical because cochineal insect population could increase after augmentative releases following rainfall events, but releasing in dry season will not result in significant increased cochineal, partly due to low recruitment and partly due to the already very high cochineal densities that are expected during dry period without augmentation. Therefore, it is better to release the cochineal after the wet season, when the conditions are likely to be favourable for cochineal and cochineal populations are low.

This study showed that the number of plants decreased in all treatment plots overtime. This reduction, however, cannot be explained by the releases of cochineal. The jointed cactus densities also decreased in the control treatment and insect exclusion treatment. This shows that reduced jointed cactus densities were most likely strongly influenced by climatic conditions such temperature and precipitation rather than releases. This explains the significant difference between months and the lack of any consistent significant differences between treatments. The strong influence of climate suggest that a thermal physiological study of the agent may be required so that sites with suitable climates in South Africa can be selected for future releases. It also suggest that climate change may have a significant influence on the future of this biological control programme.

Although jointed cactus densities were not significantly reduced after augmentation of cochineal, this does not eliminate the possibility of augmentation of cochineal insect being effective in reducing jointed cactus populations. The agent population may have reached a carrying capacity for the jointed cactus population, and if this was the case, further releases would not make any difference. This suggests that releases should be conducted at times when *D. austrinus* populations are low. Calculating a threshold levels for release (i.e. the density of cochineal under which augmentative releases should be made) should be the focus of biological control research on *O. aurantiaca* in future. Using an appropriate measure to calculate *D. austrinus* densities will be essential if an accurate threshold is to be calculated, and it is clear from this study, and the results of the laboratory study, that the number of infected cladodes is an inappropriate metric (Chapter 2). Percentage cover of cochineal, or a count of mature female cochineal individuals per cladode, may be more appropriate metrics.

Category 1 plants, which in this study were defined as jointed cactus that had single cladode attached to a tuberous root on the ground, decreased over time and few of them were recorded toward the end of study. This result may also have been affected by climatic conditions, as loose cladodes will only root if there is sufficient moisture and conditions over the period of this study were particularly dry. Loose cladodes without cochineal are expected to root and form a new plant and those with cochineal are expected not to root because of their inability to withstand cochineal colonisation. The expectation in this study was that loose cladodes in insect free treatment would root and form new plants, but that expectation wasn't met. Category 2 and category 3 plants decreased over time in all treated plots and a notable decrease was

observed after all the releases of cochineal. Category 2 plants had fewer cladodes (mostly <10), and the reduction in their density in treated plots could be the result of cochineal attack on them. The increase in total jointed cactus density recorded during December 2017 was due to an increase in category 2 plants. This could have been caused by brief summer rainfall events, which consequently favoured jointed cactus growth and briefly reduced the efficacy of cochineal. At this time, some of the loose cladodes that were not yet rooted in the ground became rooted and produced more than one cladode thus increasing the number of category 2 plants.

Damage by the cochineal insect appears to reduce the number of category 2 and category 3 plants. This decrease in larger plant numbers was most likely due to the cochineal causing cladode fall (Moran & Zimmermann 1991a). Having less large plants is perceived as a success by biological control practitioners and is likely to improve the grazing quality and capacity of lands infested by jointed cactus even if the total number of plants is not decreased. Cladodes that are infected with cochineal are very unlikely to root, and usually die, while detached cladodes without cochineal root and form new plants if climatic conditions are favourable (Zimmerman 1981; Moran & Zimmermann 1991a). In this study there was evidence for large plants dropping cladodes, but very few cladodes rooted, including in the insect exclusion plots. This result was most likely influenced by climatic conditions at the time of the study. Higher numbers of detached cladodes could have rooted if the experiment was continued after the rainfall events in the last two months of the study.

3.5. Conclusion

Post-release evaluations, such as this study, can be used to improve implementation of biological control, especially when manipulative experiments are conducted as part of the post-release evaluations (Morin *et al* 2009). The insect exclusion experiment showed that jointed cactus densities increase in the absence of cochineal. Although, there was no significant increase in jointed cactus densities, the plant is able to spread and proliferate in the absence of cochineal and therefore, it is better to have the agent present in the field than not. This study also suggested that augmentative releases of cochineal insects should be conducted when climatic conditions are correct and when natural field densities of cochineal are low in order to be effective. Under the conditions in which releases were done in this study, the releases had a very limited impact with increased cochineal densities sustained for a maximum of two or three

months at best. Sites where the *D. austrinus* is absent will therefore benefit from releases, but further releases at sites where the agent is already established will only be effective if releases are conducted when climatic conditions are correct and natural densities of cochineal are low.

Chapter 4: General discussion

This study investigated the impact of augmentative releases of *D. austrinus* on *O. aurantiaca* in South Africa. This was done by determining the impact of augmenting *D. austrinus* populations on the target weed under controlled environmental conditions in a laboratory based experiment (Chapter 2) and under field conditions (Chapter 3).

The main of objective of this study was to contribute towards the development of a reliable release strategy for the cochineal insect for biological control of jointed cactus in South Africa. Biological control is effective to a variable extent, however, jointed cactus is still a problem in many parts of South Africa. Biological control is limited by certain factors in time and space and there is evidence to suggest that biological control of jointed cactus could be improved through augmentation.

4.1 Developing a release strategy for *D. austrinus*

Ideally, a biological control agent should increase in density to levels that inflict adequate damage to the target weed to lower their reproductive output, plant growth, population increase and competitive ability. Some of the biological control agents that have been released have reduced target plant populations, however, not all of them have resulted in complete control (Schwarzländer *et al*. 2018). One possible way to improve the success rate of biological control is through the practice of augmentative biological control. Even though biological control has been practised against weeds for over a century, the release strategies that are currently used are largely based on untested assumptions (Memmott *et al*. 1997). In some cases, manipulative experiments have been conducted in the field to investigate release strategy for biological control of weeds (e.g., Memmott *et al*. 1997) but these sorts of experiments are very uncommon. There is growing pressure on biological control practitioners to conduct experiments to investigate the efficacies of releases strategies, so that the most effective and efficient release strategies are followed. After the significant allocation of resources that is required to develop a biological control agent, it is important that they are

all used to their maximum potential, and this can be achieved through implementing effective release strategies (Zachariades *et al*. 2017).

Under laboratory conditions, releases of cochineal resulted in greater damage to the target weed, but a single release event in the year was not significantly more effective than two or three releases (Chapter 2). Under field conditions, the subject plants which were directly comparable to the plants used in the laboratory based experiment, did not show the same trend, with levels of damage remaining similar for all treatments throughout the experiment (Chapter 3). The discrepancy between the laboratory and field study suggests that climatic conditions may influence the efficacy of releases. It is also possible that the population of cochineal in the field may have already reached the maximum level that it could obtain due to a combination of factors such as the weather conditions at the site, the density of cochineal that was already at the site, and the quality and quantity of plants. The laboratory study does however, indicate that under certain conditions the augmentation of cochineal populations is beneficial. This suggests that the timing of releases in the field may be important in maximising the success of augmentative releases. Releases should be conducted when jointed cactus plants are healthy and cochineal densities are low, but also when the releases are most likely to establish. The ideal times to make releases will therefore be after significant rainfall events during summer, when plants are likely to be healthy, cochineal densities will be low, and conditions for cochineal will be good.

Climatic conditions in the laboratory experiment were consistently dry and hot but plants were kept in good condition with regular watering, while in the field, fluctuating climatic conditions, including precipitation, influenced both cochineal populations and plant health. After the release of the cochineal in the laboratory experiment the favourable climatic conditions and well-watered plants allowed the cochineal population to thrive until the end of the experiment, damaging the plants on which releases were conducted to a significantly greater degree than both the control (with field levels of cochineal) and the plants on which cochineal was excluded. In the field, wet or cold weather after a release event may reduce the population of cochineal (Hosking & Deighton 1980; Zimmermann 1981; Hosking 1984), and when conditions become favourable it may be beneficial to release the agent again in order to augment the population. Even if the cochineal population would naturally increase to the same level over time, augmentative release may result in a quicker increase and this could

lead to a greater level of control by damaging the plants at a time when they would be growing most vigorously.

This study suggests that the efficacy of *D. austrinus* augmentation is not necessarily dependent on the frequency of releases, but rather on the timing. One release at the correct time may have a greater impact than multiple releases at the wrong time. Releases of *D. austrinus* during winter should be avoided as the agent is unlikely to proliferate in cold conditions. The reproduction, growth and survival of *D. austrinus* are favoured in temperatures ranging from 25 to 30 degree Celsius (Hosking 1984), so winter temperatures in South Africa are well below the most favourable conditions for the agent. Release prior to rain or the rainy season should also be avoided as the agent will be negatively affected by the rain and wet conditions (Hosking & Deighton 1980). Releases should be conducted after each significant rainfall event, and if rain is recorded soon after a release, additional release should be conducted.

Despite the presence of *D. austrinus*, *O. aurantiaca* populations may increase above acceptable thresholds at irregular intervals several years apart due to the natural cycle of populations of the plant and the biological control agent (Klein 2002). The cyclical or seasonal resurgence of target weeds is common in biological control programmes. For example, long-term monitoring of the invasive alien weed, parrot's feather (*Myriophyllum aquaticum* (Vell.) (Haloragaceae)), and the biological control agent *Lysathia* sp. (Chrysomelidae), showed that the weed was re-emerging after winter die-back and seasonal floods, and *Lysathia* sp. was present at most of the sites but in low numbers (Coetzee *et al*. 2011). Although the population of *Lysathia* is able to build up in summer, augmentative releases of the agent in the early season significantly improved the overall level of control (Coetzee *et al*. 2011). Augmenting *D. austrinus* populations on *O. aurantiaca* after rain in the summer months may have a similar impact to the augmentative releases of *Lysathia* sp. on *M. aquaticum.*

A release strategy should take the density of cochineal at field sites into account prior to augmentative releases. If very high populations of cochineal are present at a site then releases may not be necessary. Similarly, if the carrying capacity of the jointed cactus population has been reached, then further releases will not augment the agent population (Zimmermann 1981). Conducting releases at a time when cochineal densities are high will not have any

negative impact on the level of control at the release site, but time and resources would be utilised better if releases were done at sites where a greater impact is expected. Monitoring of field populations of *D. austrinus* should therefore be a key component of a management plan for *O. aurantiaca*. The population of cochineal should be consistently monitored and releases should be conducted when populations are low.

Successful biological control of *O. aurantiaca* is also positively influenced by the degree of aggregation of the weed (Zimmermann 1981). The limited dispersal ability of the agent results in greater control in dense or highly aggregated populations of *O. aurantiaca*. Highly aggregated *O. aurantiaca* infestations are often denser than the tolerable threshold level of two to three large plants per 10 m^2 and reducing these aggregated clumps of *O. aurantiaca* may reduce the overall population below this threshold at some sites (Zimmerman 1981). Similarly, the biological control agent *Trichapion lativentre* (Beguin-Billecocq) (Curculionidae) is more abundant on the invasive alien plant *Sesbania punicea* (Cav.) (Fabaceae) growing in dense aggregation than plants growing in relative isolation from conspecifics (Hoffmann & Moran 1998, Hoffmann & Moran 1999). Dense infestation of *O. aurantiaca* allows *D. austrinus* to increase rapidly and destroys most large clumps (Zimmermann 1981). For long term-control, aggregation of *O. aurantiaca* is one of the factor that can be considered in a management or release strategy by targeting aggregations or infestations that are highly aggregated. Dense and highly aggregated *O. aurantiaca* infestations should be prioritised first during augmentative releases rather than isolated plants. Previous biological control studies of *O. aurantiaca* suggested that heavy infestations (> 5 plants per 20 m^2) should require immediate attention rather than light (<1 plant per 20 m^2) and medium (between 1 and 5 plants per 20 m^2) infestations (Zimmermann 1981).

This study has contributed towards the development of a release strategy for the biological control of *O. aurantiaca* in South Africa. It is clear that the release strategy needs to be site specific and that constant monitoring of climatic conditions and agent densities is required. There is no set number of releases, or any specific time of year that is optimal for releases, so the teams that conducted releases must be flexible and able to respond relatively quickly. Monitoring of agent populations should be encouraged, and requests for releases should be made when agent populations are low and/or after significant rainfall events. These releases should also be restricted to the warmer summer months. Repeat releases should be conducted

if significant rainfall events are recorded after releases. Further research should also be conducted to calculate a threshold value of cochineal densities at which releases should be conducted. Famers and landowners could then assess the density of cochineal and request releases when densities are sufficiently low. This would reduce the number of ineffective or unnecessary releases that are conducted and optimise the level of control of the weed.

Farmers often monitor pest populations as part of management programmes and monitoring of cochineal populations could be done in a similar way. Monitoring of insect pest population can help determine when interventions, such as spraying of pesticides or releasing of biological control agents, is required, and can also determine how effective the interventions are (Hagstrum *et al*. 1998). A similar strategy could be used for the control of *O. aurantiaca*, but cochineal densities should not be calculated by counting the number of infected cladodes. The data in this study clearly indicated that the number of infected cladodes is an inappropriate method of quantifying cochineal densities (Chapter 2). The number of mature individual insects is likely to be a more suitable parameter to measure for estimating cochineal density.

In order to for an effective release strategy to be implemented against *O. aurantiaca*, it is essential that farmers have an understanding of the cyclical nature of the agent-weed system, and that they have faith that the release strategy will work. Educating farmers and managing their expectations is therefore also an important factor in implementing an effective control programme against *O. aurantiaca*.

4.2 Fluctuating *O. aurantiaca* populations and managing expectations of biological control

The first release of the cochineal insect, *D. austrinus*, in South Africa in 1935 successfully reduced jointed cactus population densities over large areas such that biological control became the only recommended control practice until 1946 (Moran & Zimmermann 1991a). Then, local resurgences of the weed, led to the reintroduction of mechanical and chemical control (Moran & Zimmermann 1991a). These control methods had little impact on the reduction of the weed due to the regrowth, re-infestation and spread from residual populations, and also resulted in reductions in cochineal populations (Moran & Zimmermann 1991a). A post-release evaluation was conducted between 1976 and 1988 and indicated that a cyclical interaction between

cochineal insect and jointed cactus over the period of 12 years (Fig 4.1) (Moran & Zimmermann 1991a). The cyclical resurgences of jointed cactus are primarily due to climatic conditions, with higher densities at times of high rainfall and lower densities during droughts. It was this cyclical relationship between the agent and the weed that caused the resurgence after initial control in 1935, so the intervention using mechanical and chemical control was premature. If no further intervention had been made the weed population would most likely have reduced to similar levels again in the next cycle (Moran & Zimmermann 1991a).

Although cladode density has been reduced, primarily due to the action of the cochineal insect, the current densities of jointed cactus in parts of the Eastern Cape Province are still considered too high by many farmers and landowners. Mechanical and chemical control are very ineffective against jointed cactus and disrupt the significant levels of control that are provided by biological control, so the most appropriate way to reduce the levels of the weed infestations further are to enhance the impact of biological control.

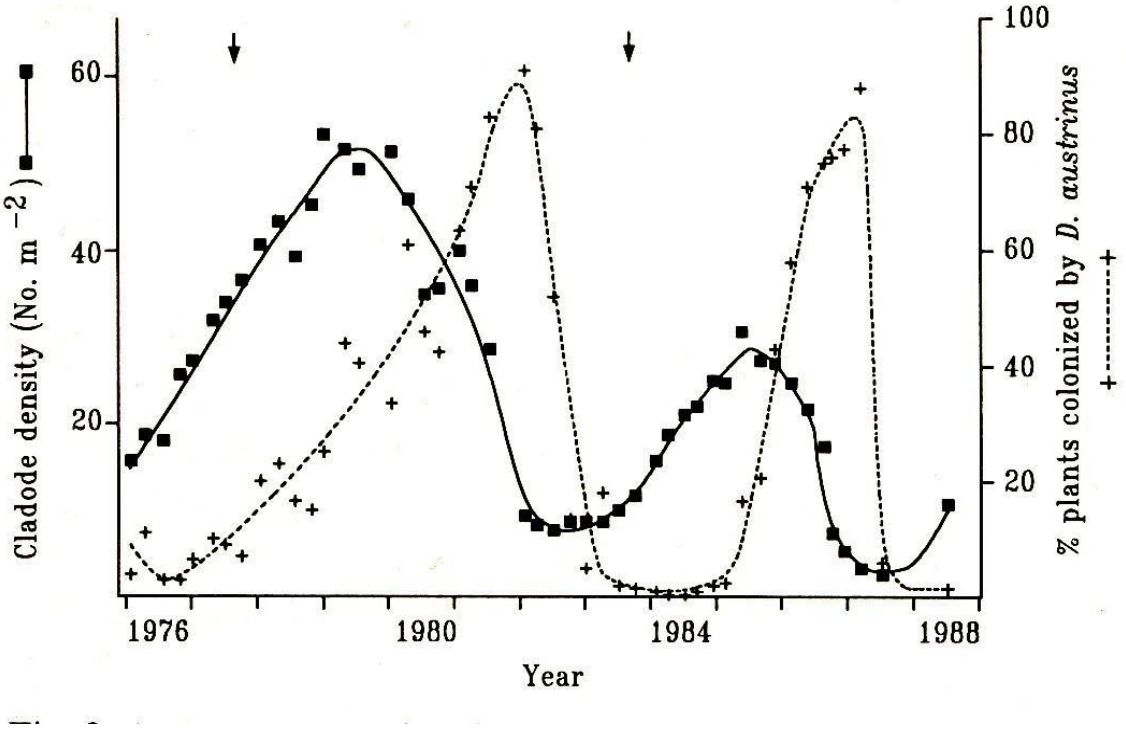

Fig 4.1 The long-term monitoring of *O. aurantiaca* and of the cochineal insect, *D. austrinus* from 1976 to 1988 on twenty 50 m^2 permanent plots (from Zimmermann & Malan 1980).

The current study was conducted over a single year and is therefore just a small snap shot of part of the cycle. Populations of *O. aurantiaca* decreased over the study period but it is likely that if the study were continued the populations would have increased, especially after summer rainfall events. It is therefore important to note that the time period over which this study was conducted was just a small part of a seasonal cycle. Population dynamics of the agent and the weed would be very different if the study was conducted over a different part of the seasonal cycle. It has also been a period of significant drought in South Africa, and populations dynamics of both the weed and the agent will differ during wetter periods. The climatic conditions at the time of this study can explain why some of the results differed from those reported in previous studies that were conducted in the same area (Zimmermann 1981).

It is important that farmers, land-managers and conservationist understand the nature of the seasonal and longer-term fluctuations of *D. austrinus* and *O. aurantiaca*. It was a lack of understanding of these fluctuations which resulted in the expensive and unnecessary herbicide campaign conducted by the South African government between 1957 and 1980 (Moran & Zimmermann 1991a). Farmers should be aware that the aim of biological control is not to eradicate *O. aurantiaca* but rather to reduce the densities of the plant so that it is less harmful to agricultural productivity and the natural ecosystem. Expectations of biological control, and particularly for cactus biological control, are often unrealistic because of some well-known successes (Hoffmann 1995; Walton 2005; Raghu & Walton 2007). Farmers need to accept that *O. aurantiaca* will always be present on their land and that populations of the weed will fluctuate. Mechanical and chemical control are ineffective and new biological control agents are very unlikely to be released, so the use of *D. austrinus* is the most effective way to control *O. aurantiaca* and will provide the greatest possible level of control (Moran & Zimmermann 1991).

4.3 Conclusions

Dactylopius austrinus has contributed significantly towards to the control of *O. aurantiaca* but, with an appropriate release strategy, this level of control can be improved. Long-term monitoring of *D. austrinus* populations must be conducted at sites where *O. aurantiaca* is

problematic, and when populations are low requests for augmentative releases should be made. The release team must respond rapidly, releasing soon after requests are received. Climatic conditions must also be taken into account, with releases being prioritised after significant rainfall events during summer. Repeat releases are required if heavy rain is recorded soon after release events. The scale of the *O. aurantiaca* problem in South Africa makes this strategy impossible without the support of farmers and land managers, who should preferably be involved in the whole process, from monitoring to mass-rearing and releasing. There are currently only two teams, each of eight people, who mass-rear and release *D. austrinus* in South Africa. This needs to be increased significantly and should be expanded by encouraging farmers to run their own mass-rearing facilities that specifically services their farms. A collaborative approach between farmers and land managers; biological control implementation services and biological control scientists will be required to implement the changes to the release strategy that have been suggested by this study.

5. References

Adair, R.J., and Groves, R.H., 1998. Impact of environmental weeds on biodiversity: a review and development of a methodology. Environment Australia. Canberra. 55pp.

Adair, R.J., and Holtkamp, R.H., 1999. Development of a pesticide exclusion technique for assessing the impact of biological control agents for *Chrysanthemoides monilifera*. *Biocontrol Science and Technology*, 9(3), pp.383-390.

Arnold, T.H., 1977. The origin and relationships of *Opuntia aurantiaca* Lindley. In: *Proceedings of 2nd National Weeds Conference of South Africa*. pp. 269-286.

Barton, J., Fowler, S.V., Gianotti, A.F., Winks, C.J., De Beurs, M., Arnold, G.C., and Forrester, G., 2007. Successful biological control of mist flower (*Ageratina riparia*) in New Zealand: agent establishment, impact and benefit to the native flora. *Biological Control,* 40: 370-385.

Briese, D.T., 2000. Classical biological control. In: *Australian weed management systems*, (Ed. B.M. Sindel) R.G. & F.J. Richardson, Melbourne, Australia, pp.161-192.

Briese, D.T., 2005. Translating host-specificity test results into the real world: the need to harmonize the yin and yang of current testing procedures. *Biological Control*, 35(3): 208-214.

Briese, D.T., and Walker, A., 2002. A new perspective on the selection of test plants for evaluating the host-specificity of weed biological control agents: the case of *Deuterocampta quadrijuga*, a potential insect control agent of *Heliotropium amplexicaule*. *Biological control*, 25(3): 273-287.

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

Byrne M., Coetzee J.A., Mcconnachie, A., Parasam, W., and Hill, M., 2003. Predicting climatic compatibility of biological control agents in their region of introduction. In: *Proceedings of the XI International Symposium on Biological Control of Weeds*, (Eds.) J.M. Cullen, D.T. Briese,

D.J. Kriticos, W.M. Lonsadale, L. Morin & J.K. Scott JK, CSIRO Entomology, Canberra, Australia, Pp. 28-35.

Canavan, K., Paterson, I.D., and Hill, M.P., 2014. The herbivorous arthropods associated with the invasive alien plant, *Arundo donax*, and the native analogous plant, *Phragmites australis*, in the Free State Province, South Africa. *African Entomology*, 22(2): 454-459.

Carson, W.P., Hovick, S.M., Baumert, A.J., Bunker, D.E. and Pendergast, T.H., 2008. Evaluating the post-release efficacy of invasive plant Biocontrol by insects: a comprehensive approach. *Arthropod-Plant Interactions*, 2(2): 77-86.

Clewley, G.D., Eschen, R., Shaw, R.H., and Wright, D.J., 2012. The effectiveness of classical biological control of invasive plants. *Journal of Applied Ecology*, 49(6): 1287-1295.

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

Crawley, M.J., 1989. Insect herbivores and plant population dynamics. *Annual Review of Entomology*, 34(1): 531-562.

Culliney, T.W., 2007. Benefits of classical biological control for managing invasive plants. *Critical Reviews in Plant Sciences*, 24(2): 131-150.

De Lotto, G., 1974. On the status and identity of the cochineal insects (Homoptera: Coccoidea: Dactylopiidae). *Journal of The Entomological Society of Southern Africa*, 37(1): 167-193.

DiTomaso, J.M., 1997, October. Risk analysis of various weed control methods. In: *Proceedings of the California. Exotic Pest Plant Council. Symposium*, (Eds.) M. Kelly, E. Wagner, & P. Warner, California, 3: 34-39.

Downey, P.O., and Paterson, I.D., 2016. Encompassing the relative non-target risks from agents and their alien plant targets in biological control assessments. *Biocontrol*, 61(6): 615-630.

Dudley, T.L., Lambert, A., and Kirk, A., 2006. Augmentation biological control of *Arundo donax.* In: *Proceedings, V California Conference on Biological Control 25-27 July 2006*, (Eds.) M. S. Hoddle & M. W. Johnson, Riverside, California, USA. University of California, Riverside, p.141 -144.

Fowler, S.V., Syrett, P., and Hill, R.L., 2000. Success and safety in the biological control of environmental weeds in New Zealand. *Austral Ecology*, 25(5): 553-562.

Fowler, S.V., Paynter, Q., Dodd, S., and Groenteman, R., 2012. How can ecologists help practitioners minimize non-target effects in weed Biocontrol? *Journal of Applied Ecology*, 49(2): 307-310.

Goolsby, J.A., De Barro, P.J., Makinson, J.R., Pemberton, R.W., Hartley, D.M., and Frohlich, D.R., 2006. Matching the origin of an invasive weed for selection of a herbivore haplotype for a biological control programme. *Molecular Ecology*, 15(1): 287-297.

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

Grevstad, F.S., 1999a, Experimental invasion using biological control introductions: the influence of release size on the chances of population establishment. *Biological Invasions*, 1: 313-323.

Grevstad, F.S., 1999b, Factors influencing the chance of population establishment: implications for release strategies in Biocontrol. *Ecological Applications*, 9: 1439-1447.

Groves, R.H., Burdon, J.J., and Marshall, D. R., 1979. Weed biology in relation to control CSIRO Division Plant Industry Report 1979-1980, CSIRO, Canberra, pp15-19.

Gunn, B.H., 1979. Dispersal of the cochineal insect *Dactylopius austrinus* De Lotto (Homoptera : Dactylopiidae). PhD Thesis, Rhodes University.

Hagstrum, D.W., Flinn, P.W., and Subramanyam, B., 1998. Predicting insect density from probe trap catch in farm-stored wheat1. *Journal of Stored Products Research*, 34(4): 251-262.

Heard, T.A., 2000. Concepts in insect host-plant selection behavior and their application to host specificity testing. In: *Proceedings of the 10th international symposium on biological control of weeds*, (Eds.) R. G. Van Driesche, T. A. Heard, A. S. McClay, & R. Reardon, Montana State University, Bozeman, MT, pp1-10.

Henderson, L. 1995. Plant invaders of southern Africa. Plant Protection Research Institute Handbook No. 12. Agricultural Research Council. Pretoria. p87.

Hill, M.P., and Coetzee, J., 2017. The biological control of aquatic weeds in South Africa: Current status and future challenges. *African Biodiversity & Conservation*, 47(2):a2152.

Hoffmann, J.H., 1988. Pre-release assessment of *Nanaia* sp.[Lepidoptera: Phycitidae] from *Opuntia pascoensis* for biological control of *Opuntia aurantiaca* [Cactaceae]. *Entomophaga,* 33(1): 81-86.

Hoffmann, J.H., 1995. Biological control of weeds:the way forward, a South African perspective. In: Stirton, C.H. (Ed.) *British Crop Protection Council Symposium Proceedings No. 64: Weeds in a Changing World.* British Crop Protection Council, Farnham, U.K. pp77–89.

Hoffmann, J.H., and Moran, V.C., 1998. The population dynamics of an introduced tree, *Sesbania punicea*, in South Africa, in response to long-term damage caused by different combinations of three species of biological control agents. *Oecologia*, 114(3): 343-348.

Hoffmann, J.H., and Moran, V.C., 1999. A review of the agents and factors that have contributed to the successful biological control of *Sesbania punicea* (Cav.) Benth. (Papilionaceae) in South Africa. Biological Control of Weeds in South Africa (1990–1998). *African Entomology Memoir*, 1, pp.75-79.

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

Hosking, J.R., 1984. The effect of temperature on the population growth potential of *Dactylopius austrinus* De Lotto (Homoptera: Dactylopiidae), on *Opuntia aurantiaca* Lindley. *Austral Entomology*, 23(2): 133-139.

Hosking, J.R., and Deighton, P.J., 1980. Biological control of moisture stressed *Opuntia aurantiaca* using Dactylopius austrinus. In: *Proceedings of the 5th International Symposium on Biological Control of Weeds*, (Ed.) E.S. Delfosse, Brisbane, Australia, pp. 483-487.

Hosking J.R, McFadyen R.E.C, and Murray N.D., 1988. Distribution and biological control of cactus species in eastern Australia. *Plant Protection Quarterly*, 3:115–23

Keane, R.M. and Crawley, M.J., 2002. Exotic plant invasions and the enemy release hypothesis. *Trends in Ecology & Evolution*, 17(4): 164-170.

Klein, H. 1999. Biological control of three cactaceous weeds, *Pereskia aculeata* Miller, *Harrisia martinii* (Labouret) Britton and *Cereus jamacaru* De Candolle in South Africa. *African Entomology Memoir*, 1: 3–14.

Klein, H., 2002. Biological of invasive cactus species (Family Cactaceae); Plant Protection Research Institute (ARC-PPRI) Leaflet Series: Weeds Biocontrol, No 2.9.

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

Klein, H., Hill, M.P., Zachariades, C. and Zimmermann, H.G., 2011. Regulation and risk assessment for importations and releases of biological control agents against invasive alien plants in South Africa. *African Entomology*, 19(2): 488-497.

Le Maitre, D.C., Richardson, D.M. and Chapman, R.A., 2004. Alien plant invasions in South Africa: driving forces and the human dimension: working for water. *South African Journal of Science*, 100: 103-112.

Louda, S.M., Kendall, D., Connor, J. and Simberloff, D., 1997. Ecological effects of an insect introduced for the biological control of weeds. *Science*, 277: 1088-1090.

Loünsbury, C., 1915. Plant killing insects: The Indian cochineal. *Agricultural Journal of South Africa*, 11: 537-543.

Mack, R.N., Simberloff, D., Lonsdale, M., Evans, H., Cout, M. and Bazzaz, F., 2001. Biotic invasions: causes, epidemiology, global consequences, and control. *Ecological Applications*, 10: 689–710.

Mann, J., 1969. Cactus-feeding insects and mites. Bulletin of the United States National Museums 256: 1–158.

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

McFadyen, R.E.C., 1998. Biological control of weeds. *Annual Review of Entomology*, 43(1): 369-393.

McClay, A.S. and Balciunas, J.K., 2005. The role of pre-release efficacy assessment in selecting classical biological control agents for weeds—applying the Anna Karenina principle. *Biological Control*, 35(3):197-207.

McGibbon, J. 1858. Catalogue of plants in the botanical garden, Cape Town, Cape of Good hope. Saul Solomon, Cape Town, pp 36.

Memmott, J., Fowler, S.V., Harman, H.M. and Hayes, L.M., 1996. How best to release a biological control agent. In: *proceedings of the IX International Symposium on Biological Control of Weeds*, (Eds.) V.C. Moran, and J.J Hoffmann, 19-26 January; Stellenbosch, South Africa, University of Cape Town, pp.291-296.

Meyer, J.Y., Fourdrigniez, M. and Taputuarai, R., 2012. Restoring habitat for native and endemic plants through the introduction of a fungal pathogen to control the alien invasive tree *Miconia calvescens* in the island of Tahiti. *Biocontrol*, 57(2): 191-198.

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

Moran, V C., and Cobby, B.S., 1979. The life history and fecundity of cochineal insects *Dactylopius austrinus* De Lotto (Homoptera: Coccoidea: Dactylopiidae). *Bulletin of Entomological Research*, 69: 629-636.

Moran, V.C., Hoffmann, J.H., and Zimmermann, H.G., 2005. Biological control of invasive alien plants in South Africa: necessity, circumspection, and success. *Frontiers in Ecology and the Environment*, 3(2): 71-77.

Moran, V.C, Hoffmann J.H, and Zimmermann H.G., 2013. 100 years of biological control of invasive alien plants in South Africa: History, practice and achievements. *South African Journal of Science*. 2013;109(9/10), Art. a0022.

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

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

Moran, V.C., Zimmermann, H.G., and Annecke, D.P., 1976. The identity and distribution of *Opuntia aurantiaca* Lindley. *Taxon*, 25:281-287.

Morin, L., Evans, K.J., and Sheppard, A.W., 2006. Selection of pathogen agents in weed biological control: critical issues and peculiarities in relation to arthropod agents. *Australian Journal of Entomology*, 45: 349-365.

Morin, L., Reid, A.M., Sims-Chilton, N.M., Buckley, Y.M., Dhileepan, K., Hastwell, G.T., Nordblom, T.L. and Raghu, S., 2009. Review of approaches to evaluate the effectiveness of weed biological control agents. *Biological Control*, 51(1): 1-15.

Müller-Schärer, H. and Schaffner, U., 2008. Classical biological control: exploiting enemy escape to manage plant invasions. *Biological Invasions*, 10(6): 859-874.

Paterson, I.D., Hoffmann, J.H., Klein, H., Mathenge, C.W., Neser, S. and Zimmermann, H.G., 2011a. Biological control of Cactaceae in South Africa. *African Entomology*, 19(2): 230-246.

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

Paterson, I.D., Vitorino, M.D., De Cristo, S.C., Martin, G.D. and Hill, M.P., 2014. Prioritisation of potential agents for the biological control of the invasive alien weed, *Pereskia aculeata* (Cactaceae), in South Africa. *Biocontrol Science and Technology*, 24(4): 407-425.

Paynter, Q., 2005. Evaluating the impact of a biological control agent *Carmenta mimosa* on the woody wetland weed *Mimosa pigra* in Australia. *Journal of Applied Ecology*, 42(6): 1054-1062.

Paynter, Q., Waipara, N., Peterson, P., Hona, S., Fowler, S., Gianotti, A., and Wilkie, P., 2006. The impact of two introduced Biocontrol agents, *Phytomyza vitalbae* and *Phoma clematidina*, on *Clematis vitalba* in New Zealand. *Biological Control*, 36(3): 350-357.

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

Pemberton, R.W., 2000. Predictable risk to native plants in weed biological control. *Oecologia*, 125(4): 489-494.

Peterson, R.K.D., and Higley, L.G., 2001. Illuminating the black box: the relationship between injury and yield. In *Biotic Stress and yield loss*, (Eds.) R.K.D. Peterson, L.G. Higley, CRC Press. Boca Raton. FL, pp 1-12.

Pimentel, D., Mcnair, S., Janecka, J., Wightman, J., Simmonds, C., O'connell, C., Wong, E., Russel, L., Zern, J., Aquino, T., and Tsomondo, T., 2001. Economic and environmental threats of alien plant, animal, and microbe invasions. *Agriculture, Ecosystems & Environment*, 84(1): 1-20.

Raghu, S. and Walton, C., 2007. Understanding the ghost of *Cactoblastis* past: historical clarifications on a poster child of classical biological control. *BioScience*, 57(8): 699-705.

Richardson D.M., Macdonald I.A.W., Hoffman J.H., and Henderson L., 1997. Alien plant invasions. In: *Vegetation of Southern Africa*, (Eds.) R.M. Cowling, D.M. Richardson and S.M. Pierce, Cambridge University Press, Cambridge, pp. 535–570.

Robertson, M.P., Kriticos, D.J. and Zachariades, C., 2008. Climate matching techniques to narrow the search for biological control agents. *Biological Control*, 46(3): 442-452.

Sands, D. P. A., and Van Driesche, R.G., 2000. "Evaluating the host range of agents for biological control of arthropods: rationale, methodology and interpretation. In: *Proceedings of the 10th international symposium on biological control of weeds,* (Eds.) R. G. Van Driesche, T. A. Heard, A. S. McClay, and R. Reardon, Bozeman, MT: Montana State University, pp. 69-83.

Schwarzländer, M., Hinz, H.L., Winston, R.L. and Day, M.D., 2018. Biological control of weeds: an analysis of introductions, rates of establishment and estimates of success, worldwide. *Biocontrol*, 63: 319–331.

Sheppard, A.W., 2003. Prioritising agents based on predicted efficacy: beyond the lottery approach. *CRC for Australian Weed Management Technical series No, 7,* pp.11-22.

Sheppard, A.W., Van Klinken, R.D. and Heard, T.A., 2005. Scientific advances in the analysis of direct risks of weed biological control agents to nontarget plants. *Biological Control*, 35(3): 215-226.

Suckling, D.M. and Sforza, R.F.H., 2014. What magnitude are observed non-target impacts from weed Biocontrol?. *PLoS One*, 9(1): e84847.

Sutton, G.F., Paterson, I.D., Compton, S.G., and Paynter Q., 2017: predicting the risk of non-target damage to a close relative of a target weed using sequential no-choice tests, paired-choice tests and olfactory discrimination experiments, *Biocontrol Science and Technology*, 27(3):364-377.

Sutton, G.F., Klein, H. and Paterson, I.D., 2018. Evaluating the efficacy of *Hypogeococcus* sp. as a biological control agent of the cactaceous weed *Cereus jamacaru* in South Africa. *Biocontrol*, 63(4): 493-503.

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

Van Driesche, R.G., Carruthers, R.I., Center, T., Hoddle, M.S., Hough-Goldstein, J., Morin, L., Smith, L., Wagner, D.L., Blossey, B., Brancatini, V. *et al.* 2010. Classical biological control for the protection of natural ecosystems. *Biological Control*, 54:S2-S33.

Van Klinken, R.D., and Raghu, S., 2006. A scientific approach to agent selection. *Austral Entomology*, 45(4): 253-258.

Van Klinken, R.D., 2000. Host specificity testing: why do we do it and how we can do it better. In: *Proceedings of the 10th international symposium on biological control of weeds*, (Eds.) R. G. Van Driesche, T. A. Heard, A. S. McClay, & R. Reardon, Bozeman, MT: Montana State University, pp.54-68.

Van Lenteren, J.C., 2012. The state of commercial augmentative biological control: plenty of natural enemies, but a frustrating lack of uptake. *Biocontrol*, 57(1), pp.1-20.

Van Lenteren, J.C. and Bueno, V.H., 2003. Augmentative biological control of arthropods in Latin America. *Biocontrol*, 48(2): 123-139.

Van Wilgen, B.W. and Lange, W.D., 2011. The costs and benefits of biological control of invasive alien plants in South Africa. *African Entomology*, 19(2): 504-514.

Van Wilgen, B.W., De Wit, M.P., Anderson, H.J., Le Maitre, D.C., Kotze, I.M., Ndala, S., Brown, B. and Rapholo, M.B., 2004. Costs and benefits of biological control of invasive alien plants: case studies from South Africa: working for water. *South African Journal of Science*, 100(12): 113-122.

Vitousek, P.M., 1990. Biological invasions and ecosystem processes: towards an integration of population biology and ecosystem studies. *Oikos*, 57: 7-13.

Walton, C., 2005.*Reclaiming lost provinces: a century of weed biological control in Queensland*. Queensland, Australia: Department of Natural Resources and Mines. Brisbane. pp104.

Wapshere, A.J., 1974. Host specificity of phytophagous organisms and the evolutionary centres of plant genera or sub-genera. *Biocontrol*, 19(3): 301-309.

Wapshere, A.J., Delfosse, E.S. and Cullen, J.M., 1989. Recent developments in biological control of weeds. *Crop Protection*, 8(4): 227-250.

Winston, R.L., Schwarzländer, M., Hinz, H.L., Day, M.D., Cock, M.J. and Julien, M.H., 2014. Biological control of weeds: a world catalogue of agents and their target weeds. USDA Forest Service, Forest Health Technology Enterprise Team, Morgantown, 5th edition. FHTET-2014-04.

Withers, T. M., Barton-Browne, L., and Stanley, J. N., 2000. How time-dependent processes can affect the outcomes of assays used in host specificity testing. In: *Proceedings of the 10th international symposium on biological control of weeds,* (Eds.) R. G. Van Driesche, T. A. Heard, A. S. McClay, & R. Reardon, Bozeman, MT: Montana State University, pp. 27–41.

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

Zimmermann, H.G., 1979. Herbicidal control in relation to distribution of *Opuntia aurantiaca* Lindley and effects on cochineal populations. *Weed Research*, 19(2): 89-93.

Zimerrmann, H.G., 1981. The ecology and control of *Opuntia aurantiaca* in South Africa in relation to the cochineal insect *Dactylopius austrinus*. PhD. Thesis, Rhodes University, Grahamstown.

Zimmermann, H., Bloem, S. and Klein, H., 2004b. Biology, history, threat, surveillance and control of the cactus moth, *Cactoblastis cactorum*. In: *Proceedings of the Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture*. International Atomic Energy Agency, Vienna, Austria. pp. 1-40.

Zimmermann, H.G. and Granata, G., 2002. Insect pests and diseases. *Cacti: Biology and Uses. Ed. University California Press. California, USA.* pp.235-254

Zimmermann, H.G., and Malan, D.E., 1980. A modified technique for the herbicidal control of jointed cactus, *Opuntia aurantiaca* Lindley, in South Africa. *Agroplantae,* 12(4): 65-67.

Zimmermann, H.G., and Moran, V.C., 1991. Biological control of prickly pear, *Opuntia ficus-indica* (Cactaceae), in South Africa. *Agriculture, Ecosystems and Environment* 37: 29–35.

Zimmermann, H.G., Moran, V.C., And Hoffmann, J.H., 2004a. Biological control in the management of invasive alien plants in South Africa, and the role of the Working for Water Programme. *South African Journal of Science*, 100: 34-40.

Zimmermann, H.G., Moran, V.C. and Hoffmann, J.H., 2009. Invasive cactus species (Cactaceae). *Biological control of tropical weeds using arthropods. Cambridge University Press, Cambridge, UK*, pp.108-129.

Zimmermann, H.G., and Van De Venter H.A., 1981. Jointed cactus. In: Subdivision Weed Research: Plant Protection Research Institute. *Farming in South Africa*.

Zwölfer, H., and Harris, P., 1984. Biology and host specificity of *Rhinocyllus conicus* (Froel.)(Col., Curculionidae), a successful agent for Biocontrol of the thistle, *Carduus nutans* L. *Journal of Applied Entomology*, 97:36-62.