The use of the cochineal insect, *Dactylopius tomentosus* Lamarck, as a biological control agent for the invasive alien thistle cholla, *Cylindropuntia pallida* (Rose) F.M. Knuth in South Africa

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Abstract

Cylindropuntia pallida (Rose) F.M. Knuth (Cactaceae) is an invasive alien plant in South Africa indigenous in the southern U.S.A. and Mexico. Large infestations of this species can be found in the Kalahari and arid Karoo regions of South Africa, which is also present in Namibia. Because it is a very spiny cactus, dense infestations have a negative impact on agriculture and natural ecosystems. This cactus has become naturalised to the extent that eradication is impossible and the negative impacts are steadily increasing due to its increasing distribution and density.

The cochineal insect, *Dactylopius tomentosus* Lamarck (Dactylopiidae), is native in Mexico and parts of North America, such as Texas, Arizona, and New Mexico in the U.S.A. This cochineal species is highly specialized and associated only with *Cylindropuntia* species, a group of cacti that are primarily restricted to the same areas. It has been introduced into Australia and South Africa as a biological control agent to control various invasive alien *Cylindropuntia* species. This cochineal species has several biotypes specific to certain *Cylindropuntia* species hosts. A biotype is a clade that cannot be differentiated morphologically from others but has different host ranges and impacts depending on the host plant species. Included amongst biotypes that have been released in South Africa are *D. tomentosus* 'imbricata' and *D. tomentosus* 'cholla' for the biological control of *Cylindropuntia imbricata* (Haw.) F.M. Knuth (Cactaceae) and *Cylindropuntia fulgida* (Engelmann) F.M. Knuth var. *mamillata* (Schott ex Engelmann) Backeb. (Cactaceae), respectively. These biotypes have resulted in both host plants being under substantial control in South Africa.

The first part of this thesis evaluated which of the two *D. tomentosus* biotypes already in use in South Africa could be an effective biological control agent for *C. pallida*. This was done by assessing the fitness of the cochineals on the three cactus species and assessing the impact that each of the cochineals has on each of the target weed species. Should these biotypes prove ineffective, there is a third biotype, namely *D. tomentosus* 'californica var. parkerii', which researchers in Australia have worked on and have found to be suitably host-specific for release in Australia and suitably damaging to *C. pallida* in that country. Therefore, this new biotype could be released in South Africa if it is required.

Sexually compatible biological control agents, especially those closely related and occurring in close spatial proximity to one another, may interbreed and the impacts of this hybridisation are difficult to predict. It is important to understand the outcomes of the hybridisation of cochineal because it can affect the impact of the biological control agents and thus the control of the target weed.

The second part of this thesis investigated the impacts of the hybridisation of the two cochineal biotypes by assessing the damage the agents would have on the target weed, and on *C. imbricata* and *C. fulgida* var. *mamillata*, in the presence of one or both cochineal biotypes.

The ‘cholla’ biotype performed better on *C. pallida* than the ‘imbricata’ biotype, but neither biotype could control *C. pallida* to an extent similar to the control they provide for their respective target weeds, *C. imbricata* and *C. fulgida* var. *mamillata.* Both the ‘cholla’ biotype and hybrids of the two biotypes of cochineal were effective at killing *C. pallida* when both *C. imbricata* and *C. fulgida* var. *mamillata* were also present. This suggests that the ‘cholla’ or hybrids may be effective at controlling *C. pallida* when either *C. imbricata* or *C. fulgida* var. *mamillata* are also present in the field due to the high population density of cochineal that results under these circumstances. There are, however, many *C. pallida* infestations in South Africa where the plant is problematic and is isolated from other *Cylindropuntia* species, and these populations are unlikely to be controlled by the ‘cholla’ biotype or the hybrids.

Neither of the cochineal biotypes that are used for biological control in South Africa are suitably damaging to *C. pallida* to warrant their use as biological control agents for this species. *Dactylopius tomentosus* ‘californica var. parkerii’ is therefore recommended for release based on its host-specificity and impact to *C. pallida* in Australia.

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Chapter 1

Introduction

1.1. Invasive Alien Plants

Included amongst the most severe threats to the conservation of native biodiversity and the functioning of ecosystems worldwide are invasive alien plants (Brooks *et al.* 2004; van Wilgen 2012). Invasive alien plants are plants found outside their indigenous distribution and become over-abundant where they are not wanted (Richardson *et al.* 2000). These plants can negatively affect biodiversity native to the areas of their invasion either directly or indirectly (Brooks *et al.* 2004). Directly, they affect the native biota by dominating or monopolizing limiting resources such as nutrients, space, water and light (Brooks *et al.* 2004). Indirectly, they affect them by colonizing open areas, advancing erosion, altering soil stability, affecting the accumulation of litter, changing soil chemistry, and promoting or suppressing fire (Vitousek 1990; Richardson *et al.* 2000; Brooks *et al.* 2004).

South Africa is amongst many countries with a long history of alien plant invasion and management programs for controlling these invasions (Richardson & van Wilgen 2004; Moran *et al.* 2013). Species from close to thirty families have been classified as invasive alien plants in South Africa (Moran *et al.* 2005). Included amongst these are woody trees, subtropical shrubs, and vines. (Moran *et al.* 2005). Most of the invasive alien plants that have invaded African regions are from North and South America (Pyšek *et al.* 2017). To combat invasive alien plants and manage their negative impacts, methods including mechanical, chemical and biological control (biocontrol) are used (Wittenberg & Cock 2001). These management interventions are implemented on a national scale in South Africa through the Working for Water (WfW) Programme with the ultimate goal of lessening their impacts (Marais *et al.* 2004; Richardson & van Wilgen 2004; Moran *et al.* 2013). Biocontrol is used as a long-term solution by the WfW Programme and is considered one of the most cost-effective ways of managing invasive alien plants (McQueen *et al.* 2001).

1.1.1. Biological control of invasive alien plants

The success of alien plants outside of their indigenous distributions can be explained by the Enemy Release Hypothesis. This hypothesis states that the alien plants, having been displaced from their indigenous region, experience a release or escape from their natural enemies (Keane

& Crawley 2002). This leads to their populations increasing and rapidly spreading in non-native areas to the detriment of native plant species which are often outcompeted (Maron & Vilà 2001; van Wilgen *et al.* 2004).

Biocontrol of invasive alien plants is a management tool that uses the target plant's natural enemies to reduce its invasiveness where it is alien (McFadyen 1998; Hoffmann *et al.* 2019). It therefore rectifies the imbalance caused by enemy release and reduces the competitive advantage that alien plants have in their novel ecosystems. The natural enemies of plants that regulate plant populations in the indigenous distribution are usually herbivorous insects, pathogens and mites (McFadyen 1998). Biocontrol of weeds has been utilised in 130 countries worldwide (Winston *et al.* 2014). It is considered to have contributed in a significant way to the management and protection of native biodiversity worldwide (Barton *et al.* 2007; Winston *et al.* 2014). Compared to the other control methods used against invasive alien plants, biocontrol has arguably been the most successful and beneficial in the longterm (McQueen *et al.* 2001). It is considered the most sustainable control methods because the released agents become part of the ecosystem and follow-ups are rarely needed (McFadyen 1998). Biocontrol is the only permanent control option besides complete eradication of a weed, which is usually impossible (McFadyen 1998). It is also environmentally friendly, cost-effective and safe (McFadyen 1998; Moran *et al.* 2013).

The primary concern using biocontrol is the safety of agents in terms of non-target impacts on plants that the agent is not intended to damage (Hinz *et al.* 2020). In many countries and regions, but particularly in Europe and the U.S.A., a fear of non-target impacts has resulted in limitations in the support and utilisation of biocontrol of weeds (Moran *et al.* 2013; Barrett *et al.* 2018). However, less than 1% of the over 400 biocontrol agents released globally have had significant non-target impacts, and all of these were predicted in pre-release studies (Paynter *et al.* 2004; Suckling & Sforza 2014; Moran & Hoffmann 2015; Hinz *et al.* 2019). Three agents are often cited as examples of weed biocontrol gone wrong. These are *Rhinocyllus conicus* Frölich (Curculionidae), *Larinus carlinae* Olivier (= *L. planus* Fabricius) (Curculionidae) and *Cactoblastis cactorum* Berg. (Pyralidae) (Hinz *et al.* 2019). All three were released when research into biological control agents did not seriously consider the impact of agents on native non-target species (Hinz *et al.* 2020). The host range of the three agents, and therefore the damage that has occurred on non-target species, was predicted prior to release, and, at the time of release, the damage from the invasive alien plants targeted for control was considered much more of a risk than that posed by the damage to non-target plants (Downey & Paterson 2016). The methods used by biocontrol practitioners can accurately predict which plants will be utilised by biocontrol agents if they are released, which is primarily the reason for the excellent safety record of biocontrol globally (Paynter *et al.* 2020). Biocontrol is therefore an environmentally safe method when modern standards and practices are adhered to.

The key to the safety of biocontrol is the host-specificity of the biocontrol agents. If a herbivore is host-specific, it can feed and complete its life cycle on only one or a few closely related species of host plants (Zimmermann *et al.* 2004). The level of specificity that is required before a herbivore can be released as a biocontrol agent depends on the composition of indigenous and valued flora where it is intended to be released, such as the number of close relatives of the target weed that are indigenous or crop species (van Klinken & Edwards 2002; Zimmermann *et al.* 2004). Internationally recognised and standardised host-specificity testing procedures are used to determine whether agents are suitably host-specific.

Wapshere (1974) proposed the centrifugal phylogenetic method for selecting host-specificity test plants. Using this method, plants selected for testing are closely related to the target plant; these include plants within the same genus, family and closely related families (Wapshere 1974; Wheeler *et al.* 2017). Closely related species are tested, followed by more and more distantly related test plant species, until the host range of the natural enemy in question is circumscribed. The physical and chemical properties that make a given host plant an acceptable host to the natural enemy are more likely to be similar in closely related plant species than in more distantly related plants. Therefore, the more distantly related a plant is to the primary host plant, the less likely it is to be a suitable host. More closely related species are therefore tested, with fewer and fewer representatives of more distantly related test plant taxa.

Briese *et al.* (2002) and Briese (2003) proposed a greater inclusion of phylogenies in host plant selection. Both the genetic relatedness and taxonomic relatedness of test plant species are considered in test plant selection. Biogeographic overlap of the related plants and their ecological similarity were also suggested as factors for consideration in selecting of plants to be considered in host-specificity tests (Briese 2003).

Calls have continued to include more factors into host-specificity testing to better predict the risk of non-target attack (Paynter *et al.* 2020). The similarity of plant secondary metabolites amongst potential host plants is one of the factors which has recently been suggested for inclusion in risk assessment methods (Barrett *et al.* 2021; Musengi *et al.* 2021). These are argued to sometimes be more accurate in explaining the agent's potential to select a host plant than the phylogenetic relationships shared by those plants (Kergoat *et al.* 2005; Rapo *et al.* 2019). The cues of plant volatiles, which are metabolites released by plants into the air, can be similar between some plants, and can be included in host-specificity testing, especially when agents which are adapted to use these for selecting their hosts are being tested (Baldwin 2010; Wheeler 2014; Wheeler *et al.* 2021). Host-specificity testing has been successfully used to accurately predict the host ranges of agents and avoid non-target impacts. Modern improvements have increased the accuracy of host-specificity testing, as can be seen by the decreasing number of non-target effects that have been recorded in the field in recent years (Hinz *et al.* 2020).

Promising candidate agents are selected from the suite of natural enemies associated with the target weed in the indigenous distribution and imported into quarantine in the country of intended introduction for host-specificity testing (Paterson *et al.* 2014). The protocol for host-specificity testing under quarantine conditions, includes choice and no-choice tests, which help researchers determine the fundamental and realised host range of the candidate agents (van Klinken 2000). The fundamental host range of an agent is all the plants that the agent can use as a host under any circumstances, whilst the realised host range includes only the plants that will be used by the agent in a particular environment, such as in the field after release (van Klinken & Edwards 2002). No-choice tests are when an agent is placed in an environment with no other alternative but to feed and oviposit on the plant selected for testing (van Klinken 2000). This test helps determine the fundamental host range of an agent, which is the host range the agent displays in the laboratory (van Klinken 2000; van Klinken & Heard 2000; van Klinken & Edwards 2002). Choice tests are when the agent has several plants to choose from, and its behaviour in that situation is observed (Hopper 2001). These can be divided into paired-choice and multi-choice tests, where either a single test plant and the target weed, or multiple test plants and the target weed, are exposed to the potential agent (Mphephu & Mukwevho 2019). Choice tests determine the realised host range of an agent, which is the host range the agent is expected to display in the field (Kuhlmann *et al.* 2000; van Klinken 2000; van Klinken & Edwards 2002). Parameters measured in host-specificity tests include fecundity, feeding damage and/or feeding damage structure abundance (e.g. galls), and the survival and duration of development of the potential agent (Volchansky *et al.* 1999; Post *et al.* 2010). These parameters are measured on non-target test plant species and the target weed and compared.

These rigorous steps are necessary to ensure that released biocontrol agents are safe and will not result in negative non-target impacts after release (van Klinken 2000). Host-specificity testing is therefore the fundamental step in weed biocontrol that ensures safety and the excellent safety record of weed biocontrol is a testament to the success of these protocols (Hinz *et al.* 2020; Paynter *et al.* 2020).

Biocontrol agents must not only be host-specific; they must also be sufficiently damaging to control the target weed succesfully. Globally, biocontrol of weeds has a high success rate in the impact agents have on their target weeds. A study that assessed the success of 468 biocontrol agents released to control 175 invasive species in 90 countries worldwide determined the overall levels of successes of weed biocontrol globally (Schwarzländer *et al.* 2018). This study showed that 70.9% of the intentionally released agents became established on their target weeds. The impact of the established agents was placed into the following categories: 'none', 'slight', 'medium', 'heavy', 'variable', 'too early post-release', 'unknown', and 'compromised' (Schwarzländer *et al.* 2018). Medium, or variable to heavy damage was observed for 53.5% of the releases (Schwarzländer *et al.* 2018; Hinz *et al.* 2020). Only 7.0% had no impact whatsoever (Schwarzländer *et al.* 2018). The highest proportion of heavy impact agents was seen for agents released and established in biocontrol programs conducted in Africa (Schwarzländer *et al.* 2018).

Pre-release impact assessments must be done to determine the impact of a potential biocontrol agent. These can be carried out in several ways which include field studies in the native range of the invasive plant and quarantine based studies (Balciunas 2004; Goolsby *et al.* 2004). Plant and insect parameters are measured to determine the impact of the potential agent on the plant, allowing for the most damaging potential agent, or combination of potential agents, to be selected for release (Balciunas 2004; McClay & Balcianus 2005). This ensures that the most damaging biocontrol agents are released and guards against the release of ineffective agents, which should be avoided.

South Africa is one of the top five countries worldwide concerning the biocontrol of invasive alien plant research (Cock *et al.* 2010; Moran & Hoffmann 2015). Sixty-six invasive alien plants have biocontrol agents established on them (Zachariades *et al.* 2018). Fifty-four of these invasive alien plant species targeted by biocontrol from 14 families were recently assessed by Moran *et al.* (2021). The extent of biocontrol success was determined using the parameters of weed density, area, biomass, and rate of spread (Moran *et al.* 2021). A majority of the results for the control of these species show favourable outcomes, many have been reduced and are being kept below tolerable thresholds (Moran *et al.* 2021). It is reported that, for at least one of the measured parameters, the invasion levels of 39 of the 54 plants have been reduced to levels below the level that existed before biocontrol was undertaken (referred to as the reversal threshold), and 15 of these 39 plants have been reduced to levels where they are no longer problematic. Thus they are under complete control and are maintained at levels below the tolerable threshold (Zachariades 2018; Moran *et al.* 2021).

In some cases, successful biocontrol programs have led to fiscal benefits outweighing the cost of implementing the biocontrol programme and lasting decades after the first success of the program was noticed (Gurr *et al.* 2000). The biocontrol program for *Xanthium occidentale* Bertol. (Asteraceae) in Australia, by *Podosphaera xanthii* (Castagne) U. Braun & Shishkoff (Erysiphaceae) and *Epiblema strenuana* Walker (Tortricidae) led to significant financial benefits (Chippendale 1995; McFadyen 1998). The successful control of *X. occidentale* resulted in an annual benefit of $720,000 in 1991 (McFadyen 1998). The control of *Salvinia molesta* D.S. Mitchell (Salvineaceae) in Sri Lanka by the *Cyrtobagous salviniae* Calder & Sands (Curculionidae) weevil resulted in a benefit-cost ratio of 1675 (Doeleman 1989; McFadyen 1998). In South Africa, financial benefits in the control of *Sesbania punicea* (Cav.) Benth. (Fabaceae) and *Opuntia aurantiaca* Lindley (Cactaceae) have also been realised; the benefit to cost ratios for these were 8:1 and 709:1, respectively (van Wilgen *et al.* 2004; Zachariades *et al.* 2017). These successes are the reason why many pervasive invasive plants are, at an increasing rate, are becoming targets for biocontrol.

1.1.2. Biocontrol of Cactaceae

Plants from the Cactaceae family are endemic to the Americas (Anderson 2001). Many Cactaceae which have been introduced into areas where they are not native, have become invasive (Novoa *et al.* 2015). These plants are detrimental to people, native flora and fauna (Shackleton *et al.* 2017). They inhibit wildlife and livestock movement, diminish the value of grazing land and have and negatively impact livestock health and ecological processes (Taylor & Whitson 1999; Shackleton *et al.* 2017) mainly because of their barbed spines and because they become over-abundant. Cactus species such as *Pereskia aculeata* Miller (Cactaceae) can grow over and kill indigenous plants and even cause large trees to collapse under the weight of the plant and die (Moran & Zimmermann 1991a; Paterson *et al.* 2011a, b). Many cacti species, including those of the *Opuntia* genus, invade arid and semi-arid areas of the world (Shackleton *et al.* 2017). There are invasive alien cacti in India and Sri Lanka in Asia, Yemen and Saudi Arabia in the Middle East, France, Italy and Spain in Europe, many countries in Africa, and Australia (Shackleton *et al.* 2017). Fifty-seven cactus species have been identified as invasive globally (Novoa *et al.* 2015). Species within the two closely related genera, *Opuntia* and *Cylindropuntia,* are amongst the most widely introduced, cultivated and invasive alien cacti (Novoa *et al.* 2015). The most widespread invasive species are *Opuntia ficus-indica* (L.) Miller (Cactaceae) followed by *Opuntia stricta* (Haworth) Haworth (Cactaceae) and *Opuntia monacantha* Haworth (Cactaceae) (Novoa *et al.* 2015). Australia and South Africa have the most invasive cactus species, with 39 and 35 species, respectively (Novoa *et al.* 2015).

Biocontrol is widely regarded as the most effective method of controlling invasive alien cacti (Paterson *et al.* 2011b). The most successful cactus biocontrol agents for *Opuntia* and *Cylindropuntia*, the most widespread and problematic of the invasive cacti, are cochineal insects (*Dactylopius* spp. Hemiptera: Dactylopiidae) (Mathenge *et al.* 2009a; Paterson *et al.* 2011b; Paterson *et al.* 2019). These insects are highly specialized to particular cactus host plants and cannot survive on other plants besides their host plant species and a few closely related species within the same genus (Moran 1980; Mathenge *et al.* 2009a). For example, the family Dactylopiidae, which includes the cochineals, is restricted to feeding on members of the Opuntioideae subfamily of Cactaceae, and the cochineal species *Dactylopius opuntiae* Cockerell (Dactylopiidae) is restricted to the genus *Opuntia*, while the cochineal species *Dactylopius tomentosus* Lamarck (Dactylopiidae) is restricted to the genus *Cylindropuntia* (Moran 1980; Paterson *et al.* 2019).

Biocontrol in South Africa began in 1913 with the intentional release of a cochineal insect, *Dactylopius ceylonicus* Green (Dactylopiidae), to control the cactus weed, *O. monacantha* (Moran *et al.* 2013). This biocontrol program was successful and led to a dramatic and permanent reduction in the density of the target weed (Paterson *et al.* 2011b; Paterson *et al.* 2019; Hill *et al.* 2020). Biocontrol of invasive alien cacti was also extensively conducted in the 1930s and 1940s with biocontrol agents released against *O. ficus-indica, O. aurantiaca,* and *Opuntia engelmanii* Salm-Dyck ex Engelmann (Cactaceae) (Pettey 1948; Annecke & Moran 1978; Moran & Annecke 1979; Moran *et al.* 2013). Significant reductions in the density of *O. aurantiaca* were recorded after a cochineal insect, *Dactylopius austrinus* De Lotto (Dactylopiidae)*,* was released in 1935 in the Eastern Cape Province and Karoo semi-desert region of South Africa (Moran & Zimmermann 1991b; Zimmermann *et al.* 2004). This history shows us the pivotal role played by cochineal insect species in the biocontrol of cactus species in South Africa.

Research on the biocontrol of invasive alien cacti, and releases of agents on these cacti, continued into the 1950s with the release of *D. tomentosus* on *Cylindropuntia imbricata* (Haworth) F.M. Knuth (Cactaceae) and *Cylindropuntia leptocaulis* (DC.) F.M. Knuth (Cactaceae) achieved substantial and complete control, respectively (Zimmermann *et al.* 2004; Walters *et al.* 2011; Moran *et al.* 2013). The 1980s saw a release of two biocontrol agents on *O. stricta,* which established and achieved complete control of this species (Zimmermann *et al.* 2004). This momentum grew to the extent that there were ten new agents released on cactus targets between 2000 and 2003 (Zimmermann *et al.* 2004). Of the 20 invasive alien cacti reported on by Moran *et al.* (2021), nine are considered under complete control with reductions in density, biomass, area, and rate of spread to below tolerable thresholds. Therefore, compared to other invasive alien plants, cactus biocontrol is more frequently successful.

While many cacti have been brought under good levels of control, there are constantly new cacti being discovered in South Africa due to the commercial cactus trade (Novoa *et al.* 2015), and many of these do not yet have effective biocontrol agents. In many cases, new cochineal biocontrol agents are likely to be the most effective way of controlling these pest plants.

1.1.3. Cochineal insects as biocontrol agents

The Coccoidea, commonly called “scale insects”, “mealybugs”, and “cochineal insects”, are made up of a specialised group of phytophagous insects which measure a few millimetres in length and display distinct sexual dimorphism and a high degree of specialization to a parasitic lifestyle on plants (Claps & de Haro 2001; Gullan & Martin 2003). Some species are polyphagous and utilise plants from several families as their hosts, others are oligophagous and have a more restricted number of hosts, and others are monophagous, with a high degree of host plant specialisation to just a single plant species (Claps & de Haro 2001). They display sexual dimorphism in that the females reach sexual maturity while retaining some juvenile characteristics and remain apterous for their entire life cycle (Claps & de Haro 2001). The females have a globose, elongated or pyriform body with a cephalothorax that can be distinguished from the abdomen, they have short legs and are mostly sedentary as adults (Claps & de Haro 2001). The males are usually smaller than the females and after pupation, emerge as winged adults with non-functional mouthparts (Claps & de Haro 2001). The juvenile stages of both sexes feed on plant saps by inserting their long stylets into the host plant (Claps & de Haro 2001).

Coccoidea are economically important because some of them are agricultural pests, especially those within the Diaspididae and Pseudococcidae families, others are important because of their uses as natural dye and resin producers and as biocontrol agents, especially those within the Dactylopiidae family (Claps & de Haro 2001; Miller *et al.* 2005). Some examples of economically important pest Coccoids are *Pseudococcus* sp. (Pseudococcidae) which are pests of fruit crops in areas with warm climates; *Miscanthicoccus miscanthi* Takahashi (Pseudococcidae), a pest of miscanthus grass; *Diaspidiotus perniciosus* Comstock (Diaspididae), a pest of stone fruit crops; *Aulacaspis tubercularis* Newstead (Diaspididae), a pest of mango and *Paracoccus marginatus* Williams and Granara de Willink (Pseudococcidae) a pest of papaya and hibiscus, among many others (Baranyovits 1978; Miller *et al.* 2005).

The Dactylopiidae family shows high host specialization to cacti, which makes them useful biocontrol agents of invasive alien cacti around the globe (Moran 1980; Moran & Zimmermann 1984; Julien & Griffiths 1998; Claps & de Haro 2001). This family has ten species belonging to the *Dactylopius* genus (van Dam & May 2012; Campana *et al.* 2015). All these species are native to North and South America and specialize in feeding on Cactaceae (De Lotto 1974; Mathenge *et al.* 2009b). Five South American species have been described, these are *D. austrinus*, *D. ceylonicus*, *Dactylopius confertus* De Lotto, *Dactylopius salmianus* De Lotto, and *Dactylopius zimmermanni* De Lotto (Claps & de Haro 2001). The origin of another species, *Dactylopius coccus* Costa is debated, with some researchers saying it originated in Peru and spread to Mexico, while others argue the opposite (Campana *et al.* 2015). This species is domesticated and has been used as a source of natural dyes for U.S.A. thousands of years, making its origin difficult to determine and controversial (Claps & de Haro 2001; Rodríguez *et al.* 2001). This dye was used for cosmetics, medicine and as a textile dye and was very valuable prior to produce synthetic dyes in the 1800s (Baranyovits, 1978; Claps & de Haro 2001). *Dactylopius confusus* Cockerell, *D. opuntiae* and *D. tomentosus* are North American species, native to the southern U.S.A. and Mexico (Chávez-Moreno *et al.* 2011). Another North American species, the recently discovered *Dactylopius gracilipilus* van Dam & May, is only known from a single location and host plant, *Corynopuntia* Knuth (Cactaceae), in Texas, U.S.A. (van Dam & May 2012).

Dactylopius are sessile and form female colonies which feed on cacti cladodes (modified stem segments that function as leaves) (Claps & de Haro 2001). They can, however, feed on the other parts of the plants, such as roots, flowers and fruits (Claps & de Haro 2001). They generally measure between 3 to 5 mm long and have purple bodies covered by a white cotton-like secretion (Claps & de Haro 2001). *Dactylopius* species such as *D. austrinus*, *D. ceylonicus*, *D. coccus*, and *D. opuntiae*, have been studied and exhibited similar life-history strategies and general morphologies (Gunn 1978; Gilreath & Smith 1987; Sullivan 1990; Guerra & Kosztarab 1992; Flores-Hernández *et al.* 2006). These species lay eggs singly or in chains and hatch within a day into motile, first-instar crawlers (Gunn 1979; Mathenge *et al.* 2009a). Sexual dimorphism becomes apparent in the late phases of the first-instar onwards (Mathenge *et al.* 2009a). The crawlers are adapted behaviourally and morphologically for dispersal; female crawlers have stiff, elongate bristles used for wind dispersal while the males disperse by flying short distances using their wings when they are adults (Moran & Cobby 1979; Moran *et al.* 1982; Washburn & Washburn 1984).

Dactylopius tomentosus lays eggs singly in a ball-shaped mesh. These are attached to the female's posterior end of the abdomen by waxy threads. The eggs average 17 days before hatching into first instar crawlers (Mathenge *et al.* 2009a). Newly hatched crawlers are bright red and are adapted behaviourally and morphologically for dispersal, they become mobile a few minutes to hours after hatching and begin looking for suitable sites where they can settle and start feeding (Mathenge *et al.* 2009a). Many tend to settle at the base of spines away from light (Mathenge *et al.* 2009a). The average time from hatching to the first moult is 18 days (Mathenge *et al.* 2009a). Second-instar female crawlers secrete wax which initially looks like white dust on their bodies and gradually becomes white coils (Mathenge *et al.* 2009a). Gravid females are bigger than non-gravid ones (Mathenge *et al.* 2009a). Once they have settled, the wax increases in quantity until it covers the female, the second moult happens under the wax and the white exuvia is displaced to the edge of the waxy covering (Mathenge *et al.* 2009a). A fertilized female develops a sub-globular body shape, while a non-fertilized one remains the same body size and shape (Mathenge *et al.* 2009a). The average time from egg to oviposition of a female is 63.3 days (Mathenge *et al.* 2009a).

Second-instar male crawlers do not become sedentary like their female counterparts, they move about from one feeding position to another until they are ready to pupate then settle at their chosen pupation site (Mathenge *et al.* 2009a). Similar to the females, the incubation of a male egg is about 17 days, after that the first instar period is about 18 days (Mathenge *et al.* 2009a).

On average, males take 21.7 days from the first moult to pupation (Mathenge *et al.* 2009a). The pupal period lasts for about 13 days and when the males emerge, they have white wings, which they use for dispersing by flying short distances. The average life span of a male from egg to death is 57.5 days, but they only live for 5.1 days as adults (Mathenge *et al.* 2009a).

Dactylopius tomentosus is native in Mexico and parts of North America, such as Texas, Arizona, and New Mexico in the U.S.A. (Rodríguez *et al.* 2001). This species is highly specialized and associated with only *Cylindropuntia* species, a group of cacti that are primarily restricted to the same area (Zimmermann & Granata 2002). It has been introduced into Australia and South Africa as a biocontrol agent to control various invasive alien *Cylindropuntia* species (Dodd 1940; Mann 1969; De Lotto 1974; Moran & Zimmermann 1991a, Paterson *et al.* 2019).

1.1.4. Selecting the most damaging cochineal biotype

Many cactus species in South Africa are considered under complete control, mainly due to biocontrol using cochineals and the galling mealy-bug *Hypogeococcus* sp., which is used for the control of columnar cactus species in the subtribe Cereanae (Klein 2011). It is characteristic of cochineal species to feed and develop on a small group of closely related cactus species within the Opuntioidea (Moran 1980), and in some cases, intraspecific lineages of cochineal insect species, called biotypes, are adapted to certain host plant species better than others (Volchansky *et al.* 1999, Mathenge *et al.* 2009b, Jones *et al.* 2015).

A biotype is a clade that cannot be differentiated morphologically from others but has a different host range and produces a different impact depending on the host plant species (Diehl & Bush 1984; Drès & Mallet 2002; Mathenge *et al.* 2010a). Biotypes have been observed to exist within *D. opuntiae,* which has sub-specific lineages, each with a restricted host range and host-adapted to a single *Opuntia* species (Githure *et al.* 1999; Volchansky *et al.* 1999; Mathenge *et al*. 2009b). *Dactylopius tomentosus* also has different biotypes (Mathenge *et al.* 2010a; Jones *et al.* 2015). The biotypes of *D. tomentosus* are also each highly adapted to different *Cylindropuntia* species (Zimmermann & Granata 2002; Mathenge *et al.* 2009b, Mathenge *et al.* 2010b). In South Africa, two *D. tomentosus* biotypes, namely, *D. tomentosus* ‘cholla’ and *D. tomentosus* ‘imbricata’ are used as biocontrol agents for *Cylindropuntia fulgida* var. *fulgida* Engelmann (Cactaceae), *Cylindropuntia fulgida* (Engelmann) F.M. Knuth var. *mamillata* (Schott ex Engelmann) Backeb. (Cactaceae), and *C. imbricata*, respectively (Mathenge *et al*. 2009b; Jones *et al.* 2015). *Cylindropuntia fulgida* var. *fulgida* and *C. fulgida* var. *mamillata* are varieties of the same species, but are morphologically very different (Pinkava 1999). The 'cholla' biotype effectively controls both *C. fulgida* varieties, and it has less impact on *C. imbricata*, while the 'imbricata' biotype effectively controls *C. imbricata* and not *C. fulgida* (Paterson *et al.* 2011b). These species are close relatives of *Cylindropuntia pallida* (Rose) F.M. Knuth (Cactaceae) and are currently under successful biocontrol by cochineal biotypes in South Africa (Moran and Zimmermann 1991b; Klein *et al.* 2020).

Unlike these species, *C. pallida* has no biocontrol agent released in South Africa. In some cases, the same cochineal species and biotype can be used to control multiple target cactus weeds, in contrast others, a particular biotype is needed for one target invasive alien plant species and it is ineffective against other species (van Steenderen *et al.* 2021). For example, *Opuntia humifusa* (Raf.) Raf. and *O. stricta* are controlled by the cochineal biotype *Dactylopius opuntiae* 'stricta'; and *C. leptocaulis* and *C. imbricata* are controlled using *D. tomentosus* 'imbricata' (Walters *et al.* 2011; Rule & Hoffmann 2018). Contrary to this, some biotypes successful on one cactus species cannot be used to control another species and their level of impact on the non-host cacti is limited (Mathenge *et al.* 2009b). This has been observed for the biotypes of *D. opuntiae;* the 'stricta' biotype can develop, albeit unsatisfactorily, on *O. ficus-indica*, while it thrives on its host plant *O. stricta* (Githure *et al.* 1999; Volchansky *et al.* 1999). On the other hand, the 'ficus' biotype thrives on *O. ficus-indica*, while it can only survive poorly on *O. stricta* and it could not be used to control *O. stricta* in the Kruger National Park (Moran & Zimmermann 1991a; Githure *et al.* 1999; Hoffmann *et al.* 1999; Volchansky *et al.* 1999; Paterson *et al.* 2021). There was also an unsuccessful attempt to control *C. fulgida* using the 'imbricata' biotype in South Africa (Moran & Zimmermann 1984; Moran & Zimmermann 1991a). After this attempt with the incorrect biotype, the 'cholla' biotype was released on *C. fulgida* var. *fulgida* in 2008 (Paterson *et al.* 2011b; Paterson *et al.* 2019; Klein *et al.* 2020). This biotype led to the cactus being reduced to very low numbers in a short time and providing complete control (Paterson *et al.* 2011b; Klein *et al.* 2020). In 2011 the 'cholla' biotype was released against *C. fulgida* var. *mamillata* and was also very successful (Klein *et al.* 2020).

In recent years, several new biotypes of *D. tomentosus* have been imported into quarantine in Australia for studies to determine their potential as biocontrol agents for various *Cylindropuntia* species (Jones *et al.* 2015). Each of the biotypes is named after the host plant species of collected in the indigenous distribution in Mexico or the southern U.S.A. One of the new biotypes, the 'californica var. parkerii' biotype, was collected from *Cylindropuntia* *californica* var. *parkerii* in Baja California, Mexico (Paterson *et al.* 2021). It was imported into quarantine in Australia for host-specificity and efficacy tests and it was found to be the most damaging biotype to *C. pallida* in Australia and therefore deemed suitable for release in that country (Paterson *et al.* 2021).

Since biotypes cannot be distinguished from one another using morphological traits, molecular techniques are used for identification (Mathenge *et al.* 2015; Jones *et al.* 2016; van Steenderen *et al*. 2021). It is essential to ensure that the correct biotype is used to control the correct target weed species so that the highest level of control is achieved. Only the most effective biotype should be released for the control of any given target weed, and biotypes that are not suitably damaging should not be released. Even if an agent is safe for release, it should only be released once proven to be suitably damaging to avoid the release of ineffective agents. This is because there is, despite of all the precautions, an unavoidable inherent risk associated with the release of alien species into new environments (Louda 2000; Sheppard *et al.* 2003; McClay & Balciunas 2005; Paterson 2010). For this reason, in environments where there are already agents that could be biocontrol agents for other emerging invasive species, such as cochineal insects that may feed on several different cactus species, it is advisable to test those on the new invasive species before finding and releasing novel agents.

This thesis assessed the efficacy of both *D. tomentosus* biotypes, currently in use against *C. imbricata* and *C. fulgida* in South Africa, on *C. pallida* in an endeavour to find a suitably damaging biocontrol agent for this cactus in southern Africa. Suppose either of the cochineal biotypes already used for biocontrol in South Africa are suitably damaging to *C. pallida*. In that case no new agent is required, but if they are not, then the agent used in Australia, *D. tomentosus* ‘californica var. parkerii’ should be considered.

1.1.5. Hybridisation of cochineal biotypes

Sexually compatible biocontrol agents, especially those closely related and occurring in close spatial proximity to one another, may interbreed and thus hybridise. Understanding the hybridisation of biocontrol agents is important, since there can be several outcomes for the fitness of the host-adapted agents, which means there will be an impact on their performance (Mathenge *et al.* 2010b). Hybridisation has been observed for agents such as the *Diorhabda* beetle species, which are used for the biocontrol of the invasive alien *Tamarix* species in North America. Hybridisation between the *Diorhabda* species led to increased egg viability in the F_1

generation, but this decreased as backcrossing in the F_2 and subsequent generations occurred (Bean *et al.* 2013).

Once agents hybridise, there could be an outbreeding enhancement or outbreeding depression (Lynch 1991; Burton *et al.* 1999). Outbreeding is hybridisation with members of a distant population and often leads to F_1 hybrids gaining increased fitness due to gene dominance which masks harmful alleles, called outbreeding enhancement (Lynch 1991; Mathenge *et al.* 2010b). Outbreeding depression occurs when the offspring experience reduced performance in fitness-related traits (Blows 1993; Stouthamer *et al.* 1996; Edmands 2002; Gilk *et al*. 2004; Mathenge *et al.* 2010b). While outbreeding enhancement may benefit biocontrol, outbreeding depression may have negative impacts on biocontrol. The various cochineal biotypes used for biocontrol interbreed freely with other biotypes of the same species, so hybridisation is inevitable if they occur in sympatry. Therefore, the potential impacts of hybridisation on the level of control provided by cochineal biotypes are important to understand.

For cochineal insects, hybridisation change the host-specificity of the biotypes. The host-specificity of hybrid offspring is dependent on whether the parents are highly or maladapted to their host plants (Edmands 2002; Spitzer 2006; Mathenge *et al.* 2010b). As a result, crossbreeding may lead to hybrid offspring that interact differently with the parents' host plants (Mathenge *et al.* 2010b). Due to the dominance, of certain genes or maternal effects some hybrids may have higher host-specificity to one or the other parental host (Craig *et al.* 2001; Dambroski *et al.* 2005). With no gene dominance the hybrids can have host-specificity equal to that of their parents (Fry 1999). Alternatively, hybrid offspring may have host-specificity intermediate to both parents because of additive effects (Nitao *et al.* 1991; Caroll *et al.* 2001). In other cases, there may be a loss of host-specificity in the hybrids (Hoffmann *et al.* 2002).

Many cacti species are similar in physiology, pathways of spread and preferred habitats (Kaplan *et al.* 2017) therefore, they can occur in sympatry. The occurrence of *O. ficus-indica* and *O. stricta* in close proximity and their biocontrol agent biotypes, the 'ficus' and 'stricta' biotypes of *D. opuntiae,* led to their interbreeding in the field in South Africa (Hoffmann *et al.* 2002; Hoffmann 2004). The 'ficus' biotype, which is used to control *O. ficus-indica*, when bred with the 'stricta' biotype used for controlling *O. stricta*, produces hybrid F_1 progeny, which are less host-specific in that they develop well on both host plants (Hoffmann 2004). Since cochineal is wind-borne and mostly depend on passive spread through the dispersal of the immature crawler stage, the chances of the hybrid offspring surviving are safeguarded in areas infested by either cactus species (Moran *et al.* 1982; Hoffmann 2004). These hybrid nymphs could benefit biocontrol because they would ensure higher population densities and less loss of crawlers during passive dispersal (Hoffmann 2004). These benefits may be nullified by purebreds produced as offspring of the F_2 and subsequent generations since they still retain the pure strain parents' host-specificity (Hoffmann 2004). This would especially be the case if these would be produced in areas where their preferred hosts are unavailable (Hoffmann 2004). There is also an expectation that in subsequent generations of crossbreeding between these biotypes, the dominant one in a site would be the one that is host-specific to the dominant host plant at that site (Hoffmann *et al.* 2002; Paterson *et al.* 2011b; Paterson *et al.* 2021). If at a site where both *O. ficus-indica* and *O. stricta* are present, the 'ficus' biotype occurs in high numbers on *O. ficus-indica,* then it would be difficult to control *O. stricta* at that site because the subsequent generation offspring would be dominated by the 'ficus' biotype specific to the *O. ficus-indica* host (Paterson *et al.* 2021).

Sympatric association has also been observed for *C. fulgida* var. *fulgida* and *C. imbricata* in some areas of their range (Henderson 2001). It has been observed that the 'cholla' and 'imbricata' biotypes of *D. tomentosus*, respectively used in the biocontrol of these cacti, are reproductively compatible and can hybridise under laboratory conditions, producing viable offspring (Mathenge *et al.* 2010b). Therefore, the sympatric association of their host cacti would mean a high potential for hybridisation between the biotypes, which could impact the biocontrol impact by the biotypes on their hosts. Similar to the *D. opuntiae* hybrids, the F_1 hybrids of the 'cholla' and 'imbricata' biotypes displayed increased fitness, meaning that both *C. fulgida* and *C. imbricata* are suitable for the reproduction, development and survival of the hybrids (Mathenge *et al.* 2010b). This means that the population growth of these would be safeguarded and there would be less loss of crawlers during dispersal in areas where both cacti occur (Moran *et al.* 1982; Hoffmann 2004; Mathenge *et al.* 2010b). Crosses of the F_2 and subsequent generations may lead to some offspring returning to similar levels of host-specificity as the pure strains of the biotype (Mathenge *et al.* 2010b). However, negative impacts on the control of *C. fulgida* and *C. imbricata* are unlikely if that *D. tomentosus* 'cholla' and 'imbricata' purebreds return to their pure strain parents' host-specificity levels. This is because the 'cholla' biotype would impact *C. imbricata* plants and the 'imbricata' purebreds would be able to kill small *C. fulgida* plants, like their parents (Mathenge *et al.* 2010b; Klein *et al.* 2020).

Due to the unpredictability of the results from hybridisation (Mathenge *et al.* 2010b), there is a need to assess the possible implications of hybridisation and make necessary interventions prior to the release of new cochineal biotypes for biocontrol. The outcomes of hybridisation are important to study, because they give us insight into how effective the agents will be in controlling the invasive cacti, which serve as their hosts. It is therefore important that the implications of hybridisation of the two *D. tomentosus* biotypes already used for biocontrol in South Africa on their potential to control the newly emerging weed *C. pallida* is understood before a new biotype is considered.

1.2. *Cylindropuntia pallida* (Rose) F.M. Knuth (Cactaceae)

Cylindropuntia pallida belongs to the Opuntioideae subfamily which has 33 species (Al-Robai *et al.* 2018). The genus to which this species belongs has cylindrical stems and sheathed spines (Fig. 1.1) (Al-Robai *et al.* 2018). Most plants in this species are low growing, some grow to 1.6m in height and 3m wide (Hosking *et al.* 2007; Johnson *et al.* 2009; Al-Robai *et al.* 2018). The cladodes are cylindrical, green-grey and grow up to 90cm long and 4cm wide (Johnson *et al.* 2009). The plant is extremely spiny (Fig.1.2.) and the spines, which grow up to 4.5cm long on outer segments of the plant, have white, papery sheaths that separate from the spine during the first year of development (Johnson *et al.* 2009). The flowers are approximately 5cm wide with pink-purple petals (Johnson *et al.* 2009). The fruit are wide towards the apex, yellow when ripe, about 2 to 4.5cm long, and young fruits have more spines than older ones. Although there are seeds in the fruit, these do not separate from the fruit and are non-viable (Hosking *et al.* 2007; Johnson *et al.* 2009).

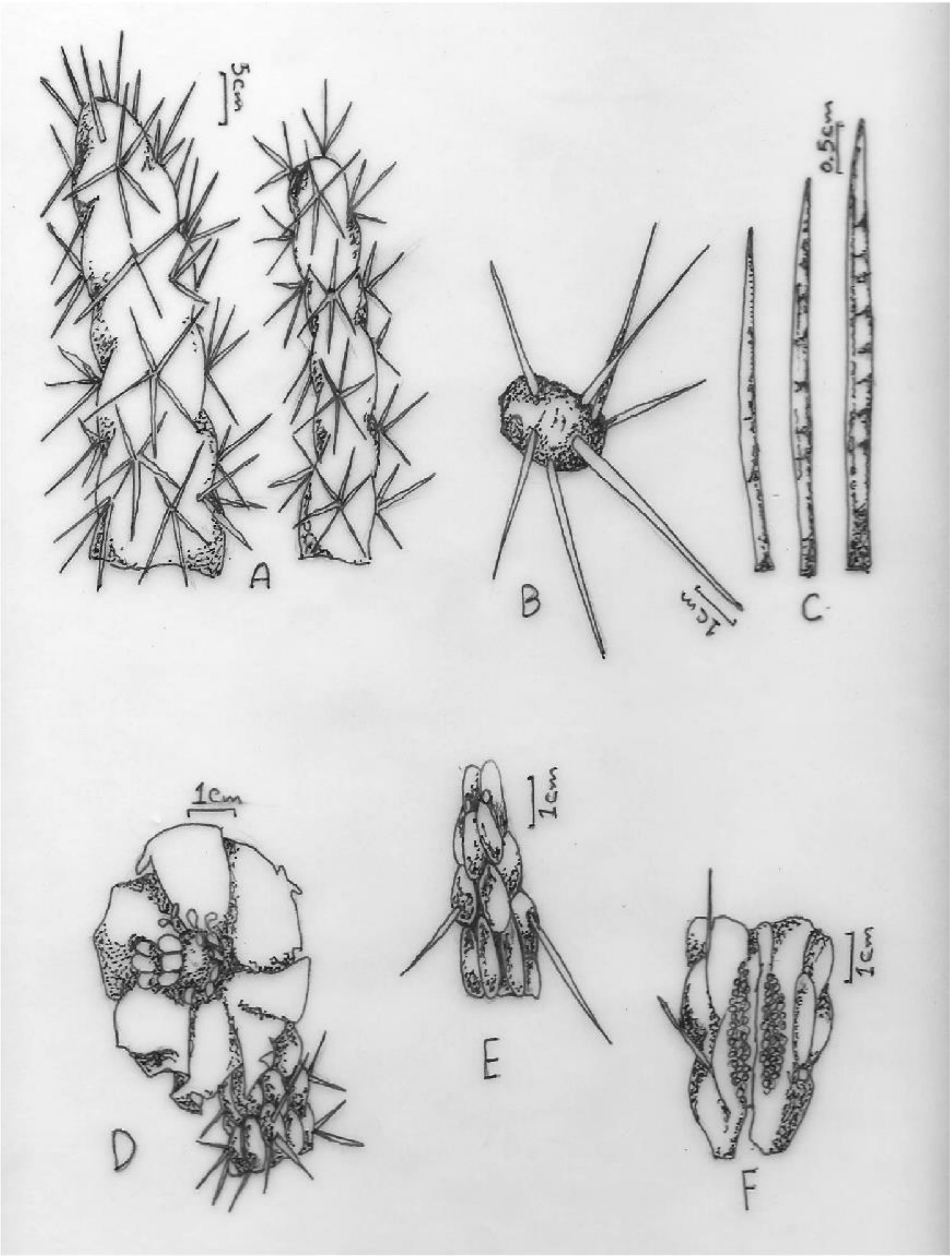

Fig. 1.1. *Cylindropuntia pallida*. A: phylloclade; B: cushion with spines; C: spines; D: flower; E: fruit; F: longitudinal section (L.S.) in the fruit (from Al-Robai *et al.* 2018).

Fig. 1.2. *Cylindropuntia pallida* plant (from The State of Queensland, Department of Agriculture and Fisheries, 2018.)

1.2.1 Etymology

The "*pallida"* species name is derived from the Latin adjective, "*pallidus*" which refers to the pale colour of the flowers (Crook and Mottram 2001). Its synonyms include: *Cactus subquadriflorus* Moc. & Sessé ex DC. *Cylindropuntia rosea* (DC.) Backeb. *Cylindropuntia rosea* var. *atrorosea* Backeb. *Opuntia rosea* DC., *Grusonia rosea* (DC.) G.D. Rowley and *Opuntia pallida* Rose (Al-Robai *et al.* 2018). The species' phylogenetic origins are unclear, but is thought to be a hybrid between *Cylindropuntia tunicata* Grawin and *C. imbricata* (Johnson *et al.* 2009; Laguna *et al.* 2013; Al-Robai *et al.* 2018). The *C. rosea* synonym is often used in Europe (Laguna *et al.* 2013; Al-Robai *et al.* 2018).

1.2.2 Distribution

The *Cylindropuntia* genus is native to the Chihuahuan, Mohave, Sonoran Deserts of the U.S.A and Mexico (Pinkava 1999). The states of Baja California, Baja California Sur and Sonora in Mexico and Arizona in the U.S.A. are rich with many *Cylindropuntia* species (Pinkava 1999). *Cylindropuntia pallida* in its native range can be found in the provinces of Hidalgo in Mexico, Puebla and Tlaxcala in central Mexico (Walters *et al.* 2011). It has invaded countries worldwide such as Spain, Saudi Arabia, Australia, South Africa and Namibia (Jones *et al.*

2016; Sáez *et al.* 2016; Al-Robai *et al.* 2018; Paterson *et al.* 2019). In South Africa, the current distribution is in the interior of the country, with a lot of the plant occurring in the Northern Cape, Western Cape and Eastern Cape provinces (Fig. 1.3). It has also been found in the North West and Free State provinces, but there are less localities in these provinces (Fig. 1.3).

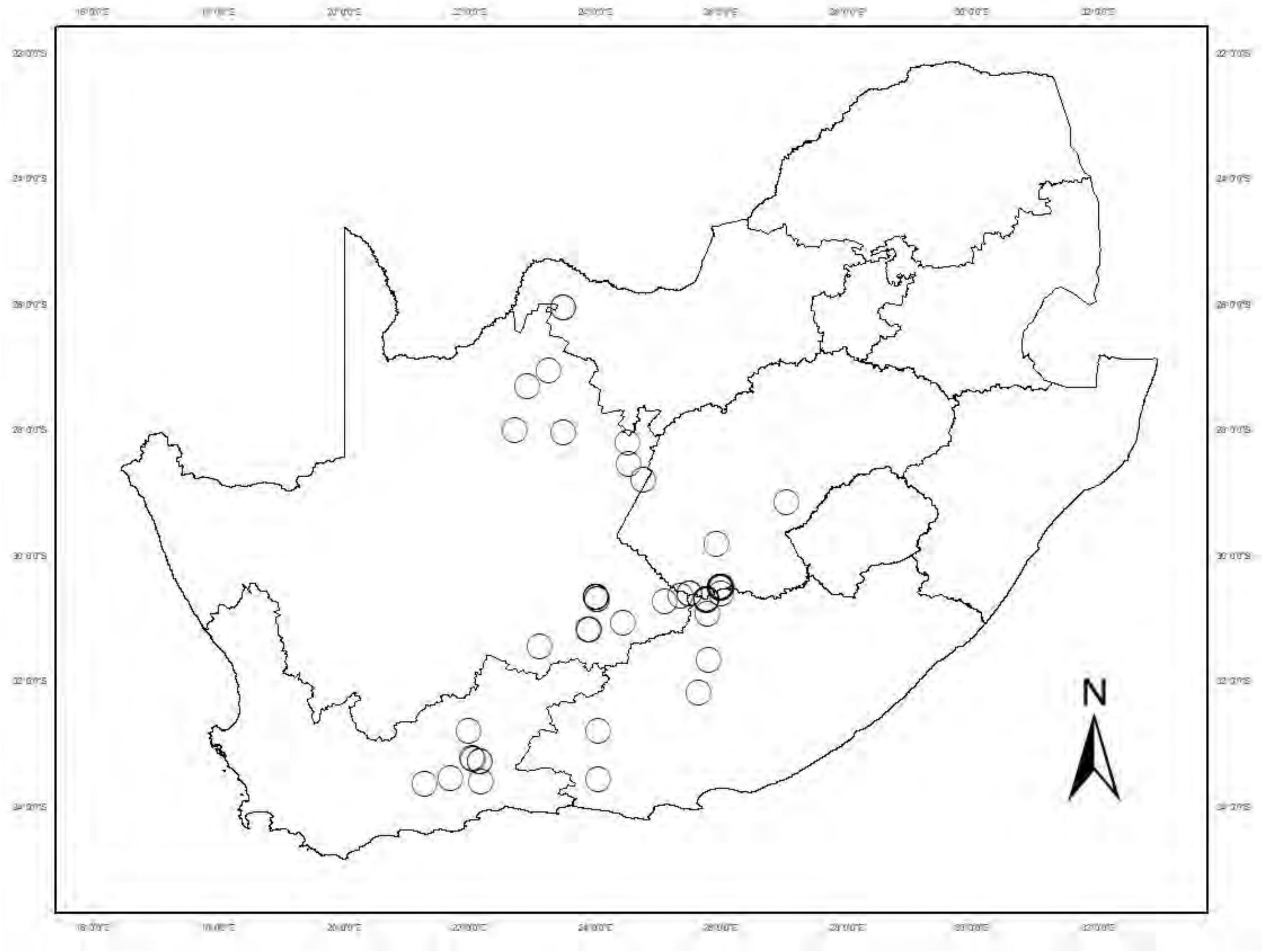

Fig. 1.3. *Cylindropuntia pallida* distribution in South Africa.

1.2.3 Invasion biology of *C. pallida*

Cylindropuntia pallida has been recorded as one of the world's most invasive and problematic plants (Deltoro *et al.* 2013; Al-Robai *et al.* 2018). It can reproduce vegetatively by detached stem segments and is dispersed by humans, animals and vehicle tyres onto which detached stem segments attach (Hosking *et al.* 2007; Deltoro *et al.* 2013). The detached segments take root when they come into contact with the ground (Deltoro *et al.* 2013).

Infested areas are are not safe for grazing animals because of the plant's dangerous long spines (Hosking *et al.* 2007; Al-Robai *et al.* 2018). Small mammals can be impaled on spiny cacti like *C. pallida* (Deltoro *et al.* 2013). Infested areas restrict animal and human movement (Hosking *et al.* 2007; Al-Robai *et al.* 2018). Native, endangered and rare plants have been placed at higher risk due to competition for space with *C. pallida* in some countries (Deltoro *et al.* 2013). Another negative impact of this plant is the decrease in the value of infested areas, reducing the appeal of those landscapes for tourism (Deltoro *et al.* 2013). It also hinders infested land for recreational purposes (Deltoro *et al.* 2013).

Dactylopius tomentosus 'californica var. parkerii' was collected and tested for host-specificity and impact in Australia (Paterson *et al.* 2021). It was shown to be suitably host-specific and damaging to *C. pallida* (Paterson *et al.* 2021). It was then released in Australia in areas with large infestations of *C. pallida* and data currently available from long-term monitoring of the infestations shows that this biotype is likely to lead to complete control of the plant in Australia in the long-term (Paterson *et al.* 2019; Paterson *et al.* 2021). In South Africa, there are no biocontrol agents used for its control.

1.3. *Cylindropuntia imbricata* (Haw.) F.M. Knuth (Cactaceae)

Cylindropuntia imbricata was probably introduced into South Africa during the first decade of the 20th century, since its earliest herbarium records date back to 1913 (Moran & Zimmermann 1991a). It is indigenous to the south-western U.S.A. and northern Mexico (Pinkava 1999). *Cylindropuntia imbricata* is a large shrub or tree that grows up to 3m tall; its stem segments are grey-green and cylindrical; the spines occur at most areoles and are usually tan to brown (Pinkava 1999). The flowers are dark pink to magenta and obovate in shape; the fruit are yellow, fleshy, obovoid and spineless with yellow to tan seeds (Pinkava 1999).

By the end of the 19th century, *C. imbricata* was already considered the most problematic *Cylindropuntia* species in South Africa (Moran & Zimmermann 1991a). It was a problem both for South Africa and Australia and biocontrol work began in 1925 in Australia using *D. tomentosus* (Dodd 1940; Mann 1969). The biocontrol of *C. imbricata* in South Africa began in 1970 with the release of the same *D. tomentosus* imported to South Africa from Australia, which is referred to as the 'imbricata' biotype (Moran & Zimmermann 1991a; Moran *et al.* 2005). It was also successful in South Africa and resulted in a cessation of herbicides against this plant species (De Beer 1986; Moran & Zimmermann 1991a; Paterson *et al.* 2011b). The cochineal is particularly successful in hot and dry conditions; it kills small plants, defoliates large ones and lessens regeneration from loose cladodes (Moran & Zimmermann 1991a; Paterson *et al.* 2021). The cochineal does not kill the large plants because of the sufficient resources the plant has in its stem for regeneration (Paterson *et al.* 2021). Though cochineal could kill large plants after many defoliation and regeneration cycles, this process can be quickened by felling large trees after defoliation (Paterson *et al.* 2021).

1.2.1 Distribution

Cylindropuntia imbricata can be found in the Karoo and Kalahari areas of the Eastern, Western and Northern Cape provinces of South Africa (Paterson *et al.* 2021; Fig. 1.4). Localities of the plant have also been recorded in the Free State and Limpopo provinces, with a few also occurring in KwaZulu-Natal and Gauteng provinces (Fig. 1.4). *Cylindropuntia imbricata* overlaps with *C. pallida* in the arid Karoo and Kalahari regions of the Eastern, Western and Northern Cape (Fig. 1.3, 1.4).

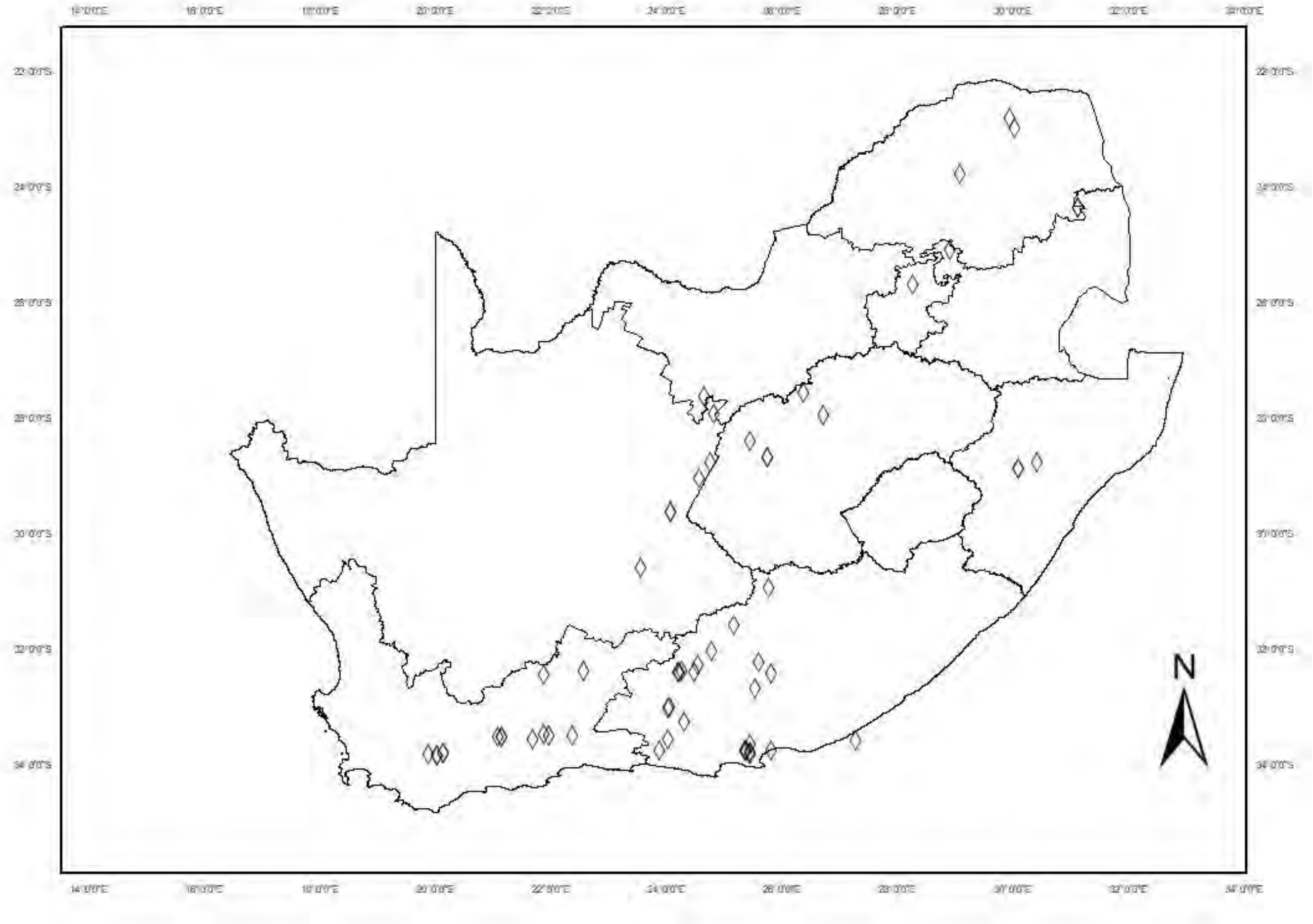

Fig. 1.4. *Cylindropuntia imbricata* distribution in South Africa

1.4. *Cylindropuntia fulgida* (Engelmann) F.M. Knuth.

Cylindropuntia fulgida is considered among the most problematic invasive alien cactus species in South Africa (Klein *et al.* 2020). It is native to Mexico and the south-western U.S.A. (Paterson *et al.* 2011b). The two varieties of *C. fulgida* occurring in South Africa are *C. fulgida* var. *fulgida*, commonly known as chain-fruit cholla and *C. fulgida* var. *mamillata,* commonly known as boxing-glove cactus (Klein *et al.* 2020). *Cylindropuntia fulgida* trees can be 1 to 3 m tall, the trunk has side-branching stems and the crown is also branched, its stem segments are grey-green and the terminal ones can be easily dislodged (Pinkava 1999). The spines are yellowish or pale pink with whitish baggy sheaths (Pinkava 1999). The flowers are pink with pale pink to magenta filaments and whitish anthers; the fruit are grey-green and form long chains; they are also fleshy and spineless (Pinkava 1999). *Cylindropuntia fulgida* var. *fulgida* has interlaced stem spines, the largest ones measuring 2.5-3 cm long, and the stems of this variant appear spiny from afar, concealing the mammillate tubercles beneath (Pinkava 1999). The stem spines of *C. fulgida* var. *mamillata* are not interlaced or are only slightly interlaced, its largest spines measure 1-2 cm long with tight-fitting, rather than baggy sheaths, and its stem segments appear spineless or nearly spineless from afar, thus exposing the strongly mammillate tubercles (Pinkava 1999).

Cylindropuntia fulgida var. *fulgida* was probably introduced into South Africa as an ornamental plant during the 1940s (Klein *et al.* 2020). It was restricted to the Douglas/Campbell area of the Northern Cape for many years until it was found in Musina in Limpopo and the Beit Bridge area of Zimbabwe in the 1990s (Klein *et al.* 2020). *Cylindropuntia fulgida* var. *mamillata* is more widely distributed than *C. fulgida* var. *fulgida* though it has a shorter invasion history (Klein *et al.* 2020). This so-called ‘boxing-glove cactus’ was first recorded in South Africa in 2000 as an unusual form of *C. fulgida* called ‘forma *monstrosa*’, and is now widely distributed in the country (Henderson & Wilson 2017).

Earlier mechanical, chemical and biocontrol programs against this plant were, for the most part, ineffective (Klein *et al.* 2020). In endeavours to control *C. fulgida* var. *fulgida*, the ‘imbricata’ biotype was used in the 1970s, it was successful against small plants, but not against large ones, and the populations of the cactus were uninhibited in their growth and expansion (Moran & Zimmermann 1984; Moran & Zimmermann 1991a; Paterson *et al.* 2011b). During 2008 and 2011, the first releases of the ‘cholla’ biotype on *C. fulgida* var. *fulgida* and *C. fulgida* var. *mamillata*, respectively (Klein *et al.* 2020). Mathenge *et al*. (2009b) observed that the ‘cholla’ biotype was the most damaging of the available biotypes on *C. fulgida* var. *fulgida*. Compared to other biotypes, this biotype's crawlers thrived on *C. fulgida* var. *fulgida*, they settled faster, had larger numbers surviving each development stage, female development time was shorter, and the females were more fecund (Mathenge *et al*. 2009b). The 'cholla' biotype effectively controls *C. fulgida* var. *fulgida* in Limpopo without further interventions, whilst in the Northern Cape, additional measures may be needed due to differences in climate, such as the cooler winter temperatures than in Limpopo (Klein *et al.* 2020). The same cochineal has also done excellent work in controlling *C. fulgida* var. *mamillata*, it is effective against old and young plants and is regarded as one of the most successful biocontrol programmes against any plant in South Africa through the entire distribution of *C. fulgida* var. *mamillata* (Klein *et al.* 2020).

1.4.1. Distribution

Of the two *C. fulgida* varieties, *C. fulgida* var. *mamillata* has the widest distribution (Paterson *et al.* 2021). It can be found in all of the nine provinces of South Africa except Gauteng, Mpumalanga and KwaZulu-Natal provinces, with the largest infestation occurring in the Northern Cape (Klein *et al.* 2020; Paterson *et al.* 2021). Its distribution overlaps with *C. pallida* in four provinces, namely Eastern, Western and Northern Cape and Free State provinces (Fig. 1.3, 1.5).

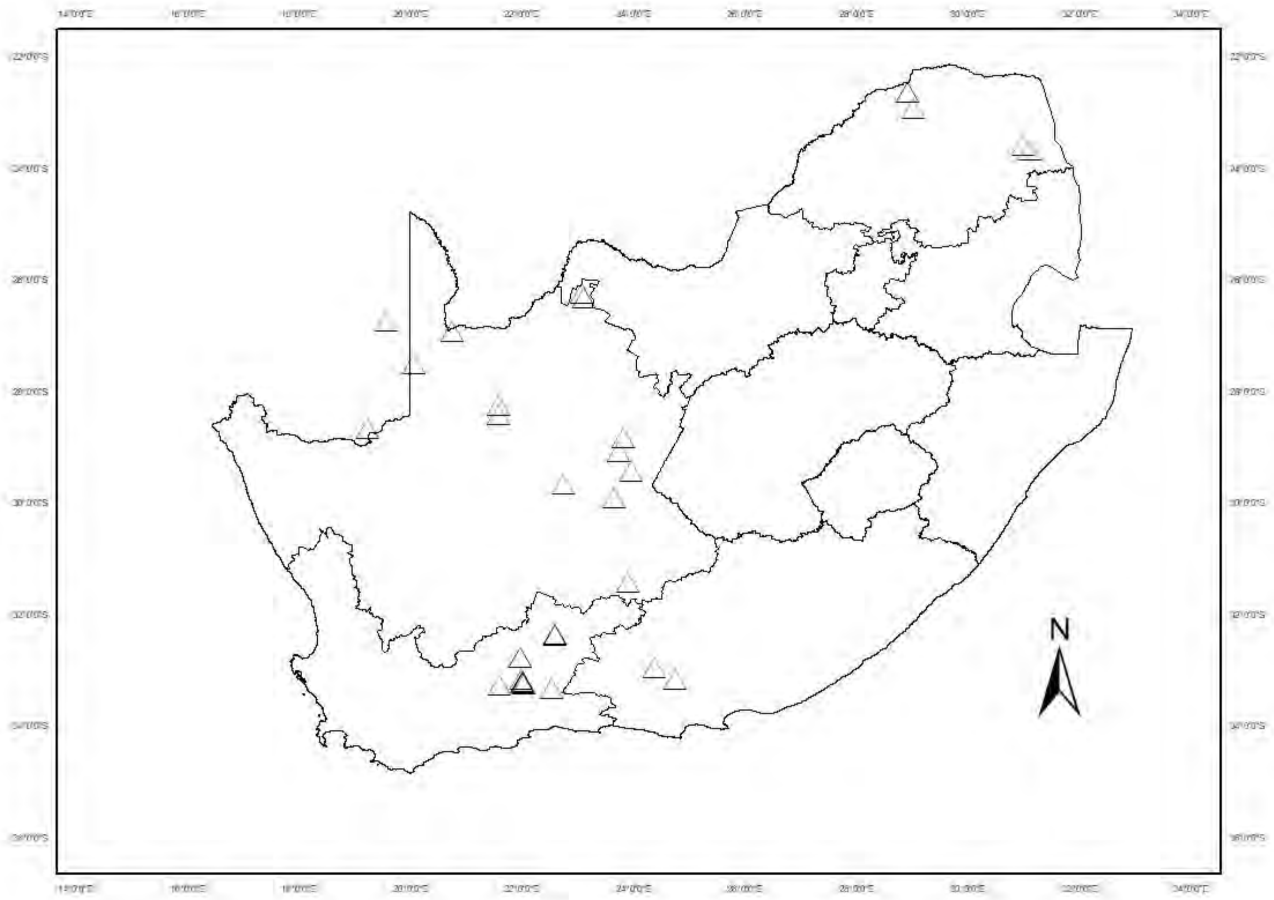

Fig. 1.5. *Cylindropuntia fulgida* var. *mamillata* distribution in South Africa.

1.5. Aims and rationale

The two *D. tomentosus* biotypes already used in South Africa to control *C. imbricata* and *C. fulgida*, are likely to feed on *C. pallida* and could be suitably damaging to be effective agents, negating the need for the importation of new biotypes for *C. pallida* control. In addition to this, the primary host plants of the two *D. tomentosus* biotypes in South Africa have overlapping distributions with *C. pallida*, and the cochineal biotypes are likely to hybridise. If a new biotype of *D. tomentosus* is required to control *C. pallida* in South Africa, then the most appropriate candidate agent would be *D. tomentosus* 'californica var. parkerii,' which is effective in controlling the target weed in Australia. The aims of this study were, therefore, to: (1) evaluate which of the two *D. tomentosus* cochineal biotypes already being used as biocontrol agents of *Cylindropuntia* species in South Africa, namely *D. tomentosus* 'imbricata' and *D. tomentosus* 'cholla', is most effective for the biocontrol of *C. pallida* in South Africa by assessing how damaging these biotypes are to *C. pallida* relatives to their target weeds; and (2) to determine the impact of cochineal hybridisation of these two cochineal biotypes on the biocontrol of *C. pallida*. This information is required to inform decision-makers whether *D. tomentosus* ‘californica var. parkerii’ should be considered for release for the control of *C. pallida* in South Africa or not.

Chapter 2

Materials and Methods

2.1. Types of data collected to evaluate the efficacy

There are various parameters that can be measured in order to evaluate the efficacy of a biocontrol agent on a target weed, these include agent parameters and plant parameters (Morin *et al.* 2009). Female fecundity (counting the number of eggs produced), insect density (counting the number of individuals of each, or one, life stage on the plant) are parameters that can be measured to assess the efficacy of a potential biocontrol agent (Morin *et al.* 2009). The parameters of cochineal, such as female fecundity, insect density, settling rate (determining the number of crawlers emerging and attaching to a feeding site), and crawler development over time can be measured to assess the efficacy of a potential cochineal agent (Jones *et al.* 2015). Short development times indicate that the host plant is suitable for the insect, it will grow at a faster rate on the host and likely provide better control (Jones *et al.* 2016).

Individual plant parameters that can be measured include growth—by measuring plant size and change in biomass of above-ground parts and survival of the plant (Morin *et al.* 2009). For cacti species, parameters that can be measured are growth and number of detached/dead cladodes (Klein *et al.* 2020). The time of plant death can also be visually determined. Jones *et al.* (2015) defined plant death as the time when the main trunk and roots could not support any more growth. The time taken until plant death was reached, is also important evidence of cochineal efficacy.

2.2. Impact Assessments

a. Agent related parameters

The aim of this experiment was to determine which of the cochineal biotypes were better suited to *C. pallida* and to determine the fitness of the two cochineal biotypes on *C. pallida* relative to the other host plants. This experiment was conducted as a laboratory-based bioassay experiment. Two cladodes of each cactus (*C. imbricata*, *C. fulgida, and C. pallida*) were placed separately in 2 litre plastic containers that were sealed but had a fine mesh window to allow for ventilation but would prevent crawlers from escaping. Cladodes were thoroughly cleaned with a paintbrush to remove predators (e.g. spiders) and ensure no cochineal was already on the cladode prior to placing them in the container.

Gravid females from each cochineal biotype (‘imbricata’ and ‘cholla’) were dewaxed to ensure that the eggs they laid afterwards were new ones and not ones already attached to the wax. Dewaxing was done using a pin onto which their wax was spun. These were placed in Petri dishes on top of blotting papers to lay eggs. The Petri dishes were closed to prevent mobile crawlers from escaping. About 30 days later, 30 newly-hatched crawlers (less than 24 hours old) from each cochineal biotype were transferred onto the cactus cladodes so that there were 30 crawlers in each plastic container. Each cochineal biotype was placed on its primary target weed, as well as the alternate target weed and *C. pallida*, and this was replicated five times (Table 1). The transfer was done using a fine paintbrush, dipped in water, then had the water blotted onto paper, to minimize damage whilst picking up the crawlers.

Table 1. Development trial treatments. Each treatment was replicated five times.

	‘imbricata’ biotype	**‘cholla’ biotype**
C. pallida	IoP	CoP
C. imbricata	IoI	CoI
C. f.* var. *mamillata	IoF	CoF

The experiment was left for 14 days to allow the crawlers to settle and develop. After this time, observation under a light microscope of the crawlers’ development began. Observations were conducted at two-day intervals. The dates of moulting, the number of nymphs in 1st, 2nd and 3rd instars, the number surviving to 2nd moult and the number of males present was recorded.

Observations were done until the females developed to the adult stage. Then when a female started to produce eggs, she was removed and dewaxed, she was weighed on a microbalance, and placed, with her eggs, in a Petri dish on top of blotting paper. The readability of the microbalance was up to 0.0001g. On one side of a small piece of paper, the treatment and replicate number (e.g. CoP 1) and, below that, the number allocated to that female (e.g. female 2) from that replicate was recorded. The date on which the female was removed from the plant, her weight in milligrams (mg) and, after 30 days, the number of her hatched crawlers were also recorded.

Using the number of initial crawlers which survived to reproduce another generation, the number of progeny they reproduced and the development time, a Fitness Index for the cochineal biotypes was calculated for each treatment. The following formula was used:

$$\textbf{Fitness index}\ (\text{FI}) = S \times P/T$$

Here, S is the proportion of crawlers surviving to first moult, P is the average number of progeny per female and T is the average female developmental time (Mathenge *et al.* 2009b).

The procedure above was repeated for all the cochineal biotypes housed in a Controlled Environment (CE) room and was replicated five times. The CE room was kept at temperatures above 25°C and below 30°C the optimum temperature range for cochineal survival. The humidity was 45%. The photoperiod was 12 hours; from 7:00am to 19:00pm daily.

This bioassay allowed for the comparison of cochineal development on the host plant they already control in South Africa, their alternative host plant, as well as on *C. pallida.* The relative performance on each of the target weeds allowed for the level of control that would be achieved on *C. pallida* to be predicted.

b. Plant related parameters

This experiment intended to assess the impact the different cochineal biotypes had on the *C. pallida* relative to their target weeds. *Cylindropuntia pallida*, *C. imbricata* and *C. fulgida* var. *mamillata* were planted in 23cm pots and housed at the Rhodes University Zoology and Entomology Department greenhouses. The plants were collected by digging up small plants from field sites within the Eastern Cape Province. These were transplanted in plant pots. These were watered and allowed to grow sufficiently. When used for the experiment, they were cleaned thoroughly using water to remove any cochineal, other insects or predators.

In this assessment, potted *C. pallida, C. imbricata* and *C. fulgida* var. *mamillata* were exposed to the two different cochineal biotypes to assess the impact the cochineal has on the plants. There were therefore six treatments mirroring the agent related assessment ('imbricata' on *C. pallida,* 'imbricata' on *C. imbricata,* 'imbricata' on *C. fulgida* var. *mamillata,* 'cholla' on *C. pallida,* 'cholla' on *C. imbricata,* and 'cholla' on *C. fulgida* var. *mamillata*) each of which was replicated five times (Table 1). Each individual treatment was placed in a cage. The cochineal biotypes were introduced on each plant by placing two cochineal-infested cladodes, on the plant, in each cage.

Data was collected once every two weeks when plant and insect parameters were measured. The parameters recorded were the number of cochineal clusters established on the plant as a whole and the number of days until plant death. The same definition of plant death as Jones *et al.* (2015), which is the time when the main trunk and roots could not support any more growth, was used. The damage done by the biotypes on each cactus species was compared to determine whether *C. pallida* was sufficiently impacted relative to the other plant species which are known to be under control in South Africa (Moran *et al.* 2021). The 'cholla' biotype on *C. fulgida* var. *mamillata* was considered a benchmark for complete control, as it is an example of complete control in the field (Jones *et al.* 2015; Klein *et al.* 2020). The 'imbricata' biotype on *C. imbricata*, on the other hand, is successful but results in substantial control (Moran *et al.* 2021). The experiment was left running for 365 days.

In this study, the efficacies of the 'imbricata' and 'cholla' biotypes of *D. tomentosus* on *C. pallida* were assessed by 1) comparing the fitness indices of the different agent biotypes as a measure of the agent's performance on different host plants relative to their performance on *C. pallida* (agent related parameter), and 2) the damage of the plant above-ground biomass, leading to plant death, which can be observed and noted by visually documenting the rate at which the plant deteriorates, as a measure of how impacted the different host plants are by the two biotypes (plant-related parameter).

2.2.1. Statistical analyses

Statistical analyses were conducted using the R Statistics software R version 4.0.3 in the integrated development environment RStudio Version 1.4.1103 (R Core Team 2020). Correlations between female weight, number of progeny and time to maturity were computed using R to assess the relationship between female weight, fecundity and developmental time as they developed on each cactus species. These were done to assess whether female weight and time to maturity were good predictors of fitness for these cochineal biotypes. The percentage of crawlers reaching the first moult in each treatment were normally distributed, while the number of days to first moult, the number of days to female maturity, the average weight of mature females, the number of crawlers per female, the number of cochineal clusters settled on a feeding spot and the number of days until plant death were not normally distributed. The normally distributed parameters were analysed using an ANOVA while the parameters that were not normally distributed were analysed using a Kruskal-Wallis rank sum test. A post-hoc Tukey HSD test was done to observe the differences between the normally distributed data. For the average weight of mature females, a post-hoc Multiple Pairwise Wilcoxon Rank Sum Test was performed because there were significant differences between the treatments. Several insect parameters are needed to determine the suitability of a host for a biocontrol agent because one parameter is not sufficient for host suitability determination (Paterson *et al.* 2012). These parameters include adult or juvenile mass, percentage survival and development time duration, Maw (1976) combined into a host suitability index (Maw 1976; Paterson *et al.* 2012). A host selected as suitable by an agent will supply more nutrients needed for its development, which would lead to shorter development times, and bigger, more gravid females (Maw 1976; Czypionka & Hill 2007).

2.3. Impact of hybridisation on potted plants

This experiment was conducted to assess the impact of hybridisation on the damage of *C. pallida* by the 'imbricata' and 'cholla' biotypes. There is significant overlap with the distribution of the three cacti species in South Africa, so hybridisation would be possible if either biotype were acceptable against *C. pallida*. Hybridisation is likely to occur in the field at present in areas where both *C. imbricata* and *C. fulgida* occur.

There were four treatments for this experiment. These were i) the controls that had no cochineal, ii) both biotypes of cochineal, iii) 'imbricata' cochineal only and, iv) 'cholla' cochineal only. The control had *C. imbricata*, *C. fulgida* var. *mamillata* and *C. pallida* plants in cages with no cochineal introduced. Both biotype treatments had *C. imbricata*, *C. fulgida* var. *mamillata* and *C. pallida* plants and five cochineal-infested cladodes of the 'imbricata' and 'cholla' biotypes, each on their hosts, while *C. pallida* had no cochineal-infested cladodes. The 'imbricata'-biotype-only treatment had all three cacti species, with only *C. imbricata* having five 'imbricata' biotype infested cladodes. The 'cholla'-biotype-only treatment also had all three cacti, but only the *C. fulgida* var. *mamillata* plants had 'cholla' biotype infested cladodes. These treatments were each replicated five times. In total, there were 60 plants in 20 cages; this was made up of 20 plants from each species.

Data was collected once every two weeks when plant and insect parameters were measured. The parameters recorded for each plant species in each cage was the number of cochineal clusters established and plant survival/mortality. This data collection made it possible to determine the number of days it took for each plant species to die using the same definition of plant death as Jones *et al.* (2015). The damage done by the biotypes on the plants within each treatment and between treatments was compared to determine which combination led to significant damage of *C. pallida*. The experiments were left running for 290 days.

2.3.1. Statistical analyses

The number of cochineal clusters established and the number of days until plant death were not normally distributed, therefore a Kruskal-Wallis rank sum tests were performed for both. Thereafter post-hoc Multiple Pairwise Wilcoxon Rank Sum Tests between treatments were conducted. Similarly to the impact assessment, statistical analyses were conducted using the R Statistics software R version 4.0.3 in the integrated development environment RStudio Version 1.4.1103 (R Core Team 2020).

Chapter 3

Results

3.1. Impact Assessments

a. Agent related parameters

Correlation analysis showed significant correlations ($p<0.05$) for all accessions (i.e. female weight, number of progeny and time to maturity). All accessions also showed positive correlations: time to maturity and number of progeny ($r = 0.53$, $p = 0.0027$), which indicates that individuals that take longer to mature will produce more progeny (Fig. 3.1); time to maturity and female weight at maturity ($r = 0.69$, $p < 0.001$), which shows us that the longer it takes for a female to develop the heavier she will be (Fig. 3.2); and weight against the number progeny ($r = 0.65$, $p = 0.00011$), which shows that the females with higher weight tend to be more fecund than low weight females (Fig. 3.3). Although all these trends were significant, the r values indicate that the correlations were weak, so one of these parameters will not accurately predict the others in all instances.

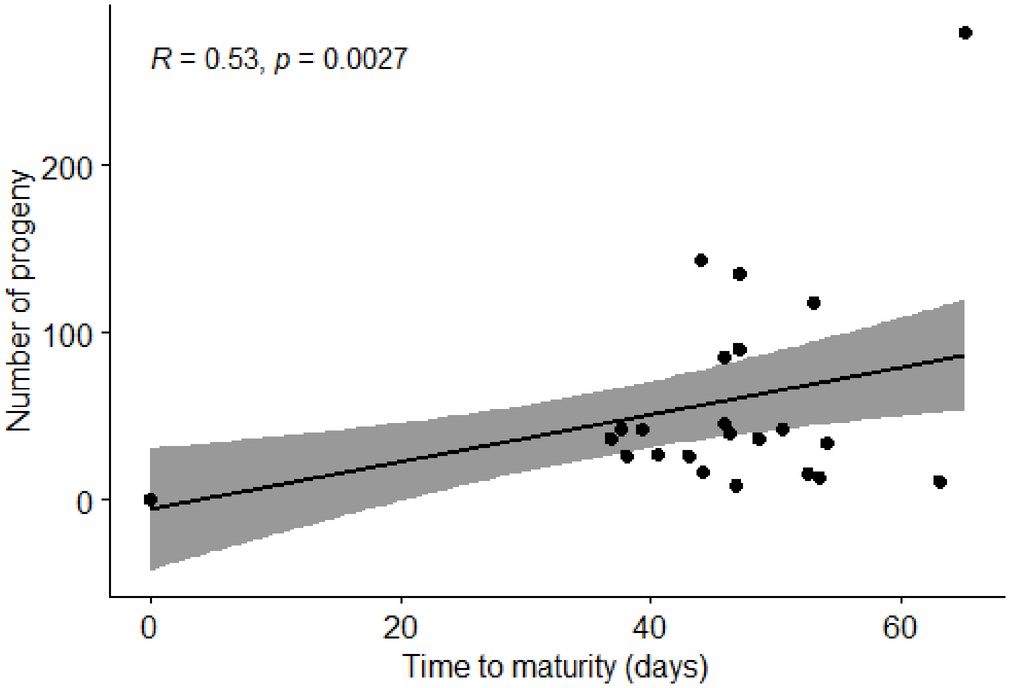

Fig. 3.1. Correlation between time to maturity and number of progeny. These data are for females of the biotypes ‘imbricata’ and ‘cholla’ reared on *C. imbricata* (*Ci*) *C. fulgida* var. *mamillata* (*Cfm*) and *C. pallida* (*Cp*).

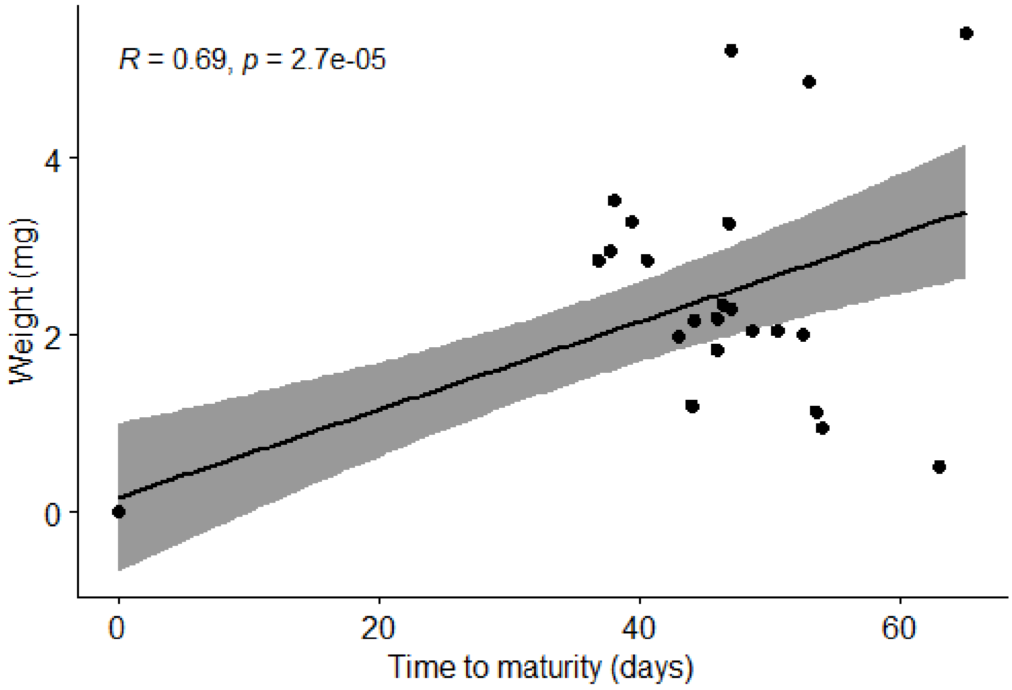

Fig. 3.2. Correlation between time to maturity and weight at maturity. These data are for females of the biotypes 'imbricata' and 'cholla' reared on *C. imbricata* (*Ci*) *C. fulgida* var. *mamillata* (*Cfm*) and *C. pallida* (*Cp*).

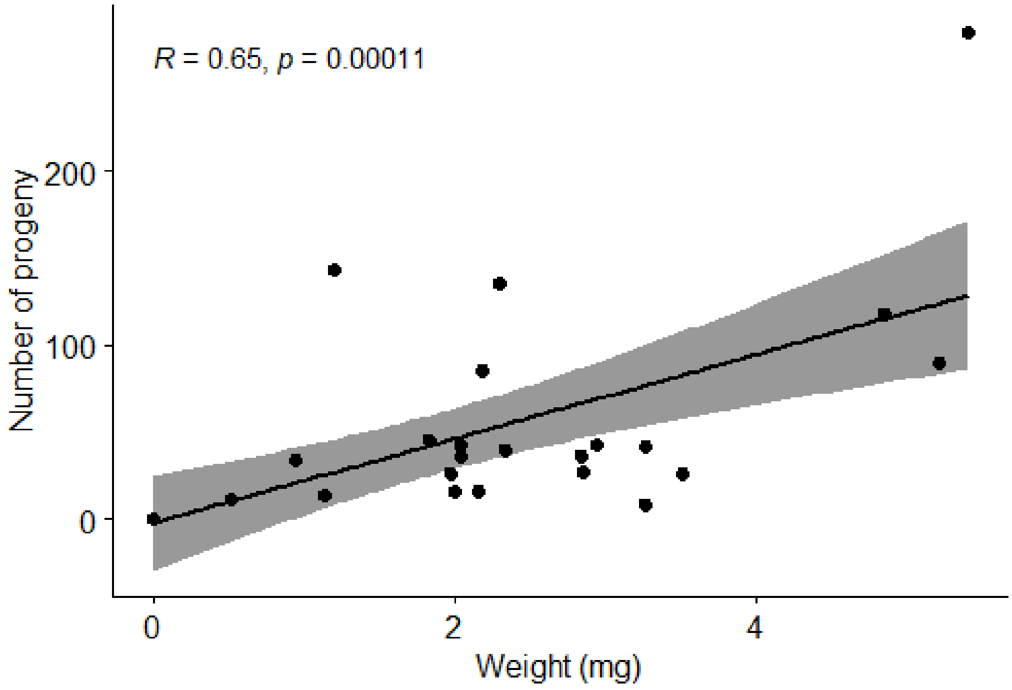

Fig. 3.3. Correlation between weight at maturity and number of progeny. These data are for females of the biotypes 'imbricata' and 'cholla' reared on *C. imbricata* (*Ci*) *C. fulgida* var. *mamillata* (*Cfm*) and *C. pallida* (*Cp*).

Correlation analysis for each of the parameters (time to maturity vs progeny, time to maturity vs weight and weight vs progeny) were also done for each biotype (Table 2). Time to maturity and female progeny were significantly correlated for the 'imbricata' cochineal biotype on *C. imbricata* (r = 0.889, p = 0.044), the 'imbricata' cochineal biotype on *C. pallida* (r = 1, p < 0.001) and the 'cholla' cochineal biotype on *C. imbricata* (r = 0.977, p = 0.004). For the time to maturity and weight parameter the 'imbricata' cochineal biotype on *C. imbricata* (r = 0.983, p = 0.003), the 'imbricata' cochineal biotype on *C. pallida* (r = 1, p < 0.001), and the 'cholla' cochineal biotype on *C. imbricata* (r = 0.977, p = 0.004), had significant correlations. The weight and number of progeny analysis had significant correlations for the 'imbricata' cochineal biotype on *C. pallida* (r = 1, p < 0.001) and the 'cholla' cochineal biotype on *C. imbricata* (r = 0.946, p = 0.015).

Table 2. Correlations between days to maturity, the number of progeny at maturity and the weight (mg) at maturity of the 'imbricata' and 'cholla' biotype females of *D. tomentosus*, cultured on *C. imbricata* (*Ci*) *C. fulgida* var. *mamillata* (*Cfm*) and *C. pallida* (*Cp*).

		Days vs. progeny		Days vs. weight		Weight vs. progeny	
		r	*P*	r	*P*	r	*P*
'imbricata'	*Ci*	0.889	0.044	0.983	0.003	0.804	0.101
'imbricata'	*Cfm*	0.596	0.289	0.719	0.171	0.734	0.158
'imbricata'	*Cp*	1	<0.001	1	<0.001	1	<0.001
'cholla'	*Ci*	0.977	0.004	0.977	0.004	0.946	0.015
'cholla'	*Cfm*	-0.329	0.589	-0.003	0.996	-0.211	0.733
'cholla'	*Cp*	-0.485	0.407	-0.464	0.431	-0.368	0.542

The percentage of crawlers surviving the first moult was significantly different between treatments (F (5, 24) = 10.02, P< 0.001). The 'cholla' cochineal biotype on *C. fulgida* var. *mamillata* was significantly different to the 'cholla' cochineal biotype on *C. imbricata;* and the 'imbricata' cochineal biotype on *Cylindropuntia pallida* treatment was different from 'cholla' on *C. imbricata,* 'cholla' on *C. pallida*, and 'imbricata' on *C. imbricata* (Fig. 3.4). The 'cholla' cochineal biotype on *C. imbricata* had a significantly higher percentage of crawler survival whilst the 'imbricata' cochineal biotype on *C. pallida* had significantly lower survival.

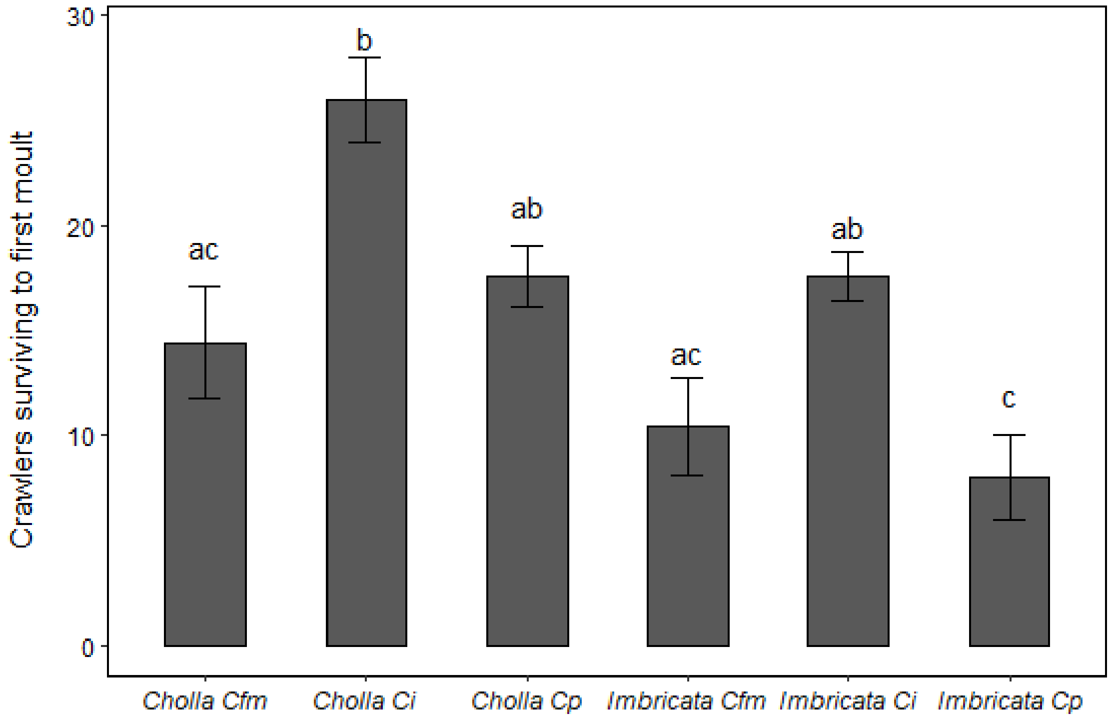

Fig. 3.4. Mean (± SE) number of crawlers surviving from first to second instar of 'imbricata' and 'cholla' biotypes on *C. fulgida* var. *mamillata* (*Cfm*), *C. imbricata* (*Ci*), and *C. pallida* (*Cp*). Different letters indicate significant differences (Tukey HSD test; $p < 0.05$).

There was a significant difference in the number of days to first moult of the cochineal biotypes between the treatments (H = 16.303 (5), p = 0.006). Post-hoc comparisons indicated that the mean of 15.4 days (SE ± 0.4) for the 'cholla' cochineal biotype on *C. imbricata* was significantly different from that of 17.0 days (SE ± 0.0) for the 'cholla' cochineal biotype on *C. pallida* and that of 18.0 days (SE ± 0.0) for the 'imbricata' biotype on *C. pallida*. The 'cholla' cochineal biotype on *C. pallida* all took 17 days to the first moult, which was significantly different from that of 14 days and 18 days for the 'imbricata' cochineal biotype on *C. imbricata* and *C. pallida*, respectively (Fig. 3.5). All 'imbricata' cochineal biotypes on *C. imbricata* reached the first moult at 14 days which was significantly different from 18 days for the same cochineal biotype on *C. pallida*. The mean number of days to first moult of the 'imbricata' cochineal biotype on *C. pallida* was significantly higher than that of the 'cholla' cochineal biotype on *C. fulgida* var. *mamillata*, the 'cholla' cochineal biotype on *C. imbricata* and the 'imbricata' cochineal biotype on *C. imbricata* (Fig. 3.5).

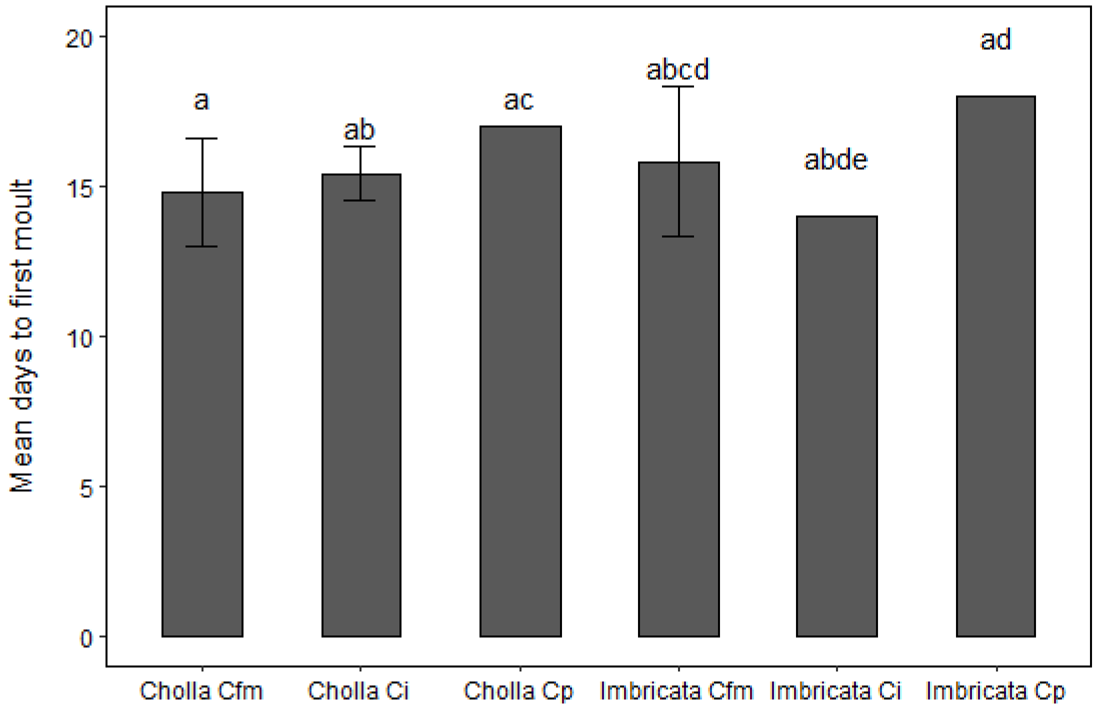

Fig. 3.5. Mean (± SE) number of days to first moult of crawlers of the 'imbricata' and 'cholla' biotypes on *C. fulgida* var. *mamillata* (*Cfm*), *C. imbricata* (*Ci*), and *C. pallida* (*Cp*). Different letters indicate significant differences. Error bars indicate the standard error around each mean.

There was no significant difference between the treatments in the average time to female maturity (H = 6.0788 (5), p-value = 0.299). The 'imbricata' cochineal biotype females on *C. pallida* took the shortest time, 12.6 days (SE ± 12.6), to mature while the 'cholla' cochineal biotype females on *C. pallida* took the longest time, 48.0 days (SE ± 2.14), yet these were not significantly different from one another or the other treatments (Fig. 3.6).

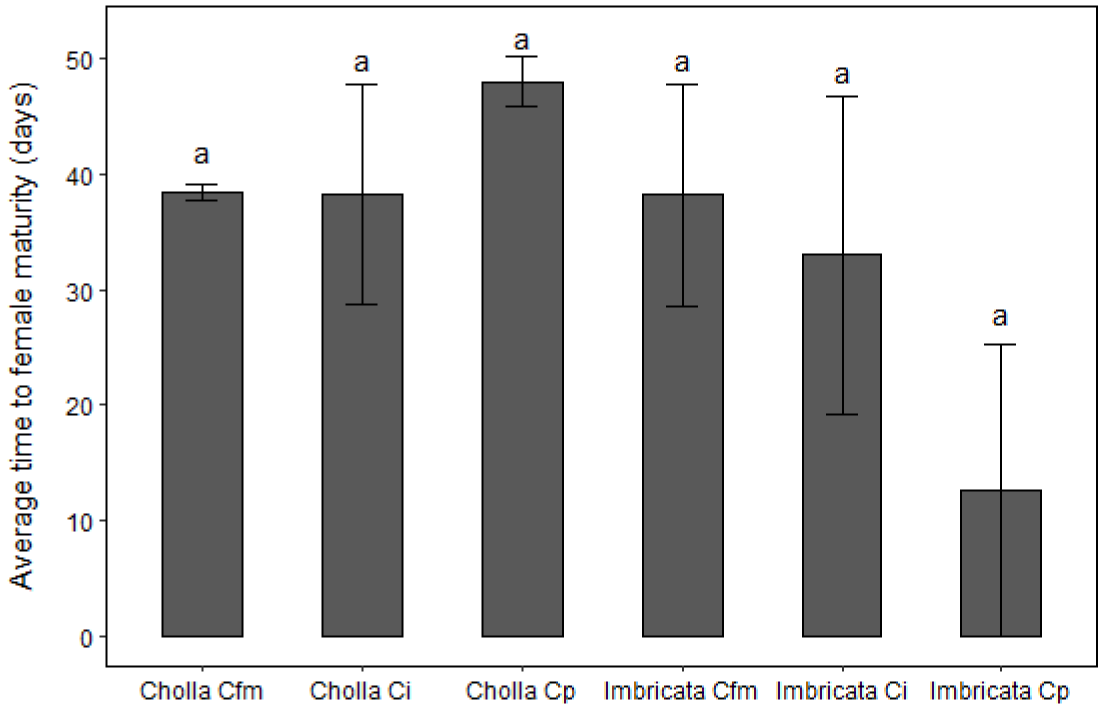

Fig. 3.6. Mean (± SE) days to female maturity of the 'imbricata' and 'cholla' biotypes from *C. fulgida* var. *mamillata* (*Cfm*), *C. imbricata* (*Ci*), and *C. pallida* (*Cp*). The same letters indicate a lack of significant differences. Error bars indicate the standard error around each mean.

There was a significant difference between the treatments in the average weight of females (H = 13.367 (5), p-value = 0.020). The Multiple Pairwise Wilcoxon Rank Sum Test between treatments indicated a significant difference between the mean of 2.11 mg (SE ± 0.340) for the 'cholla' biotype on *C. pallida* and the mean of 0.104 mg (SE ± 0.104) 'imbricata' biotype on the same species (Fig. 3.7). Other significant differences occurred between the average weight of 3.08 mg (SE ± 0.133) for the 'cholla' cochineal biotype on *C. fulgida* var. *mamillata* and the mean of 1.65 mg (SE ± 0.421) for the 'cholla' cochineal biotype on *C. imbricata* (Fig. 3.7). There was also a significant difference between the mean of the 'cholla' cochineal biotype on *C. fulgida* var. *mamillata* and that of 1.33 mg (SE ± 0.425) for the 'imbricata' cochineal biotype on *C. fulgida* var. *mamillata* (Fig. 3.7). Another significant difference was between the mean of the 'cholla' cochineal biotype on *C. fulgida* var. *mamillata* and that of 0.10 mg (SE ± 0.104) for the 'imbricata' cochineal biotype on *C. pallida* (Fig. 3.7). A significantly higher average female weight was observed for the 'cholla' cochineal biotype on *C. fulgida* var. *mamillata* females and a significantly lower one for the 'imbricata' cochineal biotype females on *C. pallida* (Fig. 3.7).

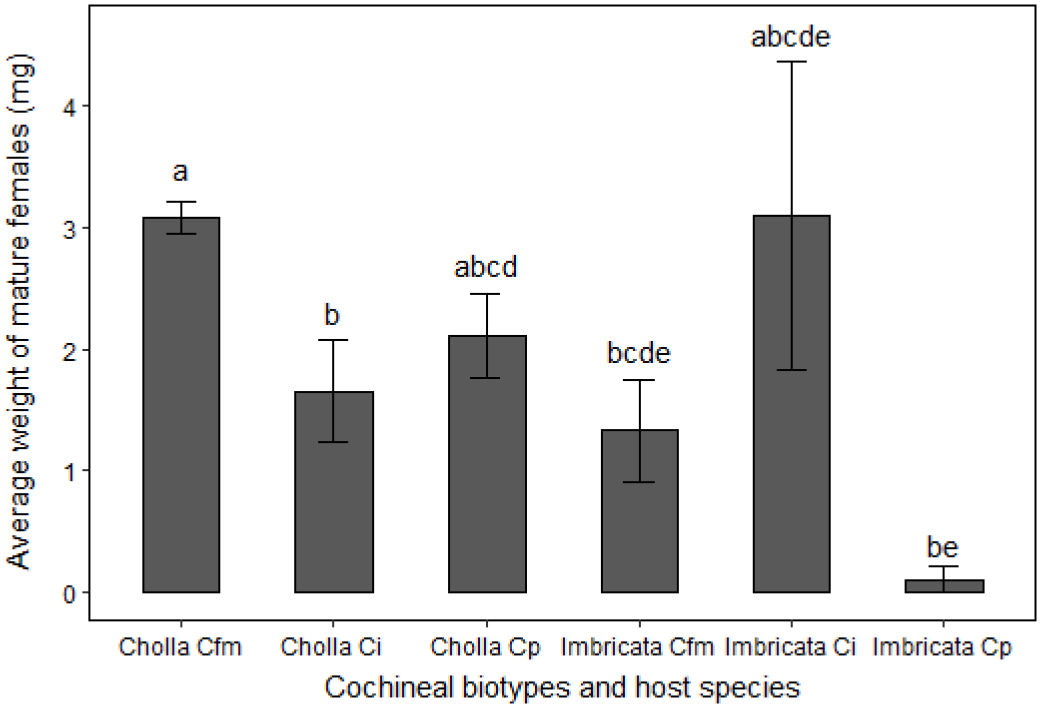

Fig. 3.7. Mean (± SE) weight of mature females of the 'imbricata' and 'cholla' biotypes reared on *C. fulgida* var. *mamillata* (*Cfm*), *C. imbricata* (*Ci*), and *C. pallida* (*Cp*). Different letters indicate significant differences (Multiple Pairwise Wilcoxon Rank Sum Test; $p < 0.05$). Error bars indicate the standard error around each mean.

There was no significant difference between the treatments in the average number of crawlers per female (H = 10.511 (5), p-value = 0.062). The 'imbricata' cochineal biotype on *C. imbricata* had the highest mean number of crawlers per female with 79.5 crawlers (SE ± 27.9), and the 'imbricata' cochineal biotype on *C. pallida* had the lowest, but these were still not significantly different from one another and the other treatments (Fig. 3.8).

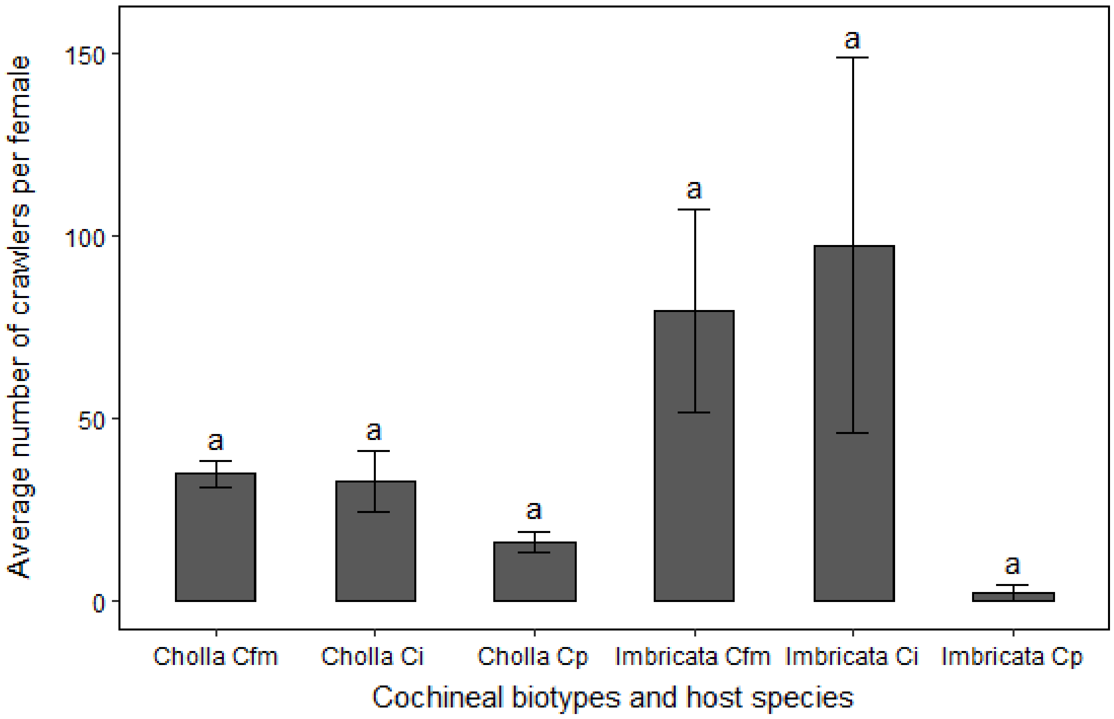

Fig. 3.8. Mean (± SE) number of crawlers per female of the 'imbricata' and 'cholla' biotypes reared on *C. fulgida* var. *mamillata* (*Cfm*), *C. imbricata* (*Ci*), and *C. pallida* (*Cp*). The same letters indicate lack of significant differences. Error bars indicate the standard error around each mean.

There was no significant difference between the fitness indices of the biotypes on the three cactus species (H = 9.362 (5), p-value = 0.095). None reached FI = 1, where a biotype is considered thriving on its host, but the 'imbricata' cochineal biotype on *C. imbricata* came very close (0.971) (Fig. 3.9). Both the 'imbricata' and 'cholla' cochineal biotypes on *C. pallida* had low FIs (0.016 and 0.191, respectively) (Fig. 3.9).

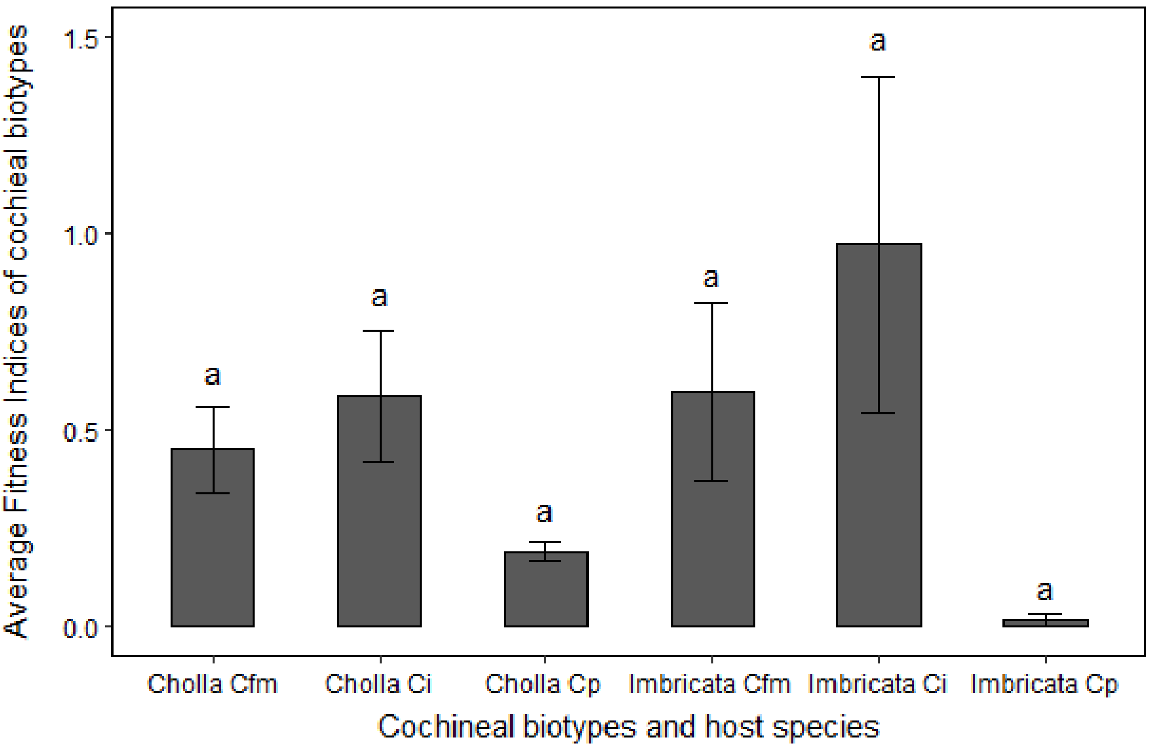

Fig. 3.9. Mean (± SE) of fitness indices of the biotypes **'imbricata'** and **'cholla'** cultured on *C. fulgida* var. *mamillata* (*Cfm*), *C. imbricata* (*Ci*) and *C. pallida* (*Cp*). The same letters indicate lack of significant differences. Error bars indicate the standard error around each mean.

b. Plant related parameters

There was a significant difference between the treatments in the settling rate of female clusters by day 108 (H $^{=}$ 20.166 (5), p-value = 0.001). The Multiple Pairwise Wilcoxon Rank Sum Test between treatments indicated that there was no significant difference between the settling rate of 102 females (SE ± 47.4) for the 'cholla' biotype on *C. pallida* and that of 16 females (SE ± 7.12) for the 'imbricata' biotype on the same species (Fig. 3.10). Significant differences were between 314 females (SE ± 81.4) of the 'cholla' biotype on *C. fulgida* var. *mamillata* and the 25 females (SE ± 18.4), 72.4 females (SE ± 25.3), 16 females (SE ± 7.12) of the 'imbricata' biotype on *C. fulgida* var. *mamillata, C. imbricata* and *C. pallida*, respectively (Fig. 3.10). There were also significant differences between the mean of 446 females (SE ± 70.1) of the 'cholla' biotype on *C. imbricata* and the mean of 25 females (SE ± 18.4), 72.4 females (SE ± 25.3), 16 females (SE ± 7.12) of the 'imbricata' biotype on *C. fulgida* var. *mamillata, C. imbricata* and *C. pallida*, respectively (Fig. 3.10).

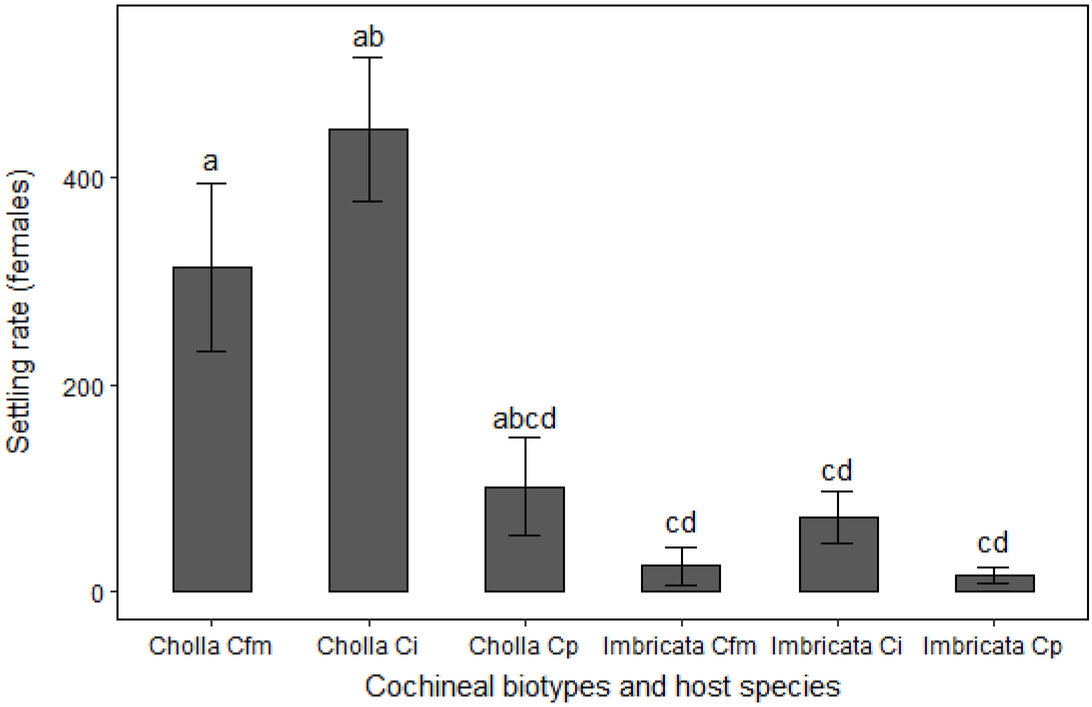

Fig. 3.10. Mean (± SE) females of the 'imbricata' and 'cholla' biotypes settled on a feeding spot on *Cylindropuntia fulgida* var. *mamillata* (*Cfm*), *Cylindropuntia imbricata* (*Ci*) and *Cylindropuntia pallida* (*Cp*). Day 108 was the common day compared across all biotypes and hosts. The same letters indicate significant differences. Error bars indicate the standard error around each mean.

The number of weeks taken for both biotypes to settle differed between the cactus species, except for the 'cholla' biotype on *C. imbricata* and *C. fulgida* var. *mamillata* (Table 3). Both cochineal biotypes took longer to settle on *C. pallida* compared to other cacti species tested (Table 3).

Table 3. Time taken (in weeks) for colony establishment of each of the two *D. tomentosus* biotypes ('imbricata', 'cholla') on the screened *Cylindropuntia* spp. during the impact trials.

	D. tomentosus biotype	
Cylindropuntia spp.	'imbricata'	'cholla'
C. imbricata	4.4	10
C. fulgida var. *mamillata*	1.6	10
C. pallida	8.2	17

The percentage of plants that died from each biotype by the end of the experiment differed between the plant species. The 'imbricata' cochineal biotype on *C. fulgida* var. *mamillata* and *C. pallida* and the 'cholla' biotype on *C. pallida* had not killed any plants by the end of the experiment (Table 4). The ‘imbricata’ cochineal biotype killed 20% *C. imbricata* plants, while the ‘cholla’ cochineal biotype killed 20% of *C. fulgida* var. *mamillata* plants (Table 4). Surprisingly, the ‘cholla’ biotype on *C. imbricata* resulted in 100% mortality (Table 4).

Table 4. Percentage of *Cylindropuntia fulgida* var. *mamillata* (*Cfm*), *Cylindropuntia imbricata* (*Ci*) and *Cylindropuntia pallida* (*Cp*) plants which died from the impact of the ‘imbricata’ and ‘cholla’ biotypes by day 365.

Treatment	Percentage plants dead
‘imbricata’ cochineal on *C. imbricata*	20%
‘imbricata’ cochineal on *C. f.* var. *mamillata*	0%
‘imbricata’ cochineal on *C. pallida*	0%
‘cholla’ cochineal on *C. imbricata*	100%
‘cholla’ cochineal on *C. f.* var. *mamillata*	20%
‘cholla’ cochineal on *C. pallida*	0%

There were no significant differences between the treatments in the days to plant death (F-value = 0.874(5), p-value = 0.484) (Fig. 3.11). The ‘imbricata’ cochineal biotype took the shortest time, 232 days, to kill all *C. imbricata*.

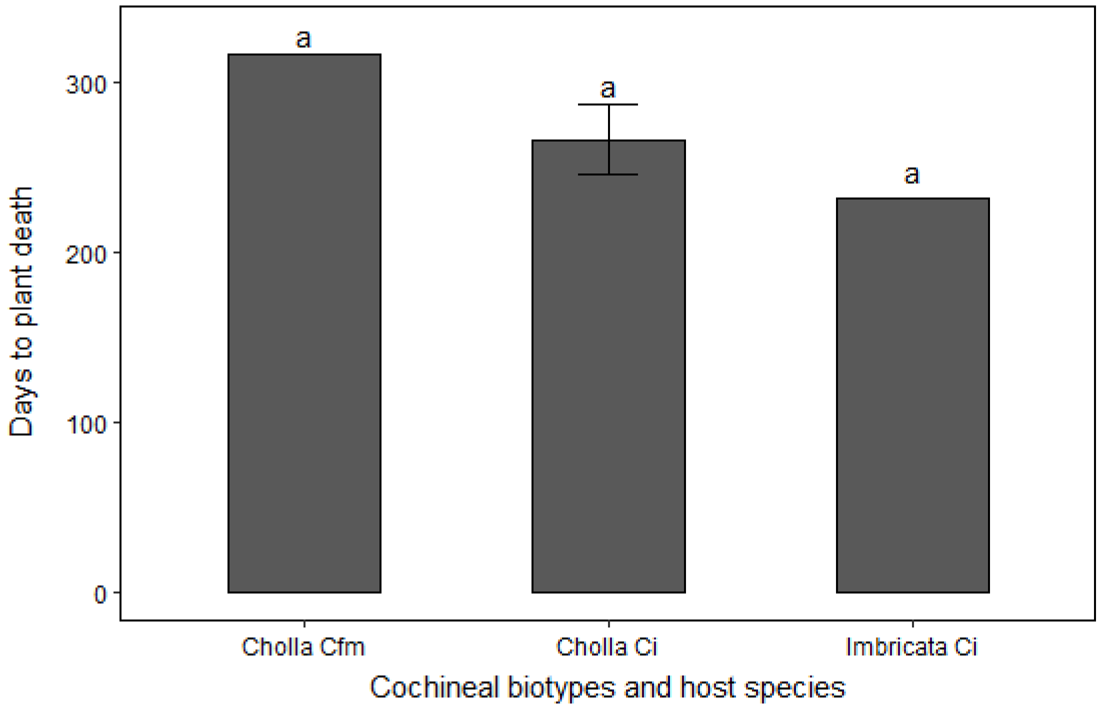

Fig. 3.11. Mean (± SE) days until the death of *Cylindropuntia fulgida* var. *mamillata* (*Cfm*) and *Cylindropuntia imbricata* (*Ci*) under the 'imbricata' and 'cholla' biotypes. The maximum number of days used for all plants was 365 days. The same letters indicate lack of significant differences. Error bars indicate the standard error around each mean.

The 'imbricata' biotype killed *C. imbricata* and *C. pallida* plants by week 71, no *C. fulgida* var. *mamillata* plants were killed by this biotype. The 'cholla' cochineal biotype killed plants from the *C. imbricata* by week 32, and some *C. fulgida* var. *mamillata* and *C. pallida* plants by week 64.

3.2. Impact of hybridisation on potted plants

The percentage of *C. pallida* plants that died from the 'cholla' biotype and the 'imbricata x cholla' biotype were the same (100%), but the percentage of *C. pallida* dead was low for the 'imbricata' biotype (40%). *Cylindropuntia imbricata* plants were also killed by the 'imbricata x cholla' biotype (100%) and their biotype (80%). There were minimal impacts on the *C. fulgida* var. *mamillata* plants.

Table 5. Percentage of plants dead at the end of the hybridisation impact analysis from each treatment.

	Control	**Both cochineals**	**‘cholla’ cochineal**	**‘imbricata’ cochineal**
C. pallida	0%	100%	100%	40%
C. imbricata	0%	100%	20%	80%
C. fulgida* var. *mamillata	0%	40%	40%	0%

There was no significant difference between the treatments of time until plant death (H = 7.55 (7), p-value = 0.3743). The ‘cholla’ cochineal biotype on *C. imbricata* 59 days (SE ± 59), took the shortest time to kill 20% of the plants, while the same biotype on *C. pallida* 272 days (SE ± 10.6) together with the ‘imbricata’ x ‘cholla’ biotype on *C. imbricata* 271 days (SE ± 11.4) took the longest time yet killed all the plants (Fig. 3.12).

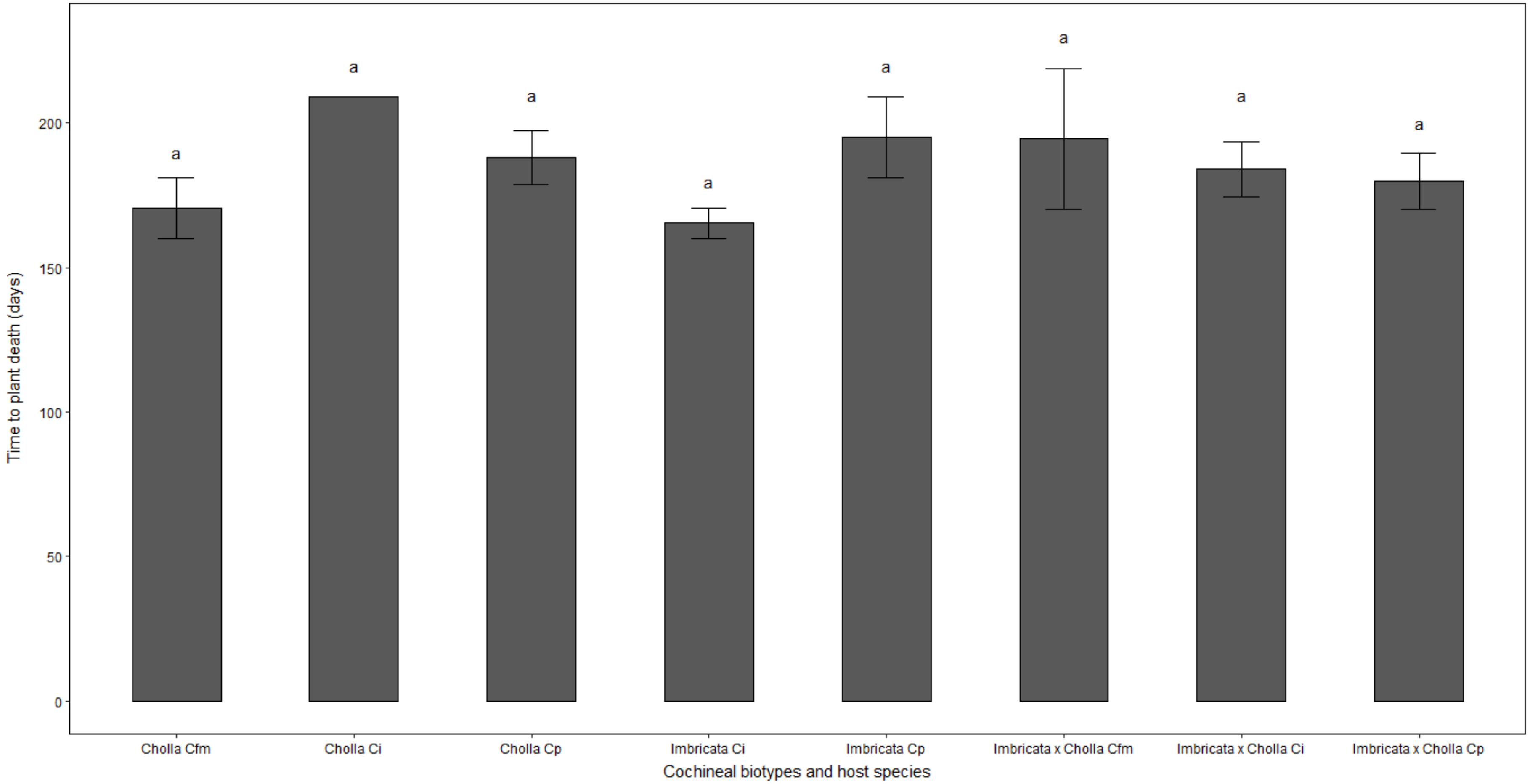

Fig. 3.12. Mean (± SE) days until death of *Cylindropuntia fulgida* var. *mamillata* (*Cfm*), *Cylindropuntia imbricata* (*Ci*) and *Cylindropuntia pallida* (*Cp*) under each the 'imbricata'-only, 'cholla'-only and 'imbricata' x 'cholla' treatments. The same letters indicate lack of significant differences. Error bars indicate the standard error around each mean.

The 'cholla x imbricata' cochineal killed *C. imbricata* and *C. fulgida* var. *mamillata* by week 31, the *C. pallida* plants were killed by week 24. The 'cholla' biotype killed plants from all three cacti by week 30. The 'imbricata' biotype killed *C. imbricata* and *C. pallida* plants by week 26, no *C. fulgida* var. *mamillata* were killed by this biotype by week 30.

Chapter 4

Discussion

The pre-release assessments conducted in Australia by Jones *et al.* (2015) indicated that the 'imbricata' biotype had no impact on *C. fulgida* var. *fulgida,* which necessitated a release of the 'cholla' biotype on *C. fulgida* var. *fulgida* and *C. fulgida* var. *mamillata*. Mathenge *et al.* (2009b) indicated that a combination of the following factors made the 'cholla' cochineal thrive on *C. fulgida* var. *fulgida*, making it the best candidate for the control of that particular cactus: the fast settling and development of crawlers, larger numbers surviving each developmental stage, short female developmental time, early commencement of reproduction and large, fecund, females that produced many crawlers. In this study, we observed this combination of traits for the 'cholla' biotype on *C. pallida*, while the 'imbricata' biotype on the same cactus lacked these traits. We also observed that the 'cholla' biotype was more effective on *C. imbricata*. The 'cholla' biotype therefore appears to be better at controlling several cacti species and is, therefore, deemed one of the best candidates for new *Cylindropuntia* targets (Jones *et al.* 2015).

Findings from this study indicate that the 'cholla' cochineal biotype performs better on *C. pallida* compared to the 'imbricata' biotype. This can be seen from the fact that a larger percentage of crawlers of the 'cholla' than the 'imbricata' biotype surviving on *C. pallida*. Also, the 'cholla' crawlers took a relatively shorter time to reach the first moult on *C. pallida* than the 'imbricata' crawlers. Though the 'imbricata' biotype female crawlers took a shorter average time to mature and begin reproduction on *C. pallida*, these weighed less than the 'cholla' females, which matured over a relatively longer period on *C. pallida*. Since female weight is positively correlated to the number of progeny the female reproduces, this trade-off of weight for a shorter development time might not be beneficial for this biotype to effectively control *C. pallida* since it will produce smaller females with fewer offspring.

The trade-off between developmental time and body size or weight has also been observed in other insect species. Females with a larger body size benefit from it because they produce a higher number of eggs and larger eggs (Rohde *et al.* 2015). This observation was made for two grasshopper species, *Chorthippus montanus* Charpentier (Acrididae) and *Chorthippus brunneus* Thunberg (Acrididae) (Rohde *et al.* 2015; Hassall *et al.* 2006). These species' nymphal stages undergo long developmental times which enabling the females to be larger and thus reproduce more and larger eggs (Rohde *et al.* 2015). The long developmental time is especially exhibited during times of high population densities of the species, which means it is likely exhibited to avoid the increased competition (Wall & Begon 1987; Rohde *et al.* 2015). The large eggs produced by these females produce larger and more fecund adults and have a higher chance of survival through the nymphal stages (Carrière *et al.* 1997; Hassall *et al.* 2006).

Other studies on *Gratiana spadicea* Klug (Chrysomelidae), have observed strong correlations between females' weight and the total number of eggs she produced (Czypionka & Hill 2007). This positive correlation between weight and number of progeny has also been established in other studies for both *D. tomentosus* (Mathenge *et al.* 2009b) and other cochineal species (Moran & Cobby 1979; Githure *et al.* 1999; Volchansky *et al.* 1999). Although results show the number of progeny vs. weight showed a positive correlation, similar to what was obtained by Mathenge *et al.* (2009b), weight vs. time to maturity differ from that by Mathenge *et al.* (2009b). This study showed that weight and time to maturity were also positively correlated in the cactus species studied, indicating that bigger females took longer to mature, producing more offspring. This establishes the importance of including traits such as developmental time, body weight and fecundity to understand cochineal host preferences and fitness and corroborates Maw's (1976) that an increase in female weight leads to an increase in fecundity . This index and others, however, as Czypionka & Hill (2007) correctly point out, cannot be used as sole indicators of host suitability and agent fitness; they need to be used with other tools before agent release can be done.

Both biotypes had a fitness index greater than one but less than zero, which means that they survived on all hosts, they took a long time to settle, developed slowly through the life stages and were not highly fecund, in comparison to previous studies (Mathenge *et al.* 2009b). This was especially so for the cochineal biotypes on *C. pallida* since these had the lowest FI compared to either cochineal on both other plant species. Mathenge *et al.* (2009b) reported that 'cholla' thrived on *C. fulgida* var. *fulgida*, which was not tested in this study. Despite the low FI for the 'cholla' on *C. fulgida* var. *mamillata* in the present study, the FI was very similar to that calculated for *C. imbricata,* indicating that they were both almost equally acceptable hosts for the cochineal. Although the FI that we calculated was less than one, the relative performance on the different species can be used to determine whether the cochineals are likely to be sufficiently damaging to *C. pallida*. The relatively high FI for 'cholla' cochineal on *C. fulgida* var. *mamillata* is corroborated by the findings of Sheppard *et al.* (2012); Mathenge *et* *al.* (2009a); Mathenge *et al.* (2010a) and Mathenge *et al.* (2010b) who showed that 'cholla' thrives on *C. fulgida* var. *fulgida* and *C. fulgida* var. *mamillata* in South Africa and Zimbabwe. The field-based assessment by Klein *et al.* (2020) of the 'cholla' biotype also showed the effectiveness of this biotype for *C. fulgida* var. *fulgida* and *C. fulgida* var. *mamillata*. Since we know that the 'cholla' cochineal is effective in the field, we can assume that a similar FI to this cochineal would suggest an effective agent. Therefore, the results suggest that the 'cholla' cochineal should be effective against both *C. fulgida* var. *mamillata* and *C. imbricata,* but not against *C. pallida,* which had a lower FI.

The 'imbricata' biotype FI on *C. imbricata* shows that this biotype performed well on its host. This was also found to be the case in other studies where the FI of 'imbricata' on *C. imbricata* was greater than one (Mathenge *et al.* 2009a; Mathenge *et al.* 2010a, b). The FI of 'imbricata' on *C. pallida* and *C. fulgida* var. *mamillata* shows that this biotype can only survive, but not thrive, on these species. This result is corroborated by Mathenge *et al*. (2009b) who also tested this biotype of cochineal against *C. fulgida* and found that it was not particularly effective. The low fitness of the 'imbricata' cochineal was especially evident on *C. pallida*. From this, we can deduce that the 'imbricata' biotype will be much less damaging and less effective at controlling *C. pallida* than it is on the original target weed, *C. imbricata* (Mathenge *et al.* 2010a).

The discrepancies between the FI of the biotypes in this study and those calculated in previous studies (Mathenge *et al.* 2009a; Mathenge *et al.* 2010a; b) could be due to abiotic conditions such as temperature and soil fertility. These differences could also be because Sheppard *et al.* (2012); Klein *et al.* (2020); Mathenge *et al.* (2009a); Mathenge *et al.* (2010a); Mathenge *et al.* (2010b) did not use *C. fulgida* var. *mamillata* as one of the species they tested in their studies, but used the other variety of the same species, *C. fulgida* var. *fulgida*. Our temperatures could have been cooler in the CE room, which meant that some of our biotype-host mixes did not result in high fitness indices. Soil fertility, which affects the nutrient richness of plants is important for agents, since factors such as palatability are used by the agents in selecting the hosts they use (Paterson *et al.* 2012). This palatability is affected by the availability and levels of nitrogen, carbon and other trace elements for use by the potential agent and the potency and amount of defence compounds that the host produces (Awmack & Leather 2002).

The 'cholla' biotype had a broader host range than to the 'imbricata' biotype, because it survived on all three species and had a good impact on two of three target species. These outcomes were also observed by Jones *et al.* (2015), in which they found that out of the eight invasive alien *Cylindropuntia* species they screened, four could potentially be effectively controlled by the 'cholla' biotype; these were *C. fulgida*, *C. imbricata*, *C. kleiniae* and *C. tunicata* (Jones *et al.* 2015).

The development of the 'cholla' biotype on *C. pallida* had some of the factors necessary for a successful biocontrol agent relative to the 'imbricata' cochineal. These include larger numbers of crawlers surviving, which take shorter times to reach first moult and larger females producing more progeny. However, compared to the other factors necessary for success, those being short female developmental time, early commencement of reproduction, a FI > 1 and greater impact at a shorter time, the 'cholla' biotype is unlikely to be suitably damaging to *C. pallida*. The 'cholla' biotype also had a fast-settling rate on all three host species, including *C. pallida*, compared to the 'imbricata' biotype. It is characteristic of the 'cholla' biotype to have higher settling rates than other biotypes, even on host species that are not its own (Jones *et al*. 2015). This fast settling rate also shows the broad host range of this biotype compared to the others (Jones *et al*. 2015). These characteristics would make this biotype a better candidate for *C. pallida* control than the 'imbricata' biotype, but given the fact that the level of damage inflicted on *C. pallida* by the 'cholla' and 'imbricata' biotype was less than the damage which each of these caused on their host species, we can assume a lesser degree of control on *C. pallida* than what has been observed for *C. fulgida* and *C. imbricata* in the field (Klein *et al.* 2020; Paterson *et al.* 2021). Jones *et al.* (2015) also showed that the 'cholla' biotype on Australian *C. pallida* was ineffective, and South Africa *C. pallida* plants are likely to be very similar.

Therefore, the evidence from this study strongly suggests that both the 'cholla' and 'imbricata' cochineal will be less effective on *C. pallida* than on the target weeds that they are intended to control (*C. fulgida* and *C. imbricata*, respectively). Both cochineal biotypes had the lowest fitness index values when feeding on *C. pallida*, and neither the 'cholla' biotype nor the 'imbricata' biotype resulted in *C. pallida* mortality, while mortality of plants was recorded for both *C. fulgida* and *C. imbricata* when exposed to the correct cochineal species. In most cases, the results of these experiments were not statistically significant due to a large amount of variance in the data. This suggests that further replication would be beneficial. However, considering all the results, it is evident that *C. pallida* would not be controlled to the same extent as either *C. imbricata* or *C. fulgida* var. *mamillata,* using the two biotypes of *D. tomentosus* that are already available in South Africa.

Evidence from Australia, in the form of both pre-release efficacy assessments conducted under quarantine conditions, as well as observations in the field after the release of the agent, suggest that *D. tomentosus* 'californica var. parkerii' is sufficiently damaging against *C. pallida* to warrant release in South Africa (Paterson *et al.* 2021). The data collected in this thesis therefore confirms that *D. tomentosus* 'californica var. parkerii' is likely to be more damaging for *C. pallida* than any agents we already have available and should therefore be considered for release.

One final consideration before the release of the agent that should be investigated is the possible damage done by hybrids of the 'cholla' and 'imbricata' cochineals. The data from this thesis suggests that when both plants with both cochineals are present, good control of *C. pallida* is likely to be achieved. This may be due to the hybrid cochineals being more vigorous on *C. pallida*, or due to the high numbers of cochineal that can build up when both primary host plants of both cochineals, as well as the cochineals themselves, are present. Further experiments should be conducted by hybridizing the 'cholla' and 'imbricata' cochineals and then testing the hybrids on *C. pallida* while controlling cochineal density. This would determine whether hybrid cochineals could be used as biocontrol agents against *C. pallida* at sites where only *C. pallida* is present, the hybrids will only achieve the control if both primary host plants, *C. fulgida* and *C. imbricata*, are present at the same locality in the field.

Suppose hybrids are damaging to *C. pallida* without the primary host plants being present. In that case, the hybrids of the cochineal that are already present in the country could be used to control *C. pallida* rather than a new cochineal. However, if both the primary host plants must be present, then hybrids would only be damaging at very few sites, as there are very few sites in South Africa where all three plants are present, and many sites where *C. pallida* is problematic, and not growing with other cacti. If both primary host plants must be present to achieve control, then *D. tomentosus* 'californica var. parkerii' should be released.

In the rest of this chapter, I discuss some of the important issues relevant to the continuation of the *C. pallida* biocontrol programme in South Africa, including i) the importance of releasing the most damaging agent, which in this case is *D. tomentosus* 'californica var. parkerii'; ii) the benefits of conducting transfer projects, where much of the work that is required has already been developed elsewhere, such as the pre-release and post-release assessments of *D. tomentosus* 'californica var. parkerii' in Australia; iii) the hybridisation of biocontrol agents;

and iv) the importance of implementing biocontrol on emerging weeds such as *C. pallida*, before they become severely problematic on a landscape scale.

4.1. The importance of releasing the most damaging agent for *Cylindropuntia pallida* in South Africa

Most pre-release studies in biocontrol programs focus on the host-specificity of potential agents, but the potential efficacy of the agent is in many ways equally as important. If an agent is not sufficiently damaging to the target plant, it should not be released, and if some potential agents are more damaging than others, then only the most damaging of the agents, or the most damaging combination, should be released (Balciunas 2004, Sutton *et al.* 2021). Closely related target invasive alien plant species may be susceptible to the same biocontrol agents. In some cases, the same agent can be used to control multiple closely related plant target weeds (Walters *et al.* 2011; Rule & Hoffmann 2018). Suppose it is possible to use a biocontrol agent that is already present in the region of introduction to control a target weed. In that case, this could avoid the unnecessary release of new agents. The release of every agent comes with an intrinsic risk, and should therefore only be done if necessary (Sheppard *et al*. 2003, McClay & Balciunas 2005).

Some biocontrol agents become abundant but are not damaging to their target plants (Sands & Schotz 1985; Peschken & Derby 1992; McClay *et al.* 2002). Lack of agent effectiveness in damaging the target plants, whilst they become abundant in number, could lead to some agents exhibiting indirect non-target impacts (Holt & Hochberg 2001). Abundant agent populations have large consequences to food weds, providing food for generalist predators and parasitoids and thus have knock-on effects to the whole food web. For damaging agents, this effect will be temporary, because when the target weed populations decrease the agent population will follow. However, for agents that are not damaging the effect is permanent because the target weed population is never reduced. Therefore, ineffective agents have a much greater potential for indirect non-target impacts than effective agents.

The costs involved in researching and releasing biocontrol agents also make it necessary to release the most damaging agents (Morin *et al.* 2009). A benefit-cost analysis is a ratio that shows the return obtained from investing in a biocontrol program (Morin *et al.* 2009). This ratio could be expected to be great when a highly damaging biocontrol agent is used. The effectiveness of agents in damaging the target invasive alien plants and affecting their populations can be ascertained by conducting various field studies, together with laboratory or glasshouse studies and demographic modelling, when possible (Briese 2006; Morin *et al.* 2009).

It is difficult to accurately predict the success of the potential agent in the field, especially when the pre-release assessments are done in a laboratory or quarantine setting, so pre-release efficacy studies should be interpreted carefully. The success of a biocontrol agent depends on many different factors, including the damage of the agent on the plant, the ecology of the agent, which determines the population density of the agent in the area of release; and the ecology of the invasive plant, which determines whether the damage by the agent is significant enough to reduce the plant population (Cullen 1995; McFadyen 1998). The agent damage on the weed is relatively easy to determine, but the impact on the plant population is not (McFadyen 1998). Despite this, there are examples of studies that have measured the impact on the plant population, and some measure of efficacy or predicted damage is now a pre-requisite for all biocontrol agent releases. Goolsby *et al.* (2004) conducted a field study on the impact of a mite, *Floracarus perrepae* Knihinicki and Boczek (Eriophyidae), on the invasive plant, *Lygodium microphyllum* (Cav.) R. Br. (Schizaeaceae). This study, conducted in the native country of the weed and mite, showed that the mite caused significant damage to the plant, destroying both above- and below-ground biomass (Goolsby *et al.* 2004). This study concluded that this mite was likely to be a damaging agent if released (Goolsby *et al.* 2004). Although the agent is established in the U.S.A., its impact is variable (Winston *et al.* 2014), so pre-release assessments in the laboratory could not accurately predict the impact in the field. Another quarantine-based study on the impact of two potential agents, *Tetramesa romana* Walker (Eurytomidae) and *Rhizaspidiotus donacis* Leonardi (Diaspididae), on the invasive alien *Arundo donax* L. (Poaceae) showed that the agents working together led to significant damage of the leaves and branches of the weed (Goolsby *et al.* 2009). Both these agents were subsequently released in the U.S.A., and while it is too soon to assess the damage by *R. donacis*, which was released later and has a relatively slow life-cycle and rate of increase, the stem mining wasp *T. romana* is effective in at least some parts of the country (Marshall *et al.* 2018; Braman *et al.* 2021). So in other cases, pre-release efficacy tests have accurately predicted that an agent will be damaging.

To avoid unnecessary biocontrol program costs, it may be better to conduct impact assessments prior to host-specificity testing for cochineals, then test the host-specificity of the most damaging agent(s). Screening multiple, potentially damaging agents for efficacy would be less costly than conducting host-specificity tests on many agents, some of which might end up not being released. The level of specificity of cochineal, and the fact that the Cactaceae are endemic to the Americas, means that all cochineals are suitably host-specific for release in Africa, and it is therefore the efficacies of the cochineal that should be tested first (De Lotto 1974; Gunn 1979). Host-specificity testing must still be conducted, but as all cochineals are expected to be host-specific, the efficacy should be used to select the particular biotypes for release. Releasing the most damaging agent is important to avoid unnecessary costs and risks posed by the introduction of an ineffective agent, which would not bring any benefit (Balciunas 2004; McClay & Balciunas 2005; Balciunas & Smith 2006). Pre-release damage assessments should then be rigorously conducted to ensure that the detrimental non-target effects which may occur if an agent is released without thorough research are avoided (Ewel *et al.* 1999; Louda *et al.* 2003).

One significant advantage for the *C. pallida* biocontrol programme is that pre-release efficacy testing to assess the relative impacts of various potential agents was done in Australia prior to the release of *D. tomentosus* ‘californica var. parkerii’ in that country, and that evidence from the field suggests that it is sufficiently damaging after release in Australia. While the pre-release data collected under laboratory conditions is valuable, the post-release data from Australia is very good evidence that *D. tomentosus* ‘californica var. parkerii’ will be effective in South Africa. A high benefit-cost ratio is likely to be realised once *D. tomentosus* ‘californica var. parkerii’ has been released in the country. The level of control achieved is also unlikely to be reduced due to climate, since the climates of South Africa and Australia are similar (Richardson & Thuiller 2007; van Wilgen *et al.* 2020).

4.2. The benefits of conducting transfer projects

The sharing of biocontrol agents, which have been successful in other countries, is beneficial because it saves costs that would have been incurred by the country to which the biocontrol agent is being transferred (McFadyen 1998; Zachariades *et al.* 2017). Biocontrol agents which have been successful in one country should be shared, since they are likely to succeed in other countries (Peschken & McClay 1995; McFadyen 1998; Paterson *et al.* 2021). Sharing agents also fosters much needed collaboration between countries, for example, if South Africa receives a biocontrol agent from Australia, it can in turn share this with its neighbouring countries which also have the same invasive alien species within their borders (Zachariades *et al.* 2017; Paterson et al. 2019). Transfer projects also create an opportunity for research into targeting highly problematic invasive alien species in some countries which might only be emerging as new problems in other countries (Zachariades *et al.* 2017). This may enable them to be controlled quickly and with negligible costs before they become problematic and have severe ecological consequences.

During the early decades of biocontrol work in South Africa, the agents introduced for the control of Cactaceae were all 'transfer projects' using biocontrol agents that had already been introduced and were successful in other countries, particularly Australia (Zachariades *et al.* 2017). Therefore, sharing biocontrol agents has been happening for many years in the biocontrol of weeds. The 1950s and 1960s saw transfer or piggy-back projects started on *Lantana camara* L. (Verbenaceae) and *Hypericum perforatum* L. (Clusiaceae) in South Africa, and this was followed by several cactus piggy-back projects (Zachariades *et al.* 2017).

The *D. tomentosus* 'cholla' biotype biocontrol agent was shared between South Africa and Australia (Paterson *et al.* 2019). In South Africa, it was used to control *C. fulgida* var. *fulgida* and *C. fulgida* var. *mamillata*, while in Australia, it was used to control *C. fulgida* var. *mamillata* (Paterson *et al.* 2019; Klein *et al.* 2020). The 'imbricata' cochineal was also initially imported to South Africa from Australia (Zimmermann & Moran 1991a). Sharing biocontrol agents, and information necessary for the control of invasive alien plants, between countries, has helped foster and develop important collaborations. These have been beneficial to all stakeholders involved in advancing and making successful biocontrol endeavours. Likely, these collaborations will still be essential for future biocontrol work and they are needed now to ensure the success of biocontrol of *C. pallida* in South Africa.

4.3. Hybridisation of biocontrol agents

Another consideration before the release of a new agent is the impact of hybridisation. The 'cholla' and 'imbricata' biotypes are reproductively compatible and, therefore, can hybridise (Mathenge *et al.* 2010b). When in the same cages these biotypes highly likely hybridised because they do not reproduce parthenogenetically (Mathenge *et al.* 2009a). The cochineal are also not highly mobile; the males mate locally with females who are sedentary (Mathenge *et al.* 2010b). The biotypes' overlapping generations also increase the chances of hybridisation and intergenerational breeding (Cook 2001; Cook & Rowell 2007; Mathenge *et al.* 2010b). Our study indicates that the potential hybrids outperformed the pure 'imbricata' biotype in killing *C. pallida* plants. This potential hybrid also outdid both biotypes in killing *C. imbricata* plants. These findings agree with those that showed the improved performance of 'cholla' and ‘imbricata’ biotypes’ hybrid offspring due to release from negative inbreeding effects (Burton *et al.* 1999; Mathenge *et al.* 2010b). Together with this, since F_1 hybrid offspring are not host-specific, they appear to have utilized all the species in their environment in our study (Hoffmann *et al.* 2002; Mathenge *et al.* 2010b).

The ‘cholla x imbricata’ cochineal appears to have taken longer to kill *C. pallida* plants compared to the ‘cholla’ and ‘imbricata’ biotypes on their hosts. All the *C. imbricata* plants were also killed by the ‘cholla x imbricata’ cochineal, whilst the impact of the hybrid offspring on *C. fulgida* var. *mamillata* was comparable to that of the ‘cholla’ biotype on this host. These findings suggest that hybridisation resulted in increased fitness, known as hybrid vigour, a phenomenon that has been observed mainly for F_1 hybrid offspring of *D. tomentosus* and is compounded due to reduced host-specificity in these hybrids, which allows large populations to build up on multiple host plants (Mathenge *et al.* 2010b). This decreased host-specificity is beneficial for biocontrol because the offspring are able to develop on and contribute to the biocontrol of these cacti and increase the population density of the agent in the field (Mathenge *et al.* 2010b).

Hybrid breakdown or reduced fitness has been observed for F_2 and subsequent backcross generations of cochineal (Blows 1993; Breeuwer & Werren 1995; Armbruster *et al.* 1997; Aspi 2000; Gilk *et al.* 2004; Peer & Taborsky 2005). The recombination of genes in F_2 and backcross generations leads to the reversal of host-specificity in some progeny; this was seen in the crosses between *D. opuntiae* cochineal biotypes (Hoffmann *et al.* 2002). This was seen as negative for biocontrol using *D. opuntiae* because the F_2 offspring, which were host-specific, did not survive (Hoffmann *et al.* 2002). However, Mathenge *et al.* (2010b) argue that the effects may not be as negative for the biocontrol of *C. fulgida* and *C. imbricata* using *D. tomentosus* because host-specific hybrids return to the parents’ preferred host of the ‘cholla’ biotype would still impact *C. imbricata* and host-specific ‘imbricata’ biotype can kill small *C. fulgida* plants (Mathenge *et al.* 2010b).

The sympatric association of *C. pallida*, *C. imbricata* and *C. fulgida* var. *mamillata*, which would likely lead to the hybridisation of the cochineal biotypes they host, could be beneficial for the biocontrol of these cacti. This is especially true of the F_1 hybrid offspring for the control of *C. pallida* and *C. imbricata*. It could be beneficial to intentionally release these cochineals together even in areas where the cacti do not occur in sympatry. Studies on the characteristics displayed by F_2 and subsequent generations would be beneficial to see how they would impact biocontrol. However, it could be expected that backcrosses have minimal negative impacts on the control of these plants, as argued by Mathenge *et al.* (2010b).

The hybridisation experiment carried out is a practical way of observing what would happen in areas that these would grow together. This sympatric association would increase the chances of hybridisation between the biotypes. However, although it is likely that hybridisation did occur, we cannot be certain that hybridisation resulted to the increased mortality of *C. pallida*. Another possibility is that each biotype used the cactus species it is adapted to, then excess crawlers settled on the non-host cacti, which resulted in the death of those plants due to high population densities of the agent. Therefore, though our results did seem to indicate better control of the *C. pallida* plants, they do not mean that releasing both the 'imbricata' and 'cholla' at a site with *C. pallida* growing alone would result in the plants being controlled. Further experiments, to determine whether the hybrids are effective without alternative host plants, are required.

Hybridisation experiments in the lab could be conducted following the methods outlined by Mathenge *et al.* (2010b). They took 30, one-day-old crawlers of the 'imbricata' biotype and placed them on each of the four cladodes of *C. imbricata* and 30 one-day-old crawlers from the 'cholla' biotype and placed them on each of four *C. fulgida* cladodes. Each cladode was placed in a cage separate from the other cladodes. After male pupation, all females were removed from two cladodes of each experimental group, leaving only males. On the other two cladodes, of each experimental group, the males were removed leaving only females. The cladodes containing 'cholla' females were paired with the cladodes containing 'imbricata' males; reciprocal crosses containing 'cholla' males paired with cladodes containing 'imbricata' females, were also done. The control treatments to produce pure lineages were also done by pairing males and females of the same biotype. All insects in these treatments were allowed to mate freely and produce F_1 generation crawlers. In repeating these experiments, the hybrid F_1 generation crawlers could be introduced onto *C. imbricata*, *C. fulgida* var. *mamillata* and *C. pallida* to see if they will lead to enhanced control of the cacti. The releases of hybrid F_1 generation crawlers cultured in the lab could be done on potted plants in the lab and the field. This would enable us to see how hybrids control *C. pallida* without the other host plants.

4.4. The importance of implementing biocontrol on emerging weeds such as *C. pallida*

The endeavour to control *C. pallida* is part of targeting emerging invasive alien plants in biocontrol research. Emerging weeds are invasive alien plants still in the early stages of invasion (Olckers 2004). Swift work to deal with these means more likelihood for success and is more beneficial than targeting them when they are well established (Olckers 2004; Zachariades *et al.* 2017). *Salvinia molesta*, *Pistia stratiotes* L., *Ageratina riparia* (Regel) R. M. King & H. Rob. (Asteraceae), *Ageratina adenophora* (Spreng.) R.M. King & H. Rob. (Asteraceae), *Cirsium vulgare* (Savi) Ten. (Asteraceae), *H. perforatum* and some *Opuntia* species are examples of invasive alien species which were emerging weeds when targeted for control and might have become more problematic if they had not been targeted early (Olckers 2004; Zachariades *et al.* 2017). These programmes targeting emerging weeds have largely been successful, with 36% under complete control and 24% under substantial control (Olckers 2004). For example, *H. perforatum* was decreased to insignificant levels in South Africa because of being targeted while it was still an emerging weed, using two biocontrol agents, namely, *Chrysolina quadrigemina* Suffrian (Chrysomelidae) and *Zeuxidiplosis giardi* Kieffer (Cecidomyiidae) (Olckers 2004). The success of these biocontrol agents led to chemical control of this plant being suspended, and such outcomes led to economic and environmental benefits (Olckers 2004).

Cylindropuntia pallida is also an emerging weed, its legal status in South Africa has changed over the years, moving from being unlisted in 2011 (Walters *et al*. 2011), to being listed in the SUSPECT (Species Under Surveillance – Possible Eradication or Containment Targets) list in 2013, when it was suspected as a potential risk (Wilson *et al.* 2013). During this time, it was targeted for eradication as part of the South African National Biodiversity Institute's Early Detection Rapid Response (EDRR) programme (Paterson *et al.* 2021). Plants targeted for eradication are in their early stages of invasion because widespread plants cannot be eradicated (Wilson *et al.* 2013). Chemical control was used, which was effective in reducing the plant densities at some sites, but significant regrowth was recorded even after many follow-up chemical treatments, and it has become clear that the plant cannot be eradicated from South Africa (Paterson *et al.* 2021). It is now perceived as a threat, becoming increasingly problematic, since it is spreading and forming dense infestations (Paterson *et al.* 2019). Large populations of this cactus can now be found in South Africa and Namibia's arid Karoo and

Kalahari regions (Paterson *et al.* 2021). The inability of chemical control to eradicate *C. pallida* and its increasing populations led SANBI to request the South African Cactus Working Group to look into the prospects of biocontrol for this plant (Paterson *et al.* 2021). Although the distribution and abundance of *C. pallida* in South Africa is rapidly expanding, it can still be considered in the early stages of the invasion. Targeting this plant with an effective biocontrol agent readily available as a piggy-back project from Australia, would ensure that the target weed is controlled before it becomes more widespread and problematic, thus increasing the chances of effective and permanent control, and minimising the negative ecological consequences of the weed.

Conclusion

At present, the release of *D. tomentosus* 'californica var. parkerii' is considered the best option for controlling *C. pallida* in South Africa. However, as I have suggested previously, lab and field experiments could be done to see whether a 'cholla x imbricata' hybrid would be able to control *C. pallida*. If hybrids are effective at sites where only *C. pallida* are present, then the new cochineal is not required, while if the presence of the other cacti is needed for the hybrids to be effective, then the new cochineal is needed. *Dactylopius tomentosus* 'californica var. parkerii' is available from Australia, where all the relevant host-specificity studies have already been conducted. Transfer projects such as this one, where biocontrol agents and knowledge are shared between countries, have been very successful in the past (Canavan *et al.* 2021). Since the effects of hybridisation between the three cochineal biotypes are not yet known, it would be safe to release the 'californica var. parkerii' biotype in areas where there is no chance of it cross-breeding with another biotype, but hybridisation studies should be conducted using the new cochineal biotype before releasing at other sites. Although the agent is known to be safe and effective, these studies would supply more information about the biotypes and the possible interactions which would be the outcome of releasing this new agent into the field in South Africa, where both the 'cholla' and 'imbricata' biotypes are present.

None of the cochineal biotypes that are used for biocontrol in South Africa are suitably damaging to *C. pallida* to warrant their use as biocontrol agents for this species.Therefore, *D. tomentosus* 'californica var. parkerii' is recommended for release based on its host-specificity and impact to *C. pallida* in Australia. This release should be conducted pending studies to determine whether hybrids of the 'cholla' and 'imbricata' biotypes are likely to be effective against *C. pallida*, and studies to evaluate the implications of hybridisation of the new cochineal with those already present in the country.

References

AL-ROBAI, S.A., HOWLADAR, S.M., MOHAMED, H.A. & AHMED, A.A. 2018. *Cylindropuntia rosea* (DC.) Backeb, (Cactaceae): a new generic alien record in the flora of Saudi Arabia. *Journal of Asia-Pacific Biodiversity* **11**: 320-323.

ANDERSON, E.F. 2001. The cactus family. Timber Press, Portland, Oregon, U.S.A..

ANNECKE D.P. & MORAN V.C. 1978. Critical reviews of biological pest control in South Africa. 2. The prickly pear, *Opuntia ficus-indica* (L.) Miller. *Journal of the Entomological Society of Southern Africa* **412**: 161-188.

ARMBRUSTER, P., BRADSHAW, W.E. & HOLZAPPEL, C.M. 1997. Evolution of the genetic architecture underlying fitness in the pitcher-plant mosquito, *Wyeomyia smithii*. *Evolution* **51**: 451–458.

ASPI, J. 2000. Inbreeding and outbreeding depression in male courtship song characters in *Drosophila montana*. *Heredity* **84**: 273–282.

AWMACK, C.S. & LEATHER, S.R. 2002. Host Plant Quality and Fecundity in Herbivorous Insects. *Annual Review of Entomology* **47**: 817-844.

BALDWIN, I.T. 2010. Plant volatiles. *Current Biology*, **20** (9): R392–R397.

BARANYOVITS, F.L.C. 1978. Cochineal carmine: an ancient dye with a modern role. *Endeavor* **2**:85-92.

BARTON, J., FOWLER, S.V., GIANOTTI, A.F., WINKS, C.J., DE BEURS, M., ARNOLD, G.C. & FORRESTER, G. 2007. Successful biocontrol of mist flower (*Ageratina riparia*) in New Zealand: Agent establishment, impact and benefits to the native flora. *Biological Control* **40**: 370–385.

BALCIUNAS, J.K. 2004. Are mono-specific agents necessarily safe? The need for pre-release assessment of probable impact of candidate biocontrol agents, with some examples. In: Cullen, J.M., Briese, D.T., Kriticos, D.J., Lonsdale, W.M., Morin, L. & Scott, J.K. (Eds.) *Proceedings of the XIth International Symposium on Biocontrol of Weeds*. 252-257. CSIRO Entomology, Canberra, Australia.

BALCIUNAS, J. & SMITH, L. 2006. Pre-release efficacy assessment, in quarantine, of a tephritid gall fly being considered as a biocontrol agent for Cape-ivy (*Delairea odorata*). *Biological Control* **39**: 516-524.

BARRATT, B.I.P., MORAN, V.C., BIGLER, F. & VAN LENTEREN, J.C. 2018. The status of biocontrol and recommendations for improving uptake for the future. *BioControl* **63**: 155–167.

BARRETT, D.P., FOWLER, S.V., SUBBARAJ, A.K., GROENTEMAN, R. & CLAVIJO-MCCORMICK, A. 2021. Metabolomic analysis of host plant biochemistry could improve the effectiveness and safety of classical weed biocontrol. *Biological Control* **160**: 104663.

BEAN, D.W., KAZMER, D.J., GARDNER, K., THOMPSON, D.C., REYNOLDS, B.P., KELLER, J.C. & GASKIN, J.F. 2013. Molecular Genetic and Hybridization Studies of *Diorhabda* spp. released for Biocontrol of *Tamarix*. *Invasive Plant Science and Management* **6**: 1–15.

BLOWS, M.W. 1993. The genetics of central and marginal populations of *Drosophila serrata*. ii. Hybrid breakdown in fitness components as a correlated response to selection for desiccation resistance. *Evolution* **47**: 1271–1285.

BRAMAN, C.A., LAMBERT, A.M., ÖZSOY, A.Z., HOLLSTIEN, E.N., SHEEHY, K.A., MCKINNON, T., MORAN, P., GASKIN, J.F., GOOLSBY, J.A. & DUDLEY, T.L. 2021. Biology of an Adventive Population of the Armored Scale *Rhizaspidiotus donacis*, a Biocontrol Agent of *Arundo donax* in California. *Insects* **12**, 588.

BREEUWER, J.A.J. & WERREN, J.H. 1995. Hybrid breakdown between two haplodiploid species: the role of nuclear and cytoplasmic genes. *Evolution* **49**: 705–717.

BRIESE, D.T. 2003. The centrifugal phylogenetic method used to select plants for host-specificity testing of weed biocontrol agents: Can and should it be modernised? In: Spafford, H.J. & Briese, D.T. (Eds.) *Proceedings of the CRC for Australian Weed Management Biocontrol of Weeds Symposium and Workshop*. 23-33. University of Western Australia, Perth, Western Australia.

BRIESE, D.T. 2006. Can an *a priori* strategy be developed for biocontrol? The case of *Onopordum* spp. thistles in Australia. *Australian Journal of Entomology* **45**: 317–323.

BRIESE, D.T., WALKER, A., PETTIT, W. & SAGLIOCCO, J-L. 2002. Host-specificity of candidate agents for *Onopordum* spp. thistles in Australia: An assessment of testing procedures. *Biocontrol Science & Technology* **12**: 149-163.

BROOKS, M.L., D'ANTONIO, C.M., RICHARDSON, D.M., GRACE, J.B., KEELEY, J.E., DITOMASO, J.M., HOBBS, R.J., PELLANT, M. & PYKE, D. 2004. Effects of Invasive Alien Plants on Fire Regimes. *BioScience* **54**(7): 677-688.

BURTON, R.S., RAWSON, P.D. & EDMANDS, S. 1999. Genetic architecture of physiological phenotypes: empirical evidence for coadapted gene complexes. *American Zoologist* **39**: 451–462.

CAMPANA, M.G., GARCÍA, N.M.R & TUROSS, N. 2015. America's red gold: multiple lineages of cultivated cochineal in Mexico. *Ecology and Evolution* **5**(3): 607–617.

COCK, M.J.W., VAN LENTEREN, J.C., BRODEUR, J., BARRATT, B.I.P., BIGLER, F., BOLCKMANS, K., CÔNSOLI, F.L., HAAS, F., MASON, P.G. & PARRA, J.R.P. 2010. Do new access and benefit sharing procedures under the convention on biological diversity threaten the future of biocontrol? *BioControl* **55**: 199–218.

CANAVAN, K., PATERSON, I.D., IVEY, P., SUTTON, G.F. & HILL, M.P. 2021. Prioritisation of targets for weed biocontrol III: A tool to identify the next targets for biocontrol in South Africa and set priorities for resource allocation. *Biocontrol Science and Technology* **31**: 584-601.

CARRIÈRE, Y., MASAKI, S. & ROFF, D.A. 1997. The coadaptation of female morphology and offspring size: a comparative analysis in crickets. *Oecologia* **110**: 197–204.

CHÁVEZ-MORENO, C.K., TECANTE, A., CASAS, A. & CLAPS, L.E. 2011. Distribution and Habitat in Mexico of *Dactylopius* Costa (Hemiptera: Dactylopiidae) and their Cacti Hosts (Cactaceae: Opuntioideae). *Neotropical Entomology* **40**(1): 62-71.

CHIPPENDALE, J.F. 1995. The biocontrol of Noogoora burr (*Xanthium occidentale*) in Queensland: an economic perspective. In: Delfosse, E.S. & Scott, R.R. (Eds.) *Proceedings of the VIIIth International Symposium on Biocontrol of Weeds*. 185–192. DSIR/CSIRO, Melbourne, Australia.

CLAPS, L.E. & DE HARO, M.E. 2001. Coccoidea (Insecta: Hemiptera) associated with Cactaceae in Argentina. *Journal of the Professional Association for Cactus Development* **4**: 77–83.

COOK, L.G. 2001. Extensive chromosomal variation associated with taxon divergence and host-specificity in the gall-inducing scale insect *Apiomorpha munita* (Schrader) (Hemiptera: Sternorrhyncha: Coccoidea: Eriococcidae). *Biological Journal of the Linnean Society* **72**: 265–278.

COOK, L.G. & ROWELL, D.M. 2007. Genetic diversity, host-specificity and unusual phylogeography of a cryptic, host-associated species complex of gall-inducing scale insects. *Ecological Entomology* **32**: 506–515.

CRAIG, T.P., HORNER, J.D. & ITAMI, J.K. 2001. Genetics, experience, and host-plant preference in *Eurosta solidaginis*: implications for host shifts and speciation. *Evolution* **55**: 773–782.

CROOK, R. & MOTTRAM, R. 2001. *Opuntia* Index Part 7: Nomenclatural note and P–Q. *Bradleya* **19**: 91-116.

CULLEN, J.M. 1995. Predicting effectiveness: fact and fantasy. In: Delfosse, E.S. & Scott, R.R. (Eds.) *Proceedings of the Eighth International Symposium on Biocontrol of Weeds*. 103–109. DSIR/CSIRO, Melbourne, Australia.

CZYPIONKA, K. & HILL, M.P. 2007. The relationship between female pupal mass and fecundity of *Gratiana spadicea* (Klug, 1829) (Coleoptera: Chrysomelidae). *African Entomology* **15**(2): 380–382.

DAMBROSKI, H.R., LINN, C., BERLOCHER, S.H., FORBES, A.A., ROELOFS, W. & FEDER, J.L. 2005. The genetic basis for fruit odor discrimination in *Rhagoletis* flies and its significance for sympatric host shifts. *Evolution* **59**: 1953–1964.

DE BEER, H. 1986. Imbricate cactus. Farming in South Africa. Weeds A. 14. Pretoria, South Africa, Government Printer.

DE LOTTO, G. 1974. On the status and identity of the cochineal insects (Homoptera: Coccoidea: Dactylopiidae). *Journal of the Entomological Society of South Africa* **37**: 167–193.

DELTORO, V., BALLESTER, G., OLTRA, J.E., PÉREZ-BOTELLA, J., PÉREZ-ROVIRA, P., GÓMEZ-SERRANO, M.A. & JIMÉNEZ, J. 2013. The practicalities of eradicating an extremely invasive cactus: Hudson pear *Cylindropuntia rosea* in the Valencia region (East Spain). In: Alonzi, A. (Ed.) *Aliens* 23-27.

DIEHL, S.R. & BUSH, G.L. 1984. An evolutionary and applied perspective of insect biotypes. *Annual Review of Entomology* **29**: 471–504.

DODD, A.P. 1940. *The Biological Campaign against Prickly Pear*. 177 pp. Brisbane, Australia, Commonwealth Prickly Pear Board Bulletin.

DOELEMAN J.A. 1989. Biocontrol of *Salvinia molesta* in Sri Lanka: an assessment of costs and benefits. *Australian Centre for International Agricultural Research Technical Report* **12**, Canberra.

DOWNEY, P.O. & PATERSON, I.D. 2016. Encompassing the relative non-target risks from agents and their alien plant targets in biocontrol assessments. *BioControl* **61**: 615–630.

DRÈS, M. & MALLET, J. 2002. Host races in plant-feeding insects and their importance in sympatric speciation. *Philosophical Transactions of the Royal Society of London, Series B* **357**: 471–492.

EDMANDS, S. 2002. Does parental divergence predict reproductive compatibility? *Trends in Ecology & Evolution* **17**: 520–527.

EWEL, J.J., O'DOWD, D.J., BERGELSON, J, DAEHLER, C.C., D'ANTONIO, C.M., GÓMEZ, L.D., GORDON, D.R., HOBBS, R.J., HOLT, A., HOPPER, K.R., HUGHES, C.E., LAHART, M., LEAKEY, R.R.B., LEE, W.G., LOOPE, L.L., LORENCE, D.H., LOUDA, S.M., LUGO, A.E., MCEVOY, P.B., RICHARDSON, D.M., & VITOUSEK, P.M. 1999. Deliberate Introductions of Species: Research Needs Benefits can be reaped, but risks are high. *BioScience* **49**(8): 619-620.

FLORES-HERNÁNDEZ, A., MURILLO-AMADOR, B., RUEDA-PUENTE, E.O., SALAZAR-TORRES, J.C., GARCÍA-HERNÁNDEZ, J.L. & TROYO-DIÉGUEZ, E. 2006. Reproduction of wild cochineal *Dactylopius opuntiae* (Homoptera: Dactylopiidae). *Revista Mexicana de Biodiversidad* **77**: 97–102.

FRY, J.D. 1999. The role of adaptation to host plants in the evolution of reproductive isolation: negative evidence from *Tetranychus urticae* Koch. *Experimental and Applied Acarology* **23**: 379–387.

GILK, S., WANG, I., HOOVER, C., SMOKER, W., TAYLOR, S.G., GRAY, A. & GHARRETT, A.J. 2004. Outbreeding depression in hybrids between spatially separated pink salmon, *Oncorhynchus gorbuscha*, populations: marine survival, homing ability, and variability in family size. *Environmental Biology of Fishes* **69**: 287–297.

GILREATH, M.E. & SMITH, J.W. 1987. Bionomics of *Dactylopius confusus* (Homoptera: Dactylopiidae). *Annals of the Entomological Society of America* **80**: 768–774.

GITHURE, C.W., ZIMMERMANN, H.G. & HOFFMANN, J.H. 1999. Host-specificity of biotypes of *Dactylopius opuntiae* (Cockerell) (Hemiptera: Dactylopiidae): prospects for biocontrol of *Opuntia stricta* (Haworth) Haworth (Cactaceae) in Africa. *African Entomology* **7**: 43–48.

GOOLSBY, J.A., SPENCER, D., & WHITEHAND, L. 2009. Pre-release assessment of impact on *Arundo donax* by the candidate biocontrol agents, *Tetramesa romana* (Hymenoptera: Eurytomidae) and *Rhizaspidiotus donacis* (Homoptera: Diaspididae) under quarantine conditions. *Southwestern Entomologist* **34**: 359–376.

GOOLSBY, J.A., ZONNEVELD, R. & BOURNE, A. 2004. Prerelease Assessment of Impact on Biomass Production of an Invasive Weed, *Lygodium microphyllum* (Lygodiaceae: Pteridophyta), by a Potential Biocontrol Agent, *Floracarus perrepae* (Acariformes: Eriophyidae). *Environmental Entomology* **33**(4): 997-1002.

GUERRA, G.P. & KOSZTARAB, M. 1992. Biosystematics of the family Dactylopiidae (Homoptera: Coccineae) with emphasis on the life cycle of *Dactylopius coccus* Costa: studies on the morphology and systematics of scale insects No. 16. Bulletin No. 92–1. Blacksburg, Virginia, Virginia Agricultural Experiment Station, Virginia Polytechnic Institute and State University.

GULLAN, P.J. & MARTIN, J.H. 2003. Sternorrhyncha. Jumping plant lice, whiteflies, aphids and scale insects. In: Resh, V.H. & Cardé, R.T. (Eds.) *Encyclopaedia of Insects*. 1079–1089. Academic Press, London, UK.

GUNN, B.H. 1978. Sexual dimorphism in the first instar of the cochineal insect *Dactylopius austrinus* De Lotto (Homoptera: Dactylopiidae). *Journal of Entomological Society of Southern Africa* **41**: 333–356.

GUNN, B.H. 1979. Dispersal of the cochineal insect *Dactylopius austrinus* De Lotto (Homoptera: Dactylopiidae). PhD Dissertation. Rhodes University, Grahamstown, South Africa.

GURR, G.M., BARLOW, N.D., MEMMOTT, J., WRATTEN, S.D., & GREATHEAD, D.J. 2000. A History of the Methodological, Theoretical, and Empirical Approaches to Biocontrol. In: Gurr, G. & Wratten, S.D. (Eds.) *Biocontrol: Measures of Success*. 3-36. Springer Science & Business Media, Dodrecht.

HARRIS, P. 1971. Biocontrol of Weeds: Weeds biocontrol. *Environmental Letters* **2**(2): 75-88.

HASSALL, M., WALTERS, R.J., TELFER, M., HASSALL, M.R.J. 2006. Why does a grasshopper have fewer, larger offspring at its range limits? *Journal of Evolutionary Biology* **19**: 267–276.

HENDERSON, L. 2001. *Alien Weeds and Invasive Plants: A Complete Guide to Declared Weeds and Invaders in South Africa. Handbook No. 12*. 300 pp. Roodeplaat, South Africa, ARC, Plant Protection Research Institute.

HENDERSON, L. & WILSON, J.R.U. 2017. Changes in the composition and distribution of alien plants in South Africa: An update from the Southern African Plant Invaders Atlas. *Bothalia* 47(2): a2172.

HILL, M.P., MORAN, V.C., HOFFMANN, J.H., NESER, S., ZIMMERMANN, H.G., SIMELANE, D.O., KLEIN, H., ZACHARIADES, C., WOOD, A.R., BYRNE, M.J., PATERSON, I.D., MARTIN, G.D. & COETZEE, J.A. 2020. Chapter 19: More than a century of biocontrol against invasive alien plants in South Africa: A synoptic view of what has been accomplished. In: van Wilgen, B.W., Measey, J., Richardson, D.M., Wilson, J.R. & Zengeya, T.A. (Eds.) *Invading Nature - Springer Series in Invasion Ecology: Biological Invasions in South Africa*. 553-572. (0; 18).

HINZ, H.L., WINSTON, R.L. & SCHWARZLÄNDER, M. 2019. How safe is weed biocontrol? A global review of direct non-target attack. *Quarterly Review of Biology* **94**: 1-27.

HINZ, H.L., WINSTON, R.L. & SCHWARZLÄNDER, M. 2020. A global review of target impact and direct nontarget effects of classical weed biocontrol. *Current Opinion in Insect Science* **38**: 48–54.

HOFFMANN, J.H. 2004. Biotypes, hybrids and biocontrol: Lessons from cochineal insects on *Opuntia* weeds. In: Cullen, J.M., Briese, D.T., Kritikos, D.J., Lonsdale, W.M., Morin, L. & Scott J.K. (Eds.) *Proceedings of the XI international symposium on biocontrol of weeds*. 238–286. CSIRO Entomology, Canberra, Australia.

HOFFMANN, J.H., IMPSON, F.A.C. & VOLCHANSKY, C.R. 2002. Biocontrol of cactus weeds: implications of hybridization between control agent biotypes. *Journal of Applied Ecology* **39**: 900–908.

HOFFMANN, J.H., MORAN, V.C. & HILL, M.P. 2019. Conceptualizing, categorizing and recording the outcomes of biocontrol of invasive plant species, at a population level. *Biological Control* **133**: 134–137.

HOFFMANN, J.H., MORAN, V.C. & ZIMMERMANN, H.G. 1999. Integrated management of *Opuntia stricta* (Haworth) Haworth (Cactaceae) in South Africa: enhanced role of two, renowned, insect agents. In: Olckers, T. & Hill, M.P. (Eds.) *Biocontrol of weeds in South Africa (1990–1998)*. 15–20. African Entomology Memoir.

HOLT, R.D., HOCHBERG, M.E. 2001. Indirect interactions, community modules and biocontrol: a theoretical perspective. In: Wajnberg, E., Scott, J.K., & Quimby, P.C. (Eds.) *Evaluating Indirect Ecological Effects Of Biocontrol*. 13–37. Wallingford, UK, CABI Publishing.

HOPPER, K.R. 2001. Research needs concerning non-target impacts of biocontrol introductions. In Wajnberg, E., Scott, J.K. & Quimby, P.C. (Eds.) *Evaluating indirect ecological effects of biocontrol*. 39–56. Wallingford, New Zealand: CABI Publishing.

HOSKING, J.R., CONN, B.J., LEPSCHI, B.J. & BARKER, C.H. 2007. Plant species first recognized as naturalised for New South Wales in 2002 and 2003, with additional comments on species recognized as naturalised in New South Wales in 2000-2001. *Cunninghamia* **10**:139-166.

JOHNSON, S.B., HOSKING, J.R., CHINNOCK, R.J. & HOLTKAMP, R.H. 2009. The biology of Australian weeds 53: *Cylindropuntia rosea* (DC.) Backeb. and *Cylindropuntia tunicata* (Lehm.) FM Knuth. *Plant Protection Quarterly* **24**(2): 42.

JONES, P., HOLTKAMP, R. & DAY, M. 2016. The host range of four new biotypes of *Dactylopius tomentosus* (Hemiptera: Dactylopiidae) from southern U.S.A. and their potential as biocontrol agents of *Cylindropuntia* spp. (Cactaceae) in Australia: Part II. *Biocontrol Science and Technology* **26**(8): 1033–1047.

JONES, P.K., HOLTKAMP, R.H., PALMER, W.A. & DAY, M.D. 2015. The host range of three biotypes of *Dactylopius tomentosus* (Lamarck) (Hemiptera: Dactylopiidae) and their potential as biocontrol agents of *Cylindropuntia* spp. (Cactaceae) in Australia. *Biocontrol Science and Technology* **25**(6): 613–628.

JULIEN, M.H. & GRIFFITHS, M.W. 1998. Biocontrol of Weeds: A World Catalogue of Agents and their Target Weeds. 223 pp. Wellingford, UK, CAB International.

KAPLAN, H., WILSON, J.R.U., KLEIN, H., HENDERSON, L., ZIMMERMANN, H.G., MANYAMA, P., IVEY, P., RICHARDSON, D.M. & NOVOA, A. 2017. A proposed national strategic framework for the management of Cactaceae in South Africa. *Bothalia - African Biodiversity & Conservation* **47**(2): 1-12.

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

KERGOAT G.J., DELOBEL, A., FEDIERE, G., LE RÜ, B. & SILVAIN, J. 2005. Both host-plant phylogeny and chemistry have shaped the African seed-beetle radiation. *Molecular Phylogenetics and Evolution* **35**(3): 602–611.

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

KLEIN, H., ZIMMERMANN, H.G. & XIVURI, T. 2020. Exceptional biocontrol of two varieties of *Cylindropuntia fulgida* (Cactaceae) in South Africa using a recently-identified different biotype of the cochineal insect, *Dactylopius tomentosus* (Dactylopiidae), *Biocontrol: Theory and Applications in Pest Management* **149**:Not Available.

KUHLMANN, U., MASON, P.G. & FOOTTIT, R.G. 2000. Host-specificity Assessment of European *Peristenus* Parasitoids for Classical Biocontrol of Native *Lygus* species in North America: Use of Field Host Surveys to Predict Natural Enemy Habitat and Host Ranges. In: Van Driesche, R.G., Heard, T.A., McClay, A. & Reardon, R. (Eds.) *Proceedings: Host-specificity Testing of Exotic Arthropod Biocontrol Agents: The Biological Basis for Improvement in Safety*. 84-95. USDA Forest Service, Morgantown, WV.

LAGUNA, E., DELTORO, V.I., FERRER, P.P., NOVOA, A. & GUILLOT, D. 2013. About the binomen *Cylindropuntia rosea* (Cactaceae) and its invasive individuals recorded in the Valencian Community (Spain). *Bouteloua* **16**:40-51.

LOUDA, S.M. 2000. *Rhinocyllus conicus* - Insights to improve predictability and minimize risk of biocontrol of weeds. In: Spencer, N.R. (Ed.) *Proceedings of the X International Symposium on Biocontrol of Weeds*. 187 -193. Montana State University, Bozeman, Montana, U.S.A.

LOUDA, S.M., PEMBERTON, R.W., JOHNSON, M.T., & FOLLETT, P.A. 2003. Nontarget Effects — The Achilles' Heel Of Biocontrol? Retrospective Analyses to Reduce Risk Associated with Biocontrol Introductions*. *Annual Review of Entomology* **48**:365–396

LYNCH, M. 1991. The genetic interpretation of inbreeding depression and outbreeding depression. *Evolution* **45**: 622–629.

MANN, J. 1969. *Cactus-feeding insects and mites*. Smithsonian Institution Bulletin 256, 158 pp. Washington, DC, Smithsonian Institution.

MARAIS, C., VAN WILGEN, B.W. & STEVENS, D. 2004. The clearing of invasive alien plants in South Africa: a preliminary assessment of costs and progress. *South African Journal of Science* **100**: 97-103.

MARON, J.L. & VILÀ, M. 2001. When do herbivores affect plant invasion? Evidence for the natural enemies and biotic resistance hypotheses. *Oikos* **95**: 361-373.

MARSHALL, M., GOOLSBY, J.A., VACEK, A.T., MORAN, P.J., KIRK, A.A., CORTES, MENDOZA, E., CRISTOFARO, M., BOWNES, A., MASTORAS, A., KASHEFI, J., CHAS KOPOULOU, A., SMITH, L., GOLDSMITH, B., & RACELIS, A.E. 2018. Densities of the arundo wasp, *Tetramesa romana* (Hymenoptera: Eurytomidae) across its native range in

Mediterranean Europe and introduced ranges in North America and Africa. *Biocontrol Science and Technology* **28**: 772–785.

MATHENGE, C.W., HOLFORD, P., HOFFMANN, J.H., SPOONER-HART, R., BEATTIE, G.A.C. & ZIMMERMANN, H.G. 2009a. The biology of *Dactylopius tomentosus* (Hemiptera: Dactylopiidae). *Bulletin of Entomological Research* **99**: 551–559.

MATHENGE, C.W., HOLFORD, P., HOFFMANN, J.H., ZIMMERMANN, H.G. & BEATTIE, G.A.C. 2009b. Distinguishing suitable biotypes of *Dactylopius tomentosus* (Hemiptera: Dactylopiidae) for biocontrol of *Cylindropuntia fulgida* var. *fulgida* (Caryophyllales: Cactaceae) in South Africa. *Bulletin of Entomological Research* **99**: 619–627.

MATHENGE, C.W., HOLFORD, P., HOFFMANN, J.H., ZIMMERMANN, H.G., SPOONER-HART, R. & BEATTIE, G.A.C. 2010a. Determination of biotypes of *Dactylopius tomentosus* (Hemiptera: Dactylopiidae) and insights into the taxonomic relationships of their hosts, *Cylindropuntia* spp.. *Bulletin of Entomological Research* **100**(3): 347–358.

MATHENGE, C.W., HOLFORD, P., HOFFMANN, J.H., ZIMMERMANN, H.G., SPOONER-HART, R.N. & BEATTIE, G.A.C. 2010b. Hybridization between *Dactylopius tomentosus* (Hemiptera: Dactylopiidae) biotypes and its effects on host-specificity. *Bulletin of Entomological Research* **100**(3): 331–338.

MATHENGE, C.W., RIEGLER, M., BEATTIE, G.A.C., SPOONER-HART, R.N. & HOLFORD, P. 2015. Genetic variation amongst biotypes of *Dactylopius tomentosus*. Insect Sci. **22**(3): 360–374.

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

MCFADYEN, R.E.C. 1998. Biocontrol of Weeds. *Annual Review of Entomology* **43**: 369-393.

MCCLAY, A.S. & BALCIUNAS, J.K. 2005. The role of pre-release efficacy assessment in selecting classical biocontrol agents for weeds—applying the Anna Karenina principle. *Biological Control* **35**: 197–207.

MCCLAY, A.S., HINZ, H.L., DE CLERCK-FLOATE, R.A., & PESCHKEN, D.P. 2002. *Matricaria perforata* Mérat, scentless chamomile (Asteraceae). In: Mason, P.G. & Huber, J.T.

(Eds.) *Biocontrol Programmes in Canada, 1981–2000*. 395–402. CABI Publishing, Wallingford, UK.

MCQUEEN, C., NOEMDOE, S. & JEZILE, N. 2001. The WORKING FOR WATER Programme. *Land Use and Water Resources Research* **1**(4): 1–4.

MILLER, D.R., MILLER, G.L., HODGES, G.S. & DAVIDSON, J.A. 2005. Introduced scale insects (Hemiptera: Coccoidea) of the United States and their impact on U.S. agriculture. *Proceedings of the Entomological Society of Washington* **107**(1): 123-158.

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

MORAN V.C. & ANNECKE D.P. 1979. Critical reviews of biological pest control in South Africa. 3. The jointed cactus, *Opuntia aurantiaca* Lindley. *Journal of the Entomological Society of Southern Africa* **42**(2): 299–329.

MORAN, V.C. & COBBY, B.S. 1979. On the life-history and fecundity of the cochineal insect, *Dactylopius austrinus* De Lotto (Homoptera: Dactylopiidae), a biocontrol agent for the cactus *Opuntia aurantiaca*. *Bulletin of Entomological Research* **69**: 629-636.

MORAN, V.C., GUNN, B.H. & WALTER, G.H. 1982. Wind dispersal and settling of first-instar crawlers of the cochineal insect *Dactylopius austrinus* (Homoptera: Coccoidea: Dactylopiidae). *Ecological Entomology* **7**: 409-419.

MORAN, V.C. & HOFFMANN, J.H. 2015. The Fourteenth International Symposium on Biocontrol of Weeds, 1969–2014: Delegates, demographics and inferences from the debate on non-target effects. *Biological Control* **87**: 23–31.

MORAN, V.C., HOFFMANN, J.H. & ZIMMERMANN, H.G. 2005. Biocontrol 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. & ZIMMERMANN, H.G. 2013. 100 years of biocontrol of invasive alien plants in South Africa: History, practice and achievements. *South African Journal of Science* **109**(9/10): 1-6.

MORAN, V.C., ZACHARIADES, C. & HOFFMANN, J.H. 2021. Implementing a system in South Africa for categorizing the outcomes of weed biocontrol. *Biological Control* **153**: 104431.

MORAN, V.C. & ZIMMERMANN, H.G. 1984. The biocontrol of cactus weeds: achievements and prospects. *Bicontrol News and Information* **5**: 297–320.

MORAN, V.C. & ZIMMERMANN, H.G. 1991a. Biocontrol of cactus weeds of minor importance in South Africa. *Agriculture, Ecosystems and Environment* **37**: 37–55.

MORAN V.C. & ZIMMERMANN H.G. 1991b. Biocontrol of jointed cactus, *Opuntia aurantiaca* (Cactaceae), in South Africa. *Agriculture Ecosystems and Environment* **37**: 5–27.

MORIN, L., REID, A.M., SIMS-CHILTON, N.M., BUCKLEY, Y.M., DHILEEPAN, K., HASTWELL, G.T., NORDBLOM, T.L. & RAGHU, S. 2009. Review of approaches to evaluate the effectiveness of weed biocontrol agents. *Biological Control* **51**: 1-15.

MPHEPHU, T.E. & MUKWEVHO, L. 2019. Pre-release evaluation of absolute spillover impact risk of *Physonota maculiventris* (Chrysomelidae: Cassidinae) on nontarget plant species *Helianthus annuus* (Asteraceae) and *Zea mays* (Poaceae) in South Africa. *Biocontrol Science and Technology* **29**(8): 804-816.

MUSENGI, K., MBONANI, S. & BYRNE, M.J. 2021. Host suitability of three *Opuntia* taxa for the *Dactylopius opuntiae* (Hemiptera: Dactylopiidae) ‘stricta’ lineage. *Biocontrol Science and Technology*, DOI: 10.1080/09583157.2021.1932747.

NOVOA, A, LE ROUX, J.J., ROBERTSON, M.P., WILSON, J.R.U. & RICHARDSON, D.M. 2015. Introduced and invasive cactus species: a global review. *AoB Plants* **7**: 1-14.

NITAO, J.K., AYRES, M.P., LEDERHOUSE, R.C. & SCRIBER, J.M. 1991. Larval adaptation to lauraceous hosts: geographic divergence in the spicebush swallowtail butterfly. *Ecology* **72**: 1428–1435.

OLCKERS, T. 2004. Targeting emerging weeds for biocontrol in South Africa: the benefits of halting the spread of alien plants at an early stage of their invasion. *South African Journal of Science* **100**: 64-68.

PATERSON, I.D. 2010. Biocontrol of *Pereskia aculeata* Miller (Cactaceae). A Thesis Submitted in fulfilment of the requirements for the degree DOCTOR OF PHILOSOPHY, Rhodes University, Grahamstown.

PATERSON, I.D., COETZEE, J.A., HILL, M.P. & DOWNIE, D.A. 2011a. 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**(1): 59-65.

PATERSON, I.D., COETZEE, J.A., WEYL, P., GRIF, T.C., VOOGT, N. & MARTIN, P. 2019. Cryptic species of a water hyacinth biocontrol agent revealed in South Africa: host-specificity, impact, and thermal tolerance. *Entomologia Experimentalis et Applicata* **167**: 682–691.

PATERSON, I.D., HILL, M.P., CANAVAN, K. & DOWNEY, P.O. 2021. Prioritisation of targets for weed biocontrol II: The South African Biocontrol Target Selection system. *Biocontrol Science and Technology* **31**: 566-583.

PATERSON, I.D., HOFFMANN, J.H., KLEIN, H., MATHENGE, C.W., NESER, S. & ZIMMERMANN, H.G. 2011b. Biocontrol of Cactaceae in South Africa. *African Entomology* **19**: 230–246.

PATERSON, I.D., KLEIN, H., MUSKETT, P.C., GRIFFITH, T.C., MAYONDE, S., MOFOKENG, K., MNQETA, Z. & VENTER, N.C. 2021. Biocontrol of Cactaceae in South Africa. *African Entomology*. 29: 713-734.

PATERSON, I.D., MANHEIMMER, C.A. & ZIMMERMANN, H.G. 2019. Prospects for biocontrol of cactus weeds in Namibia, *Biocontrol Science and Technology* 1-7.

PATERSON, I.D., VITORINO, M.D., DE CRISTO, S.C., MARTIN, G. & HILL, M.P. 2014. Prioritization of potential agents for the biocontrol of the invasive alien weed, *Pereskia aculeata* (Cactaceae), in South Africa. *Biocontrol Science and Technology* **24**: 407-425.

PAYNTER, Q.E., FOWLER, S.V., GOURLAY, A.H., HAINES, M.L., HARMAN, H.M., HONA, S.R., PETERSON, P.G., SMITH, L.A., WILSON-DAVEY, J.R.A., WINKS, C.J. & WITHERS, T.M. 2004. Safety in New Zealand weed biocontrol: A nationwide survey for impacts on non-target plants. *NZ Plant Protection* **57**: 102–107.

PAYNTER, Q., PATERSON, I.D. & KWONG R.M. 2020. Predicting non-target impacts. *Current Opinion in Insect Science* **38**: 79–83.

PEER, K. & TABORSKY, M. 2005. Outbreeding depression, but no inbreeding depression in haplodiploid ambrosia beetles with regular sibling mating. *Evolution* **59**: 317–323.

PETTEY, F.W. 1948. The biocontrol of prickly pears in South Africa. *Science Bulletin. Department of Agriculture and Forestry, Union of South Africa* **271**: 1–163.

PESCHKEN, D.P. & DERBY, J.L. 1992. Effect of *Urophora cardui* (L.) (Diptera: Tephritidae) and *Ceutorhynchus litura* (F.) (Coleoptera: Curculionidae) on the weed Canada thistle, *Cirsium arvense* (L.). Scop. *The Canadian Entomologist* **124**: 145–150.

PESCHKEN, D.P. & MCCLAY, A.S. 1995. Picking the target: a revision of McClay's scoring system to determine the suitability of a weed for classical biocontrol. In: Delfosse, E.S. & Scott, R.R. (Eds.) *Proceedings of the VIIIth International Symposium on Biocontrol of Weeds*. 137–143. DSIR/CSIRO, Melbourne, Australia.

PINKAVA, D.J. 1999. Cactaceae Cactus Family: Part Three: Cylindropuntia (Engelm.) Knuth Chollas. *Journal of the Arizona-Nevada Academy of Science* **32**(1): 32-47

POST, J.A., KLEINJAN, C.A., HOFFMANN, J.H. & IMPSON F.A.C. 2010. Biocontrol of *Acacia cyclops* in South Africa: The fundamental and realized host range of *Dasineura dielsi* (Diptera: Cecidomyiidae). *Biological Control* **53**: 68–75.

PYŠEK, P., PERGL, J., ESSL, F., LENZNER, B., DAWSON, W., KREFT, H., WEIGELT, P., WINTER, M., KARTESZ, J., NISHINO, M., ANTONOVA, L.A., BARCELONA, J.F., CABEZAS, F.J., CÁRDENAS, D., CÁRDENAS-TORO, J., CASTAŃO, N., CHACÓN, E., CHATELAIN, C., DULLINGER, S., EBEL, A.L., FIGUEIREDO, E., FUENTES, N., GENOVESI, P., GROOM, Q.J., HENDERSON, L., INDERJIT, KUPRIYANOV, A., MASCIADRI, S., MAUREL, N., MEERMAN, J., MOROZOVA, O., MOSER, D., NICKRENT, D., NOWAK, P.M., PAGAD, S., PATZELT, A., PELSER, P.B., SEEBENS, H., SHU, W., THOMAS, J., VELAYOS, M., WEBER, E., WIERINGA, J.J., BAPTISTE, M.P. & VAN KLEUNEN, M. 2017. Naturalised alien flora of the world: species diversity, taxonomic and phylogenetic patterns, geographic distribution and global hotspots of plant invasion. *Preslia* **89**: 203–274.

RAPO, C.B., SCHAFFNER, U., EIGENBRODE, S.D., HINZ, H.L., PRICE, W.J., MORRA, M., GASKIN, J. & SCHWARZLÄNDER, M. 2019. Feeding intensity of insect herbivores is associated more closely with key metabolite profiles than phylogenetic relatedness of their potential hosts. *PeerJ* **7**: e8203.

R Core Team. 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

RICHARDSON, D.M., PYŠEK, P., REJMÁNEK, M., BARBOUR, M. G., PANETTA, F. D. & WEST, C. J. 2000. Naturalization and Invasion of Alien Plants. *Diversity and Distributions* **6**: 93–107.

RICHARDSON, D.M. & THUILLER, W. 2007. Home away from home – objective mapping of high-risk source areas for plant introductions. *Diversity and Distributions* **13**: 299–323.

RICHARDSON, D. M. & VAN WILGEN, B. W. 2004. Invasive alien plants in South Africa: How well do we understand the ecological impacts? *South African Journal of Science* **100**: 45–52.

RODRÍGUEZ, L.C., MÉNDEZ, M.A. & NIEMEYER, H.M. 2001. Direction of dispersal of cochineal (*Dactylopius coccus* Costa) within the Americas. *Antiquity* **75**: 73–77.

ROHDE, K., DREHER, E. & HOCHKIRCH, A. 2015. Sex-specific phenotypic plasticity in response to the trade-off between developmental time and body size supports the dimorphic niche hypothesis. *Biological Journal of the Linnean Society* **115**: 48–57.

RULE, N.F. & HOFFMANN, J. 2018. The performance of *Dactylopius opuntiae* as a biocontrol agent on two invasive *Opuntia* cactus species in South Africa. *Biological Control* **119**: 7–11.

SÁEZ, L., SERAPIO, J., GÓMEZ-BELLVER, C., ARDENGHI, N.M.G., GUILLOT, D. & JOAN, R. 2016. New records in vascular plants alien to the Balearic Islands. *Orsis* **30**: 101-131.

SANDS, D.P.A. & SCHOTZ, M. 1985. Control or no control: a comparison of the feeding strategies of two salvinia weevils. In: Delfosse, E.S. (Ed.) *Proceedings of the VI International Symposium on Biocontrol of Weeds*. 551–556. Agriculture Canada, Vancouver, Canada.

SCHWARZLÄNDER, M., HINZ, H.L., WINSTON, R.L., DAY, M.D. 2018. Biocontrol of weeds: an analysis of introductions, rates of establishment and estimates of success, worldwide. *BioControl* **63**: 319–331.

SHACKLETON, R.T., WITT, A.B.R., PIRORIS, F.M. & VAN WILGEN, B.W. 2017. Distribution and socio-ecological impacts of the invasive alien cactus *Opuntia stricta* in eastern Africa. *Biological Invasions* **19**: 2427–2441

SHEPPARD, A., DAY, M., GRICE, T., & NESER, S. 2012. Assessment of the status, impact and management of weeds in Zimbabwe, with particular focus on biocontrol, in the context of food security and sustainable production from smallholdings and water use in lakes and rivers. Canberra: Australian Centre for International Agricultural Research.

SHEPPARD, A.W., HILL, R., DECLERCK-FLOATE, R.A., MCCLAY, A., OLCKERS, T., QUIMBY Jr. P.C. & ZIMMERMANN, H.G. 2003. A global review of risk-benefit-cost analysis for the introduction of classical biocontrol against weeds: a crisis in the making? *Biocontrol News and Information* **24**(4): 91N-108N.

SPITZER, B. 2006. Local maladaptation in the soft scale insect *Saissetia coffeae* (Hemiptera: Coccidae). *Evolution* **60**: 1859–1867.

STOUTHAMER, R., LUCK, R.F., PINTO, J.D., PLATNER, G.R. & STEPHENS, B. 1996. Non-reciprocal cross-incompatibility in *Trichogramma deion*. *Entomologia Experimentalis et Applicata* **80**: 481–489.

SUCKLING, D.M. & SFORZA, R.F.H. 2014. What magnitude are observed non-target impacts from weed biocontrol? *PLoS One* **9**: e84847.

SULLIVAN, P.R. 1990. Population growth potential of *Dactylopius ceylonicus* Green (Hemiptera: Dactylopiidae) on *Opuntia vulgaris* Miller. *Journal of Australian Entomological Society* **29**: 123–129.

SUTTON, G.F., CANAVAN, K., DAY, M.D. & PATERSON, I.D. 2021. Field based ecological studies to assess prospective biocontrol agents for invasive alien plants: an example from giant rat's tail grass. *Journal of Applied Ecology* **58**: 1043-1054.

TAYLOR, W.R. & WHITSON, T.D. 1999. Plains prickly pear cactus control. University of Wyoming, Cooperative Extension Service, Bulletin No. B-1074

THE STATE OF QUEENSLAND, DEPARTMENT OF AGRICULTURE AND FISHERIES. 2018. Hudson pear *Cylindropuntia rosea* (Syn. *Cylindropuntia pallida*) and *Cylindropuntia tunicate*. Department of Agriculture, Fisheries and Forestry, Biosecurity Queensland. Available at: https://www.daf.qld.gov.au/__data/assets/pdf_file/0011/1395920/hudson-pear.pdf. (Accessed: 12 March 2019)

VAN DAM, A.R. & MAY, B. 2012. A new species of *Dactylopius* Costa (*Dactylopius gracilipilus* sp. nov.) (Hemiptera: Coccoidea: Dactylopiidae) from the Chihuahuan Desert, Texas, U.S.A. *Zootaxa* **3573**: 33–39.

VAN KLINKEN, R.D. 2000. Host-specificity testing: why do we do it and how we can do it better. In: Van Driesche, R.G., Heard, T.A., McClay, A. & Reardon, R. (Eds.) *Proceedings: Host-specificity Testing of Exotic Arthropod Biocontrol Agents: The Biological Basis for Improvement in Safety*. 54–68. USDA Forest Service, Morgantown, WV.

VAN KLINKEN, R.D. & EDWARDS O.R. 2002. Is host-specificity of weed biocontrol agents likely to evolve rapidly following establishment? *Ecology Letters* **5**: 590–596.

VAN KLINKEN, R.D. & HEARD, T.A. 2000. Estimating the fundamental host range of *Evippe* sp. #1, a potential biocontrol agent for *Prosopis* species (Leguminosae). *Biocontrol Science and Technology* **10**(3): 331-342.

VAN STEENDEREN, C.J.M., PATERSON, I.D., EDWARDS, S. & M.D. DAY. 2021. Addressing the red flags in cochineal identification: The use of molecular techniques to identify cochineal insects that are used as biocontrol agents for invasive alien cacti. *Biocontrol* **152**: 104426.

VAN WILGEN, B.W. 2012. Evidence, perceptions, and trade-offs associated with invasive alien plant control in the Table Mountain National Park, South Africa. *Ecology and Society* **17**(2): 23.

VAN WILGEN, B.W., DE WIT, M.P., ANDERSON, H.J., LE MAITRE, D.C., KOTZE, I.M., NDALA, S., BROWN, B. & RAPHOLO, M.B. 2004. Costs and benefits of biocontrol of invasive alien plants: case studies from South Africa. *South African Journal of Science* **100**: 113-122.

VAN WILGEN, B.W., MEASEY, J., RICHARDSON, D.M., WILSON, J.R. & ZENGEYA, T.A. 2020. Biological Invasions in South Africa: An Overview. In: van Wilgen, B.W., Measey, J., Richardson, D.M., Wilson, J.R. & Zengeya, T.A. (Eds.) *Biological Invasions in South Africa.* 3-31. Springer Nature, Cham, Switzerland.

VITOUSEK, P.M. 1990. Biological invasions and ecosystem processes: Toward an integration of population biology and ecosystem studies. *Oikos* **57**: 7–13.

VOLCHANSKY, C.R., HOFFMANN, J.H. & ZIMMERMANN, H.G. 1999. Host-plant affinities of two biotypes of *Dactylopius opuntiae* (Homoptera: Dactylopiidae): enhanced prospects for biocontrol of *Opuntia stricta* (Cactaceae) in South Africa. *Journal of Applied Ecology* **36**: 85–91.

WAPSHERE, A.J. 1974. A strategy for evaluating the safety of organisms for biological weed control. *Annals of Applied Biology* **77**: 201–211.

WALTERS, M., FIGUEIREDO, E., CROUCH, N.R., WINTER, P.J.D., SMITH, G.F., ZIMMERMANN, H.G. & MASHOPE, B.K. 2011. Naturalised and invasive succulents of southern Africa. *Abc Taxa* **11**: i-x, 360 pp.

WALL, R. & BEGON, M. 1987. Population density, phenotype and reproductive output in the grasshopper *Chorthippus brunneus*. *Ecological Entomology* **12**: 331–339.

WASHBURN, J.O. & WASHBURN, L. 1984. Active aerial dispersal of minute wingless arthropods: exploitation of boundary-layer velocity gradients. *Science* **223**: 1088–1089.

WHEELER, G.S. 2014. Plant chemistry-based host-specificity of *Oxyops vitiosa*, a weevil introduced for weed biocontrol of *Melaleuca quinquenervia*. In: Impson, F.A.C., Kleinjan, C.A. & Hoffmann, J.H. (Eds.) *Proceedings of the XIV International Symposium on Biocontrol of Weeds*. 116. University of Cape Town, South Africa

WHEELER, G.S., David, A.S. & Lake, E.C. 2021. Volatile chemistry, not phylogeny, predicts host range of a biocontrol agent of Old-World climbing fern. *Biocontrol* **159**: 104636.

WHEELER, G.S., DUNCAN, J.G. & WRIGHT, S. 2017. Predicting spillover risk to non-target plants pre-release: *Bikasha collaris* a potential biocontrol agent of Chinese tallowtree (*Triadica sebifera*). *Biological Control* **108**: 16-21.

WILCOVE, D.S., ROTHSTEIN, D., DUBOW, J., PHILLIPS, A., LOSOS, E. 1998. Quantifying threats to imperiled species in the United States. *BioScience* **48**: 607-615.

WILSON, J.R.U., IVEY, P., MANYAMA, P. & NÄNNI, I. 2013. A new national unit for invasive species detection, assessment and eradication planning. *South African Journal of Science* **109**: 1–13.

WINSTON, R.L., SCHWARZLÄNDER, M., HINZ, H.L., DAY, M.D., COCK, M.J.W. & JULIEN, M.H. (Eds.). 2014. Biocontrol of weeds: A world catalogue of agents and their target weeds. 5th edition. FHTET-2014-04, USDA Forest Service, Forest Health Technology Enterprise Team, Morgantown, West Virginia.

WITTENBERG, R & COCK M.J.W. (Eds). 2001. Invasive Alien Species: A Toolkit of Best Prevention and Management Practices. CAB International, Wallingford, Oxon, UK.

ZACHARIADES, C. 2018. Biocontrol of invasive alien plants in South Africa: a list of all insects, mites and pathogens released as biocontrol agents from 1913-2018. http://www.arc.agric.za/arc-ppri/Documents/Table2- NaturalEnemiesReleased.pdf.

ZACHARIADES, C., PATERSON I.D., STRATHIE, L.W., HILL, M.P. & VAN WILGEN, B.W. 2017. Assessing the status of biocontrol as a management tool for suppression of invasive alien plants in South Africa. *Bothalia* **47**(2): 1-19.

ZIMMERMANN, H.G. & GRANATA, G. 2002. Insect pests and diseases. In: Nobel, P.S. (Ed.) *Cacti: Biology and Uses*. 235–254. University of California Press, Berkeley, CA.

ZIMMERMANN, H.G., MORAN, V.C. & HOFFMANN, J.H. 2004. Biocontrol 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.