

Host plant associations of two cochineal insect species, *Dactylopius ceylonicus* and *D. opuntiae* (Dactylopiidae: Hemiptera), on the invasive cactus species *Opuntia monacantha*, *O. ficus-indica* and a possible hybrid cactus, in South Africa

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Abstract

Several cochineal species have been used with great success for biological control of invasive cactus species, both in South Africa and elsewhere. Although most cochineal species are oligophagous, they are all exclusively associated with opuntoid cacti and therefore pose minimal risk of non-target effects in the Old World where there are no native Cactaceae. However, the ability of cochineal to use more than one host plant species has validated reports of inadvertent displacement of *Dactylopius ceylonicus* (the original agent released in 1913) by *D. opuntiae* (which was originally released on *Opuntia ficus-indica* in 1938) on *O. monacantha* in South Africa. There are also reports that the two cactus hosts, *O. monacantha* and *O. ficus-indica*, have hybridized in South Africa, but there are uncertainties as to which of the two cochineal species is associated with the alleged hybrid. The primary aim of this study was to determine the present status of *D. ceylonicus* and *D. opuntiae* on *O. monacantha* and on the possible hybrid. Cochineal samples were collected off cactus plants from sites in the Eastern Cape and Western Cape and identified using bioassays and morphological features. The performance of each cochineal species on each of the three cactus hosts (*O. monacantha*, *O. ficus-indica* and the hybrid) was determined through measures of female developmental rates, survival and mass at reproductive maturity. Results showed that *D. ceylonicus* remains present on *O. monacantha* at each of seven study sites, while *D. opuntiae* did not occur on *O. monacantha* at any of the sites. However, *D. opuntiae* is capable of establishing on *O. monacantha*, *O. ficus-indica* and the possible hybrid cactus under laboratory conditions. *Dactylopius ceylonicus* is also compatible with both *O. monacantha* and the possible hybrid, although it did not establish on *O. ficus-indica*. These findings showed that *D. ceylonicus* has not been displaced by *D. opuntiae* on *O. monacantha* in South Africa and it is expected that *O. monacantha* will continue to be kept under excellent biological control into the future. Results also indicated that possible hybridisation between the two *Opuntia* species has not produced a new taxon that is immune to either of the two control agents. However, further research is required to improve our understanding of this new plant taxon and confirm it will be suppressed by one or both of the cochineal species.

Key words: ‘Invasive species’, ‘biological control’, ‘cactus hybridization’, ‘displacement’

1. Introduction

1.1. The status of invasive species in Africa

Alien species exist across the African continent, with magnitudes that vary between countries and ecosystems (Chenje and Mohamed-Katerere, 2006). However, the number of alien species continues to grow in virtually all countries (McNeely et al., 2001; Hulme, 2009). These species can include disease organisms, agricultural weeds and insect pests (often labelled as alien invasive species, abbreviated as AIS). The increase in severity and geographic extent of invasions by these organisms arises as a result of the acceleration in global trade and travel, allowing for faster dissemination of propagules (Hulme, 2009; van Wilgen et al., 2013), as well as from mounting human mediated disturbances that make ecosystems more susceptible to invasions by AIS (Hobbs, 1989; Saunders et al., 1991).

Invasions can, directly and indirectly, result in fundamental ecosystem-level changes (Vitousek, 1990; Chenje and Mohamed-Katerere, 2006). These changes arise when AIS significantly alter the flow, availability and quality of resources (such as light, oxygen or water) and/or nutrients (such as nitrogen) within the invaded ecosystem (Ehrenfeld, 2002; Richardson and van Wilgen, 2004). These modifications affect biogeochemical cycles, as well as the physical environment and trophic interactions within food webs (Crooks, 2002; Richardson and van Wilgen, 2004), which ultimately impact entire ecosystem functioning (Heywood, 1989; D'Antonio and Dudley, 1995; Mack et al., 2000).

It is well documented that these ecosystem changes resulting from invasions threaten biodiversity (Courchamp et al., 2003; Vilà et al., 2011; Pyšek et al., 2012). Invaders are also recognised for their negative economic and social implications (Perrings et al., 2002; Pimentel et al., 2005; Pejchar and Mooney, 2009) as they affect the supply of environmental goods (to which agriculture, forestry and fisheries are explicitly linked) and environmental services (to which human health and well-being are linked) (Mooney, 2005). In fact, estimates revealed that the global costs of AIS exceed the total economic output of the entire African continent (Pimentel, 2002), and what is more, predicted global climate change is expected to substantially increase these costs (Dukes and Mooney, 1999; Hobbs and Mooney, 2005; Thuiller et al., 2007).

1.2. Managing invasions

It is often the duty of conservation biologists and environmental managers to tackle the difficult task of preventing invasions and to eradicate or control existing invasions (Richardson and van Wilgen, 2004; Buckley, 2008). The ultimate goal of these management approaches is to prevent the loss of native biodiversity or re-establish biodiversity and restore ecosystem functioning (Hulme, 2006). The three successive steps (prevention, eradication and control) form the cornerstones of the best management practices available (Wittenberg and Cock, 2001). Of these approaches, prevention of the initial introduction of AIS is obviously the most cost-effective and environmentally sound management tactic (DiTomaso, 2000; Leung et al., 2002; Müller-Schärer, 2002). However, due to the high number of candidate AIS and limited inspection capacity, detecting incoming AIS and separating them from non-invasive alien species becomes a difficult task (D'Antonio et al., 2004; Hulme, 2006). If existing prevention techniques fail, early detection and rapid response become the next most desirable options. Although this approach is hindered by the difficulty to detect AIS in low abundances, when discovered in time, rapid responses can facilitate the eradications of harmful AIS (Wittenberg and Cock, 2001). The larger the area invaded, the harder the task of eradication becomes, and in general successful eradications have mostly taken place on islands or in small areas where rapid responses were made (Courchamp et al., 2003). For invasions that become too wide spread, control is the only remaining management strategy (van Wilgen et al., 2013). Control aims to facilitate long-term suppression of the invader to levels that reduce impacts and contain the spread of AIS from invaded areas into uninvaded areas (Hulme, 2006).

To minimise the impacts of invasions it is often necessary to integrate these three management approaches (Buckley, 2008; Pyšek and Richardson, 2010). Within each approach numerous tools exist, including chemical control, mechanical control and biological control, where classic biological control is the intentional introduction of an exotic, usually co-evolved, living organism for permanent establishment on the target pest (Eilenberg et al., 2001). In general, the aim of chemical and mechanical control is to remove the target AIS (Hulme, 2006), while that of classical biological control is normally not to eradicate the target, but to reduce population sizes to a point where they no longer threaten biodiversity and ecosystem functioning (Waage et al., 1988).

1.2.1. Biological control

Worldwide, the practice of biological control on weeds has been highly beneficial, with more than 50% of cases achieving complete or substantial control of target species (Moran et al., 2005; van Wilgen et al., 2013). In cases where complete success is achieved, formerly costly weeds are suppressed permanently, without requiring additional management interventions (Moran et al., 2005), making this one of the most cost-effective tools available (van Wilgen et al., 2001; McConnachie et al., 2003).

However, concerns about biological control have been raised in the literature, specifically regarding the safety of adding more exotic species into a system, given the possibility of “host-shifting” (where non-target organisms are attacked by the introduced agent) (Louda et al., 1997). As such, increasingly demanding risk-adverse regulatory testing is required in the modern practice of weed biological control (van Wilgen et al., 2013). However, despite concerns, “host-shifts” have not occurred in the over 350 recorded cases where weed biological control agents have been used worldwide (van Wilgen et al., 2004).

1.2.2. Biological control in practice

Weed biological control began inadvertently as a result of a series of unexpected events. The agent involved was a species of *Dactylopius* (Hemiptera: Dactylopiidae), a distinctive genus of soft-bodied scale insects, called “cochineal insects” (Zimmermann et al., 2009). Cochineal live and feed almost exclusively on cacti of the genus *Opuntia* (De Lotto, 1974; Pérez-Guerra and Kosztarab, 1992; Claps and de Haro, 2001). Adult female cochineal produce eggs which hatch almost immediately into first instar nymphs known as “crawlers” (Pérez-Guerra and Kosztarab, 1992). Female crawlers then experience two additional life stages (second instar nymph and adult), while males undergo four additional life stages (second instar nymph, prepupa, pupa and adult) to reach adult form. Cochineal exhibit sexual dimorphism which is especially pronounced in the adult life stages (Pérez-Guerra, 1991; Claps and de Haro, 2001); adult males are winged and mobile, while females are sessile and have neotenus characteristics.

Although males are mobile, dispersal of cochineal insects occurs during crawler phase. Crawlers tend to climb up their host plant and are then carried away by wind currents, landing inadvertently on neighbouring plants (Pérez-Guerra, 1991; Pérez-Guerra and Kosztarab, 1992). Once crawlers land on a suitable host plant they will insert their stylet into the phloem and feed.

In the case of females they then remain sessile for the rest of their life cycle. *Dactylopius* females tend to aggregate in colonies on the surface of the cactus plant, and when colonies become dense, most species overwhelm and kill their host (van Wilgen et al., 2013).

In the late 18th century, *Dactylopius ceylonicus* (Green), one of the 11 species in the family Dactylopiidae, was introduced into India, onto extensive infestations of the alien cactus, *Opuntia monacantha* (Wildenow), Haworth (Cactaceae) (Tryon, 1910; Rao et al., 1971). The aim of this introduction was to establish a dye production industry, as the carminic acid extracted from these cochineal insects was expected to produce high quality red pigments (Greenfield, 2006; Zimmermann et al., 2009). However, within a few years of the introduction, India's dye-production industry was no longer viable as the cochineal had virtually eliminated all their host plants, *O. monacantha*. Following this, *D. ceylonicus* was redistributed to other areas where problematic infestations of *O. monacantha* occurred, thus facilitating the control of the weed and giving rise to the first successful biological control project (Zimmermann et al., 2009).

1.3. *Opuntia* and *Dactylopius*

Over the last 500 years, inter-cultural and economic exchanges of both *Opuntia* (Cactaceae: Opuntioideae) and *Dactylopius* species have played a pivotal role in the expansion of both genera across the globe (Chávez-Moreno et al., 2009). To this day cacti of the *Opuntia* genus are still valued as food, for both humans and livestock, and as a source of alcoholic beverages and live fences (Badii and Flores, 2001; Griffith, 2004). *Opuntia* are capable of surviving in a wide range of ecological conditions as a result of their ability to propagate both sexually and vegetatively, and their capacity to withstand arid conditions (Chávez-Moreno et al., 2009). Due to their success these species have become the primary invasive weeds in many xeric and subtropical habitats outside of their native home ranges (Hunt and Taylor, 2002).

South Africa is an example of one such country, where species within the *Opuntia* genus have become established and spread aggressively through its landscapes (Zimmermann et al., 2004; Chávez-Moreno et al., 2009). Dense infestations of *Opuntia* cacti obstruct land utilization, displace native flora, and consequentially give rise to devastating economic and ecological impacts in the country (Vosloo, 1906; van der Merwe, 1931; van Sittert, 2002; Richardson and van Wilgen, 2004).

The spread of one such *Opuntia* species, *O. monacantha*, triggered the initiation of the first biological control project in South Africa (Zimmermann et al., 2009). In 1913, the herbivorous cochineal insect, *D. ceylonicus*, was released onto *O. monacantha*, following its successful control of the weed in India (Lounsbury, 1915). Within a few years of its introduction into South Africa, *O. monacantha* was considered to be under complete control (Neser and Annecke, 1973), that is, they continue to exist in low densities, but no other control methods are needed to reduce the weed populations to acceptable levels (Zimmermann et al., 2004).

Since the early success of *D. ceylonicus* on *O. monacantha* an additional 14 biological control agents have been released, with the aim of controlling 15 of South Africa's cactaceous weed species (Paterson et al., 2011). Originally, these agents were introduced onto eight target species, however, due to the oligophagous nature of some of the agent species, they subsequently became established on seven additional cactus types (Paterson et al., 2011). Oligophagous insects are not normally suitable biological control agents (due to their capacity to feed on a range of hosts). However, oligophagous cactophages have been used for biological control in South Africa, with no risk to non-target native or commercial plants because there are no native Cactaceae in the Old World, with the possible exception of *Rhipsalis baccifera* (J.Müller) Stern, (Moran, 1980; Zimmermann et al., 2009). Although the risk of non-target effects is limited in the case of the introduced cactus insects, their ability to use hosts other than the target species may influence the efficacy of other biological control agents that are already established on the 'adopted' species. Indeed, competitive displacement of closely related biological control agent species has been shown to occur in a number of studies (Wilson and Lindow, 1992; Murdoch et al., 1996; Reitz and Trumble, 2002) .

1.4. South Africa's control program against *O. monacantha*: The status of control agents and the target host plant

Circumstantial evidence suggests that competitive displacement may have occurred on South Africa's *O. monacantha* cactus weed. Informal surveys of the weed populations suggest that *D. ceylonicus* is no longer common on *O. monacantha*, and that *Dactylopius opuntiae* (Cockerell), has replaced it (H.G. Zimmermann pers. com.). The introduction of *D. opuntiae* into South Africa, in 1938, facilitated the successful control of the invasive alien cactus, *Opuntia ficus-indica* (L.) Miller (Petty, 1948; Githure et al., 1999). *Dactylopius opuntiae* has also featured in

the control of *Opuntia* species in several other countries (Moran and Zimmermann, 1984; Hosking et al., 1994) due to its capacity to thrive on different host species (for a list of all hosts see Perez-Guerra, 1991). However, despite the excellent success of *D. opuntiae* on *O. ficus-indica*, its efficacy as a control agent of *O. monacantha* is unknown. The possibility exists that *D. opuntiae* is an inferior agent, and as such, the displacement of *D. ceylonicus* would have severe negative implications for the country's *O. monacantha* control initiative.

In addition to concerns regarding the prospect of competitive exclusion of one or other of the cochineal on *O. monacantha* and *O. ficus-indica*, recent reports also suggest that these two host cactus species have hybridized (J.H. Hoffmann pers. com.). Although no genetic analyses have been performed to confirm or refute this, natural hybridisations between sympatric *Opuntia* cactus groups have been known to occur in the past (Benson, 1969a, 1969b; Grant and Grant, 1979).

As in previous cactus hybrids, features of the current possible hybrid cactus deviate from both parental taxa (Grant and Grant, 1979). Hybrid cladodes (modified succulent stems) are bright green and shiny, as is typical of *O. monacantha* (Henderson, 2001), however, the cladode's area is substantially larger than those of typical *O. monacantha*, and the cladode's thickness is intermediate between that of *O. monacantha* and *O. ficus-indica*. In addition, spines on the possible hybrid cactus appear longer than those of the parental taxa. While spines on *O. monacantha* are typically singular and those on *O. ficus-indica* vary between two or three spines per cluster (Henderson, 2001), spines on the hybrid are frequently arranged in clusters of two (Appendix 1).

Limited additional data are available on the status of the possible hybrid cactus and its compatibility with the cochineal biological control agents. However, in many documented cases hybrid weeds have become competitively superior to their parental taxa, and even more invasive (Ellstrand and Schierenbeck, 2006). This can result from a change in the phenotypic mean that enhances hybrid fitness and confers beneficial traits (Perry et al., 2002; Ellstrand and Schierenbeck, 2006). Hybrid success can also stem from increased phenotypic variation (following recombination) that generates new phenotypes with superior abilities to adapt to their environment (Facon et al., 2005).

The interactions between natural enemies and hybrid variants are complex and difficult to predict (Müller-Schärer and Schaffner, 2008). Empirical evidence from previously-studied hybridisation events suggests that hybrids experience reduced insect and pathogen resistance (Fritz et al., 1999); however, the subject is understudied (Müller-Schärer and Schaffner, 2008) and the possibility remains that biological control agents fail to accept hybrid hosts or have lower levels of performance on these hosts (Müller-Schärer and Steinger, 2004; Müller-Schärer et al., 2004; Müller-Schärer and Schaffner, 2008). As such, it is important to evaluate the compatibility of biological control agents with the possible hybrid cactus in order to assess the risk of further establishment and invasion.

1.5. Aims of the study

This was a pilot study to assess the status and insect-host compatibility of biological control agents on *O. monacantha* and the possible hybrid cactus in South Africa. The primary aim was to verify the absence of *D. ceylonicus* on *O. monacantha*, within the country, and further experimentally analyse the compatibility of *D. opuntiae* on *O. monacantha*. The secondary aim was to evaluate the performance of biological control agents on the possible hybrid cactus.

2. Materials and methods

2.1. Study design

The aims of this study were accomplished through experimental analyses that evaluated the developmental success of each cochineal type, grown on each cactus type. This approach exploits the characteristic fidelity of *D. ceylonicus* and *D. opuntiae* to their cactus hosts to enable identification. *Dactylopius ceylonicus* can only develop on *O. monacantha* (Perez-Guerra, 1991; Ben-Dov, 2006), while *D. opuntiae* is capable of developing on both *O. monacantha* and *O. ficus-indica* (Perez-Guerra, 1991; Ben-Dov, 2006). In addition, morphological comparisons were made to confirm the developmental patterns that were observed. Although distinguishing species within the genus of Dactylopiidae, using morphological keys, is notoriously difficult and depends on the quality of specimens mounted on microscope slides (Perez-Guerra, 1991; Gullan and Kosztarab, 1997; Portillo and Viguera, 2006), *D. ceylonicus* and *D. opuntiae* have distinct setae which can be used to differentiate the two species (De Lotto, 1974; Perez-Guerra, 1991).

2.2. Experimental set-up

Cladodes of the study cacti and their associated cochineal were collected from seven sites within South Africa's Eastern Cape and Western Cape Provinces (Fig. 1). The sites represented the primary distribution of *O. monacantha* populations within the country (J.H. Hoffmann, pers. com.) and included Stellenbosch, Kenton-on-Sea, Zunney De Mond, Salem, Klipfontein, Bathurst and Port Alfred (Fig. 1; Table 1). Supposed hybrid cacti were sampled from Klipfontein and Colchester, two regions where possible hybrid populations are known to occur (J.H. Hoffmann, pers.com.) (Fig. 1). Cladodes of *O. ficus-indica* were collected from Stellenbosch and Grahamstown, where the cactus is known to host *D. opuntiae*.

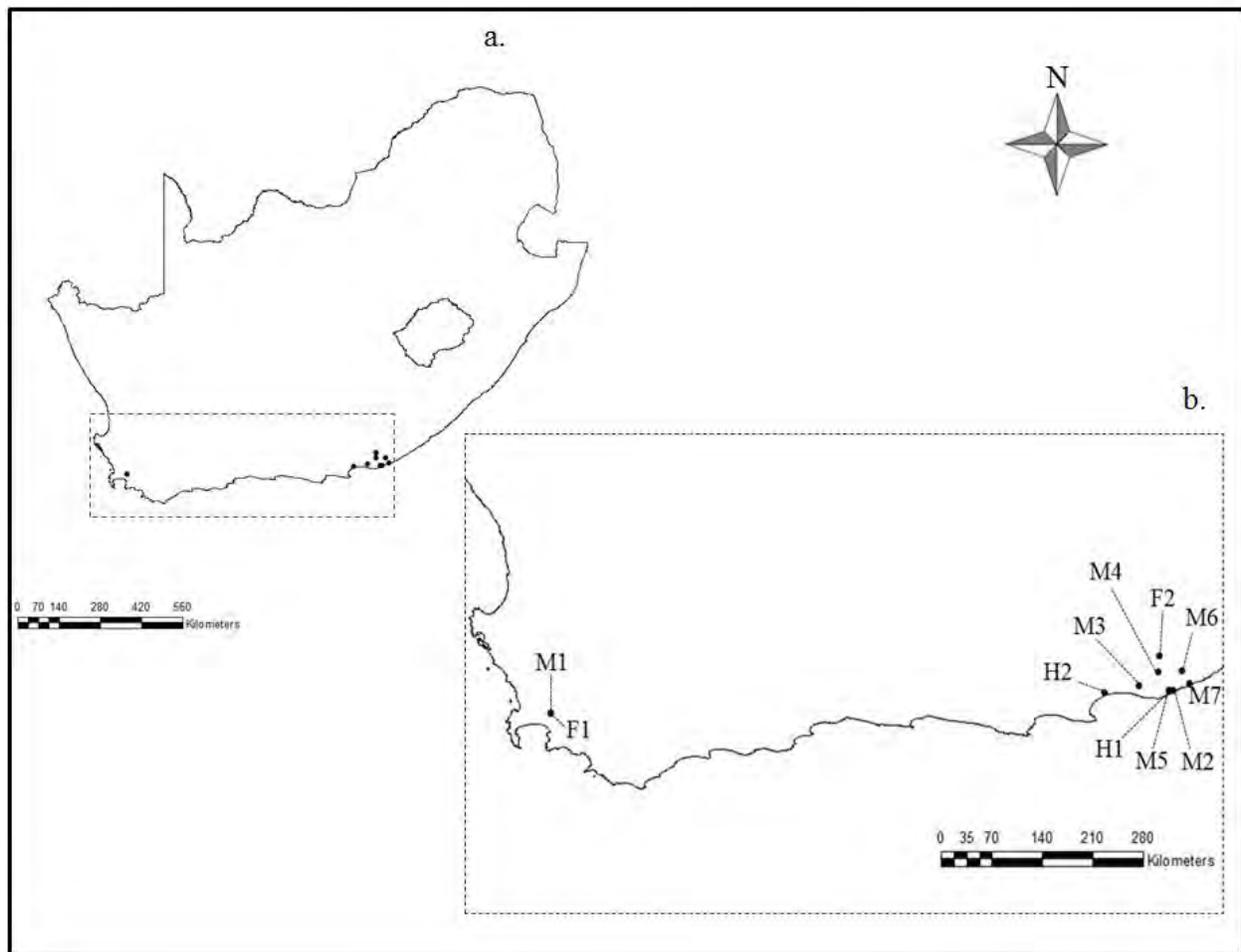


Fig. 1: Sampling sites for the three cactus types *O. monacantha* (M1-M7), *O. ficus-indica* (F1-F2), and a possible hybrid cactus (H1-H2) (a.), collected within South Africa (b.)

Table 1: GPS location of sample sites where *O. monacantha*, *O. ficus-indica* and possible hybrid cacti were collected, within South Africa

Cactus type	Site name	Site ID	Latitude	Longitude
<i>O. monacantha</i>	Stellenbosch	M1	-33.950867	18.878589
<i>O. monacantha</i>	Kenton-on-Sea	M2	-33.677017	26.659147
<i>O. monacantha</i>	Zunney De Mond	M3	-33.626539	26.227931
<i>O. monacantha</i>	Salem	M4	-33.462825	26.472089
<i>O. monacantha</i>	Klipfontein	M5	-33.683434	26.616936
<i>O. monacantha</i>	Bathurst	M6	-33.450195	26.766904
<i>O. monacantha</i>	Port Alfred	M7	-33.600004	26.866782
<i>O. ficus-indica</i>	Stellenbosch	F1	-33.950867	18.878589
<i>O. ficus-indica</i>	Grahamstown	F2	-33.283426	26.483438
Hybrid	Klipfontein	H1	-33.686103	26.617881
Hybrid	Colchester	H2	-33.703161	25.805489

The containers of cladodes were brought back to laboratories at the University of Cape Town, where the experimental study was performed. Cladodes of each cactus type were held in separate rooms, to ensure that cochineal insects remained on their respective host cacti and no cross contamination occurred.

A select number of cladodes of each cactus type were then thoroughly washed with a jet of water under high pressure to dislodge any cochineal insects. The washed cladodes were left for ten days and then re-inspected. Those found with cochineal were washed again to ensure that the cladodes were completely free of insects.

Clean cladodes of each of the three cactus types were seeded with crawlers, obtained from five gravid females that were collected off each of the three harvested cactus types, from all of the sampled sites (Fig. 2). The females were removed from their original host cladodes and kept in isolation in vials while they produced crawlers which were harvested daily. Ninety crawlers were removed from each of the five females. This cohort of siblings was divided into three groups of 30, each of which was placed on one of the clean cladodes from each of the three cactus types. Crawlers were transferred one-by-one onto the cladodes using a fine paintbrush. They were transferred as they became available so that only crawlers that were less than 24 hours old were used. It took a maximum of three days to get 30 crawlers onto each cladode.

The final experimental set-up therefore contained five lots of sibling crawlers on each of the three host cacti, for each of the sampled sites (Fig. 2). The decision to use 30 crawlers on each cladode was made to ensure that there was no overcrowding and that each individual insect would have an adequate food supply available (Moran and Cobby, 1979). Studies have shown that the sex-ratio amongst crawlers of *D. ceylonicus* is approximately 63% females (Sullivan, 1990), while in *D. opuntiae* it is approximately 50% females (Hoffmann et al., 2002). Therefore random selection of the 30 crawlers was expected to represent the prevailing sex ratio for each species on each of the clean cladodes.

Inoculated cactus cladodes were placed on isolated polystyrene blocks and supported on pins inserted in the blocks. The cladodes were laid horizontally on the pins, allowing crawlers to access and settle on almost its entire surface area. The cladodes with crawlers were retained in insectaries at 30°C on a 12-hour daylight cycles while the insects developed to maturity and mated. The three cochineal types were kept in separate insectaries to ensure that females only mated with males of the same type.

Cladodes were then routinely examined. As soon as crawlers were observed around any female all female adults on that cladode were detached. The wax-covering of each female adult was then carefully removed and her weight was measured using an OHAUS Adventurer balance. Weight measurements were used as a proxy for female condition at maturity. The total numbers of adult females removed from each cladode was recorded and the time (in days) from inoculation to reproductive maturity was noted. These measures represented the proportion of crawlers that survived to maturity and their rate of development. Following this, each female was retained in a separate vial and allowed to produce all her offspring. The numbers of crawlers produced were then counted for a subset of females which were specifically chosen to represent as wide a range in weights as possible. No measurements were made on males due to their disparate life cycle.

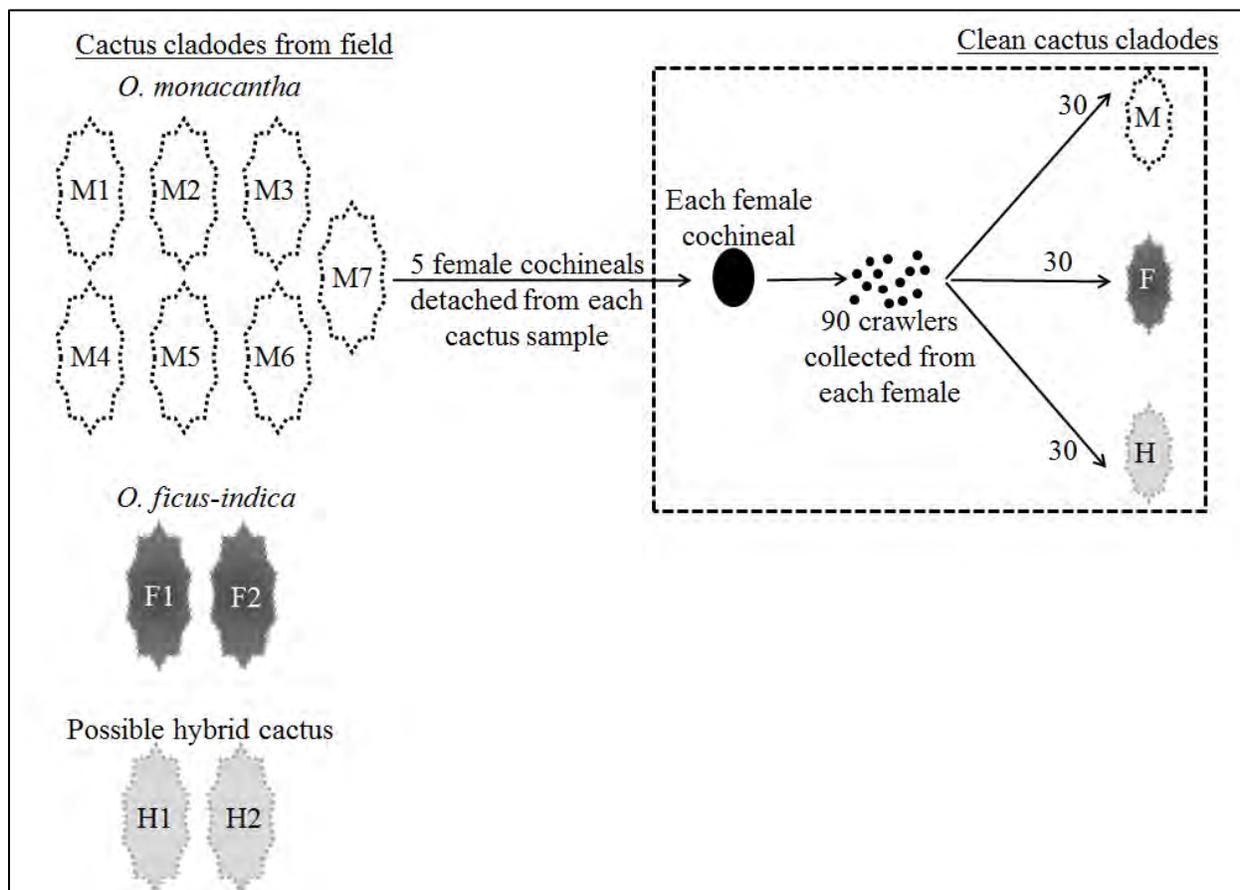


Fig. 2: Experimental set-up of this study. M1-M7 represents *O. monacantha* cacti and their associated cochineal samples from Stellenbosch, Kenton-on-Sea, Salem, Zunney De Mond, Klipfontein, Port Alfred and Bathurst, respectively. F1 and F2 represent *O. ficus-indica* cacti and their associated cochineal samples from Stellenbosch and Grahamstown. H1 and H2 denote possible hybrid cacti and their associated cochineal samples from Klipfontein and Colchester. Five female cochineal insects were collected from each cactus sample and allowed to produce crawlers. Ninety crawlers were removed from each of these females and split into three groups of 30. Each group of 30 crawlers was then placed onto clean cladodes of *O. monacantha* (M), *O. ficus-indica* (F) and the possible hybrid cactus (H).

2.3. Morphological examination

Setae structures (length and basal width) were examined for a subset of harvested cochineal females, using a Phenom proX desktop scanning electron microscope, after specimens had been freeze dried. Measurements of setae were taken from both the anterior and posterior segments of each insect.

2.4. Statistical analyses

2.4.1. Insect-cactus compatibility

All analyses were run using the statistical software R 3.02 (R core team, 2013) in the integrated development environment RStudio (RStudio, 2012). Following the preliminary identification of cochineal species, differences in rate of development, survival and weight at reproductive maturity of each insect-cactus combination were quantified using Generalised Linear Mixed Models (GLMMs). In these analyses, all sources of cochineal of the same species were grouped as one. The majority of data in each of the measures of developmental success were skewed and variances were often heteroskedastic. The decision to use GLMMs was therefore deemed appropriate, as they provide a more flexible approach for analysing non-normal data when random effects are present (Bolker et al., 2008).

Generalised Linear Mixed Models were run using the package 'lme4' (Bates et al., 2014). Models were fitted using log likelihood maximum estimates. The outputs from each model were analysed using the Anova function, from the 'car' package (Fox and Weisber, 2011) and post-hoc tests were then performed using the package 'phia' (De Rosario-Martinez, 2013).

Analyses were performed separately on each measure of developmental success. Therefore, for each analysis the rate of development, survival or weight at reproductive maturity was set as the response variable. In order to account for the over-dispersion, a poisson error structure was fitted to the rate of development data, while a binomial and gamma error structure were fitted to the survival and weight data, respectively. Random effects terms were used in all the models, so as to incorporate the variation amongst the separate sampling sites for each of the cochineal types, as well as to encompass the variation in developmental success of the offspring from individual female insects. The key categorical predictor variables in each of the three models were the cochineal species, the cactus species and the interaction between cochineal and cactus species. Significant predictor variables in the models were identified using Type II Wald chi-squared tests. Interactions found to be significant were further analysed using the holm adjusted Tukey method, with pairwise comparisons across the fixed effects.

2.4.2. Weight as a proxy for fecundity

The relationship between the weight of adult females and the number of crawlers they produced was analysed, to determine patterns of correlation between female weight and fecundity. The appropriate fitted model was determined and used to predict the relationship, through the ‘nls’ function (in the package ‘stats’ (R Core Team, 2013)), as suggested by Bolker et al., (2008). This function determines the non-linear (weighted) least-square estimates of parameters in non-linear models (Bolker et al., 2008). Following this, a Generalised Linear Model was run with the number of offspring as the response variable and weight of the female cochineal insect, cochineal species and the cactus type as predictor variables. Outputs of the model were then interpreted using the same methods as described for developmental rate, survival and weight models. The objective of the Generalised Linear Model was to determine if the predicted relationship between weight and fecundity held true across the different cochineal species, grown on different cactus host plants.

3. Results

3.1. Overall survival

The cochineal derived from *O. ficus-indica* were all capable of surviving to reproductive maturity on both *O. monacantha* and *O. ficus-indica* showing that they were all *D. opuntiae*. The cochineal derived from each of the seven *O. monacantha* populations only survived to reproductive maturity on *O. monacantha* and never on *O. ficus-indica*, showing they were all *D. ceylonicus*.

When each of the provenances of cochineal from Colchester and Klipfontein hybrids were placed on *O. monacantha* and *O. ficus-indica*, the patterns of survival for Klipfontein were the same as those for *D. ceylonicus* cochineal insects, i.e. they only survived on *O. monacantha* plants. On the other hand, the cochineal derived from Colchester showed a *D. opuntiae* survival pattern, i.e. they survived on both *O. monacantha* and *O. ficus-indica*. It was therefore apparent that each hybrid cactus population hosted a different cochineal species: *D. ceylonicus* at Klipfontein and *D. opuntiae* at Colchester. All of the provenances were capable of developing to maturity on both sources of possible hybrid cactus.

Analyses of setae structure confirmed the above findings, as the ratios of length to width differed between samples. Anterior and posterior setae from *D. ceylonicus* (*O. monacantha*'s cochineal) had approximately equal length to width ratios (Fig. 3; Table 2). This was significantly different from the length to width ratios of *D. opuntiae* ($\chi^2=10.62$, DF=1, $p<0.01$), where anterior and posterior setae had greater lengths than widths. The cochineal from Klipfontein had setae structurally characteristic of those on *D. ceylonicus* ($\chi^2=0.27$, DF=1, $p>0.05$), while the cochineal from Colchester had setae that were characteristic of those on *D. opuntiae* ($\chi^2=0.47$, DF=1, $p>0.05$) (De Lotto, 1974; Perez-Guerra, 1991) (Table 2).

Table 2: The average (\pm standard error) lengths and widths (μm) of setae on the anterior and posterior segments of cochineal derived from four cactus types. N represents sample size.

Cochineal species from:	Anterior segment (μm)		Posterior segment (μm)	
	Length (\pm SE)	Width (\pm SE)	Length (\pm SE)	Width (\pm SE)
<i>O. monacantha</i> (<i>D. ceylonicus</i>)	15 (± 0.5) (N=28)	17 (± 0.6)	14 (± 0.5) (N=32)	15 (± 0.5)
<i>O. ficus-indica</i> (<i>D. opuntiae</i>)	16 (± 0.5) (N=31)	12 (± 0.3)	16 (± 0.2) (N=34)	11 (± 0.6)
Hybrid cactus (Klipfontein)	13(± 0.3) (N=44)	13 (± 0.3)	14 (± 0.2) (N=33)	15 (± 0.2)
Hybrid cactus (Colchester)	17 (± 0.2) (N=20)	12 (± 0.2)	16 (± 0.3) (N=24)	13 (± 0.2)

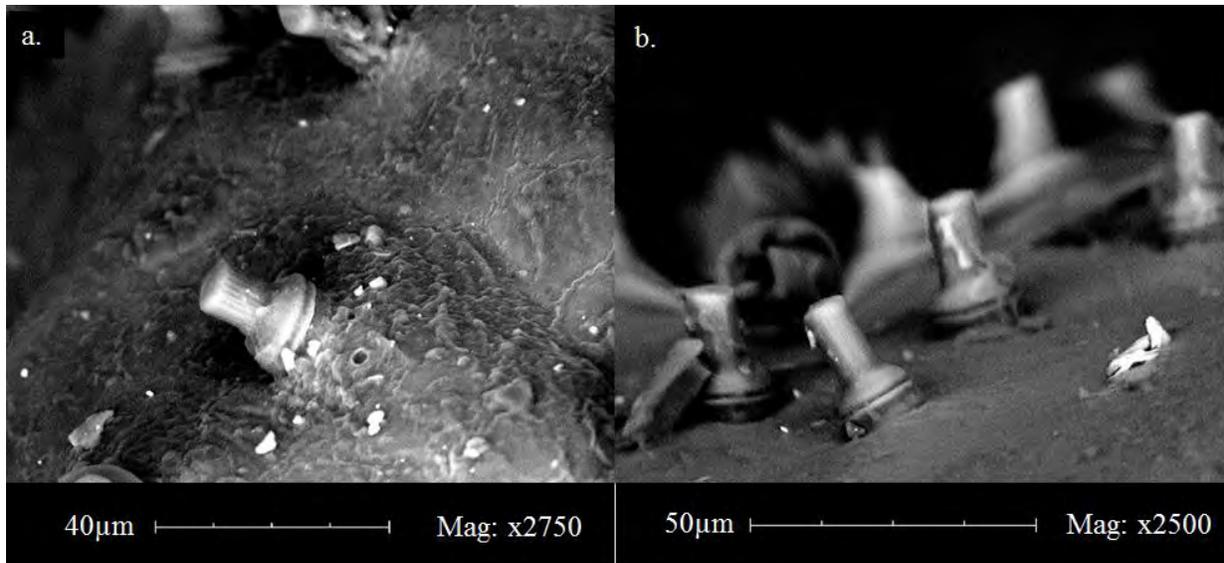


Fig. 3: Scanning electron micrographs of the setae from *D. ceylonicus* (a) and *D. opuntiae* (b).

3.2. Contrasting developmental success

3.2.1. Rate of development

Overall, *D. ceylonicus* reached reproductive maturity in less time than *D. opuntiae*, on both *O. monacantha* ($\chi^2=11.03$, DF=1, $p<0.01$) and the possible hybrid cactus hosts ($\chi^2=23.09$, DF=1, $p<0.001$) (Fig. 4). However, the Generalised Linear Mixed Model indicated no significant effect of host cactus type on the developmental rate ($\chi^2=1.91$, DF=2, $p>0.05$) of either *D. ceylonicus* or *D. opuntiae*. *Dactylopius ceylonicus* females took approximately 30 days to reach reproductive maturity on both *O. monacantha* and the possible hybrid cactus, while *D. opuntiae* females required approximately 39 days (Fig. 4).

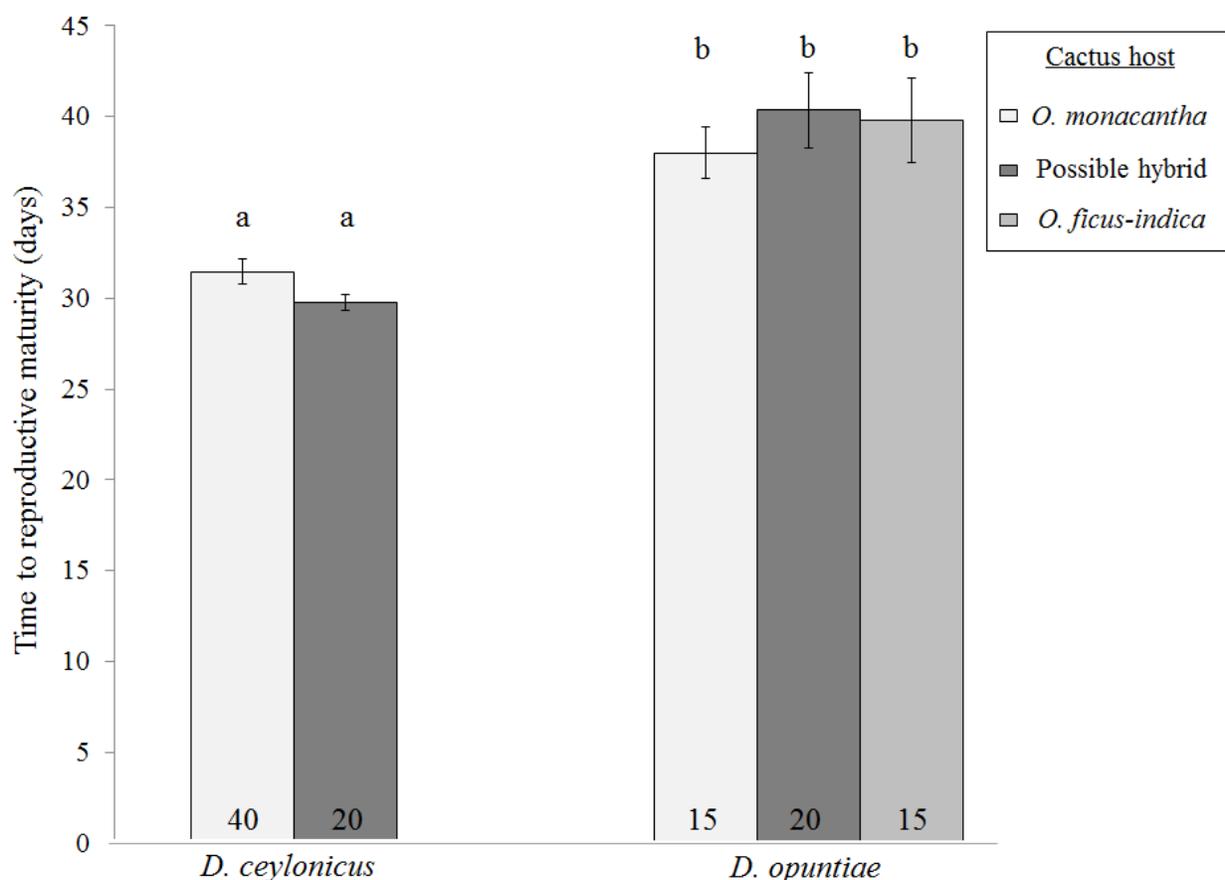


Fig. 4: Mean (\pm standard error) days from crawler to reproductive maturity for female *D. ceylonicus* and *D. opuntiae* reared on different *Opuntia* host plants in the laboratory. **a** and **b** represent significant difference in rate of development ($p < 0.05$). Numerals in bars show the number of experimental cladodes on which crawlers were grown in each category.

3.2.2. Survival

Generalised Linear Mixed Models revealed that cactus type ($\chi^2=21.95$, DF =1, $p < 0.01$) and the interaction between cactus type and cochineal type ($\chi^2=12.78$, DF =1, $p < 0.01$) were significant predictors of survival. Pair-wise post-hoc comparisons of survival across cochineal species indicated no significant differences between *D. ceylonicus* and *D. opuntiae* when each species was grown on *O. monacantha* ($\chi^2=0.04$, DF =1, $p > 0.05$) (Table 3). There was also no significant difference in survival between *D. ceylonicus* and *D. opuntiae* when grown on the possible hybrid cacti ($\chi^2=1.75$ DF=1, $p > 0.05$) (Table 3). In addition, pair-wise comparisons of survival across cactus types revealed that *D. ceylonicus* survived equally well on *O. monacantha* and the possible hybrid cactus host ($\chi^2=0.89$, DF=1, $p > 0.05$), however, it was not capable of surviving

on *O. ficus-indica*. The number of *D. opuntiae* females that survived to adulthood on *O. monacantha* was significantly higher than those grown on both the possible hybrid cactus ($\chi^2=12.27$, DF=1, $p<0.001$) and *O. ficus-indica* ($\chi^2=19.33$, DF=1, $p<0.001$); on the other hand survival between the possible hybrid cactus and *O. ficus-indica* was not significantly different ($\chi^2=6.3$, DF=1, $p=0.08$) (Table 3).

Table 3: Mean (\pm standard error) percentage of female *D. ceylonicus* and *D. opuntiae* that survived from crawler to reproductive maturity on different *Opuntia* host plants in the laboratory. N represents sample size of experimental cladodes, each of which were inoculated with 30 crawlers.

	<i>O. monacantha</i>	<i>O. ficus-indica</i>	Presumed hybrid of <i>O. monacantha</i> and <i>O. ficus-indica</i>
<i>D. ceylonicus</i>	44 \pm 2 (N = 40)	0 (N = 40)	51 \pm 5 (N = 20)
<i>D. opuntiae</i>	45 \pm 8 (N = 15)	24 \pm 4 (N = 15)	35 \pm 4 (N = 20)

3.2.3. Mass of females at maturity

A significant cochineal type effect ($\chi^2=234.77$, DF=1, $p<0.001$) and cochineal-cactus type interaction effect ($\chi^2=34.30$, DF=1, $p<0.001$) was shown in the Generalised Linear Mixed Model when mass of reproductive adult females was the dependent variable. *Dactylopius ceylonicus* females attained greater mass than *D. opuntiae* when grown on both *O. monacantha* ($\chi^2=96.04$ DF=1, $p<0.001$) and on the possible hybrid cactus ($\chi^2=193.56$, DF=1, $p<0.001$) (Table 4). Pair-wise post-hoc comparisons indicated that the mass of adult *D. ceylonicus* females grown on *O. monacantha* and the hybrid cactus did not differ significantly ($\chi^2=0.19$, DF=1, $p>0.05$). However, the masses of *D. opuntiae* females were significantly different on all three host plants. Females on *O. monacantha* attained a higher average mass than those on both the possible hybrid ($\chi^2=59.00$, DF=1, $p<0.001$) and *O. ficus-indica* ($\chi^2=34.52$, DF=1, $p<0.001$). *Opuntia ficus-indica* produced females with the lowest mass at reproductive maturity ($\chi^2=36.24$, DF=1, $p<0.001$) (Table 4).

Table 4: Mean (\pm standard error) mass (mg) of female *D. ceylonicus* and *D. opuntiae* that survived from crawler to reproductive maturity on different *Opuntia* host plants in the laboratory. N represents sample size of experimental cladodes, each of which were inoculated with 30 crawlers.

	<i>O. monacantha</i>	<i>O. ficus-indica</i>	Presumed hybrid of <i>O. monacantha</i> and <i>O. ficus-indica</i>
<i>D. ceylonicus</i>	19 \pm 0.40 (N = 40)	0 (N = 40)	18 \pm 0.53 (N = 20)
<i>D. opuntiae</i>	9 \pm 0.40 (N = 15)	4 \pm 0.42 (N = 15)	6 \pm 0.35 (N = 20)

The differences in average female mass directly translate into differences in fecundity as the mass of the females had a positive exponential relationship with the number of offspring produced (Fig. 5). This relationship did not differ significantly between the two cochineal species ($\chi^2=0.457$, DF=1, $p>0.05$). The modelled relationship between mass and fecundity also remained the same for each species of cochineal across the two cactus hosts ($\chi^2=1.479$, DF=1, $p>0.05$). This model explained approximately 56% of the variation in the data for number of offspring produced.

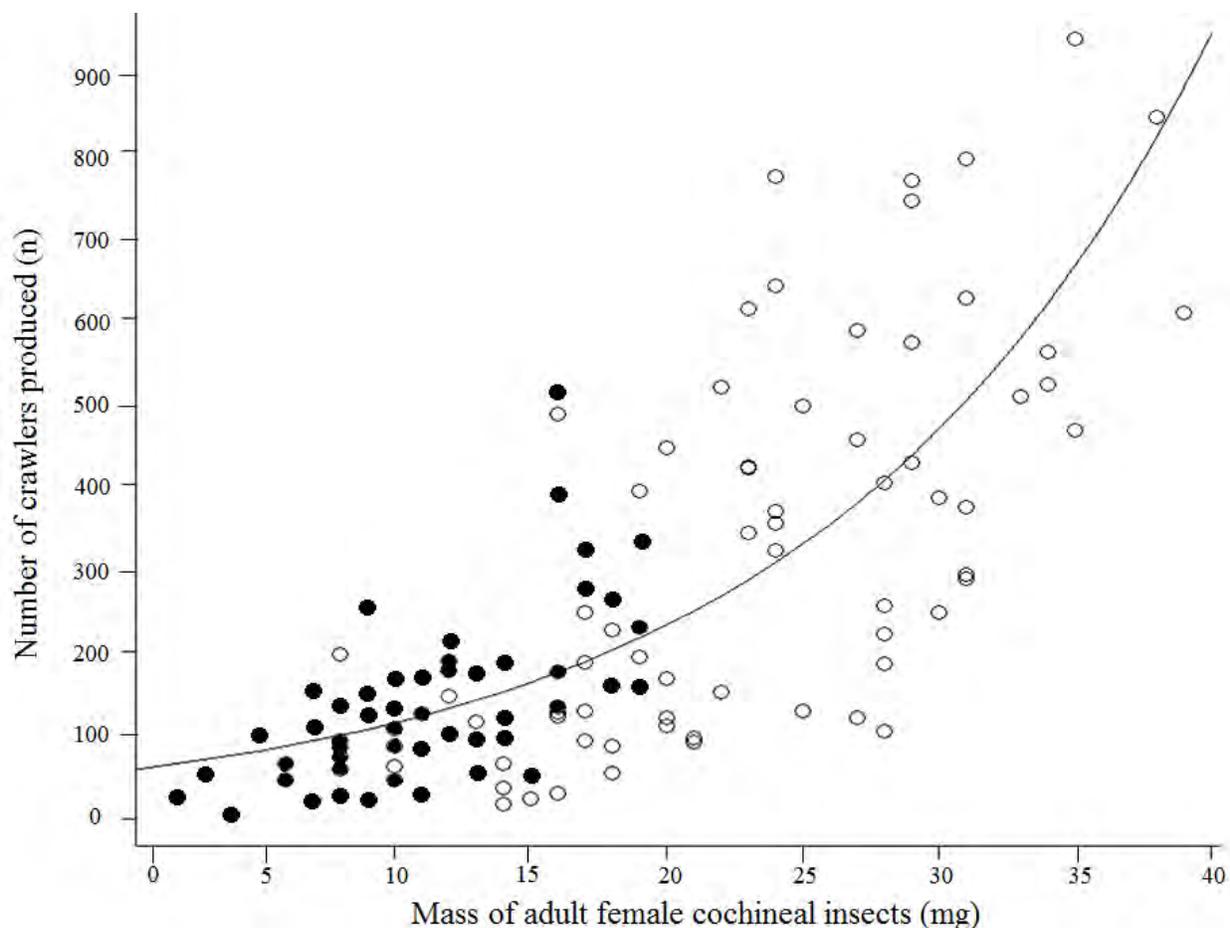


Fig. 5: Modelled relationship (trend line) between adult female mass (mg) and number of crawlers produced, for both *D. ceylonicus* (open circles; N=67) and *D. opuntiae* (closed circles; N=57) females, grown on *O. monacantha* and the possible hybrid cactus hosts. Model (Offspring number = $3.57 * \text{Weight of mother}^{1.45}$) explained 56% of the variation for combined data.

4. Discussion

4.1. The status of *O. monacantha*, a possible hybrid cactus and their biological control agents

The self-dispersing and self-perpetuating nature of biological control agents, that make projects so cost-effective, also make the consequences of their release irreversible. As a result, a lot of attention has been devoted to understanding the threats that biological control agents could pose to non-target native taxa (Howarth, 1991; Louda et al., 2003; Simberloff and Stiling, 1996a; Simberloff and Stiling, 1996b; Strong and Pemberton, 2000). In recognition of these undesirable

effects modern biological control programs emphasize the importance of host specificity in introduced agents (Pearson and Callaway, 2005). In cases where exotic target weeds have no equivalent native taxa, the demand for high host-fidelity is less stringent as host-shifting between exotic taxa is assumed to be less risky (Paterson et al., 2011). Yet oligophagous control agents that include non-target weeds as hosts may interfere with biological control agents already present on the host. Interference may reduce the efficacy of the original control agent or even result in a loss of the agent, ultimately jeopardizing the viability of the biological control program already in place (Denno et al., 1995; Ehler and Hall, 1982).

The results of this study show that any extension of *D. opuntiae* from *O. ficus-indica* onto *O. monacantha* is unlikely to impinge upon the success of *D. ceylonicus* as a biological control agent of *O. monacantha*, in South Africa. *Dactylopius ceylonicus* continues to be predominant on *O. monacantha*, as all cochineal females from the seven sampled populations were identified as *D. ceylonicus*. This finding shows that *D. opuntiae* has not displaced *D. ceylonicus* on *O. monacantha*, even though both agents can develop on the host and have co-existed in South Africa for over 70 years. The prevalence of *D. ceylonicus* on *O. monacantha* may result from the fact that *D. ceylonicus* females reach reproductive maturity in less time than *D. opuntiae* and mature females attain a higher mass and are thus more fecund than *D. opuntiae*. Even though both species' offspring have a similar probability of survival, the higher reproductive output of *D. ceylonicus* will result in a higher number of reproductively mature adults surviving to the next generation, giving them an advantage over *D. opuntiae* on *O. monacantha*.

Although the dominance of *D. ceylonicus* on *O. monacantha* can be explained by performance disparities between the agents, the complete absence of *D. opuntiae* from *O. monacantha* is puzzling. According to the preference-performance hypothesis, female insects should preferentially associate with hosts that maximise the success of their offspring (Clark et al., 2011; Gripenberg et al., 2010). Although the preference-performance hypothesis is not directly applicable to cochineal insects, as dispersal is passive, it is still expected that *D. opuntiae* would associate with *O. monacantha*, since developmental success is higher on this host plant than on *O. ficus-indica*. Yet, this study showed that *D. opuntiae* is scarce, if not entirely absent, on *O. monacantha* in the field. This apparent contradiction shows that performance experiments under laboratory conditions may not be a true representation of the capacity of *D. opuntiae* to develop

on *O. monacantha* under natural conditions. Environmental factors may override physiological adaptations which are apparent in the laboratory and this could explain the absence of *D. opuntiae* on *O. monacantha* in the field (Grevstad, 1999; Newman et al., 1998). As such, reported findings of *D. opuntiae* on *O. monacantha* are likely to be the exception, or, may be due to misidentification. If the former is the case, larger sample sizes from each *O. monacantha* population should detect the presences of *D. opuntiae* individuals, but this can only be verified through extended field studies. Additional experimental analyses could also be performed to determine the capacity of *D. opuntiae* to develop on *O. monacantha* in the presence of *D. ceylonicus* both under laboratory and field conditions.

In effect, this study highlights the inherent unpredictability and complexity in trying to predict the consequences of interactions between weed biological control agents. Yet, due to the current concern for indirect impacts associated with classical biological control, there is an increasing demand for more stringent, all-encompassing pre-release evaluations (Barratt et al., 2010). Rigorous screening programs that assess the risk of interactions between agents would circumvent interruption of existing biological control initiatives; however, they would also be associated with increased costs. Oligophagous agents would be particularly costly to evaluate due to the high number of potential hosts and hence high number of possible interactions. Although oligophagous agents are rarely used in biological control (McEvoy and Coombs, 2000; Sheppard, 2003), antagonistic interactions between host-specific agents may also arise when multiple agents are deployed on the same target weed (Pearson and Callaway, 2005; Wang and Messing, 2003).

The release of multiple species on a single target weed is common in the practice of biological control, and relies on the assumption that eventually the most effective agent, or combination of agents, is likely to emerge (Pearson and Callaway, 2005). Although this strategy increases the risk of interruptive interactions between agents, thus far there is no published evidence for the replacement of a successful control agent by an unsuccessful agent on an invasive weed (Barratt et al., 2006; Crawley, 1989; Denoth et al., 2002). In fact, the literature suggests that higher numbers of control agents on weeds generally result in either a neutral or positive effect on the success of biological control programs (Denoth et al., 2002). Essentially, negative interactions between weed control agents rarely play a significant role in undermining the viability of control

programs on plants. Therefore, while the value of risk assessments should not be understated, there is an equal risk that overly stringent regulations could unnecessarily increase pre-release testing costs and preclude the use of promising control agents (McCoy and Frank, 2010). For example, if the current study had been undertaken before *D. opuntiae* was released in South Africa, a cautious practitioner may have discouraged the use of *D. opuntiae*, given its capacity to grow on *O. monacantha* and potentially interfere with *D. ceylonicus*. Such a decision would have forfeited control of *O. ficus-indica* in South Africa with unnecessary and immeasurable losses for the nation.

In addition to assessing the status of control agents on *O. monacantha*, this study investigated the effects of the possible hybridisation between *O. monacantha* and *O. ficus-indica*. The primary aim was to evaluate whether hybrid plants experience increased insect pathogen resistance. Indeed, results suggest that hybridisation alters the plant's compatibility with one of the cochineal agents, *D. opuntiae*. Differences in performance (sibling survivorship and mass at reproductive maturity) of *D. opuntiae* on the possible hybrid cactus and its parental taxa demonstrate that this agent is less compatible with the hybrid than *O. monacantha*, but more compatible with the hybrid than *O. ficus-indica*. Conversely, the compatibility of *D. ceylonicus* on the possible hybrid was equivalent to that on *O. monacantha*. This interspecific difference in the level of agent-hybrid compatibility is unexpected, given that *D. opuntiae* is capable of surviving on both parental taxa of the hybrid plant, while *D. ceylonicus* is only compatible with *O. monacantha*; as such it was thought that *D. ceylonicus* would show lower compatibility with the hybrid than *O. monacantha*. A fuller understanding of the mechanisms driving these differences may be gained through genetic analyses performed on the possible hybrid host.

Differences in the performance of the two cochineal species on the possible hybrid cactus reflect the same patterns as on *O. monacantha*, that is, *D. ceylonicus* is superior to *D. opuntiae*, with a faster rate of development and higher mass at maturity. The suggestion is therefore that *D. ceylonicus* is more compatible than *D. opuntiae* on the possible hybrid, however, in order to assess which species is the superior control agent, further experimental and field analyses are needed. Ideally, these studies should assess the damage each species inflicts upon the hybrid cactus.

Nevertheless, within an ecological context, the threat posed by the possible hybrid cactus appears limited, as both *D. ceylonicus* and *D. opuntiae* are capable of establishing on the cactus host. Furthermore, superficial examinations indicated that both control agents are capable of damaging this possible hybrid, although this was not specifically quantified in the study. Therefore, it appears that hybridisation between the two *Opuntia* cactus weeds does not facilitate a complete release from natural enemies. However, this study was performed on one generation of cochineal insects and one generation of the possible hybrid cactus under laboratory conditions. Interaction between the agents and cactus plants may present different outcomes under natural conditions. Previous studies also demonstrated that hybridisation may only confer benefits, such as pathogen resistance, in later generations (Hails, 2000). As such, it is important that additional long-term studies are pursued, to ensure that hybridisation does not promote the spread and proliferation of this *Opuntia* hybrid. In addition to evaluating the risks associated with hybridisation and natural enemy release, further studies should also confirm that interactions between *D. ceylonicus* and *D. opuntiae* do not interfere with hybrid host suppression.

This study is one among many that show the complexities involved in managing invasive plant species. Where management projects have been initially efficacious, success can be interrupted by changes in the environment, such as the introduction of new species into the system. For plant biological control, interactions between weeds and between introduced control agents can lead to unanticipated environmental and economic risks if, for example, these interactions disrupt control initiatives. With growing concern regarding the indirect effects of biological control, practitioners are increasingly faced with the challenge of balancing the harm and risks that accrue from additional agent introductions.

In view of the global problem of invasive species, this study confirms the potential persistence of biological control. One-hundred years after the initiation of the control program against *O. monacantha* the agent remains present on the target host and continues to maintain it at negligible levels.

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6. Appendix



Appendix 1: Morphological characteristics of *O. monacantha* (c), *O. ficus-indica* (a) and the possible hybrid between the two (b).