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THE IMPACT OF THE HYBRIDISATION OF *DACTYLOPIUS OPUNTIAE* COCKERELL COCHINEAL LINEAGES ON THE BIOLOGICAL CONTROL OF CACTUS WEEDS

BY

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Abstract

In an attempt to control the harmful invasive alien plant, *Opuntia megapotamica* Arechav. (Cactaceae)*,* a population of the cochineal insect *Dactylopius opuntiae* Cockerell (Dactylopiidae) (known as the 'engelmannii' lineage) is being considered as a potential biological control agent*.* This lineage of cochineal and the already established 'ficus' and 'stricta' lineages of the same cochineal species will inevitably hybridise where they occur in sympatry, possibly influencing the efficacy of the lineages on their respective hosts. Laboratory studies were conducted to investigate the likely outcomes of hybridisation between the 'engelmannii' and 'ficus' lineages. Detailed hybridisation trials, during which individual insects were manipulated and crossed, were used to compare the host affinity of F_1 and F_2 hybrids between the 'ficus' and 'engelmannii' lineages with the host specificity of the two pure-bred lineages. Host affinity was determined by plotting the net rate of increase (*R*) of a cochineal population developing on one host plant species against *R* on the other host plant species. F_1 hybrids were less species-specific than the purebred lineages in both crosses. Thus, the first generation will most likely remain effective in controlling both plant species in the field. F_2 hybrids produced a mixture of purebred and hybrid genotypes, with a higher net rate of increase when compared to purebred nymphs on their alternative host. Biological control of both *Opuntia ficus-indica* (L.) Mill. (Cactaceae) and *O. megapotamica* in the Eastern Cape Province, where both invasive alien plant species occur together, could be enhanced by the less specific nature of the F_1 progeny, which developed equally well on both *O. megapotamica* and *O. ficus-indica*; but this benefit will be reduced by the loss of host specificity of F_2 progeny. The success of biological control would depend on whether the species-specific nymph encounters its target host, because the less specific nymphs will have little effect on controlling either weed. These findings indicate that only purebred *D. opuntiae* lineages should be released in monocultures of their targeted weed. Long-term consequences of hybridisation should be monitored in the field.

Declaration

I hereby declare that this thesis is an original report of my research, has been written by me and has not been submitted, either in the same or different form, to this or any other university for a degree. I know the meaning of plagiarism and declare that all of the work in the thesis, except for that which is clearly indicated and properly acknowledged, is my own.

The ethics clearance number for this project is 2021-3842-5900.

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Chapter 1 GENERAL INTRODUCTION

Opuntia megapotamica Arechav. (Cactaceae: Opuntioideae) is a harmful invasive alien cactus species in South Africa that is a target for biological control using cochineal insects. There are many other cochineals used as biocontrol agents for other invasive alien cacti in South Africa, some of which feed on *O. megapotamica* but do not result in sufficient control. This study investigates whether hybridisation between the new proposed Arizonan cochineal agent and *Dactylopius opuntiae* (Cockerell) 'ficus' lineage that has already established on *Opuntia ficus-indica* (L.) Mill. will influence the level of control of both *O. megapotamica* and *O. ficus-indica* should the new agent be released. This chapter provides background on the family Cactaceae (order Caryophyllales) and genus *Opuntia* Mill. as well as the biological control programmes that have utilised cochineal insects to control *Opuntia* cacti in the past. It also provides the background to *O. megapotamica* biological control and the history of the taxonomic confusions that have resulted in this being a particularly challenging biocontrol programme.

1.1. FAMILY CACTACEAE

The family Cactaceae is endemic to the Americas (Edwards *et al*., 2005), with the exception of one epiphytic species, *Rhipsalis baccifera* (J. S. Muell.) Stearn (mistletoe cactus) that is considered indigenous to the Old World tropics, including southern Africa (Benson, 1982; Oulo *et al.,* 2020). The indigenous distribution of the family in the Americas extends from southern Patagonia in Argentina and Chile, to Alberta and British Columbia in Canada (Anderson, 2001; Edwards *et al*., 2005).

The Cactaceae is a diverse family with varied morphology, comprising of over 1800 species (Anderson, 2001; Edwards *et al*., 2005). The smallest of the species, measuring 9 mm in diameter is *Blossfeldia liliputana* Werderm., while *Pachycereus pringlei* (S. Watson) Britton and Rose (elephant cactus/Mexican Giant cardon) stands at 20 m tall (Anderson, 2001). The family Cactaceae is characterised by shrubs, climbers, ground-covers or trees with green, fleshy, succulent and photosynthetic stems of varying shapes and sizes; with the presence of areoles and spines; and with the absence of leaves being a common feature among typical species (Obermeyer, 1976; Eggli, 1993). The stems are succulent but sometimes woody, and can be cylindrical, globose or flattened. They bear rounded, usually raised woolly areoles (Obermeyer, 1976). The areoles, which are unique to the family, are highly specialised axillary or lateral buds, short shoots or branches (Mauseth, 1983). Tufts of short barbed spines, referred to as glochids or glochidia, are sometimes present (in subfamily Opuntioideae) (Walters *et al.,* 2011). There are sometimes clusters of spines or singular spines. Spines are rudimentary leaves found in areoles of cacti (Anderson, 2001). The flowers are mostly singular and the fruit are usually an edible berry with a few to numerous seeds (Obermeyer, 1976). As a response to weather extremes, cacti have a nocturnal stomatal opening trait that allows for efficient use and storage of limited soil water, and this, along with the succulent habit of most species, makes the family particularly well adapted to arid environments (Walters *et al*., 2011).

Cactaceae possess characteristics that allow for a tolerance of temperature extremes and drought, growing at altitudes ranging from below sea level to over 4 500 m in the Andes Mountains and climates with very little rainfall to areas with over 5 000 mm of precipitation (Shetty *et al*., 2011; Shalabi, 2015). They are adapted to different habitats including hot deserts, sandy coastal areas, scrublands, dry deciduous forests, tropical rain forests and mountain slopes (Barthlott and Hunt, 1993).

The family Cactaceae has four subfamilies, viz. Pereskioideae, Cactoideae, Opuntioideae (Edwards *et al*., 2005) and Maihuenioideae (Anderson, 2001; Cuadrado and Garralla, 2009). The genus *Opuntia,* a representative of Opuntioideae, is one of the largest, most taxonomically confused and most widespread plant genera, comprising between 180 and 200 species (Weniger, 1969, Anderson, 2001). *Opuntia* is believed to have originated in south western South America and diversified after a dispersal event northward into the arid regions of western North America (Chávez-Moreno *et al.,* 2009; Majure *et al.,* 2012). As a result, *Opuntia* has a broad distribution, high morphological and species diversity and low molecular marker divergence (Majure *et al*., 2012).

1.2. USES OF CACTI

The uses for cacti are generally categorised into the following: 1) ornamental (horticulture), 2) food and fodder, 3) medicinal, 4) hedging and 5) other (religious or furniture) (Novoa *et al.,* 2015). Members of the Cactaceae form one of the most popular groups of horticultural plants in the world, and have been spread globally as ornamentals, curiosities, and collectors' items (Zimmermann *et al*., 2009; Walters *et al.,* 2011; Novoa *et al.,* 2015). In South Africa, the commercial trade in ornamental cacti is a highly profitable industry, and Novoa *et al*. (2017) state that approximately 300 cacti species are imported into South Africa for ornamental horticultural purposes annually. Some of the cactus species with dense, sharp spines and glochids, including *Opuntia* spp. and *Cylindropuntia* (Engelm.) F.M. Knuth spp., and those that grow very tall, such as *Cereus* spp., are planted widely as barriers and living fences (Le Houérou, 1996; Inglese *et al*., 2002; Larsson, 2004; Majure *et al.,* 2012). They are also used to control erosion, aid rehabilitation of land in some countries, and protect against drought and wildfires as the cacti store high volumes of water (Le Houérou, 1996; Inglese *et al*., 2002; Majure *et al.,* 2012; Bauman & Schmidt, 2015). They also function as carbon reservoirs in regions with inconsistent and unpredictable climate (Shetty *et al*., 2011; Inglese *et al.,* 2017).

Persistent droughts, continuous desertification, and a rise in the human population coupled with misuse of natural resources, has changed and continues to change the environment and its ecological services (Inglese *et al.,* 2017). The flat-stemmed *Opuntias*, universally known as prickly pears, are highly adaptable and have a tolerance for extremes. One species in particular, *Opuntia ficus-indica* (L.) Mill., is regarded as a crop of the future as it has the qualities to survive the extremes and changes to the environment and climate and sustain human and animal populations (Shetty *et al*., 2011; Inglese *et al.,* 2017). The fruit (called tuna), flowers and cladodes (also known as leaf pads) of several cactus groups, in particular in the genus *Opuntia*, have long been valued as sources of food (both fruit and vegetable) and beverages for human consumption, and as forage and fodder for livestock and game – both in their native and adventive distribution worldwide (Weniger, 1969; Le Houérou, 1996; Inglese *et al*., 2002; Majure *et al.,* 2012; Bauman & Schmidt, 2015). In countries like Ethiopia and Madagascar that have been devastated by drought, people and animals are surviving on cacti, making it important for food security (Inglese *et al*., 2017). *Opuntia ficus-indica* is regarded as the most important agricultural cactus species in our day, and the first fruit crop used in semi-arid and non-irrigated lands (Shetty *et al.,* 2011). It is easy to grow and requires very little irrigation (Chávez-Moreno *et al*., 2009).

The *Opuntia* species contain a wide variety of nutrients and bioactive compounds including vitamins (e.g. vitamin C), minerals (e.g. manganese and magnesium) and phytochemicals (Tesoriere *et al.,* 2004; Sáenz, 2013; Osuna-Martínez *et al*., 2014; Bauman & Schmidt, 2015). Studies have found that the cladodes and fruit differ in the composition of these nutrients (Sáenz, 2013; Bauman & Schmidt, 2015). The high soluble solid content of their fruit and high concentrations of mucilage and pectin makes them suitable for processing products such as preserves, juices, nectars, dried fruit and liquors, while pickles, juices and preserves are made from the cladodes (Inglese *et al.,* 2002; Sáenz, 2013). There is a perception that there is a great potential to explore and exploit *Opuntia* spp. further in the food industry (Kaur *et al*., 2012; Sáenz, 2013; Inglese *et al.,* 2017). There is a joint effort by the Food and Agriculture Organisation (FAO) and International Centre for Agricultural Research in the Dry Areas (ICARDA) in the form of a book that details how to utilise the *Opuntia* species optimally, including the plant's genetic and physiological properties, soil preference and susceptibility to diseases and pests. The book *Crop Ecology, Cultivation and Uses of Cactus Pear* (by Inglese *et al*., 2017) also offers advice on how to put prickly pear to optimal use as food, just as it is consumed in its native Mexico. The book also goes on to give some background information on the history, origin, taxonomy and global invasions of prickly pear. The last chapter focuses on marketing the species in an effort to change the perception many have of it as a weed.

Prickly pears have numerous medicinal uses too. Traditionally, *O. ficus-indica* has been utilised by indigenous tribes in Mexico and those in central and southern Arizona, for the treatment of digestive ailments, diabetes, oedema and applied topically for burn and wound care (Castaneda *et al.,* 1997; Shetty *et al*., 2011; Osuna-Martínez *et al*., 2014; Bauman & Schmidt, 2015). Furthermore, prickly pear has been used as a diuretic, a fever reducer and helps manage vitiligo, urinary problems, tumours and inflammation (Castaneda *et al.,* 1997; Shetty *et al*., 2011; Gili *et al*., 2020). The plant extract is an anti-inflammatory, anti-viral and has anti-clotting properties. Studies on the aqueous extract of *O. megapotamica* fruit pulp have found that it is a bone anabolic agent in that it increases and improves the functioning of osteoblasts, thus having the potential to treat bone-associated diseases such as postmenopausal osteoporosis (Gili *et al.,* 2020). The flowers have been used to alleviate lung problems while the fruit has been used for whooping cough relief (Bauman & Schmidt, 2015). The fruit and cladodes of *Opuntia* spp. contain several compounds that are essential for a healthy diet, such as dietary fibre, hydrocolloids (pectin and mucilage), betalains, carotenoids, calcium, potassium and vitamin C (Sáenz, 2013). The prickly pear is highly nutritious with soluble carbohydrates that are beneficial in livestock's milk production and weight gain (Inglese *et al.,* 2017). Prickly pear cladodes are used in the production of soap, body lotions and creams (Kaur *et al*., 2012; Majure *et al.,* 2012; Bauman & Schmidt, 2015) and to extend the shelf life of strawberries and Serrano peppers when coated in their mucilage (Del-Valle *et al*., 2005; Torres *et al*., 2018). As such, *Opuntia* spp. have numerous actual and potential uses in the pharmaceutical and cosmetics industry too (Kaur *et al*., 2012; Sáenz, 2013).

In addition, *Opuntia* spp. can be used in the construction industry (e.g. the mucilage can bind compounds), the energy sector (e.g. biogas or fuelwood), and the agricultural sector (e.g. improving soil composition and drainage, controlling erosion and purifying water) (Le Houérou, 1996; Inglese *et al*., 2002; Sáenz *et al.,* 2004; Sáenz, 2013; Bauman & Schmidt, 2015). Moreover, the prickly pear is used for the propagation of cochineal insects to produce carmine dye, which is a valuable industry (Le Houérou, 1996; Inglese *et al*., 2002; Majure *et al.,* 2012; Bauman & Schmidt, 2015).

1.3. CACTACEAE IN SOUTH AFRICA

1.3.1. Introduction, spread and impact of cacti

People have been introducing, not only cacti, but also many alien species of other flora and fauna, from their native ranges into South Africa for many years (Wilson *et al*., 2009). The introduction of alien species has been either intentional or unintentional. Food crops are the most common of intentional introductions, while sometimes seeds are unknowingly transported and unintentionally introduced into receiving environments (Walters *et al*., 2011). It is important to note that only a few taxa of introduced species become invasive in the receiving environment, but the few that do become established are often very problematic (Simberloff & Rejmánek, 2011; Novoa *et al.,* 2015).

Prickly pears were amongst the earliest plants brought back to Europe by the Spanish conquerors of the Americas. From Europe, they were most likely introduced to Southern Africa during the early European settlement in the Cape in the early 1600s (Howard & Touw, 1981; Beinart & Wotshela, 2011; Walters *et al.,* 2011). In the 18th century, the earliest Afrikaner frontier farmers took *O. ficus-indica* to the former provinces of Natal and the Transvaal (Beinart & Wotshela, 2011). From there it spread ferociously, predominantly through ox-wagon transport corridors and flooding after heavy rainfall (Beinart & Wotshela, 2011). As with many other non-native plant species, Cactaceae become overabundant for a variety of reasons. They are pre-adapted to arid regions, such as the interior of South Africa, and have two effective means of dispersal: their seeds are lodged in conspicuous, tasty fruit and are thus dispersed by birds and mammals; and effective vegetative propagation through the detachment of stem fragments (Chávez-Moreno *et al*., 2009; Majure *et al.,* 2012; Novoa *et al.,* 2015). Importantly, their new environment lacks those natural enemies that normally keep cactus numbers under control in their native range (Mann, 1969; Walters *et al*., 2011). Most cactophagous insects do not feed outside the Cactaceae family (Mann, 1969; Zimmermann *et al.,* 2004; Walters *et al*., 2011), and since South Africa has only one native Cactaceae, which is morphologically very different and spineless and grows in sub-tropical forests rather than arid regions, there were no cactus-feeding insects in the environment that could have adopted the cacti as new hosts. This gave the arriving cacti a competitive advantage over the natural South African vegetation (Walters *et al.,* 2011). This is an example of the Enemy Release Hypothesis in that the introduced plants do not have to build up a resistance and/or defence to their natural enemies because there are none; instead, they pull all their energy towards rapid growth and reproduction (Schulz *et al*., 2019). There is a decrease in regulation by herbivores and other natural enemies, which in turn results in an increase in distribution and abundance of the introduced plant species (Keane & Crawley, 2002; Schulz *et al*., 2019). All the while, specialist natural enemies attack indigenous plants, giving the alien species a competitive advantage (Keane & Crawley, 2002).

Cacti form dense stands through vegetative propagation and spread rapidly because cladodes are easily dislodged and will readily root. This results in substantial changes to the composition of vegetation in invaded areas, and transformation of the landscape (Zimmerman *et al*., 2009; Walters *et al*., 2011). High abundance of cacti is associated with a loss of biodiversity and ecological functioning and decreased agricultural productivity (Novoa *et al.,* 2015). The large and prolific spines of many cactus species result in direct injuries to livestock and wildlife, and reduce access to pastures, water sources and shade (Walters *et al*., 2011). The cost of controlling cactus has not been quantified, but control efforts between 1995 and 2008 are estimated to be close to R100 million (about 6.2 million USD) (Novoa *et al*., 2017).

Invasions, defined as plants spreading over extensive distances from the original introduction sites (Richardson *et al.,* 2000), by cacti were the first plant invasions to be recognised and regulated worldwide due to their impact as agricultural and environmental weeds (Novoa *et al.,* 2019). Regulations were legislated to mitigate the impacts of cacti in their non-native distribution and reduce further introductions and spread. Cacti are the most widespread and dominant groups of invasive alien plants in the world, and South Africa specifically (van Wilgen *et al*., 2012). Globally, fifty-seven cactus species have been recorded as invasive (Zimmermann, 2017). *Opuntia* was one of the first invasive taxa to be regulated under the Agricultural Pest Act No. 11 of 1911 (Kaplan *et al.,* 2017). Invading about 22 countries outside of its native range, *Opuntia ficus-indica* is the most widespread cactus invader species (Novoa *et al*., 2015) but is also a beneficial plant depending on the context (Inglese *et al*., 2017). Australia has the largest number of invasive cactus species (39), followed by South Africa (35) and Spain with 24 (Novoa *et al*., 2015; Novoa *et al*., 2019). The genus *Opuntia* now has a widespread distribution in South Africa (Figure 1-1).

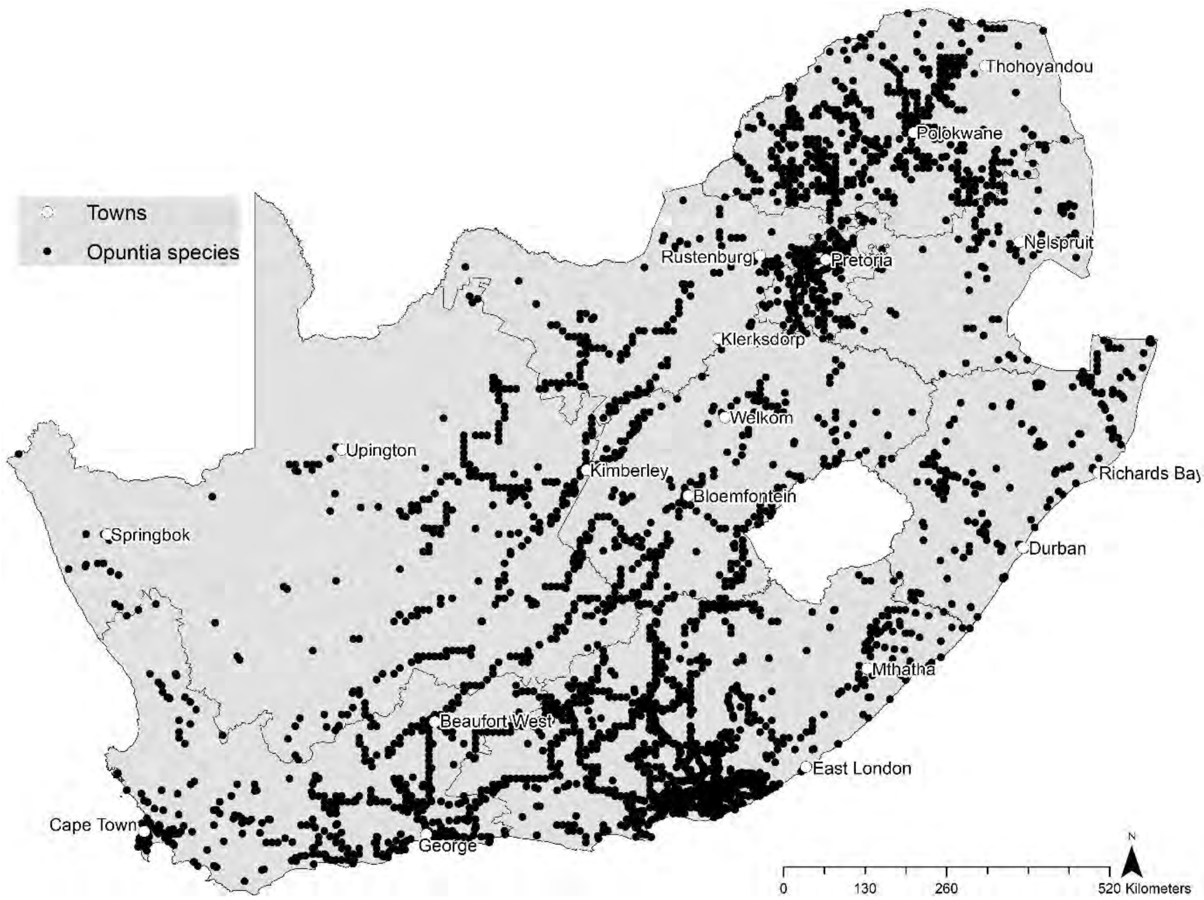


Figure 1-1: The distribution of *Opuntia* spp. in South Africa (sources GBIF; SAPIA, ARC).

1.3.2. Legislation applicable to invasive cacti in South Africa

Cactus invasions have had severe impacts on agriculture in South Africa dating over 150 years, but there was no legislation that prevented or controlled their introduction or movement prior to 1911 (Walters *et al.,* 2011). Between 1652 and 1911, there were periods and several events identified to have been responsible for the introduction of many alien plant invaders (Walters *et al.,* 2011). Described in Wells *et al.* (1986), this period marked the deliberate introduction of many important cacti and at least one *Agave* L. species to beautify and establish new and beneficial plant species in the colony. Legislation pertaining to invasive plants was informed by this period. Table 1-1 shows the prominent legislation for South Africa that was or is applicable to alien plants, from 1911 to present, as reflected in Walters *et al.* (2011).

Table 1-1: Prominent alien plant legislation in South Africa (adapted from Walters *et al.,* 2011).

- Agricultural Pest Act No. 11 of 1911
- The Cape Provincial Council Ordinance No. 18 of 1928
- The Jointed Cactus Eradication Act No. 52 of 1934
- The Noxious Weeds Act No. 42 of 1937
- The Soil Conservation Act No. 76 of 1969
- Agricultural Pest Act No. 3 of 1973
- Agricultural Pest Act No. 36 of 1983
- Conservation of Agricultural Resources Act No. 43 of 1983
- The Environment Conservation Act No. 73 of 1998
- The National Environmental Management Act No. 107 of 1998
- The National Environmental Management: Biodiversity Act No. 10 of 2004

Chapter 5 of the National Environmental Management: Biodiversity Act (NEMBA), which has superseded the Weeds and Invader Plant Regulations in terms of the Conservation of Agricultural Resources Act (CARA), regulates, legislates and informs the introduction, movement and subsequent distribution of cacti within South Africa's borders. It aims to prevent the introduction of potentially invasive alien species into the country and to aid in the control and management of already established invasive alien species. NEMBA was promulgated in 2014 making it the most recent and relevant legislation. The Alien and Invasive Species (AIS) Regulations in terms of NEMBA contain the current lists of alien and invasive species, as well as the specifics of the required measures against these species. There are 381 plants listed in NEMBA, of which 33 are cacti and 14 of those are *Opuntia*.

1.4. *OPUNTIA MEGAPOTAMICA*

1.4.1. (Mis)Identification

Cacti are particularly difficult to identify and are poorly represented in herbaria, as they are a challenge to collect and curate because of their spines and succulence (Walters *et al.,* 2011; Majure *et al.,* 2012; Novoa *et al.,* 2015). Interspecific hybridisation and a lack of morphological variability make the genus *Opuntia* infamous for its taxonomic difficulty (Weniger, 1969; Anderson, 2001; Rebman & Pinkava, 2001; Beinart & Wotshela, 2011; Winter *et al*., 2011; Majure *et al.,* 2012; Srikanth & Whang, 2015). Its species can hybridise within their natural range and artificially in the horticultural trade.

Until recently, *Opuntia megapotamica* was considered a morphotype/form of *Opuntia engelmannii* Salm-Dyck in South Africa. It was referred to as the Eastern Cape form of *O. engelmannii* because it is abundant in that province. There are other taxa of cacti that are considered different forms of *O. engelmannii* in South Africa: including Free State/ Northern Cape (FS/NC), Limpopo white-spine, Limpopo red-spine and Limpopo yellow-spine. *Opuntia engelmannii* is a complex and highly variable species that is considered 'the most misunderstood species' within the notoriously difficult and species-rich *Opuntia* genus (Weniger, 1969). It is often used as a 'dustbin' species, where any other species that is similar and cannot be assigned easily to another taxon is dumped and clustered with others (Schlesak *et al.,* 2018). *Opuntia engelmannii* is the widest ranging cactus of North America, with a distribution spanning more than half the lower width of the continent extending from the desert in Chihuahua to as far south as Hidalgo (in Mexico), and from California to Texas and as far north as southern Nevada and Utah (in the United States) (Weniger 1969; Johnson, 2004; Walters *et al*., 2011). It grows on slopes, ridges, canyons, valleys, creeks and in woodland (Johnson, 2004). Because of its expansive range and habitats, there are many variations and adaptations within the species, slightly differing in form. According to Weniger (1969), there are ten varieties, others recognising at least 15, of *O. engelmannii* in the United States alone, with possibly more diversity in Mexico. Weniger (1969) lists the following varieties: *Opuntia engelmannii* var. *engelmannii*; *Opuntia engelmannii* var. *cyclodes* Engelm. & Bigelow*; Opuntia engelmannii* var. *texana* (Griffiths) Weniger*; Opuntia engelmannii* var*. alta* (Griffiths) Weniger*; Opuntia engelmannii* var. *cacanapa* (Griffiths & Hare) D.Weniger*; Opuntia engelmannii* var. *flexispina* (Griffiths) B.D. Parfitt & Pinkava*; Opuntia engelmannii* var. *aciculata* (Griffiths) D.Weniger*; Opuntia engelmannii* var. *dulcis* (Engelm.) Schumann; *Opuntia engelmannii* var. *subarmata* (Griffiths) D.Weniger and *Opuntia engelmannii* var. *linguiformis* (Griffiths) B.D. Parfitt & Pinkava. Depending on the author, these varieties can be treated as varieties, species or lumped together as a single species without distinguishing them. *Opuntia engelmannii* var. *engelmannii* is very abundant in southern Arizona, New Mexico and western Texas, while *O. engelmannii* var. *flavispina* (L.D. Benson) B.D. Parfitt & Pinkava occurs along the western margin in southern Arizona and is endemic there (Parfitt & Pinkava, 1988; Rebman & Pinkava, 2001; Johnson, 2004). *Opuntia engelmannii* var. *texana* is abundant through Texas except in the extreme west. Most of the other varieties have restricted distributions, with a huge diversity in the Rio Grande Valley in Texas, but none is as noticeable and dominant as *O. engelmannii* var. *engelmannii* and *O. engelmannii* var. *texana*.

In South Africa, *Opuntia megapotamica* was previously identified as *Opuntia engelmannii* var. *lindheimeri* Engelm., *Opuntia lindheimeri* Engelm. and *Opuntia tardospina* Griffiths (Walters *et al.,* 2011). In the 1920s or 1930s, *O. tardospina* was still considered an accepted species, and that is when E. J. Alexander of the New York Botanical Gardens misapplied the name to South African *O. megapotamica*. *Opuntia tardospina* is morphologically very similar to the South American *Opuntia* in the series Armatae into which *O. megapotamica* is placed and is possibly the most morphologically similar North American *Opuntia* to *O. megapotamica*. Obermeyer (1976) in Flora of South Africa stated that *O. tardospina* was absorbed under *O. lindheimeri* by Lyman Benson in the Flora of Texas (1969). Between 1976 and 2006, *O. lindheimeri* was in turn absorbed into *O. engelmannii*, presumably taking *O. tardospina* with it. In the current South African AIS Regulations in terms of NEMBA, *O. lindheimeri* is considered a variety of *O. engelmannii* (SAPIA 54, 2019), while *O. tardospina* is regarded as a synonym. Weniger (1969) states that all Texas forms of *O. engelmannii* were lumped together and referred to as *O. lindheimeri,* while the western forms were referred to with other names. This resulted in *O. megapotamica* mistakenly regarded as an unconfirmed variety of *O. engelmannii* until recently when genetic analysis, using similar methods described in Realini *et al.* (2015), confirmed that what was regarded as the Eastern Cape form of *O. engelmannii* is, in fact, of South American origin and is genetically very similar to *O. megapotamica*. South Africa's *O. engelmannii* Eastern Cape variety was an exact match of the TrnL-F chloroplastic intergenic spacer region to South America's *O. megapotamica*, which differs significantly from North American cacti (Paterson *et al*. 2021). South American *Opuntia* taxonomic specialists have also verified the plant's identity as *O. megapotamica* (Font, F, pers. comm.; Paterson *et al.,* 2021). The correct identification of the species has massive consequences for the biological control thereof, in that the insects that were collected, and studied herein, were not collected from *O. megapotamica* and nowhere near its native range in South America, because initially it was considered the North American species, *O. engelmannii*.

1.4.2. Morphological description

Opuntia megapotamica (Figure 1-2) is a well-defined branched succulent spreading shrub with erect stems that can reach a height of 2 m. The dull green cladodes are almost circular, are thick and fleshy, and measure between 100 and 120 mm. The spines are sparse, long, off-white and yellow-brown towards the base and slightly curved in a downward direction, usually on the upper part of the cladode. Tufts of yellow-brown glochids are prominent on upper margin of cladodes and on the fruit areoles. The plant has orange flowers and the fruit are oblong and wine-red with a purple pulp, on occasion splitting longitudinally when ripe (Anderson, 2001; Font, 2019; Klein & Zimmermann, 2020).

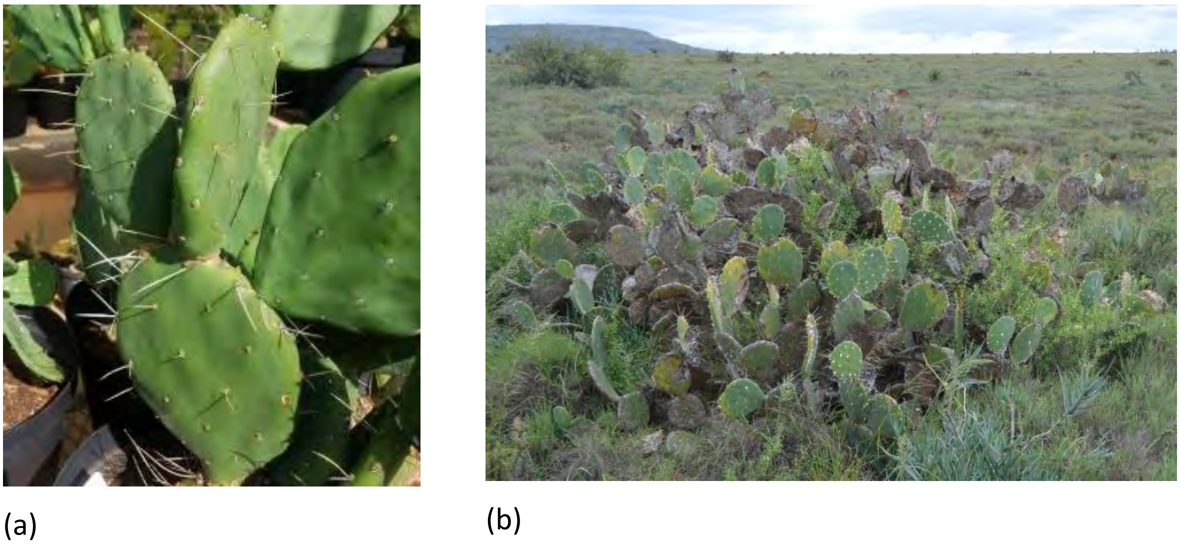

(a) (b)

Figure 1-2: *Opuntia megapotamica:* (a) potted plant and (b) infestation in Eastern Cape Province.

1.4.3. Distribution

i. Native distribution

The native distribution of *O. megapotamica* includes the south of Brazil in the state of Rio Grande do Sul and Uruguay. It also extends to northeast and central Argentina (Entre Rios, Buenos Aires, northern Rio Negro, Santa Fe and Catamarca) (Font, 2019). *Opuntia megapotamica*, along with *O. ficus-indica* and *Opuntia elata* Link and Otto var. *elata* are the most common *Opuntia* species in Argentina, even though *O. ficus-indica* is native to Mexico and thus an alien species in Argentina (Brooks *et al.,* 2012).

ii. Distribution and invasion in South Africa

Although not mentioned by name in the literature, *Opuntia megapotamica* was already present in large numbers in the Eastern Cape during the 1920s or 1930s. This is evident as seen on a photo taken by the Film Board of the Union of South Africa during that period (Figure 1-3).

Figure 1-3: *Opuntia megapotamica* in the Eastern Cape Province in the 1920s/1930s (courtesy of the Film Board of the Union of South Africa).

Dense infestations of *O. megapotamica* are present in the Eastern Cape Province (hence it was previously referred to as the Eastern Cape form of *O. engelmannii*) around Cookhouse, Bedford, Adelaide and Fort Beaufort (Figure 1-4). It is important to note that the FS/NC form of *O. engelmannii* has been identified in the Eastern Cape in isolated but dense infestations, so neither *O. megapotamica* nor the varieties of *O. engelmannii* are limited to their recognised geographic locations after which they were named. The taxonomic confusion regarding this species makes all previous surveys of its distribution misleading because it was lumped together with *O. engelmannii,* which has a broad distribution across the country. Surveys to determine the distribution of both these taxa are therefore required.

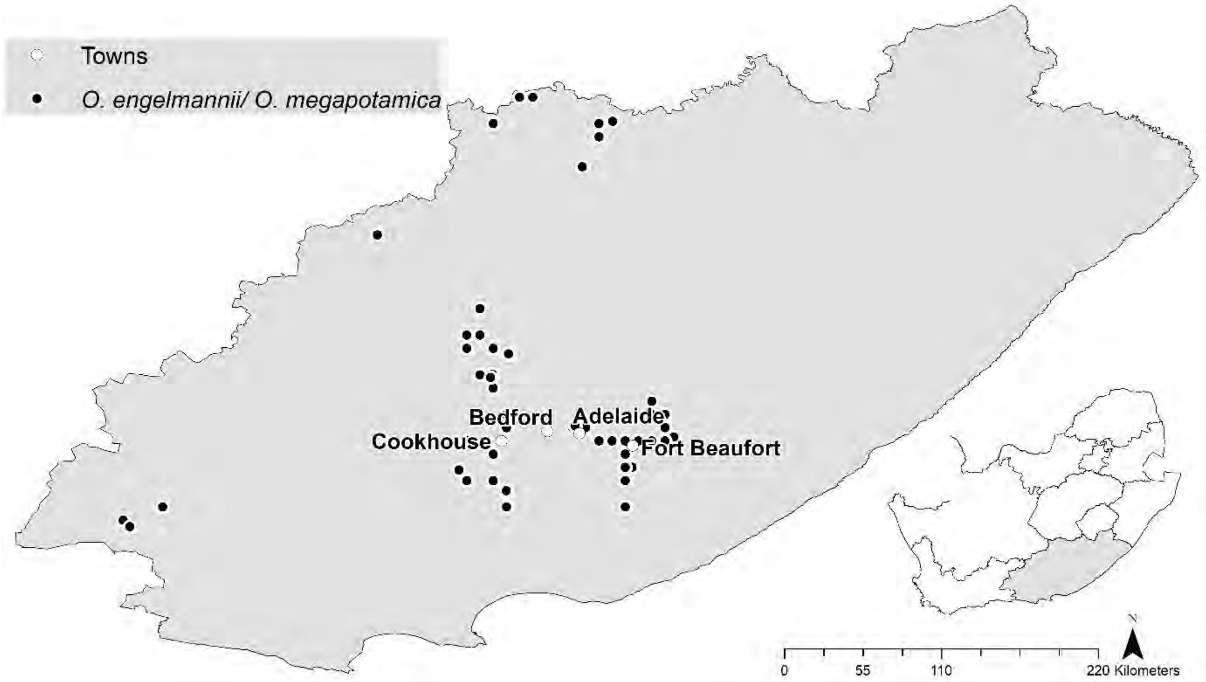


Figure 1-4: Distribution of *Opuntia megapotamica* and *Opuntia engelmannii* in the Eastern Cape (sources GBIF; SAPIA).

Opuntia megapotamica is a problematic agricultural weed in the Eastern Cape Province of South Africa. The plant forms large dense spiny thickets. These exclude indigenous flora and associated fauna, which in turn reduces biodiversity. It also reduces access to grazing, water sources and shade, thus reducing the carrying capacity of rangeland (Kaplan *et al*., 2017). With reduced rangeland carrying capacity, there is a reduction in productivity, thus leading to a loss of income. The spines and glochids are directly harmful to indigenous animals, livestock and people (Henderson, 2001; Walters *et al.,* 2011; Lloyd & Reeves, 2014). They are especially problematic in the wool and mohair industry (which is an important industry in the areas where the plant is most problematic) as a pollutant of wool products. The presence of *O. megapotamica* negatively affects the functioning of ecological services and changes the landscape, particularly the habitat structure for indigenous flora and fauna (Walters *et al.,* 2011; Lloyd & Reeves, 2014; Kaplan *et al.,* 2017).

1.5. MANAGEMENT AND CONTROL

O. lindheimeri, as it was previously referred to, was formally classified as an invader in 1984 (in terms of CARA (SAPIA 54, 2019), later declared a category 1(b) weed species in terms of NEMBA (Henderson, 2015). *Opuntia megapotamica* must therefore be removed if possible, and its spread controlled.

1.5.1. Mechanical and herbicidal control

Mechanical and herbicidal control are amongst the options for controlling the spread of *O. megapotamica*. Mechanical control generally involves labour-intensive physical removal of the weed using bulldozers, excavators, shovels and spades. This is usually followed by placing them in water so they rot, or drying them at a minimum of 2 m above the ground to avoid regrowth (Novoa *et al*., 2019). The non-specific systemic herbicide, glyphosate, is registered for use against *O. ficus-indica* while fluroxypyr/triclopyr 320/160 g/L is recommended for cover spray of *O. engelmannii* (and possibly *O. megapotamica* even though not mentioned by name) (Van Zyl, 2016; Klein & Zimmermann, 2020). Off-label use of herbicides, in collaboration with the Registrar (Act 36), has been allowed in the past. Herbicides can be used as a foliar application or stem injection. Both mechanical and chemical control methods require follow-up treatments and close monitoring (Novoa *et al*., 2019). The chemicals and implements used for these methods are costly and largely ineffective due to *O. megapotamica*'s ability to reproduce vegetatively. They offer only temporary relief as treated areas often become re-infested with rooted small plants making these methods unsustainable (Martin, 2003).

1.5.2. Biological control

"Biological control of weeds is a practice in which host-specific plant-feeding insects, mites and pathogens (that is, the plants' 'natural enemies') are transferred from their country of origin and released into a new country where the plants have become problematic" (Zimmerman *et al*., 2004). The main aim of biological control is to reduce the invasive plant's reproductive potential, removing the plant's competitive advantage over native vegetation, and ultimately suppressing the population (Harley & Forno, 1992). The success of biological control can be measured as a reduction in the plant's invasion trajectory, with a decline in the density and distribution of the target weed being the ultimate measure of success (Hoffmann *et al*., 2019). Complete eradication is not a goal of biological control, but rather a decrease in weed density to below a damage threshold (Hoffmann *et al.* 2019). Biological control agents become a permanent part of the receiving ecosystem, permanently supressing the weed populations. Since the biological control agent is a permanent addition to the ecosystem, and releases are usually irreversible, it is important that other plant species are safe from attack by biological control agents. The agents must therefore be suitably host specific to the target weed, so that other plants are not damaged. If an agent damages another species besides the target weed that it is intended to control it is referred to as a non-target attack. The extent of non-target attacks varies greatly, ranging from negligible to significant negative impacts that affect non-target plants on the population level (Hinz *et al*., 2020). Until 2008, only 13.1% of intentionally released agents globally had been found to attack non-target species (Hinz *et al.,* 2019). Approximately 75% of non-target attacks that have been recorded occurred in plant species of the same family as the target weed (Hinz *et al.,* 2019). Non-target impacts have declined over the years as host specificity testing procedures have improved due to long-term post release monitoring, experimental evolutionary studies and scientific advancements (Hinz *et al.* 2019; Hinz *et al*., 2020). Most non-target attacks were, in fact, predicted prior to the release of the biological control agent, but were deemed acceptable at the time of release (Downey & Paterson, 2016).

In order to avoid non-target attacks, host-specificity testing is conducted to determine whether the agent is suitably specific to be released where the target plant is problematic. This involves exposing non-target plant species that are closely related to the target plant to the potential biological control agent and assessing whether they are suitable hosts, this is followed by exposure to more and more distantly related non-target plants until the host range has been circumscribed (Paynter *et al.,* 2020). Host specificity testing has been shown to accurately predict the host range of insect herbivores that are used as biological control agents if it is conducted correctly (Paynter *et al.,* 2015). In the event that the agent is not monophagous and has a slightly wider host range, provided the other host plants are not native to South Africa, surrounding countries, or crop plants, it may be approved for release (Klein, 2002; Klein & Zimmermann, 2020). It is therefore possible to release biological control agents that feed on many different cactus species in areas, such as Africa, where the Cactaceae are not indigenous.

The oligophagous phycitid moth *Cactoblastis cactorum* (Berg) (Lepidoptera: Pyralidae) is native to South America (southern Brazil, Argentina, Paraguay and Uruguay) and feeds on several Opuntioideae hosts (Dodd, 1940; Mann, 1969; Hoffmann *et al.,* 1998b). The moth was introduced into the Caribbean to control an indigenous cactus and spread as a phytosanitary contaminate, probably with the cactus trade, to Florida in the United States of America, where it threatens indigenous cacti. This is widely regarded as a serious non-target impact, but it must be stressed that the host range of *C. cactorum* was always known to include many of the Opuntioideae, so the non-target impact was completely predictable (Downey & Paterson, 2016). This biological control agent should not have been released in the Caribbean where it was very likely to spread to mainland North America and feed on indigenous and valued cactus species, but it is not an example of an unpredicted host range expansion.

Biological control agents do not completely eradicate infestations (Klein, 2002). Success is reached when the weed populations have been reduced to below a tolerable threshold or another previously defined goal (Hoffmann *et al.,* 2019). Biological control agents usually reduce the reproductive potential of individual plants resulting in a slow decrease of the density of the weed population or in a reduction in invasive potential of the plant population. As the target weed population is never completely eradicated, there is always a source of the agent and if the weed population increases, the agent population will increase in response to this, thus providing permanent control.

With data that included 90 countries, compiled until 2012 by Winston *et al.* (2014) and summarised by Schwarzländer *et al*. (2018), it is reported that 468 biological agents had been released against 175 target weed species (Table 1-2). The target weed species comprise 48 plant species. Of the 468 agents released, 70.9% of them had established successfully. The impact of the agents post-release was summarised as follows: 7% of releases resulted in no impact at all on the target weed, while 55% of the agents caused heavy, medium or variable damage. Overall, 65.7% of targeted weeds are under some level of biological control (Schwarzländer *et al*., 2018).

Table 1-2: Agents released and weeds targeted for classical biological control globally (compiled by Winston *et al.* (2014) and summarised by and adapted from Schwarzländer *et al.* (2018)).

Regions	**No. of agent species introduced**	**No. of target weeds**	**No. of agents introduced per weed**	**% agents established**	**No. of target weeds controlled**	**% target weeds controlled**
Australia	202	56	3.6	67.8	39	69.6
North America	137	59	2.3	79.6	36	61
South Africa	103	51	2	69.9	38	74.5
Hawaii	87	22	4	67.8	13	59.1
Oceania	58	19	3.1	55.2	15	78.9
New Zealand	53	23	2.3	69.8	8	34.8
Asia	42	18	2.3	71.4	12	66.7
Africa (excluding South Africa)	38	15	2.5	78.9	11	73.3
Caribbean	16	13	1.2	25	6	46.2
South America	14	10	1.4	64.3	3	30
Eurasia	10	5	2	40	0	0

i. Biological control of Opuntioideae cacti

Cacti were the first plants to be targeted for classical biological control (Novoa *et al.,* 2019). In 1795, the cochineal insect, *Dactylopius ceylonicus* Green, was brought into India from Brazil to produce dye under the assumption that it was the carmine cochineal dye species *D. coccus* Costa (Tryon, 1910). It was then intentionally taken from northern to southern India in 1836 and to Sri Lanka in 1865 for the control of *O. monacantha*, once its value as a biological control agent was recognised (Goeden, 1988). Australia's first attempt at biological control for *Opuntia stricta* (Haw.) Haw. began as early as 1908 (Palmer *et al.,* 2010). In South Africa, *Opuntia monacantha* Haworth was the first plant targeted for biological control using *Dactylopius ceylonicus* in 1913 (Zimmerman *et al*., 2004; Paterson *et al.,* 2011; Moran *et al*., 2013). Since then, many cactus species have been targeted for biological control and are under different levels of control (Table 1-3). Of the 57 cactus species that have become invasive globally, 23 had been subjected to biological control by 2010 (Zimmermann *et al.,* 2009). Biological control has been particularly successful on species of *Opuntia* compared with other cactus genera. This can be attributed to the specialised nature of the evolutionary relationship between the cochineal insects and the *Opuntia* plants, as it is cochineal insects that are most frequently used as biological control agents.

Table 1-3: Biological control agents and level of biological control success on *Opuntia* and *Cylindropuntia* cacti in South Africa (adapted from Moran *et al.* (2021).

Cactus species	**Biological control agent**	**Level of control**
Opuntia aurantiaca Gillies ex Lindl.	*Dactylopius austrinus* De Lotto	Substantial
Opuntia cespitosa Raf.	*Dactylopius opuntiae* Cockerell 'stricta' lineage	Complete
Opuntia engelmannii Salm-Dyck Northern Cape/ Free State variety	*Dactylopius opuntiae* Cockerell 'stricta' lineage?	Substantial
Opuntia ficus-indica Haworth	*Dactylopius opuntiae* Cockerell 'ficus' lineage	Substantial
Opuntia megapotamica Arechav.	*Dactylopius opuntiae* Cockerell 'ficus' lineage?	Negligible
Opuntia monacantha Haworth	*Dactylopius ceylonicus* Green	Complete
Opuntia salmiana Parm. ex Pfeiff	*Cactoblastis cactorum*	Negligible
Opuntia stricta Haworth	*Dactylopius opuntiae* Cockerell 'stricta' lineage	Substantial
Opuntia tomentosa Salm-Dyck	*Dactylopius opuntiae* Cockerell 'ficus' lineage	Substantial
Cylindropuntia fulgida (Engelm.) F.M. Knuth var. *fulgida*	*Dactylopius tomentosus* Lamarck 'cholla' lineage	Complete
Cylindropuntia fulgida (Engelm.) F.M. Knuth var. *mamillata* (Schott ex Engelm.) Backeb.	*Dactylopius tomentosus* Lamarck 'cholla' lineage	Complete
Cylindropuntia imbricata (DC.) F.M. Knuth	*Dactylopius tomentosus* Lamarck 'imbricata' lineage	Substantial
Cylindropuntia leptocaulis (DC.) F.M. Knuth	*Dactylopius tomentosus* 'imbricata' lineage	Complete
Cylindropuntia pallida (Rose) F.M. Knuth	*Dactylopius tomentosus* Lamarck 'californica var. parkeri' lineage	Permission to release granted in May 2020

Generally, the biological control of cacti has an exceptionally good track record in South Africa (Paterson *et al*. 2011; Paterson *et al.* 2021). A number of reasons make cacti excellent targets for biological control. These include the fact that cacti and their natural enemies are endemic to the Americas making them highly suitable to be utilised as biological control agents outside of their native range, and allowing for the use of oligophagous cactophages such as *C. cactorum*, without the risk of non-target attack (Mann, 1969; Paterson *et al*., 2019). Most natural enemies of Cactaceae are also specialists that feed exclusively on the family Cactaceae, such as the Dactylopiidae (cochineal insects) which are usually restricted to Opuntioideae (except *D. confertus* which is associated with the genus *Trichocereus* and its relatives), while the species *Dactylopius tomentosus* Lamark is restricted to a single genus, *Cylindropuntia* (Zimmermann, 2017; Paterson *et al*., 2019; Paterson *et al.,* 2021). There are many cochineal species and lineages to choose from in the indigenous range, which gives a real opportunity to select the correct lineage to control the cactus weed, increasing the chances of finding suitably specific and damaging agents (Hoffmann, 2004; van Steenderen *et al*., 2021). The most important biological control agents against *Opuntia* species and their close relatives, such as *Cylindropuntia*, are *Cactoblastis cactorum* and *Dactylopius* species.

ii. *Cactoblastis cactorum* moth

Cactoblastis cactorum was introduced in Australia in 1926 for the control of *O. stricta* and in South Africa in 1933 for *O. ficus-indica* from stock from Australia (Moran & Annecke, 1979). The original host plant species from which the cactus moth was collected and exported to Australia remains uncertain (Varone *et al.* 2012). Its biology is detailed in Pettey (1948). Larvae tunnel into cladodes and then hollow them out, feeding on the internal tissue. This causes mucilage and frass to ooze and drip from the plant. Microbial pathogens may then enter the plant and secondary infections may occur which lead to plant death. Earlier assessments of the efficacy of *C. cactorum* on *O. stricta* in Australia were distorted by ignoring the damage produced by the cochineal insects, which was most likely the more damaging of the two agents in the control of *O. stricta* in Australia (Hoffmann *et al.,* 1999; Hoffmann *et al*., 2020). Although widespread, the famous *C. cactorum* is not a significantly damaging biological agent in South Africa (Pettey, 1948; Moran & Annecke, 1979; Starmer *et al.,* 1988; Hoffmann *et al*., 1998; 1998b; Volchansky *et al*., 1999).

Cactoblastis cactorum has a relatively broad host range, so it does feed on *O. megapotamica* in South Africa, but it is not considered an effective biological control agent. The high mucilage content of *O. megapotamica* may reduce the performance of the moth (Varone *et al.,* 2012). The primary defence mechanism of *Opuntia* spp. against the first instar moth is the mucilaginous thick cuticle on cladodes (Hoffmann & Zimmermann, 1989). As such, *C. cactorum* has had insignificant impact in controlling infestations of *O. megapotamica* in the country.

iii. *Dactylopius* cochineal species

There are ten described *Dactylopius* (Costa) species in the monogeneric family Dactylopiidae namely *Dactylopius austrinus* De Lotto, *Dactylopius ceylonicus* Green, *Dactylopius coccus* Costa, *Dactylopius confertus* De Lotto, *Dactylopius confusus* Cockerell*, Dactylopius opuntiae* Cockerell, *Dactylopius salmianus* De Lotto, *Dactylopius tomentosus* Lamarck and *Dactylopius zimmermanni* De Lotto (Chávez-Moreno *et al*., 2009) and finally, the tenth and most recently discovered species on *Corynopuntia schottii* (Engelm.) F.M.Knuth (in Texas) is *Dactylopius gracilipilus* sp. nov. (Van Dam & May, 2012). Five of the species are endemic to North America and the other five are endemic to South America, south of the Amazon Basin (De Lotto, 1974; Perez Guerra & Kosztarab, 1992). Members of the family Dactylopiidae only feed on members of the Cactaceae family, both Cactoideae and Opuntioideae (De Lotto, 1974; Perez Guerra & Kosztarab, 1992).

Cochineal insects are sedentary hemipterans that are covered by a waxy white wool-like substance that is excreted by the insect (Figure 1-5) (Zimmerman *et al.,* 2009). Their bodies are of a dark red/ purple colour due to a high content of carminic acid. The females go through two moults and develop into soft, sac-like creatures, almost without features under a protective white waxy layer that becomes denser as the individual matures. Cochineal insects present sexual dimorphism, evident in the adult life stages (Perez Guerra & Kosztarab, 1992); adult males are winged and mobile, while the females are sessile with neotenous characteristics. Males go through multiple moults. They form a cocoon and cease feeding, lose their mouthparts and then emerge as winged adults (Moran & Cobby, 1979; Van Dam & May, 2012). The winged adult males only live for a few days, solely to fertilise multiple mature females (who live up to 5 months) (Moran & Cobby, 1979; Nobel, 2002).

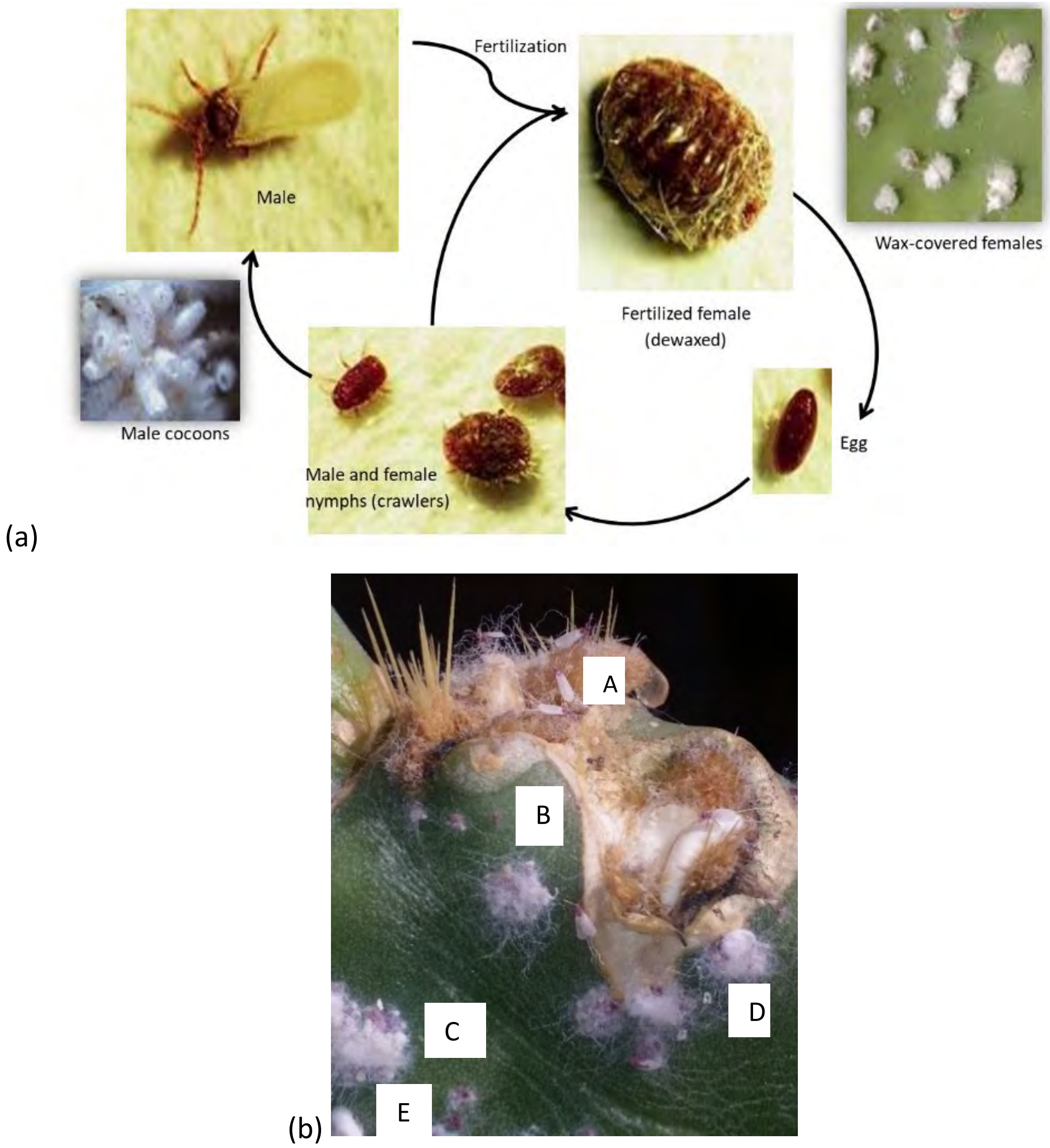


Figure 1-5: (a) Lifecycle of cochineal insects (Moran & Cobby, 1979); (b) Cochineal on *Opuntia*, showing the different life stages on a single cladode where A) is the winged adult male; B) mobile nymph; C) colony of second and third instars covered in wax; D) shed exuvia; E) cocoon.

The fertilised female produces approximately 430 eggs (in her lifetime) that are a light red colour (Moran & Cobby, 1979). The eggs hatch inside the female (ovoviviparity) or within minutes after oviposition. Ovipositon occurs at night. The eggs hatch into tiny red mobile male and female nymphs, called crawlers. The nymphs start to produce waxy filaments within an hour of hatching to aid wind dispersal (Moran *et al.,* 1982). Crawlers settle on the cladode or are dispersed by wind to new cladodes, where they insert their mouthparts and begin to feed. They moult and shed exuviae, not ceasing to produce the dense waxy layer. Males go on to form cocoons and probably moult again to then emerge as winged adults who are ready to fertilise mature females. Males live for approximately three days and do not feed. For *D. opuntiae* the process from egg to maturity takes between 40 and 50 days for females and 31-43 days for males, while *Dactylopius tomentosus* females take 63 days while the males take 57 days to mature (Moran & Cobby, 1979; Nobel, 2002; Mathenge *et al.,* 2009b).

Cochineal insects are the most effective of the biological agents that have been used to control cactus weeds worldwide (Hoffmann *et al.,* 2002). The saliva injected by the insect during feeding is toxic and is possibly responsible for causing damage to tissues of host plants (Mathenge *et al.,* 2009). Infested plants exhibit discoloured and withered cladodes that drop off and dry out, and unlike healthy cladodes, are not able to root (Hoffmann *et al.,* 2020).

1.5.3. Cochineal biotypes/lineages

An interesting (but not unique) feature of some of the *Dactylopius* species is that they have biotypes (Diehl & Bush, 1984). According to Diehl and Bush (1984), biotypes are morphologically identical clades of an insect species that differ with regard to their host adaptation. They may differ in other traits such as activity patterns, feeding preferences, development time, fecundity, feeding impact, size, shape, colour, insecticide resistance and migration (Mathenge *et al.,* 2010; Jones *et al.,* 2015). They, however, have not developed enough behavioural, physiological or genetic changes for there to be reproductive isolation (Mathenge *et al.,* 2010). In Van Dam and May (2012), it is stated that biotypes seem to represent populations that were isolated geographically, and therefore receive a negligible gene flow from other populations. In essence, they are infraspecific populations/groups. The term biotype is simplistic and has consequently misled the perception of the variations within pest populations as it is an umbrella term (Downie, 2010). For the purposes of this study, due to the criticism of the ambiguous nature highlighted by Downie (2010), contention around and interchangeability of the term, the word lineage, believed to be a more accurate description of the different intraspecific taxa, is used.

Studies have shown that host preferences of lineages of cochineal insects show unmistakeable differences between their development on preferred and alternate hosts, thus making them highly specific (Volchansky *et al*., 1999; Mathenge *et al.*, 2009; Mathenge *et al.,* 2010; Jones *et al*., 2015; Jones *et al.,* 2016; Rule & Hoffmann, 2018). Lineages occur in many insect groups, but in biocontrol, the use of several lineages of the same species has been limited to *Dactylopius* insects on cacti. Each lineage qualifies to be dealt with as a separate entity with the potential to control its own specific host plant (Githure *et al.,* 1999; Volchansky *et al*., 1999). Only the correct lineage is effective in controlling its preferred target weed, so if the incorrect lineage is utilised then control is not achieved (Githure *et al*., 1999).

Dactylopius tomentosus displays distinct host preferences when collected from different *Cylindropuntia* species (Mathenge *et al.*, 2009; Mathenge *et al.,* 2010; Jones *et al.,* 2015; Jones *et al.,* 2016). Jones *et al.* (2015) found that there were varying degrees of specificity within *Cylindropuntia*. The two studies by Jones *et al*. (2015 and 2016) found that the 'imbricata' and 'cholla' lineages had the widest range within *Cylindropuntia*, but there were differences in the development and performance amongst the various targeted host species. A series of host specificity testing and efficacy trials found that *Cylindropuntia fulgida* (Engelm.) F.M. Knuth var. *mamillata* (Schott ex. Engelm.) Backeb was the preferred host for the 'cholla' lineage. *Cylindropuntia imbricata* (Haw.) F.M. Knuth was successfully controlled by the 'imbricata' lineage (Jones *et al.,* 2015).

As a biological control agent, the 'imbricata' lineage is effective on *C. imbricata* in South Africa, but not on *Cylindropuntia fulgida* (Engelm.) F.M. Knuth var. *fulgida* and *C. fulgida* var. *mamillata* and as a result, another lineage of *D. tomentosus* ('cholla') was obtained from Mexico in 2008. The 'cholla' lineage has produced remarkable results in controlling *C. fulgida* var. *fulgida* and *C. fulgida* var. *mamillata* (Klein *et al.,* 2020). In two sites in the Northern Cape Province, spectacular results were reported, of the 'cholla' lineage on *C. fulgida* var. *mamillata* having killed 90% infestations in just under a year and a half post release (Klein *et al.,* 2020).

Matching the lineage to the host has important consequences for the biological control of cactus weeds, because only the correct host-adapted lineage will provide complete control of the target weed (van Steenderen *et al*. 2021). Four *Dactylopius* species of cochineal are used as biological control agents, controlling eight different target weeds in South Africa (Table 1-3). Of the four species, two have different lineages that control different target weeds. While in some cases a species or lineage can survive on other Cactaceae, it is only a small number of closely related target weeds that are sufficiently damaged to warrant the use of that agent for control.

1.5.4. Hybridisation in biological control

Few studies have investigated the impact of hybridisation of biological control agents, but there are some examples in the weed biocontrol literature. A lineage of the beetle *Longitarsus jacobaeae* (Waterhouse) (Coleoptera: Chrysomelidae) collected in Italy, was first introduced in western United States in 1969 and has since established and become widespread, providing effective biological control against its target weed *Jacobaea vulgaris* Gaertn. (Asterales: Asteraceae) (Szűcs *et al.* 2012). Beetles collected for redistribution in coastal Oregon did not establish in eastern Oregon and high elevation alpine regions in Montana, while those collected from high elevation sites on Mt. Hood in Oregon established well when relocated to other high elevation and moist areas (Littlefield *et al.,* 2008). In an attempt to improve establishment and control, a cold adapted lineage of the beetle was collected in the indigenous distribution in Switzerland in 2002 (Littlefield *et al*., 2008). Both the Italian and Swiss populations have a narrow and similar host range (Pelser *et al*., 2007). A study by Szűcs *et al.* (2012) investigated hybrids between the Swiss and Italian lineages of the Eurasian beetle and found that there was increased vigour in the first and second generation of hybrids with some maternal influence. The fitness of both F_2 hybrid populations was higher than that of both the Italian and Swiss parent. The conclusion was that intentional hybridisation might be beneficial in biological control strategies, in that there is a population build-up in the early years post release. There is high hybrid fitness in the earlier generations and this can accelerate biological control success as a result of the rapid population build up and high fecundity of hybrids in the initial years following release. This is the case as there may be disruptions of coadapted gene complexes that may cause outbreeding depression, which may only be apparent in later generations or manifest under field conditions (Szűcs *et al.,* 2012).

Tamarix spp. L. (Caryophyllales: Tamaricaceae) arrived in North America in the mid-1800s from Asia and Europe to combat riverbank erosion and as ornamental plants (Brock, 1994), and has colonised riparian habitats from Montana to Mexico (Gaskin & Schaal, 2002). In 2001 the Asian leaf beetle *Diorhabda elongata* (Brullé) (Coleoptera: Chrysomelidae) was released as a biological control agent against *Tamarix* (DeLoach *et al.,* 2003). *Diorhabda elongata*, initially classified as a single species, however, has a wide geographic range, spanning North Africa, Eurasia into China and Mongolia that comprised of different subspecies (Tracy & Robbins, 2009). Due to the differences in the insect's photoperiodic requirements for reproduction and diapause and the differences in its level of control of *Tamarix,* those lineages or subspecies of *Diorhabda* underwent taxonomic revision using morphological and biogeographical data which defined them as a complex comprising five species, namely: *Diorhabda elongata* ((Brullé), *Diorhabda carinulata* Desbrochers*, Diorhabda carinata* Faldermann and *Diorhabda sublineata* Lucas and *Diorhabda meridionalis* Berti & Rapilly, which is not used in *Tamarix* biological control (Tracy & Robbins, 2009). *Diorhabda carinulata* is the most widespread in North America (in Oregon, Idaho, Wyoming, Colorado, Utah, Nevada, northern Arizona and northern New Mexico) while the others have been released in Texas and are spreading (Bean *et al.,* 2013). A study on hybridisation found that hybrids between *D. carinulata* and each of the other three species produced few viable offspring due to male sterilisation in the first generation, while egg viability between the other three species is comparable with that of the parents (Bean *et al*., 2013). The effects of hybridisation of these species were quantified by a study by Bitume *et al.* (2017) which found that the life history traits of the hybrids were unchanged or improved with hybridisation when compared to the parents. Some of the crosses were positively impacted by hybridisation, which resulted in increased fecundity and reduced development time and there was no evidence of reduced fitness in any of the hybrid crosses. Further investigations showed clear differences in egg viability of the F_2 hybrid generation between the intraspecific crosses of the four species used in the biological control of tamarisk (Bean *et al*., 2013). Furthermore, they reported a high *D. carinulata* female mortality when *D. carinulata* was crossed with the other three species. The examples of the *Diorhabda* beetles and *L. jacobaeae* differ from the situation with using cochineal insects to control cactus in South Africa because there are cochineal insects that are used as agents for several cactus species that can interbreed and therefore influence the efficacy of control of several weed species to varying degrees.

Mathenge *et al.* (2010)'s study on hybridisation of *D. tomentosus* cholla and imbricata cochineal lineages found that the hybrids outperformed both purebred lineages. This meant that in the case of both *C. fulgida* var. *fulgida* and *C. imbricata* occurring in sympatry, hybridisation of the two insect lineages would not negatively influence the biological control of either host. Those authors suggested that the hybrids be released in areas with both hosts for maximum impact. Hoffmann *et al.* (2002) found that while the first generation hybrids of the *D. opuntiae* 'stricta' and 'ficus' lineages developed equally well on both *O. stricta* and *O.*

ficus-indica and were not species-specific, some individuals of the second generation hybrids and backcrosses with the F_1 hybrids were host-specific, while the others were not and would therefore feed equally on both hosts. South Africa makes use of both *D. tomentosus* and *D. opuntiae* lineages as biological control agents for invasive cacti and the extent of the impact of hybridisation on biological control in the field needs to be studied further, as extrapolation of laboratory results to the field is often challenging (Table 1-3).

Sometimes cactus infestations are not monocultures, with multiple species growing in close proximity to one another. This is the case with *Cylindropuntia* species in Australia and South Africa (Mathenge *et al.,* 2009; Jones *et al*., 2016) and *Opuntia* species in South Africa (Hoffmann *et al.,* 2002). Each of these cactus species require the correct lineage of cochineal to be present if effective control is to be achieved. When this occurs, the two insect lineages will interbreed or hybridise freely and produce viable progeny (Volchansky *et al*., 1999; Hoffmann *et al*., 2002; Mathenge *et al.,* 2010; Jones *et al*., 2016). One of three scenarios can result from hybridisation: 1) the fitness of hybrids can decrease due to hybrid breakdown or outbreeding depression which impacts the performance of individual hybrids across fitness traits such as mortality, fecundity and development time; 2) the fitness of the hybrids can increase relative to the parents through dominance of beneficial traits and by facilitating the formation of novel genotypes; or 3) there may only be a minimal impact from hybridisation, particularly when there is little genetic differences between parents (Fisher, 1930; Edmands, 2002; Mallet, 2005). The host affinity, efficacy and virulence of the biological control agents can therefore be impacted by hybridisation in a variety of ways and is difficult to predict without direct investigation (Jones *et al*., 2016).

1.6. SOURCING A POTENTIAL BIOCONTROL AGENT FOR *OPUNTIA MEGAPOTAMICA* AND THE USE OF 'NEW ASSOCIATIONS' IN CACTUS BIOLOGICAL CONTROL

In an effort to find a biological control agent to combat the problem posed by the various forms of *O. engelmannii* and *O. megapotamica* in South Africa, populations of cochineal were collected, on two different trips by researchers from Agricultural Research Council-Plant Health Protection (ARC-PHP), on *O. engelmannii* plants in various localities within Arizona and New Mexico in the United States of America. At the time, *O. megapotamica* was considered a variant of *O. engelmannii*, so it was the indigenous distribution of *O. engelmannii* in North America that was targeted for collection trips for potential agents (Table 1-4). Under quarantine conditions at the ARC's Roodeplaat quarantine facility, cochineal populations that were collected in North America were tested for their predicated efficacy by using a fitness index to determine which would be the most damaging to the various forms of *O. engelmannii*, including what is now know to be *O. megapotamica* (Paterson *et al.* 2021).

Table 1-4: Locations of cochineal population collections.

1	Flagstaff, Arizona: N 35° 13′ 36.4″; W 111° 35′ 29.9″; elev. 2117 m
2	Four Peaks Mountain (1), Az: N 33° 40′ 27.5″; 111° 30′ 22.3″; elev. 738 m Four Peaks Mountain (2), Az: N 33° 41′ 35.5″; 111° 29′ 38.4″; elev. 722 m
3	Sedona, Az: N 34° 52′ 12.3″; W 111° 43′ 42.4″; elev. 1412 m
4	Tucson, Az: N 32° 14′ 55″; W 10° 54′ 35″
5	Las Cruces, New Mexico: N 32° 16′ 18.8″; W 106° 47′ 34.3″; elev. 1164 m

A cochineal population collected on *O. engelmannii* var. *engelmannii* in Flagstaff, Arizona had the highest fitness index (FI) and proved to be the most damaging of the cochineals to *O. megapotamica* (Table 1-5). The higher the fitness index, the quicker the insect develops, and the more offspring it produces. A higher fitness index is expected to result in better control. Potted plants were also used to ascertain which population would kill the target host in the shortest period and the Flagstaff population did just that. The Flagstaff population, using genetic markers, was identified as *Dactylopius opuntiae* (van Steenderen *et al.,* 2021). This Flagstaff population will be referred to as the 'engelmannii' lineage from this point as it was collected on *O. engelmannii*.

Table 1-5: Fitness index of 5 USA populations of *Dactylopius* cochineal on *Opuntia megapotamica.*

Locality	**Fitness Index**
Tucson, Az:	0, 11
Sedona, Az:	0, 74
Four Peaks Mountain, Az	2, 01
Las Cruces, New Mexico	2, 32
Flagstaff, Az	2, 98

The *O. engelmannii* cochineal was damaging to *O. megapotamica* in efficacy assessments, but it has no evolutionary history with the host plant because it is a North American cochineal and *O. megapotamica* is indigenous to South America. The reason for its virulence could be the lack of evolutionary history between the host plant and the insect (Varone *et al.,* 2012) which suggests the possibility of a virulent new association between *O. megapotamica* and the cochineal insect (Hokkanen & Pimentel, 1984). *Opuntia megapotamica's* defences may be naïve and less capable to defend itself against this cochineal than against those with which it has co-evolved. Old associations in the science of biological control refer to target species reunited with natural enemy that they have a shared evolutionary history with (Ehler, 1995), while new associations are those that have not been exposed to the natural enemy over evolutionary time (Hokkanen & Pimentel, 1989; Ehler, 1995). The lack of commensalism between the insect herbivore and the targeted weed may result in a high level of biological control success in some instances (Hokkanen & Pimentel, 1984). Furthermore, the insect may simply have characteristics of a successful natural enemy to the point where it could provide control for more than one taxonomically related target pest. In the majority of successful new associations, the original host is closely related to the new host, making the insect oligo- or polyphagous and this could potentially add to its success regardless of its evolutionary association with the target weed (Hokkanen & Pimentel, 1984; Ehler, 1995).

There is a history of successful new associations in the biological control of cactus weeds (Hokkanen & Pimentel, 1989; Varone *et al*., 2012). The performance of *C. cactorum* was influenced significantly by the evolutionary history with different *Opuntia* spp. under optimal laboratory conditions in a study by Varone *et al.* (2012) which evaluated the biology and ecology of *C. cactorum*. Under those conditions, larvae performed best on the Northern American *O. ficus-indica* and *Opuntia robusta* H.L. Wendl. ex. Pfeiff and South American *Opuntia arechavaletae* Speg. than Mexican *O. leucotricha* D.C. and South American *O. megapotamica, O. elata* var. *elata*, and *Opuntia quimilo* K.Schum and *Opuntia sulphurea* G.Don ex Salm-Dyck which have been recorded as non-hosts of the moth (Varone *et al*., 2012). The exceptional results obtained from *D. tomentosus* 'cholla' lineage on *C. fulgida* var. *mamillata* in the Northern Cape are attributed to the new association technique as it is known to have been collected off of a different species to the target weed (Klein *et al.,* 2020). It is therefore worthwhile considering a damaging cochineal insect from North America for the control of *O. megapotamica*, despite the fact that it is a South American cactus species, based on the theory of new associations, as well as some evidence of this approach being successful in other cactus biocontrol programmes.

1.7. *DACTYLOPIUS OPUNTIAE* LINEAGES AS BIOCONTROL AGENTS FOR *OPUNTIA MEGAPOTAMICA*

Two lineages of *Dactylopius opuntiae* (‘stricta’ and ‘ficus’ lineages) have already been introduced into South Africa as biocontrol agents and successfully control three problematic *Opuntia* cactus species (*Opuntia stricta* and *O. cespitosa* by the former and *O. ficus-indica* by the latter) (Table 1-3). In trials conducted by Volchansky *et al.* (1999), it was revealed that the two strains/lineages readily mated and produced viable offspring that develop well on either parental host plant (i.e. *O. stricta* and *O. ficus-indica*) when crossed. This suggests that lineages of *D. opuntiae* could potentially interbreed freely when exposed to one another, which is not surprising since they are the same species (Hoffmann *et al.,* 2002). This may affect the host preference/affinity of the insects, and have an impact on the level of control the cochineal insects have on their target hosts (Hoffmann *et al.,* 2002; Mathenge *et al.,* 2010). Hybridisation between any new *Dactylopius* spp. that are released is likely to occur, and prior to the release of any new agents it is essential that we understand what the impact of hybridisation to the control of these species, as well as the new target weed species, will be. The ‘ficus’ lineage of *D. opuntiae* has established on *O. megapotamica* in South Africa but is not considered to provide suitable levels of control. The ‘ficus’ lineage is therefore present in the field where any new lineages of cochineal would be released, and the most damaging of the potential agents in quarantine that could be released is very likely to hybridise with it.

The ‘ficus’ lineage of *D. opuntiae* (and the cactus moth, *C. cactorum*, to a much lesser degree) has been used to reduce populations of *O. ficus-indica* with noticeable results (Pettey, 1948; Volchansky *et al*., 1999). *Cactoblastis cactorum* was released in 1933, while *D. opuntiae* ‘ficus’ lineage was released in 1938 in South Africa (Klein, 2011). There has been an estimated 90% decline in the density and distribution of *O. ficus-indica* infestations in South Africa since the biological control agents were introduced, and infestations are now stabilised and maintained permanently at the current level of control (Zimmermann & Moran, 1991, Walters *et al*., 2011). Dense infestations continue to persist in the Eastern Cape Province (Figure 1-6) and arid to semi-arid Karoo region which includes part of the Eastern, Western and Northern Cape Provinces. Cooler and wet weather conditions negatively affect biological control agents, as the efficacy of the cochineal insects is reduced (Zimmermann & Moran, 1991). In some areas of the Eastern Cape, *O. ficus-indica* and *O. megapotamica* occur in close proximity (Figure

1-6), which means the two lineages of cochineal will be exposed to one another, and likely hybridise.

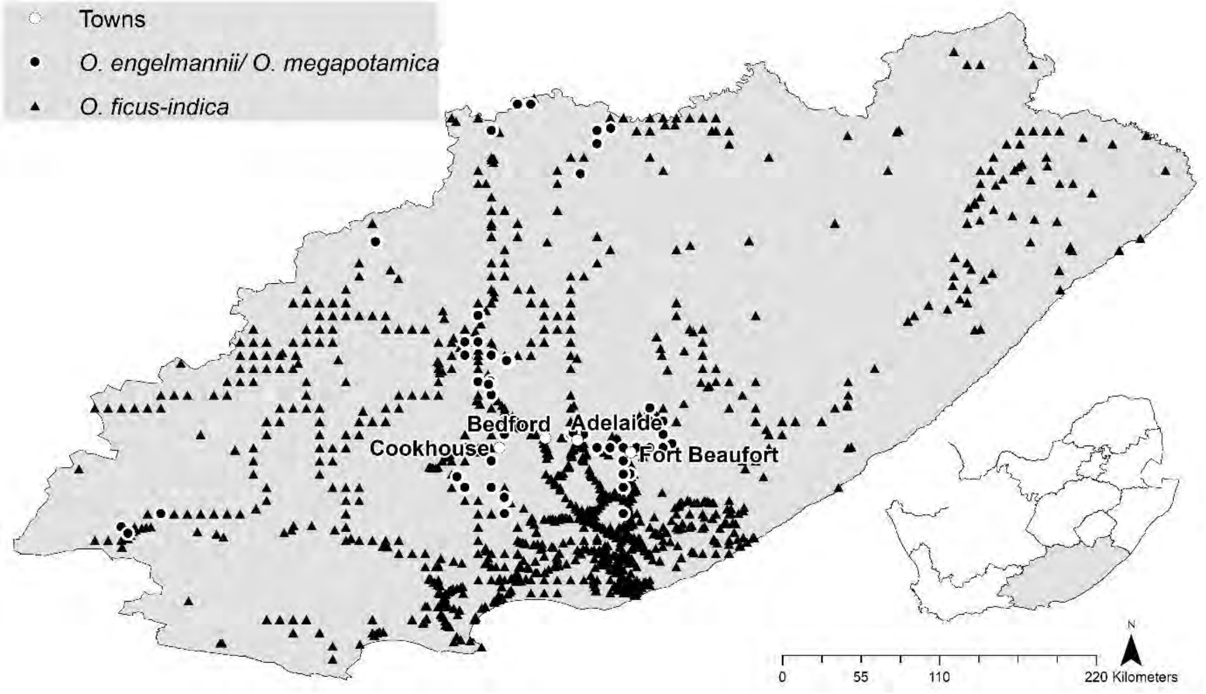


Figure 1-6: *Opuntia ficus-indica* and *Opuntia megapotamica (*and *Opuntia engelmannii)* infestations in the Eastern Cape Province (sources GBIF; SAPIA, ARC).

1.8. AIM OF THE STUDY

The main objective of this study was to determine whether the addition of yet another *Dactylopius opuntiae* lineage would disrupt the current level of control of *O. ficus-indica*, or the potential future control of *O. megapotamica,* due to hybridisation. The interactions between the 'ficus' lineage and the new 'engelmannii' lineage have the potential to influence host affinity and efficacy of the hybrids, which in turn may impact the level of control the biological control agents have on both *O. ficus-indica* and *O. megapotamica* in the Eastern Cape Province. This was investigated as a comparison of the fitness of the purebred lineages and the first and second hybrid generations.

Chapter 2 MATERIALS AND METHODS

In view of the reality that *O. megapotamica* has already been colonised by the 'ficus' lineage of *D. opuntiae* throughout its distribution in South Africa, a number of concerns about the potential release of the 'engelmannii' lineage into this environment had to be investigated. These concerns centred around, firstly, the ability of the 'engelmannii' lineage to establish and develop on and control plants already infested with the 'ficus' lineage, and secondly, the effect of cross breeding (hybridisation) between the two lineages on the existing level of biocontrol of *O. ficus-indica*. These aspects were addressed by tracking the development of individual cochineal nymphs on detached cactus cladodes of both species to determine and record the host-affinity of purebred lineages. The next step was to ascertain the efficacy of the sibling batches produced through hybridisation on both target plants.

Methods employed in this study were adapted from work by Mathenge *et al.* (2010) and Hoffmann *et al.* (2002) but were directly comparable to Hoffmann *et al.* (2002) as they studied the same species of cochineal. Mathenge *et al* (2010)'s study on hybridisation of *D. tomentosus* 'cholla' and 'imbricata' cochineal lineages found that the hybrids outperformed both purebred lineages. This meant that in the case of both cacti species occurring in sympatry, hybridisation of the two insect lineages would not negatively influence the biological control of either host. The study did not extend to the second generation of hybrids. Hoffmann *et al.* (2002) found that while the first generation of hybrids of the 'stricta' and 'ficus' lineages developed equally well on both *O. stricta* and *O. ficus-indica* and were not species-specific, some individuals of the second generation of hybrids and backcrosses with the F_1 hybrids were host-specific, while the others were not. These results illustrate that caution is required when releasing lineages on the preferred host in the presence of the alternative host in order to maximise the efficacy of the insect lineages.

Sources of plants

The *O. megapotamica* plants were originally collected in Bedford (Eastern Cape Province). *Opuntia ficus-indica* plants were obtained from the University of Pretoria's Experimental

Farm. Plants were housed and maintained in the nursery at the Agricultural Research Council's Plant Health Protection facility in Roodeplaat, Pretoria.

Sources of insects

The 'engelmannii' lineage of *D. opuntiae* was collected on *O. engelmannii* var. *engelmannii* in Flagstaff, Arizona, and was reared on *O. megapotamica* potted plants and loose cladodes in cages and maintained in the quarantine facilities of the Weeds Research Division of ARC-PHP at Roodeplaat, Pretoria. The 'ficus' lineage was field-harvested from *O. ficus-indica* plants found on the periphery of the PHP campus. It was also reared on cladodes in the quarantine facilities. All experiments were conducted under quarantine conditions in the registered quarantine facility at PHP in Pretoria as the 'engelmannii' lineage is yet to be released.

Quarantine glasshouse compartment conditions

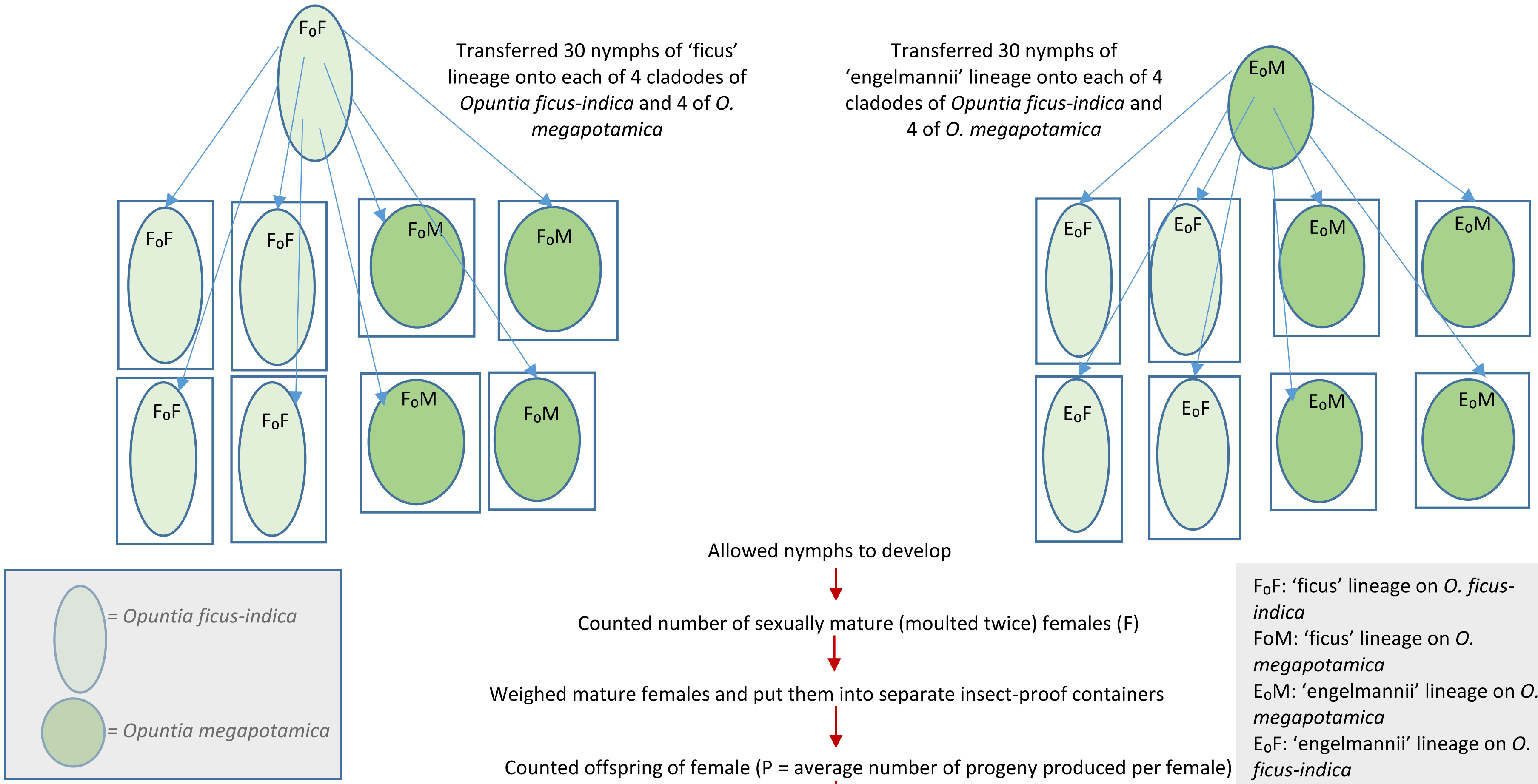
The quarantine glasshouse compartment had natural lighting and was set at a maximum of 30 °C and minimum of 12 °C, but temperatures could fluctuate between those limits based on ambient temperatures. Extremes of temperature were buffered by air conditioning and retention of daytime heat through the structure of the glasshouse. The air, cooled by evaporation, was circulated through the glasshouse when the maximum temperatures were reached, but when the temperature dropped below the minimum, the air conditioner was activated to heat the glasshouse. Atmospheric humidity was set at 50% RH. Microscope observations were made in the laboratory at a constant room temperature of 24±2 °C and +50% RH.

2.1. HOST AFFINITIES OF THE PUREBRED 'FICUS' AND 'ENGELMANNII' LINEAGES OF *DACTYLOPIUS OPUNTIAE* (Figure 2-1)

This experiment aimed to investigate how each cochineal lineage developed on the target host plant, as well as on its alternative host, i.e. to determine the host affinity of each of the two lineages. We refer to *O. megapotamica* as the target host because it was the plant targeted for biological control in this study. *Opuntia ficus-indica* and *O. megapotamica* were exposed to the 'ficus' lineage and the same two plant species were also exposed to the 'engelmannii' lineage. The development of the 'ficus' lineage on *O. ficus-indica* was compared to its development on *O. megapotamica*. Furthermore, the development of the 'engelmannii' lineage on *O. megapotamica* was compared to its development on *O. ficus-indica*. During the comparable study conducted by Hoffmann *et al.* (2002), target host *O. stricta* and alternative host *O. ficus-indica* were exposed to the 'stricta' lineage, while *O. ficus-indica* and *O. stricta* were exposed the 'ficus' lineage. Once the host affinities of the two purebred lineages are on record, this will serve as the benchmark against which the host affinities of the hybrids will be compared. This will, in turn, make it possible to determine whether and how these host affinities are affected by hybridisation.

To determine the host-plant affinities of the 'engelmannii' and 'ficus' lineages, eight terminal cladodes each of *O. megapotamica* and *O. ficus-indica* were harvested from the plant collection in the nursery and placed in separate insect-proof, well-ventilated transparent plastic containers. The rectangular plastic containers had the following dimensions: 7 litres, 355 mm long, 235 mm wide and 120 mm high with 4 locking clips on the lid. For ventilation, two circles had been cut out of each side of the containers and covered with gauze. Four of the *O. ficus-indica* cladodes were infested with 30 newly hatched 'ficus' lineage crawlers and the other four with 30 newly hatched 'engelmannii' lineage crawlers. The same was done with the *O. megapotamica* cladodes, exposing them to both lineages. Each cladode was horizontally supported on four pins on polystyrene boards to allow sufficient space underneath the cladode for crawlers to settle. The development of the nymphs was observed, and sexually mature females and males were counted. Mature females are those that have moulted twice. We removed each female only when she started producing offspring, unlike Hoffmann *et al.* (2002) who removed all females once one started producing offspring. As soon an individual female started producing offspring, she was dewaxed by winding the waxy threads around a pin, and then the insect was removed from the cladode, weighed, and placed in a separate container to allow her offspring to hatch. The number of offspring produced per female was counted, including the crawlers produced before the females were removed from the cladode.

Host affinity of the lineages was determined by using the net rate of increase (R) of the two cochineal lineages on their target host versus on the alternative host, and this was the benchmark against which all hybrids were compared.



Transferred 30 nymphs of 'ficus' lineage onto each of 4 cladodes of *Opuntia ficus-indica* and 4 of *O. megapotamica*

Transferred 30 nymphs of 'engelmannii' lineage onto each of 4 cladodes of *Opuntia ficus-indica* and 4 of *O. megapotamica*

Allowed nymphs to develop

Counted number of sexually mature (moulted twice) females (F)

Weighed mature females and put them into separate insect-proof containers

Counted offspring of female (P = average number of progeny produced per female)

Calculated *R*= (F x P)/30 on each host

F_oF: 'ficus' lineage on *O. ficus-indica*
FoM: 'ficus' lineage on *O. megapotamica*
E_oM: 'engelmannii' lineage on *O. megapotamica*
E_oF: 'engelmannii' lineage on *O. ficus-indica*

Figure 2-1: Host affinity of pure cultures of 'ficus' and 'engelmannii' lineages of *Dactylopius opuntiae*

This is calculated as follows: R= (F x P)/30 where R is the net rate of increase, F is the number of sexually mature females and P is the average number of progeny produced (Begon & Mortimer, 1981; Hoffmann *et al.,* 2002). Just as in Hoffmann *et al.* (2002), after each hybridisation event, the *R-value* for each batch of siblings from each female on *O. megapotamica* was plotted on a graph against the *R-value* for the corresponding batch on *O. ficus-indica*.

2.2. HYBRIDISATION OF LINEAGES

In the natural environment, the different cochineal lineages of *D. opuntiae* are expected to hybridise, as they are of the same species (Volchansky *et al.,* 1999; Hoffmann *et al*., 2002; Mathenge *et al*., 2010). In this species and particularly this family, paternally inherited traits are lost during spermatogenesis and males pass on only maternal ones (Bull, 1979; Gavrilov, 2007; Mathenge *et al.,* 2010; Ross *et al.,* 2010; de la Filia *et al.,* 2015). The males are haploid, with just a maternal genome while the females are diploid with both maternal and paternal genomes. Because of this Comstockiella genetic system, haploid-diploid (lecanoid) sex determination and paternal elimination, it is important to specify both the gender and lineage of each parent during the hybridisation trials. The inherited preference for host affinity not only depends on the cross, but on the preference of the maternal species (Bitume *et al.,* 2017). The levels of hybridisation were carefully set out in the experiments below to ensure that all possible F_1 hybrids that could result from the hybridisation of the different lineages through two generations were covered. Individual insects were manipulated and forced to hybridise in specific ways, resulting in controlled crosses between insects. Mathenge *et al.* (2010), who hybridised different lineages of *D. tomentosus*, only investigated the host affinity of the hybrid F_1 generation, but the current study has also tested host affinity for the hybrid F_2 generation, just as Hoffmann *et al*. (2002) did with *D. opuntiae* 'stricta' and 'ficus' lineages. Hoffmann *et al.* (2002), however, also included backcrosses between the F_1 hybrids and purebreds, which was not done in my study.

The genotypes are represented as depicted in the schematic layout of the experiments (Figure 2-1 to Figure 2-4), adapted from Hoffmann e*t al.* (2002), with the lineages represented by 'F' (for 'ficus' lineage) and 'E' (for 'engelmannii' lineage). Couplets represent lineages, with two uppercase letters representing females and an uppercase and lowercase representing a male.

F_1 hybrids are either (a) FE for females and Fe for males derived from ‘ficus’ female crossed with male ‘engelmannii’, or (b) EF for females and Ef for males derived from ‘engelmannii’ female crossed with ‘ficus’ male. F_2 hybrids follow the same convention but pairs of couplets are hyphenated to represent the lineage of each F_2 cross, the first pair representing the maternal lineage and the second the paternal, resulting in the following combinations: FE-Fe, FE-Ef, EF-Fe, EF-Ef.

Table 2-1: Parent genotypes of *Dactylopius opuntiae* used in various combinations of cross-mating experiments over three generations and the resultant genotypes and phenotypes of the progeny expected from each cross. Reference to the corresponding figures in the text is shown for each cross.

		Parent genotypes		**Progeny genotypes**		**Progeny phenotypes**	**Figure**
Generation	**Cross category**	**Female**	**Male**	**Female**	**Male**		
F_0		FF	Ff	FF	FF	‘ficus’	1a
F_0		EE	Ee	EE	Ee	‘engelmannii’	1b
F_1	i	FF	Ee	FE	Fe	Hybrid	2a
F_1	i	EE	Ff	EF	Ef	Hybrid	2b
F_2	ii	FE = EF	Ef	FE + EE	Fe + Ee	‘engelmannii’ + hybrid	3a
F_2	ii	FE = EF	Fe	FF + EF	Ff + Ef	‘ficus’ + hybrid	3b

2.2.1. Host affinity of F_1 hybrids (Figure 2-2)

On four clean *O. ficus-indica* cladodes, 30 one-day old ‘ficus’ lineage nymphs were transferred onto each cladode. Thirty nymphs of the ‘engelmannii’ lineage were transferred onto each of four clean *O. megapotamica* cladodes. Each cladode was individually placed in a plastic container on a polystyrene board. The nymphs could settle and develop on the cladodes. As soon as the sexes could be distinguished with certainty, on two of the *O. ficus-indica* cladodes, all males were removed and discarded and only females remained. The males were distinguishable by the cocoons they had formed. This was done to make certain of the maternal origin of the hybrid that would result from the cross. On the other two *O. ficus-indica* cladodes, all females were removed and discarded, and the males retained. The same procedure was followed on the *O. megapotamica* cladodes with ‘engelmannii’ lineage.

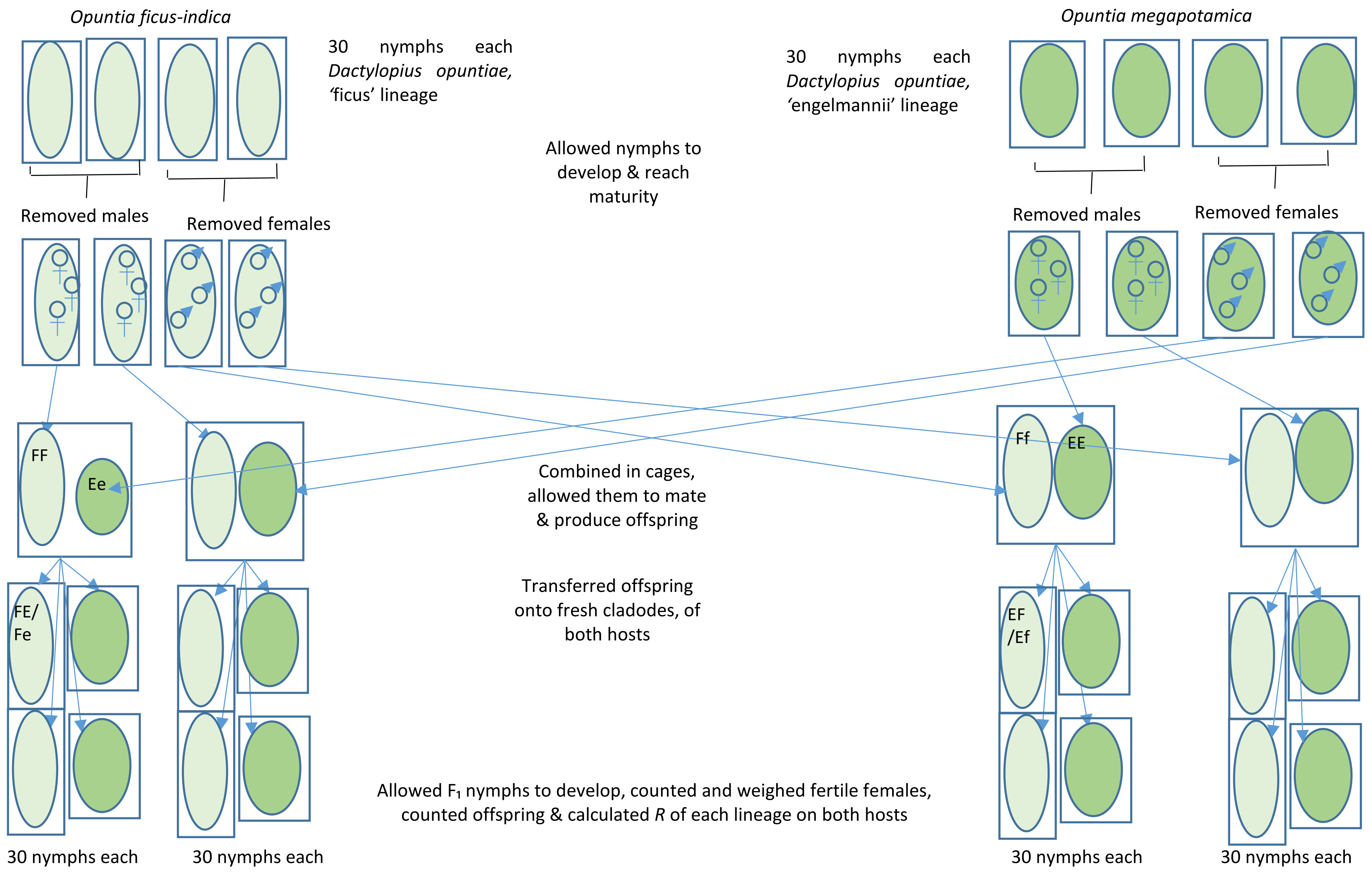


Figure 2-2: Producing F_1 hybrids

Each *O. ficus-indica* cladode containing only females was then paired with an *O. megapotamica* cladode containing only males, in an insect-proof well-ventilated container, supported on pins on a polystyrene board. Each *O. ficus-indica* cladode with males was paired with an *O. megapotamica* cladode with females. This resulted in two containers of each of the two types of crosses (FF x Ee and EE x Ff). The mature males and females could mate to produce offspring (i.e. FE and Fe, or EF and Ef).

To determine the host affinity of each type of cross, 30 newly hatched crawlers from each of the resultant hybridisation containers were transferred onto each of two cladodes of *O. ficus-indica* and another 30 onto each of two cladodes of *O. megapotamica*. The development was observed and net rate of increase (R) of both hybrid combinations, on each of the cactus hosts, was determined and analysed as described in 2.1.

2.2.2. Host affinity of F_2 hybrids (Figure 2-3 and Figure 2-4)

The same method described above and in Figure 2-2 was followed to initially produce the F_1 hybrid generation on four cladodes of each cactus host with each of the two combinations of F_1 cochineal hybrids (FE/ Fe and EF/ Ef). Each combination was placed in four containers and on two of those all males were removed and on the other all the females were also removed. Inverse F_1 offspring of different sexes were combined (producing combinations of FE-Ef and EF-Fe) and they could mate, producing F_2 hybrids. For example, a hybrid with maternal 'ficus' lineage parent was crossed with a hybrid with maternal 'engelmannii' lineage parent; and a hybrid with maternal 'engelmannii' lineage parent was crossed with maternal 'ficus' lineage parent. To determine the host affinity of the offspring, thirty newly hatched F_2 hybrid nymphs of each combination was transferred onto two fresh cladodes of both hosts, in insect-proof plastic containers, allowed to develop, mate and produced offspring. The host affinity of each sibling batch was determined. The second half of sibling batches produced was that of identical F_1 hybrids. For example, a hybrid with maternal 'ficus' lineage parent was crossed with a maternal parentage 'ficus' lineage hybrid; and a hybrid with maternal 'engelmannii' lineage parent was crossed with maternal 'engelmannii' lineage parent. The resultant sibling combinations produced were EF-Ef and FE-Fe and their host affinity was determined and analysed.

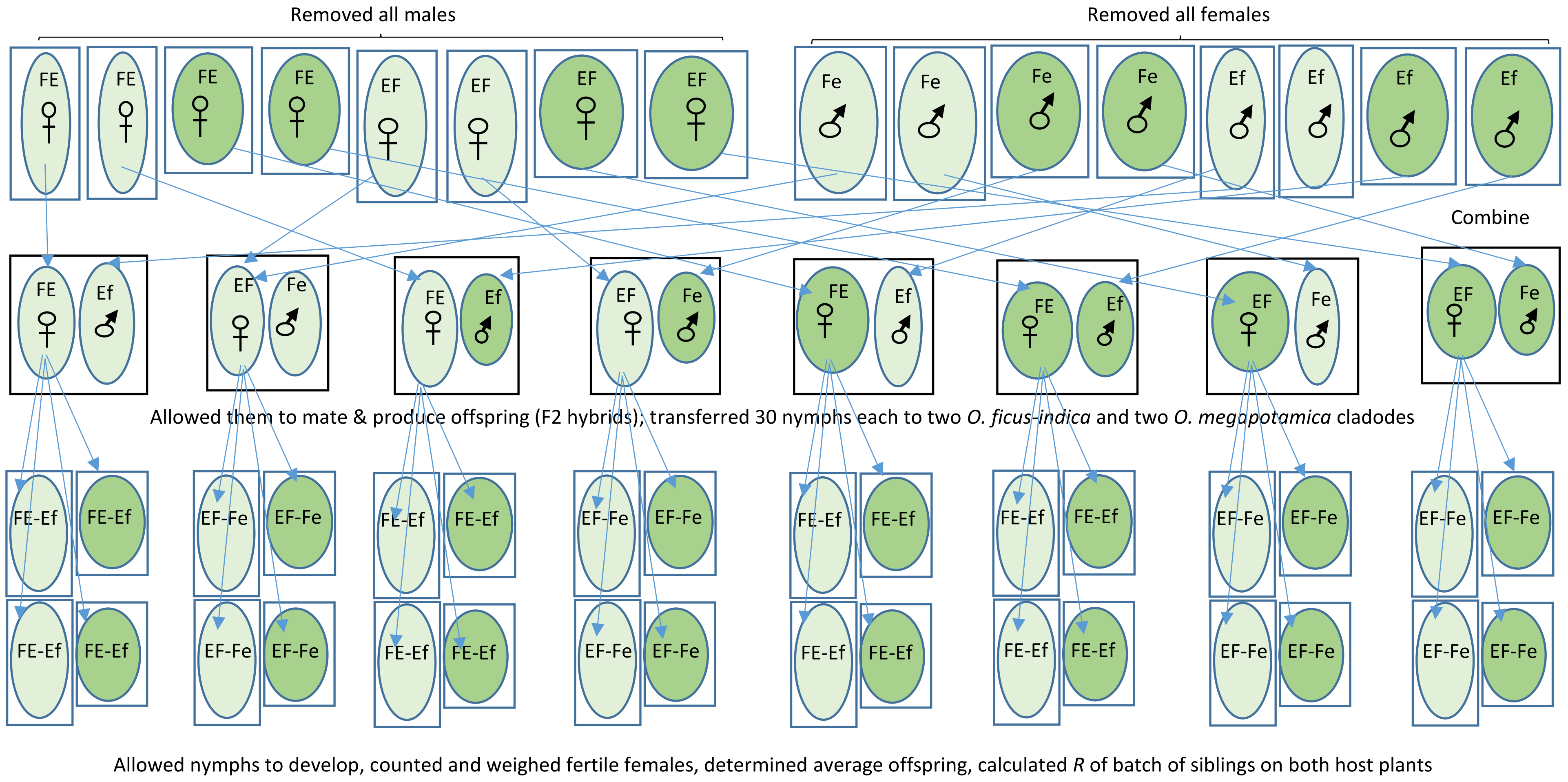


Figure 2-3: Producing F_2 hybrids (between inverse F_1s)

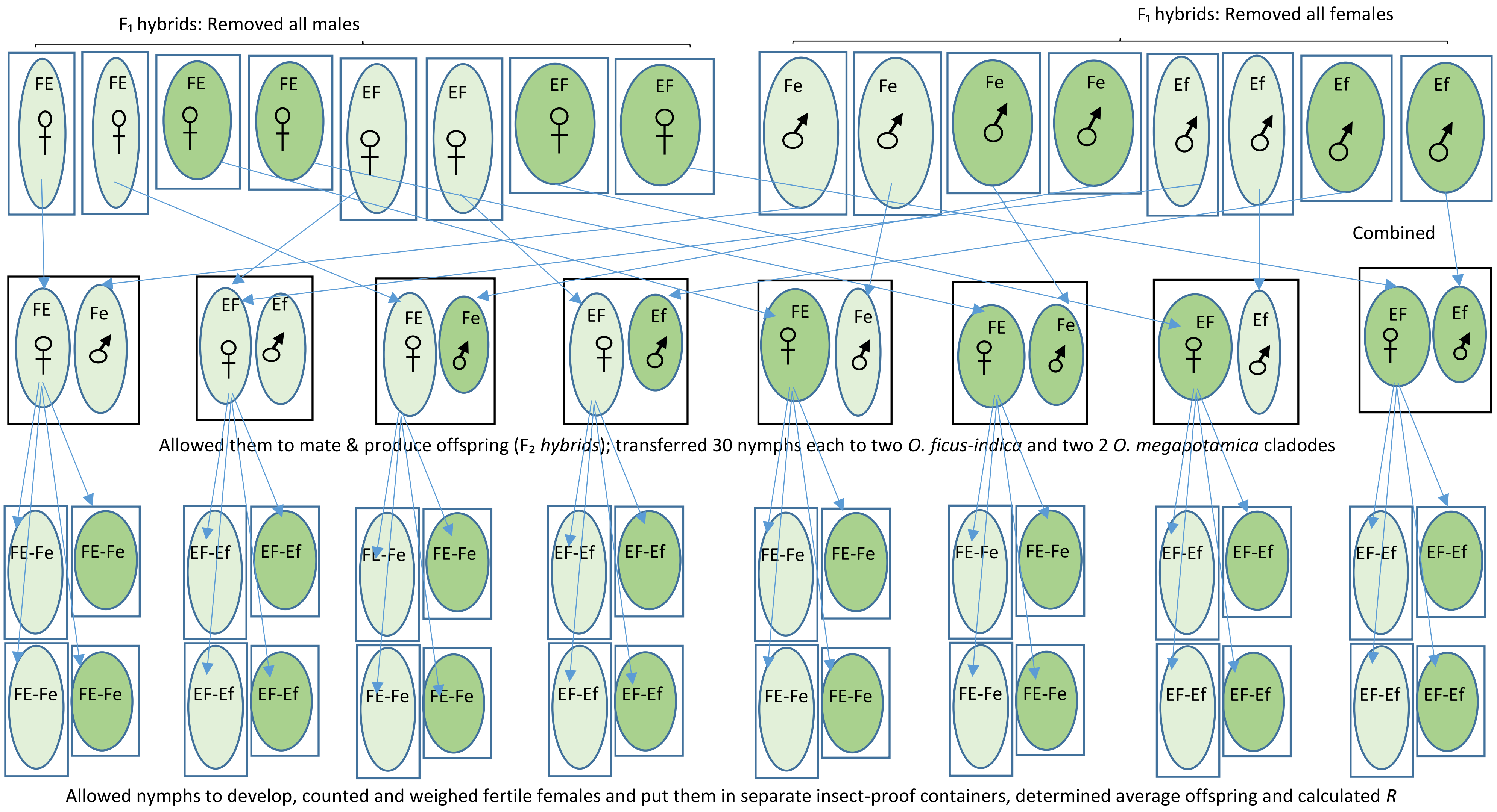


Figure 2-4: Producing F_2 hybrids (between identical F_1s)

2.3. STATISTICAL ANALYSIS

Analyses were conducted using Statistica Version 13.6 (© Stat Soft, Inc., USA.). All data were first checked for normality and were analysed using t-tests (for the *R-values*) and ANOVA (for the analysis of the averages of the females' mass, progeny produced and the time it took from egg to adult female), to determine *P* and the significant differences between the purebred and hybrid offspring.

Chapter 3 RESULTS

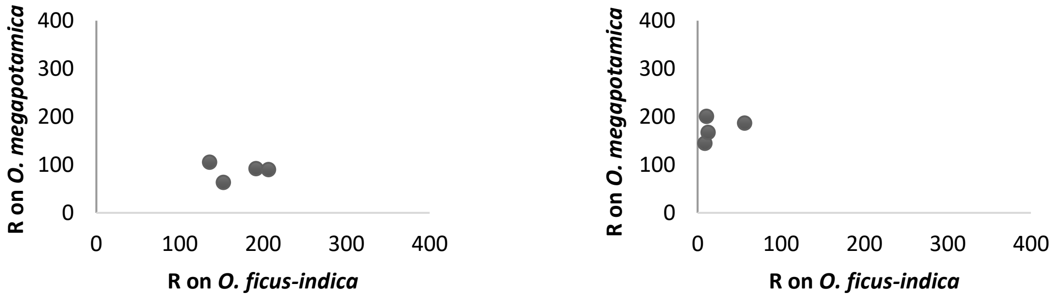
3.1. HOST AFFINITY OF THE PUREBRED ‘FICUS’ AND ‘ENGELMANNII’ LINEAGES OF *DACTYLOPIUS OPUNTIAE*

Table 3-1 lists the average female mass, number of progeny produced, number of days to maturity, the sex ratio (which is the number of females produced over the total population) and the *R-value* of the sibling batches of purebred lineages of ‘engelmannii’ and ‘ficus’. The *D. opuntiae* ‘engelmannii’ lineage produced more offspring, on average, on its target host (*O. megapotamica*) than the ‘ficus’ lineage did on its preferred host (*O. ficus-indica*). However, the ‘ficus’ lineage produced more offspring on the alternative host (*O. megapotamica*) than the ‘engelmannii’ lineage produced on its alternative host (*O. ficus-indica*). Females of the ‘engelmannii’ lineage were the heaviest on *O. megapotamica*, on average, closely followed by ‘ficus’ lineage females on their preferred host *O. ficus-indica*. The shortest development time to reach maturity was that of the ‘ficus’ lineage on *O. ficus-indica*, followed by the same lineage on *O. megapotamica*. Generally, the ‘ficus’ lineage performed better, based on the parameters studied, on *O. megapotamica* than the ‘engelmannii’ lineage did on *O. ficus-indica*. The purebred populations had more males than females, thus experiments showed a male-biased sex ratio as survival of the females (those harvested from each cladode) was variable. Overall, the averages of the mass of females, number of progeny produced and the time it took from egg to adult of the purebred lineages on the two host plants were found to be significantly different when analysed, using ANOVA. These parameters, individually analysed, further confirmed these findings. For instance, the average mass of the ‘engelmannii’ lineage females on *O. megapotamica* and the ‘ficus’ lineage females on *O. megapotamica* vs. the average mass of the ‘engelmannii’ lineage females on *O. ficus-indica* and the ‘ficus’ lineage females on *O. ficus-indica*, were statistically different (*P* values < 0.05; Table 3-1), eluding to the host affinity of the pure lineages. The ‘ficus’ lineage performed better on *O. ficus-indica* than on *O. megapotamica*, and the ‘engelmannii’ lineage performed better on *O. megapotamica* than on *O. ficus-indica*. The two lineages seem to be host-specific, distinctly preferring the one cactus species to the alternative.

Table 3-1: Summary of the means of female mass, number of progeny produced and time to reach maturity of purebred lineages. Standard errors are represented (S.E.) for each mean. ANOVA was used to calculate *P* and determine statistical significance.

Purebred (*n*=4)					
Aspect	‘engelmannii’ lineage on *O. megapotamica*	‘engelmannii’ lineage on *O. ficus-indica*	‘ficus’ lineage on *O. megapotamica*	‘ficus’ lineage on *O. ficus-indica*	ANOVA
Average ♀ mass (mg)	18.2 ± 0.8	5.6 ± 0.9	8.5 ± 0.7	16.2 ± 0.8	F (3,12) = 32.77 *P* < 0.00001
Average progeny	438.4 ± 26.7	101.3 ± 26.7	192.1 ± 16.9	381.5 ± 22.5	F (3,12) = 28.46 *P* = 0.00001
Maturity* (days)	44.5 ± 0.9	48.2 ± 1.4	44.1 ± 0.8	41.9 ± 0.2	F (3,12) = 3.53 *P* = 0.04
Sex ratio (%)	40	21.7	45.8	45	
R-value	175.4	21.9	88	171.7	

*time it took for the first offspring to appear



(a) (b)

Figure 3-1*: R-values* for purebred progeny of ‘ficus’ and ‘engelmannii’ lineages of *Dactylopius opuntiae.* (a) ‘Ficus’ females (FF) crossed with ‘ficus’ males (Ff); (b) ‘engelmannii’ females (EE) crossed with ‘engelmannii’ males (Ee).

The net rate of increase (fertile females produced multiplied by the number of progeny produced, all divided by number of parental generation nymphs), represented as the *R-values,* was used to compare the performance of the purebred ‘ficus’ and ‘engelmannii’ lineages on *O. ficus-indica* and *O. megapotamica*, and those *R-values* were used to plot the graphs (Figure 3-1, Figure *3-2* & Figure 3-3). The *R-values* for the purebred lineages of ‘ficus’ and ‘engelmannii’ showed distinct differences between the two lines (Figure 3-1) as the points along the lines were clustered closer on the primary host’s axis (Figure 3-1 (a) and (b)). In Figure 3-1 (a), the points clustered closer to the x-axis as the ‘ficus’ lineage of *D. opuntiae* developed significantly better on *O. ficus-indica* than on *O. megapotamica* (*t* = 4.47, *P* = 0.004) while in Figure 3-1 (b), the points clustered closer to the y-axis because the 'engelmannii' lineage performed better on *O. megapotamica* than on *O. ficus-indica.* The 'engelmannii' lineage seems to perform better on *O. megapotamica* than the 'ficus' lineage on *O. ficus*-indica (*t* = 9.2, *P* < 0.0001). This statistical significance of the two lineages on either host species gave rise to the distinct pattern of the clustering of points on the x-axis ('ficus' lineage offspring on the *O. ficus-indica* axis) and the y-axis ('engelmannii' lineage offspring on the *O. megapotamica* axis) on the two graphs (Figure 3-1).

3.2. HYBRIDISATION OF LINEAGES

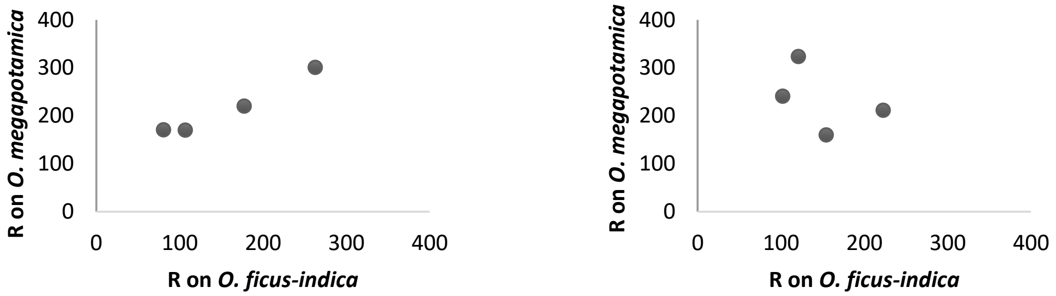
3.2.1. Host affinity of F_1 hybrids

There was no significant difference, on average, in the time it took for the first offspring to appear across the F_1 hybrids (Table 3-2). However, the 'engelmannii' lineage females crossed with 'ficus' lineage males (EE-Ff) produced the most progeny on average on *O. megapotamica* which was expected as those females were the heaviest (Table 3-2). The 'ficus' lineage females crossed with the 'engelmannii' lineage males (FF-Ee) created the second most offspring, from the second biggest females on *O. ficus-indica*. With 'ficus' lineage females crossed with 'engelmannii' lineage males (FF-Ee) on *O. ficus-indica* coming a close second with regards to the female mass, development time and progeny produced, this may be an indication that the maternal origin of the pair results in a better performance on its preferred host regardless of the male they are crossed with, eluding to haploid-diploid sex system and the elimination of the male's paternal characteristics. The *P* value indicated that neither the average mass of females nor the number of progeny produced were statistically different (*P* was 0.5 and 0.3 respectively). As with the study of purebreds (Table 3-1), the longer the time to reach maturity, the smaller the females were and the fewer the progeny they produced (Table 3-2). The cross EE-Ff took 56.7 ± 0.7 days for the females averaging 14.1 ± 1.0 mg to reach maturity and produce an average of 367.1 ± 24.7 offspring on *O. ficus-indica* compared to 53 ± 0.5 days for the females that mass an average of 19.5 ± 0.8 mg to reach maturity, producing 520.4 ± 29.3 offspring on *O. megapotamica*. FF-Ee cross produced heavier females, more progeny and in lesser time on *O. megapotamica* when compared with EE-Ff on *O. ficus-indica*. This trend continued from the purebred experiments where the pure 'ficus' lineage performed noticeably better on *O. megapotamica* than the pure ‘engelmannii’ lineage did on *O. ficus-indica*.

Table 3-2: Summary of the means of female mass, number of progeny produced and time to reach maturity of F_1 hybrids. Standard errors are represented for each mean. ANOVA was used to calculate *P* and determine statistical significance.

F_1 hybrids (n=4)					
Aspect	EE-Ff on *O. megapotamica*	EE-Ff on *O. ficus-indica*	FF-Ee on *O. megapotamica*	FF-Ee on *O. ficus-indica*	ANOVA
♀ mass (mg)	19.5 ± 0.8	14.1 ± 1.0	17.6 ± 1.0	18.2 ± 1.0	F (3,12) = 0.83 *P* = 0.5
Average progeny	520.4 ± 29.3	367.1 ± 24.7	458.8 ± 27.8	488.7 ± 31.7	F (3,12) = 1.25 *P* = 0.3
Maturity* (days)	53 ± 0.5	56.7 ± 0.7	56.6 ± 0.7	55.9 ± 0.5	F (3,12) = 1.35 *P* = 0.3
Sex ratio (%)	45	40.8	44.16	34.16	
R-value	234.2	149.9	215.9	156.8	

*time it took for the first offspring to appear



(a) (b)

Figure 3-2: *R-values* for F_1 progeny of crosses between ‘ficus’ and ‘engelmannii’ lineages of *Dactylopius opuntiae*. (a) ‘Ficus’ females (FF) crossed with ‘engelmannii’ males (Ee); (b) ‘engelmannii’ females (EE) crossed with ‘ficus’ males (Ff).

Both types of F_1 hybrids were able to develop and survive until maturity equally well on both host plant species. In both cases (i.e., FF-Ee and EE-Ff), the *R-values* of the F_1 progeny fell more or less equidistantly between the two axes of the graph, because hybrids of the two lineages were not species-specific (t = 1.15, P = 0.2, and t = 1.9, P = 0.1 for R ‘engelmannii’ compared with R ‘ficus’ in Figure 3-2 (a) and (b) respectively). These high *P* values indicate the performance of the crosses on the two plant species was not significantly different as a result of the crosses’ lack of species affinity. Figure 3-2 (a) showed that the F_1 hybrid offspring of ‘ficus’ females (FF) crossed with ‘engelmannii’ males (Ee) fared better on both *O. ficus-indica* and *O. megapotamica* than the purebred ‘ficus’ and ‘engelmannii’ lineages on their preferred hosts. The hybrids were therefore, less species-specific than the purebreds. The offspring of the initial crosses provided the hybrid populations used in the F_2 crosses that followed.

3.2.2. Host affinity of F_2 hybrids

Table 3-3: Summary of the means of female mass, number of progeny produced and time to reach maturity of F_2 hybrids. Standard errors are represented for each mean. ANOVA was used to calculate *P* and determine statistical significance.

	F_2 hybrids (*n*=8)								
Aspect	FE-Ef on *O. megapotamica*	FE-Ef on *O. ficus-indica*	EF-Ef on *O. megapotamica*	EF-Ef on *O. ficus-indica*	FE-Fe on *O. megapotamica*	FE-Fe on *O. ficus-indica*	EF-Fe on *O. megapotamica*	EF-Fe on *O. ficus-indica*	ANOVA
♀ mass (mg)	19 ± 0.6	22 ± 0.8	17.5 ± 0.7	10.5 ± 0.6	17 ± 0.6	16.1 ± 0.8	18.5 ± 0.6	20.2 ± 0.8	F (7,56) = 5.16 *P* = 0.0001
Average progeny	352.8 ± 13.3	400.1 ± 15.8	395.6 ± 17.2	228 ± 18.4	372.5 ± 14.5	363.7 ± 18.6	337.2 ± 13.2	341.7 ± 14.5	F (7,57) = 3.60 *P* = 0.003
Maturity* (days)	62.7 ± 0.4	62.5 ± 0.4	53.1 ± 0.36	61 ± 1.0	53.4 ± 0.5	54.8 ± 0.4	63.8 ± 0.4	63.1 ± 0.3	F (3,12) = 1.35 *P* = 0.3
Sex ratio (%)	47.08	47.5	42.08	37.9	45.4	36.25	42.08	37.9	
R-value	166.1	190	156.1	86.5	172.4	131.8	160.2	156.6	

*time it took for the first offspring to appear
Shaded= Inbred F_2 hybrids
Non-shaded= Crossbred F_2 hybrids

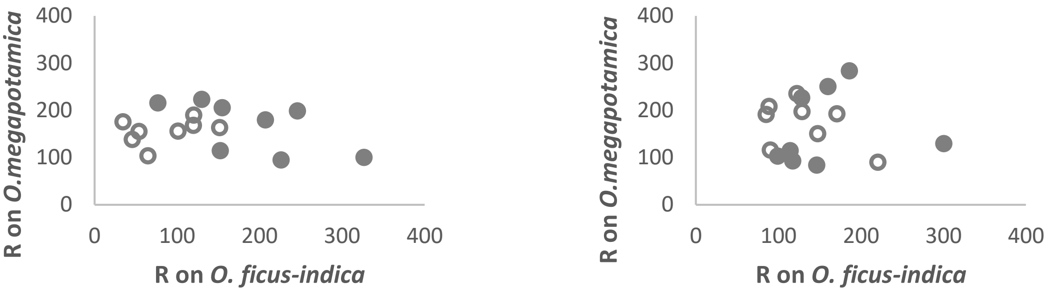
Second generation crosses comprise inbreeding and crossbreeding. Inbreeding is when a male is crossed with a female of the same genotype, while crossbreeding is when a male is crossed with a female of a different genotype. Inbred EF-Ef hybrids (Figure 3-3 (a) and Table 3-3), produced offspring that favoured *O. megapotamica* while, FE-Fe (Figure 3-3 (b) and Table 3-3) favoured *O. ficus-indica* indicated by the higher net rate of increase. The crossbred hybrids had high *R-values*, which indicate that both FE-Ef and EF-Fe fared very well on either host.

When compared, the FE-Ef cross performed slightly better (higher *R-values*) than EF-Fe on either host. All hybrid combinations survived well on either host, but Ef (males) crossed with FE (females) perform better on *O. ficus-indica* with higher *R-values* than the other hybrid crosses on either host (Table 3-3 & Figure 3-3). This combination produced the heaviest females, the most progeny and had the largest sex ratio in favour of the males, than the other crosses on both *O. megapotamica* and *O. ficus-indica*. Both crosses in Figure 3-3 (a) and (b) indicated a mix of purebred and hybrid offspring (Table 3-4), as was expected (Chapter 2, Table 2-1). The FE-Fe combination fared well on both host plants, even though there was a considerable difference in the *R-values*, thus proving to be the least specific of the two inbred F_2 hybrids (i.e. between FE-Fe and EF-Ef) in that the range in its performance on both *O. ficus-indica* and *O. megapotamica* was not as widely different as that of EF-Ef on the two host plants.

Table 3-4: T-test statistics for *R-values* for groups of F_2 hybrids (Figure 3-3) compared with the purebred (Figure 3-1) and F_1 hybrids (Figure 3-2).

Comparison	*t*	*P*
R 'ficus', Figure 3-3(a) with Figure 3-1(b)	0.88	= 0.4
R 'ficus', Figure 3-3(a) with Figure 3-2	0.57	= 0.5
R 'engelmannii', Figure 3-3(b) with Figure 3-1(a)	0.88	= 0.4
R 'engelmannii', Figure 3-3(b) with Fig. Figure 3-2	0.91	= 0.3

There was a slight mixture of genotypes (purebred and hybrid) in the F_2 progeny produced by the four crosses, evident in the scattering of the points in the graph (Figure 3-3). For instance, some of the plotted dots representing EF-Ef offspring were clustered closer to the y-axis which may indicate a host affinity towards *O. megapotamica* (Figure 3-3 (a)), while majority of the points fell more or less equidistantly between the two axes of the graph. Crossbred hybrids (male crossed with female with different genotype) FE-Ef and EF-Fe developed well on *O. ficus-indica* but there was no significant difference statistically between the two (t = 0.71, P = 0.48 and t = 0.098, P = 0.9 respectively). None of the crosses compared were significantly different from one another, with *P* values ranging between 0.3 and 0.5, which means that the *R-values* of both 'engelmannii' and 'ficus' did not differ significantly from those of the hybrid genotypes.



(a) (b)

Figure 3-3: *R-values* for F_2 progeny of crosses between hybrid females and hybrid males of D*actylopius opuntiae*. (a) FE females (●) and EF females (○) crossed with Ef males; (b) FE females (○) and EF females (●) crossed with Fe males.

There is considerable scatter in the distribution of the R-*values*, which indicated a variation in host specificity of the F_2 hybrids (Figure 3-3). Overall, the *R-values* of F_2 hybrids indicated that there was no significant difference between the siblings produced from the crosses ($t = 0.23$, $P = 0.8$). There is a slight mixture of genotypes in the progeny produced regardless of whether the F_1 females were FE or EF (closed circles compared with open circles in Figure 3-3 (a) and (b)), but with mostly hybrids than purebred individuals. There was an expectation for these crosses to produce a mixture of purebred and hybrid genotypes (Table 2-1).

Chapter 4 GENERAL DISCUSSION

The aim of the study was to determine whether the introduction of another *Dactylopius opuntiae* lineage would, through hybridisation, influence the host affinity, and ultimately the efficacy, of the resultant hybrids. This, in turn, would predict what could potentially happen with control in the field. The results have showed that the two lineages of cochineal hybridise under laboratory conditions to produce viable offspring. This was to be expected as the two lineages are of the same species. The reciprocal crosses were reproductively compatible and produced viable offspring too, as was the case in previous studies, with other cochineal species and lineages, by Volchansky *et al.* (1999), Hoffmann *et al*. (2002) and Mathenge *et al*. (2010). In the first generation of hybrids, there was hybrid vigour and the parent females performed better on their preferred host than the purebred lineages, irrespective of the male they were crossed with. The second generation saw some individual hybrids performing better than the purebred lineages but not better than the F_1 hybrids, with evidence of hybrid breakdown. In this chapter, the consequences of these results for control of *O. megapotamica* and *O. ficus-indica* in South Africa are discussed.

4.1. *OPUNTIA MEGAPOTAMICA* VERSUS *OPUNTIA FICUS-INDICA*

Opuntia megapotamica seems to be a more favourable host for cochineal survival, this is clear from all the hybridisation experiments, where the 'ficus' lineage performed better on *O. megapotamica* than the 'engelmannii' lineage on the alternative host (*O. ficus-indica*) considering the parameters observed (mass, progeny, and days to maturity). This was evident when the rate of increase of the purebred, F_1 and F_2 hybrids was higher on *O. megapotamica* cladodes than on *O. ficus-indica*. The *R-values* were higher on *O. megapotamica*, with the exception of the FE-Ef hybrid combination, which was 166.1 on *O. megapotamica* versus 190 on *O. ficus-indica*. Therefore, regardless of the genotype, *D. opuntiae* performed better on *O. megapotamica* than on *O. ficus-indica*, indicating that generally *O. megapotamica* was a more favourable host plant than *O. ficus-indica* for the insects. Studies by Githure *et al*. (1999) and Hoffmann *et al.* (2002) testing the 'stricta' and 'ficus' lineages on *O. stricta* and *O. ficus-indica* respectively, found that *O. stricta* was a more favourable host to both lineages. The deduction from these observations is that *O. ficus-indica* is perhaps less palatable to the cochineal lineages tested on it than *O. stricta* and *O. megapotamica*. Furthermore, these results suggest that the ‘ficus’ lineage may not be optimally adapted to *O. ficus-indica* and there could possibly be a better adapted lineage in the Americas that could be more damaging to, and more effective for the control of, *O. ficus-indica* (Githure *et al*., 1999).

Whether a new and more damaging cochineal lineage is required for the control of *O. ficus-indica* is debatable. The proposed ‘engelmannii’ lineage is not as damaging to *O. ficus-indica* as the ‘ficus’ lineage that has already been released and is currently controlling *O. ficus-indica* in South Africa. So, the release of the ‘engelmannii’ lineage is unlikely to result in significant changes to *O. ficus-indica* populations. High palatability of *Opuntia* species, due to their adaptation to arid and semi-arid conditions, makes them valued fodder alternatives in drier times and in arid and semi-arid regions (Matos *et al.,* 2021). *Opuntia ficus-indica* is fast becoming a very important crop in North Africa’s Mediterranean region as the earth becomes drier due to the changing climate and cactus grows relatively well in most arid and harsh regions of the world, requiring little, if any, irrigation (Nobel, 2002). Drought- and famine-stricken Madagascar and Ethiopia residents and animals often rely on cactus for sustenance when they are unable to grow anything else (Inglese *et al.,* 2002). In South Africa, the spineless varieties of *O. ficus-indica* are not listed in the alien and invasive species regulations and are grown, on a small-scale, and used as drought fodder crops for animals and for fruit from naturalised populations (Potgieter & Mashope, 2009). Its decreased weed status (due to various control interventions over the years, but primarily due to biological control) and its economic value, which has increased in recent years, has altered public perceptions of *O. ficus-indica* and the biological control agents are often considered pests (Zimmermann & Moran, 1991). The status of the plant in the NEMBA regulations needs to be reconsidered because it is under such effective biological control that increased control is not required and the plant can be used as a crop, so in under many circumstances, a higher level of control is not desirable (Zachariades *et al.,* 2017). Due to the massive reduction in the plant’s populations because of effective biological control, the plant’s negative impacts have reduced, balancing out with the positive impacts (Hill *et al.,* 2020). Greater levels of control and allocation of resources towards control is not necessary and would be a waste of effort

(Zachariades *et al.,* 2017; Hill *et al.,* 2020). However, if it were not for the 'ficus' cochineal, *O. ficus-indica* infestations would not be manageable, with the potential to revert back to infestations that covered 900000 ha in the past (Zimmermann & Moran 1991). Dense prickly pear infestations are now a rare sight in South Africa and the plant's utilisation is a valuable commodity (Paterson *et al.* 2021). Increased levels of control of *O. ficus-indica* could negatively affect the economy and exacerbate the negative public perceptions on biological control as a management tool for alien invasive plants. A more damaging cochineal lineage is therefore not desirable for *O. ficus-indica*, and the fact that 'engelmannii' lineage is less damaging to it than the 'ficus' lineage makes the 'engelmannii' lineage more suitable for control. If the 'engelmannii' lineage was more damaging to *O. ficus-indica* than the 'ficus' lineage, it would have to be rejected for release.

4.2. THE LINEAGES AS SEPARATE ENTITIES

The 'ficus' lineage survived relatively well on the alternative host, *O. megapotamica*. The 'stricta' (Volchansky *et al*., 1999; Hoffmann *et al*., 2002) and now 'engelmannii' lineage of *D. opuntiae* both performed significantly better on their target plants than the 'ficus' lineage did on *O. ficus-indica*. The performance of the purebred lineages of 'engelmannii' and 'ficus' were significantly different on their preferred host, suggesting that they were host specific. Overall, these findings infer that each *D. opuntiae* lineage must continue to be regarded as a separate entity because they have shown that they have the potential to control their particular host and perform differently on various host plant species within the genus (Volchansky *et al.,* 1999).

One important question is whether these differences warrant the use of the term biotype for each of the lineages. A key criterion of a biotype is that they should have developed enough behavioural, physiological and genetic traits for reproductive isolation as a result of geographic isolation (Mathenge *et al*., 2010). It unknown what exactly about these lineages is new in terms of the damage they cause to the plants and the classification is made on a single partially measured trait and its effect (Downie, 2010). In this regard, biotypes are classified according to their function and not their morphological and/or genetic traits. Downie (2010) goes on to mention that this biased perceptive only focuses on the observer's purposes and not on the biological entity, which could be a complex of races and strains, and this leads to misclassification and makes it pseudo-taxonomic. Mathenge *et al.* (2015) now conclude that *Dactylopius* species are a complex of biotypes, cryptic or sibling species and host races. The interchangeability in use of the term biotype synonymous with host race and strain (in Volchansky *et al*., 1999), has led to a dilution of their meanings and added to the confusion. A lineage, on the other hand, defined as ''an ancestral-descendant sequence of interbreeding populations" (Simpson, 1951), and "evolutionary unit that includes an ancestral population, all of its descendants, and only its descendants… which is also called a monophyletic group or a clade" (Freeman, 2005) is the more appropriate term to use for both the 'engelmannii' and 'ficus' lineages studied here. Furthermore, the term lineage is relative to the specific genetic markers used to differentiate between *Dactylopius* spp. clades and genotypic clusters in van Steenderen *et al*. (2021), a study which assisted to determine the classification of the 'engelmannii' lineage studied.

4.3. NEW ASSOCIATIONS

Due to the earlier misidentification of *O. megapotamica* in South Africa, the cochineal collected on *O. engelmannii* in Arizona does not occur in sympatry with *O. megapotamica* in the indigenous distribution. This lineage did not coevolve with the plant, but was virulent in its performance on *O. megapotamica*. This introduction of an exotic natural enemy against pests they did not coevolve with and therefore do not share a history of intensive interaction, is a 'novel' or 'new' association (Hokkanen & Pimentel, 1989) as opposed to 'old' associations where the pest and enemy have a long and continuous evolutionary history (Ehler, 1995). Pimentel (1963) hypothesised that the completely new associations of pest and enemy will be the most damaging to the pest as they lack an evolutionary history and ecological homeostasis. This association exploits the lack of evolved commensalism, where one species benefits from another but neither are negatively impacted by their long-term biological interaction (Abrams, 1986). New associations are therefore thought to be virulent natural enemies that are more successful in biological control of weeds than old associations (Hokkanen & Pimentel, 1989). The enemy and the pest that have coevolved have a lifelong relationship of responding to one another's attacks and defence mechanisms in an evolutionary arms race (Abrams, 1986). With an increase in armaments in one contestant in the race comes an increase in armaments in response in the other contestant (Abrams, 1986). The improvement in the insects' abilities to establish and feed on the target weed results in an evolutionary response of the plant to avoid being consumed by the insect and this is regarded as an arms race because there is never equilibrium between the insect and the plant populations (Abrams, 1986). There is improvement in both the herbivore feeding on the plant as well as the plant's defences against the insects' feeding. In new associations, a lack of coevolution may result in the plant not having the necessary defences against the herbivore because they do not share that history. This gives the cochineal insect an advantage over the plant species and explains the frequent success of new associations in biological control. Hokkanen and Pimentel (1989) suggest that successful biological control can be attained in an evolved equilibrium, which is a consequence of new associations in their lack of evolutionary arms race, and presented evidence to suggest that the rate of success with new associations in weed biological control was higher than that of old associations. Other authors have however, criticised the use of new associations and have questioned the validity of the analyses conducted by Hokkanen and Pimentel (1989), including Goeden and Kok (1986) and Waage and Greathead (1988).

The predator/exploiter in the new association should also have a different attack strategy to the naturally occurring native pests of the target plant (as is the case in the old association). The exploiter should feed or attack a different part/organ of the target plant or have a different mechanism of attack than that of its native pest such that the plant would not have evolved defences against the new exploiter (Hokkanen & Pimentel, 1989). There are several cochineal insects that are endemic to South America, and *O. megapotamica* would have developed defences against them, but not towards the 'engelmannii' lineage. This new association has a very similar mode of damage and attacks the same plant parts as natural enemies in the indigenous distribution of *O. megapotamica*, and therefore may not be considered a typical new association according to the definition of Hokkanen & Pimentel (1989). It is also important to note that, while the 'engelmannii' lineage is damaging to *O. megapotamica*, there is a possibility that cochineals from the native distribution in Argentina or Uruguay (i.e., old associations) may be even more damaging. In the interim, the new association of the cactus plant and the Arizonian cochineal has produced promising results under quarantine conditions. Opting to use an enemy that did not coevolve with the pest is also riskier in theory, and could only be used where the alternative hosts of the natural enemy are not valued (Hokkanen & Pimentel, 1989; Simberloff & Stiling, 1996).

There is more to the new associations approach than geographic isolation. A relatively rare exploiter in the native range of the victim has the potential to succeed when it is set free from competition and parasitism in its new environment. Pimentel first proposed this, as a technique, in 1963, but it had already begun in 1939 on Santa Cruz Island. This very successful program introduced *D. opuntiae* cochineal to native *Opuntia littoralis* (Engelm.) Cockerell and *Opuntia oricola* Philbrick. Other spectacular successes are mentioned in Hokkanen and Pimentel (1989), which also include new associations of biological control agents originating from completely different host genus. These host specific agents have the capability to reduce populations of species regarded as economic pests to acceptable levels, perpetuated by the fact that the agents' natural enemies are also absent in the new environment (Hokkanen & Pimentel, 1989). Hokkanen and Pimentel (1989) found there was a need for more promising biological control agents with a higher success rate than what was available at the time.

In this study, the agents were collected on host plants of the same genus but from different continents, and on that basis, the pest-enemy relationship is a new association. However, the cochineal insect can only feed on *Opuntia* spp., making it suitably host specific for release in South Africa despite the fact that it was not collected off the target weed. Unlike in the study by Klein *et al*. (2020), where the 'new' association could perhaps be ascribed to the exceptional results of the 'cholla' lineage of cochineal which was found in an isolated part of Mexico on a closely related species of *C. fulgida* var. *mamillata*; the 'engelmannii' lineage was collected from the very abundant *O. engelmannii* var. *engelmannii* in Arizona, which is a much more distantly related species to the target plant than is the case for *C. fulgida*.

Varone *et al.* (2012) studied the laboratory performance of South American *C. cactorum* on some South and North American *Opuntia* species occurring in Argentina and found that the larvae of the cactus moth performed best on North American *O. ficus-indica* and *O. robusta* and on South American *Opuntia arechavaletae*, which may be attributed to a higher nutritional quality or lack of defences of the hosts that coevolving species would have (Guzmán Loayza & Chávez, 2007; Woodard *et al*., 2012). The high performance of the moth on South America's *O. arechavaletae* was inconsistent with other results in the study, as the moth performed badly on the other South American species that were tested (Varone *et al.,* 2012). The association between *C. cactorum* and *O. ficus-indica* is a new one as the two did not coevolve and *O. ficus-indica* lacks defences against the cactus moth. Through these studies (Varone *et al.* 2012 & 2014) it was evident that *C. cactorum* would not be effective in controlling *O. megapotamica* as its performance was reduced. The cactus moth and *O. megapotamica* have coevolved and the moth was unable to counteract the plant's primary defence mechanism of high mucilage, which limits entry of solitary-feeding larvae. The association between the moth and *O. megapotamica* is an old one and that is evident in the way the presence of the moth in South Africa has had no significant impact on the management of *O. megapotamica.*

There is, therefore, significant evidence to suggest that the use of the 'engelmannii' lineage as a 'new association' should be considered for the control on *O. megapotamica* in South Africa, and the change in the identity of the target plant from *O. engelmannii* to *O. megapotamica* should not stop the release. It is however important that the cochineal that is released is the most effective agent that we have available, so before the 'engelmannii' lineage is released, surveys for other potential agents that may be more damaging should be conducted in South America, where *O. megapotamica* is indigenous.

4.4. HYBRIDISATION

Studies by Hoffmann *et al.* (2002), Githure *et al*. (1999) and Mathenge *et al.* (2010) showed the cochineal lineages hybridised and this affected the host affinity of the cochineals and therefore the efficacy of biological control on *O. ficus-indica*, *O. stricta*, *C. imbricata* and *C. fulgida* var. *fulgida* respectively.

The first generation offspring studied in Mathenge *et al.* (2010) were found to be less host specific than their parents, developing equally well on either host. That generation outperformed the parent 'cholla' and 'imbricata' lineages. The Fitness Index of the hybrids was greater on the alternative host of their maternal parents as compared to the pure lineages on their alternative host. The increased performance of F_1 hybrids could be because of the release from negative effects of inbreeding. Inbreeding of hybrids occurs in the second generation but Mathenge *et al.* (2010) did not extend to the second generation, so whether this would reverse the increase in performance of the F_1 hybrids in the F_2 generation is unknown.

The first generation which resulted from the 'stricta' and 'ficus' lineages of *D. opuntiae* and 'cholla' and 'imbricata' lineages of *D. tomentosus* were not species-specific and developed equally well on either host (Hoffmann *et al*., 2002; Mathenge *et al.,* 2010). The haploid-diploid (lecanoid) sex determination system found in *Dactylopius* species meant that the males only passed on the genes inherited from their maternal parent to their offspring. The F_2 crosses between hybrids as well as crosses between a purebred and a hybrid, resulted in some nonspecific offspring, while others produced purebred-specific offspring. Nonspecific offspring do not favour one plant species over another, they perform equally well on either host, making them effective in their control, while specific offspring have an affinity towards one plant species and do not survive on the other, making them effective on only one target weed species. The nonspecific hybrids have an advantage in that they can develop on either host plant and therefore build up large populations, while the host specific individuals are disadvantaged in that they are limited to utilising only one host plant.

In my study, I found that there was no significant difference in the time it took for the F_1 hybrids to reach maturity. Both lineages survived until maturity and developed equally well on both *O. ficus-indica* and *O. megapotamica*. There was no significant difference in the net rate of increase of the hybrids on either host. Females are fully diploid and pass both maternal and paternal chromosomes to their progeny (Bull, 1979) and this was evident in the affinity of the hybrids. Parent females perform better on their preferred host regardless of the male they are crossed with due to the haploid-diploid sex system and paternal elimination characteristic of the male. The F_1 generation illustrated superior performance to that of the purebred parents, yielding high *R-values*. This was an indication of hybrid vigour of the F_1 hybrids relative to their parents (Edmands, 2002). This theory states that the hybrids have increased fitness and this is most common in F_1 generations (Edmands, 2002). These results were similar to those of Mathenge *et al*. (2010) and Hoffmann *et al.* (2002). The F_1 generation seems to perform better than the purebred parent lineages. It also survived and developed similarly well on either host. In the event that a F_1 hybrid crawler found itself on either *O. ficus-indica* or *O. megapotamica*, it would remain effective in controlling the plant, performing better than the purebred lineages due to hybrid vigour, as was the case with the Swiss and Italian lineages of the beetle *L. jacobaeae* on *J. vulgaris* (Szűcs *et al.,* 2012).

The resultant F_2 hybrids thrived on either host. The net rate of increase fell somewhere in the middle between the purebred and the F_1 hybrids. Some of the hybrids performed better than the purebred lineages, but not as well as the F_1 hybrids. There is evidence of a hybrid breakdown in the F_2 generation (and backcrosses) as there is a possible reversal to host specificity in some offspring, as was reported by Hoffmann *et al.* (2002). This is due to the presence of inbreeding between the sibling batches produced. In line with these results, an assumption could be made that some of the second-generation hybrids have an affinity towards the host plants (like the purebred lineages), but are not species-specific. Dispersal of cochineal is primarily through wind dispersal of crawlers (Moran *et al.,* 1982). The likelihood of interplant dispersal of cochineal is increased proportionately with the occurrence of both host plants in proximity (Moran *et al.,* 1982). Subsequently, hybrid survival will be higher than either of the host specific purebred lineages because the probability of a nonspecific crawler settling on a suitable host will increase relative to the combined abundance of the two cactus species, either host would be suitable (Hoffmann *et al.,* 2002). The cochineal's population growth may be boosted by hybrid survival, all the while causing damage to *O. ficus-indica* and *O. megapotamica* (Hoffmann *et al.,* 2002). The plants may be inundated by the sheer number of crawlers. Some of the population of F_2 hybrids are, however, host specific. When those host specific individuals land on a host that is not suitable (i.e. a hybrid nymph with a preference for *O. ficus-indica* landing on *O. megapotamica* and a hybrid nymph with a preference for *O. megapotamica* landing on *O. ficus-indica*), will develop poorly and not contribute towards controlling that host plant. In this way, the crawlers' survival and development would decrease (Hoffmann *et al.,* 2002). This may negatively influence the rate at which the cochineal population will increase.

The results in this study differed from Hoffmann *et al.* (2002) in a number of ways. In both this study and in Hoffmann *et al.* (2002), the purebred lineages were statistically different from each other, while the first generation was not. The differences in the two studies are in the comparisons of the second generations with the purebred lineages in order to determine whether the F_2 crosses produce species-specific or non-species-specific offspring (Table 3-4). Hoffmann *et al*. (2002) found that the *P* values indicated that there were significant differences between the F_2 'ficus' and one of the two 'stricta' hybrids when compared with the purebred and F_1 hybrids (Table 3-4). The *P* value ($P = 0.6922$) of the other F_2 'stricta' showed no statistical difference from the hybrids and this was the exception to their study. In this study, there were no significant differences in the comparisons of the F_2 hybrids against the purebred and F_1 hybrids, with the lowest P value being 0.3. Seemingly, there was very little difference, if any, in the performance and the slight mixture of purebred and hybrid characteristics of the purebred and F_1 hybrids when compared with the F_2 hybrids. The reasons for these differences in this study and Hoffmann *et al.* (2002) are largely due to the fact that I did not have as many replicates due to space and time limitations in the quarantine laboratory. The 'engelmannii' lineage is yet to be released in field, so all experiments were conducted in quarantine and the results reflect the situation under controlled laboratory conditions as opposed to Hoffman *et al.* (2002) who studied hybridisation of already released lineages, and used a bigger sample size, with 40 replicates. As a consequence of the quarantine-bound cochineal lineage, our comparisons and analyses were over a relatively smaller sample size. Due to the high P values that indicated that there was no statistical difference amongst the crosses studied herein, the difference observed in the samples and experiments cannot be used to make an inference of effectiveness towards the entire population because the replicates were limited in number and the experiments were conducted in the laboratory. As a direct result of running experiments in the lab, there might have been a skewed sex-ratio of crawlers placed on the cladode as numbers of crawlers were limited (Nelson-Rees, 1960). We did not use paired t-test analysis as in Hoffmann *et al.* (2002) because we used crawlers from a group of females of similar lineage and not a single female's offspring to populate the respective replicates. As a result of this, ANOVA was used when data with more than two means was compared and where t-tests were used, they were not paired. Because of the limited number of females, the fertile females of similar genotypes were placed in one container and the offspring used in the experiments were sourced from that container and not sourced per female like in Hoffmann *et al.* (2002) where the offspring from one female was used to populate a complete replicate. Backcross experiments were not done due to space and time limitations and a contamination of samples in the laboratory. Backcross experiments would have afforded the opportunity to include those results and compare them directly with Hoffmann *et al.* (2002).

4.5. EXTRAPOLATION OF LABORATORY RESULTS INTO THE FIELD

The ‘engelmannii’ lineage had been in quarantine for almost five years, and this may have influenced its efficacy. It may have become adapted to the optimal conditions in the quarantine facility and might then yield very different results in the field to what is predicted. Genetic adaptations may be impacted by different growth and stress conditions (Knöppel *et al.,* 2018). Prolonged stay of organisms under laboratory conditions may cause mutations that increase fitness under the conditions they are exposed to (medium adaptation), but these mutations may not be beneficial after release (Knöppel *et al.,* 2018). Under optimal conditions, the organism puts all its energy towards reproducing. The ‘engelmannii’ lineage may not be as virulent and aggressive on *O. megapotamica* in the field as it is in the laboratory due to weather conditions as well as the presence of predators, which might affect their efficacy. Cochineal numbers are reduced by wet and cold conditions, as well as predation by generalist predators (Annecke *et al.,* 1969; Zimmermann & Moran, 1991). Similarly, to the release of any new biological control agent, it is therefore difficult to predict the efficacy of the agent and the level of success prior to release (McClay & Balciunas, 2005; Morin *et al.,* 2009).

In the field, there are many monospecific infestations of *O. megapotamica*, but in some cases, there are *O. ficus-indica* plants within *O. megapotamica* infestations. In accordance with the recommendations made by other researchers who studied hybridisation of *Dactylopius* cochineal, precaution needs to be taken when releasing the lineages in areas where more than one host plant occurs. Hoffmann *et al.* (2002) suggest that the purebred lineages be released where the target plants occur in monocultures and continue monitoring sites where the two lineages have been released and *O. stricta* and *O. ficus-indica* co-occur. Because Mathenge *et al.* (2010) had only studied the first generation of hybrids, they advised that in areas where both *C. fulgida* var. *fulgida* and *C. imbricata* occur, hybridisation would not negatively affect biological control of either cactus weed, but it is possible that this may not have been the case if the host specificity and efficacy of the F_2 generation of hybrids was investigated and then taken into consideration. The recommendation from my study is that in *O. megapotamica* monocultures that are already infested with the ‘ficus’ cochineal, the new ‘engelmannii’ cochineal lineage should be released as the results from this study suggest that good control will be achieved. Where both *O. ficus-indica* and *O. megapotamica* occur, it may be more difficult to get control so monocultures should be prioritised for the releases of the ‘engelmannii’ lineage.

A growing body of literature suggests that in herbivorous insect species, maternal parents have been shown to greatly influence host affinity (Amarillo-Suarez & Fox, 2006 in Bitume *et al.,* 2017). Host affinity and performance are traits that are influenced maternally. It was suggested that for new releases of biological control agents, results such as those found in Bitume *et al.,* 2017 and in my study, should be followed by trials run at a larger scale (e.g., field cages with growing plants and not cut plants) to evaluate their robustness under a more natural environmental setting. Furthermore, conducting experiments using cut cladodes has been reported to change insect activity and may reduce plant resistance (Palmer *et al.,* 2010), so it remains unknown how insect performance may differ (Varone *et al.,* 2012). This study, does however, allow for direct comparison with other studies that have also used cut and detached cladodes, and has therefore provided important evidence to guide decision makers in whether and how to release the ‘engelmannii’ lineage, despite the difficulties of extrapolating these results to the field.

4.6. CONCLUSION

This study demonstrated that the two lineages of *D. opuntiae* are reproductively compatible and produce hybrids with enhanced fitness. The two lineages could potentially co-exist and remain effective in controlling their preferred host species. The first generation of hybrids will remain effective in controlling the targeted weed species of *O. ficus-indica* and *O. megapotamica* in the case that the two plants’ infestations occur in close proximity, as is the case in the Eastern Cape Province, where *O. megapotamica* is most problematic. This generation will cause even more damage to *O. megapotamica* than the purebred lineages due to hybrid vigour. Its reduced host specificity is beneficial because each hybrid can survive on either host, thus contributing to effective control of both *O. ficus-indica* and *O. megapotamica* in areas where the two plants occur in close proximity. The second generation of hybrids could be less effective in controlling the target weed. In the second generation of hybrids, some individuals revert to being species specific while some are not. In this case, the individual nymphs will have to disperse to a suitable host and since dispersal of cochineal is largely mediated by wind, the number of individuals that happen to disperse to acceptable hosts will be determined by the relative abundance of each host plant species. Those nymphs that do not disperse to the correct host plant will develop poorly and contribute very little, if at all, to the next generation and the control of the target weed. This could negatively impact the level of control.

Some *O. megapotamica* plants are already infested with the ‘ficus’ lineage cochineal but the ‘engelmannii’ lineage, if released, will be able to utilise *O. megapotamica* optimally as a host. The current level of control on *O. ficus-indica* will not be drastically disrupted by the introduction of the ‘engelmannii’ lineage and the ‘engelmannii’ lineage will remain effective in controlling *O. megapotamica* in the presence of the ‘ficus’ lineage.

The ‘engelmannii’ lineage is therefore safe to release, and there is sufficient evidence to suggest that it will be damaging to the target weed, in areas where the ‘ficus’ cochineal is present and in areas where *O. megapotamica* is free from any cochineals. It is, however, possible that a more damaging cochineal, or other natural enemy, will exist in the indigenous distribution of *O. megapotamica*, because the confusion around the origin of this cactus resulted in the collection of agents from *O. engelmannii* in North America, rather than the indigenous distribution in Argentina and Uruguay. Prior to the release of the ‘engelmannii’ lineage, we should be certain that there are no damaging old associations in the indigenous distribution that could be exploited as biological control agents. This would ensure that the most damaging possible agent is released.

REFERENCE LIST

Abrams, P.A. (1986). Is predator-prey coevolution an arms race? *Trends in ecology and evolution,* 1(4), 108-110.

Algotsson, E. (2009). Biological diversity. In Strydom, H.A. and King, N.D. (Eds.) *Environmental Management in South Africa*. (2nd Ed.) Cape Town: Juta, 97-125.

Amarillo-Suarez, A.R., and Fox, C.W. (2006). Population differences in host use by a seed beetle: local adaptation, phenotypic plasticity and maternal effects. *Oecologia,* 150, 247-258.

Anderson, E.F. (2001). The cactus family. Timber Press, Portland, Oregon, USA.

Annecke, D.P., Moran, V.C., and Burger, W.A. (1969). Improved biological control of the prickly pear, *Opuntia megacantha* Salm-Dyck, in South Africa through the use of insecticide. *Phytophylactica*, 1*,* 9-13.

Barthlott, W., and Hunt, D.R. (1993). Cactaceae. In Kubitzki, K., Rohwer, J.G., and Bittrich, V. (Eds.) *The families and genera of vascular plants*. *Springer Verlag*, Berlin, Germany, 2, 161-197.

Bauman, H., and Schmidt, A. (2015). Food as medicine: Prickly pear cactus (*Opuntia ficus-indica,* Cactaceae). *HerbalEGram 12(9),* 1-4.

Bean, D.W., Kazmer, D.J., Gardner, K., Thompson, D.C., Reynolds, B., Keller, J.C., and Gaskin, J.F. (2013). Molecular genetic and hybridisation studies of *Diorhabda* spp. released for biological control of *Tamarix. Invasive plant science and management*, 6, 1-15.

Begon, M., and Mortimer, M. (1981). *Population ecology: A unified study of animals and plants*. Blackwell scientific publications, Oxford, UK.

Ben-Dov Y., and Marotta, S. (2001). Taxonomy and family placement of *Coccus bassi* Targioni Tozzetti, 1867 (Hemiptera: Coccoidea). *Phytoparasitica, 29(2),* 169-170.

Benson, L. (1982). *The cacti of the United States and Canada.* Stanford University Press, Stanford, California, USA.

Bitume, E.V., Bean, D., Stahlke, A.R., and Hufbauer, R.A. (2017). Hybridisation affects life-history traits and host specificity in *Diorhabda* spp. *Biological Control,* 111, 45-52.

Brock, J. (1994). *Tamarix* spp. (Salt cedar), an invasive exotic woody plant in arid and semi-arid riparian habitats of Western USA. *Ecological management of invasive riverside plants*, 27-43.

Brooks, C., Ervin, G., Varone, L., and Logarzo, G. (2012). Native ecotypic variation and the role of host identity in the spread of an invasive herbivore, *Cactoblastis cactorum*. *Ecology,* 93(2), 402-410.

Bull, J.J. (1979). An advantage of the evolution of male haploidy and systems with similar genetic transmission. *Heredity*, 43, 261-361.

Castaneda, A.I., Gonzalez, S.J., and Frati, M.A.C. (1997). Hypoglycaemic effect of an *Opuntia streptacantha* Lemaire dialysate. *Journal of the Professional Association for Cactus Development 2*, 73–75.

Chávez-Moreno, C.K., Tecante, A., and Casas, A. (2009). The *Opuntia* (Cactaceae) and *Dactylopius* (Hemiptera: Dactylopiidae) in Mexico: a historical perspective of use, interaction and distribution. *Biodiversity and Conservation*, 18, 3337-3355.

Cuadrado, G.A., and Garralla, S.S. (2009). Palinología de los Géneros de Cactaceae *Maihuenia* (Maihuenioideae) y *Pereskia* (Pereskioideae) de Argentina. *Bonplandia, 18*(1): 5-12.

de la Filia, A.G., Bain, S.A., and Ross, L. (2015). Haplodiploidy and the reproductive ecology of arthropods. *Current Opinion in Insect Science*, 9, 36-43.

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.

de Souza, N.R., and Hoffmann, J.H. (2015). Testing the hypothesis that a cochineal insect species (Hemiptera: Dactylopiidae) may have been displaced by a congeneric biological control agent from a different cactus host. *Biological Control, 85,* 25-29.

Del-Valle, V., Hernandez, M.P., Guarda, A., and Galotto, M.J. (2005). Development of a cactus-mucilage edible coating (*Opuntia ficus-indica*) and its application to extend strawberry (*Fragaria ananassa*) shelf life. *Food Chemistry, 91,* 751–756.

DeLoach, C.J., Lewis, P.A., Herr, J.C., Carrthers, R.I., Tracy, J.L., and Johnson, J. (2003). Host specificity of the leaf beetle, *Diorhabda elongata deserticola* (Coleoptera: Chrysomelidae) from Asia, a biological control agent for saltcedars (*Tamarix*: Tamaricaceae) in the Western United States. *Biological Control*, 27, 117-147.

Diehl, S.R., and Bush, G.L. (1984). An evolutionary and applied perspective of insect biotypes. *Annual Review of Entomology 29(1)*, 471– 504.

Dodd, A.D. (1940). *The biological campaign against prickly pear*. Commonwealth Prickly Pear Board Bulletin, Government Printer, Brisbane, Australia.

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

Downie, D.A. (2010). Baubles, bangles and biotypes: a critical review of the use and abuse of the biotype concept. *Journal of Insect Science*, 10(176), 1-18.

Edmands, S. (2002). Does parental divergence predict reproductive compatibility? *Trends in Ecology and Evolution*, 17(11), 520–527.

Edwards, E.J., Nyffeler, R., and Donoghue, M.J. (2005). Basal cactus phylogeny: Implications of Pereskia (Cactaceae) paraphyly for the transition to the cactus life form. *American Journal of Botany, 92*, 1177–1188.

Eggli, U. (1993). *Glossary of botanical terms with special reference to succulent plants.* Richmond: British Cactus and Succulent Society.

Ehler, L.E. (1995). Evolutionary history of pest-enemy associations. Delfosse, E.S., and Scott, R.R. (Eds.) DSIR/CSIRO, Melbourne, 83-91. Proceedings of the eighth international symposium of biological control of weeds 2-7 February 1992. Lincoln University, Canterbury, New Zealand.

Fisher, R.A. (1930). *The genetical theory of natural selection.* Clarendon Press, Oxford, England.

Font, F. (2019). A revision of *Opuntia* series Armatae K. Schum. (*Opuntia* ser. *Elatae* Britton & Rose (Cactaceae-Opuntioideae). In: David Hunt (Ed.). Further studies in the *Opuntioideae. Succulent plants research,* 8, 51-94.

Freeman, S. (2005). *Biological Science*. Pearson, 2nd edition.

Gaskin, J.F., and Schaal, B.A. (2002). Hybrid Tamarix widespread in U.S. invasion and undetected in native Asian range. Proceedings of the National Academy of Sciences of the United States of America, 20 August 2002, 99(17), 11256-11259.

Gavrilov, A.I. (2007). A catalog of chromosome numbers and genetic systems of scale insects (Homoptera: Coccinea) of the world. *Israel Journal of entomology,* 37, 1–45.

Gili, V., Laiuppa J.A., and Santillán, G.E. (2020). Aqueous extract from *Opuntia megapotamica* fruit pulp promotes osteoblast activity. A technical note and preliminary report. *International Journal of Spine Research 2*(1): 37-45.

Githure, C.W., Zimmermann, H.G., and Hoffmann, J.H. (1999). Host specificity of biotypes of *Dactylopius opuntiae* (Cockerell) (Hemiptera: Dactylopiidae): prospects for biological control of *Opuntia stricta* (Haworth) Haworth (Cactaceae) in Africa. *African Entomology,* 7, 43–48.

Goeden, R.D. (1988). A capsule of biological control of weeds. *Biocontrol News and Information 9(2),* 55-61.

Goeden, R.D., and L.T. Kok. 1986. Comments on a proposed "new" approach for selecting agents for the biological control of weeds. *Canadian Entomologist***,** 118, 51-58.

Guzmán Loayza, D., and Chávez, J. (2007). Bromatological study of the cladode of the nopal (*Opuntia ficus-indica*) for human consumption. *Journal of the Peruvian chemical society*, 73(1), 41-45.

Harley, K.L.S., and Forno, I.W. (1992). *Biological control of weeds: a handbook for practitioners and students*. Melbourne: Inkata Press, 1-74.

Henderson, L. (2001). *Alien Weeds and Invasive Plants. A complete guide to declared weeds and invaders in South Africa*. Handbook No. 12. Plant Protection Research Institute, Agricultural Research Council, Pretoria, South Africa.

Henderson, L. (2015). Karoo invasion: is history repeating itself? *Grassroots*, 15(4), 50-59.

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., and Coetzee, J.A. (2020). More than a century of biological control 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., and Zengeya, T.A. (Eds.) *Invading nature- Springer Series in Invasion Ecology: Biological Invasions in South Africa*, 553-572.

Hinz, H.L., Winston, R.L., and Schwarzländer, M. (2019). How Safe Is Weed Biological Control? A Global Review of Direct Nontarget Attack. *Quarterly Review of Biology*, 94(1), 1-27.

Hinz, H.L., Winston, R.L., and Schwarzländer, M. (2020). A global review of target impact and direct nontarget effects of classical weed biological control. *Current opinion in insect science*, 38, 48-54.

Hoffmann, J.H. (2004). Biotypes, hybrids and biological control: lessons from cochineal insects on *Opuntia* weeds. Proceedings of the XI International Symposium on Biological Control of Weeds, 27 April-2 May 2003, Canberra, Australia, 283-286.

Hoffmann, J.H., Impson, F.A.C., and Volchansky, C.R. (2002). Biological control of cactus weeds: implications of hybridisation between control agent biotypes. *Journal of Applied Ecology*, 39, 900-908.

Hoffmann, J.H., Moran, V.C., and Hill, M.P. (2019). Conceptualising, categorising and recording the outcomes of biological control of invasive plant species, at a population level. *Biological control,* 133, 134-137.

Hoffmann, J.H., Moran, V.C., and Zeller, D.A. (1998b). Evaluation of *Cactoblastis cactorum* (Lepidoptera: Phycitidae) as a biological control agent of *Opuntia stricta* (Cactaceae) in the Kruger National Park, South Africa. *Biological Control*, 12(1):20-24.

Hoffmann, J.H., Moran, V.C., and Zeller, D.A. (1998). Exploiting a partially successful biocontrol agent for integrated control of a weed: *Cactoblastis cactorum* (Lepidoptera: Phycitidae) on *Opuntia stricta* (Cactaceae) in South Africa. *Journal of Applied Ecology*, 35, 156-160.

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

Hoffmann, J.H., Moran, V.C., and Zimmermann, H.G., and Impson, F.A.C. (2020). Biocontrol of a prickly pear cactus in South Africa: Reinterpreting the analogous, renowned case in Australia. *Journal of Applied Ecology,* 57, 2475-2484. doi:10.1111/1365-2664.13737

Hoffmann, J.H., and Zimmermann, H.G. (1989). Ovipositional and feeding habits in cactophagous pyralids: Prediction for biological control of cactus weeds in southern Africa. In Delfosse, E.S. (Ed.), Proceedings VII International Symposium Biological Control Weeds, 6-11 March 1988, Rome, Italy. MAF, 395-399.

Hokkanen, H.M.T., and Pimentel, D. (1984). New approach for selecting biological control agents. *Canadian Entomologist,* 116, 1109-1121.

Hokkanen, H.M.T., and Pimentel, D. (1989). New associations in biological control: theory and practice. *Canadian Entomologist,* 121(10), 829-840.

Howard, R.A., and Touw, M. (1981). The cacti of the Lesser Antilles and the typification of the genus *Opuntia* Miller. *Cactus Succulent Journal,* 53, 233–237.

Inglese, P., Basile, F., and Schirra, M. (2002). Cactus pear fruit production. In Nobel, P.S. (Ed.) *Cacti: Biology and uses*. University of California Press, Berkeley, California, USA, 163-183.

Inglese, P., Mondragon, C., Nefzaoui, A., and Sáenz, C. (2017). *Crop ecology, cultivation and uses of cactus pear*. The Food and Agriculture Organisation (FAO) of the United Nations and the International Centre for Agricultural Research in the Dry Areas (ICARDA), Rome.

Jones, P.K., Holtkamp, R.H., and Day, M. (2016). The host range of four new biotypes of *Dactylopius tomentosus* (Hemiptera: Dactylopiidae) from southern USA and their potential as biological control agents of *Cylindropuntia* spp. (Cactaceae) in Australia: Part II. *Biocontrol Science and Technology*, 26(8), 1033-1047. doi: 10.1080/09583157.2016.1182620

Jones, P.K., Holtkamp, R.H., Palmer, W.A., and Day, M. (2015). The host range of three biotypes of *Dactylopius tomentosus* (Lamarck) (Hemiptera: Dactylopiidae) and their potential as biological control agents of *Cylindropuntia* spp. (Cactaceae) in Australia. *Biocontrol Science and Technology,* 25(6), 613-628. doi: 10.1080/09583157.2014.999747

Kaplan, H., Wilson, J.R.U., Klein, H., Henderson, L., Zimmermann, H.G., Manyama, P., Ivey, P., Richardson, D.M., and Novoa, A. (2017). A proposed national strategic framework for the management of Cactaceae in South Africa. Bothalia - African Biodiversity & Conservation, 47(2), 1-12.

Kaur, M., Kaur, A., and Sharma, R. (2012). Pharmacological actions of *Opuntia ficus-indica*: A review. *Journal of Applied Pharmaceutical Science, 2*(7), 15-18.

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

Klein, H. (complier) (2002). Principles on biological control. *PPRI Leaflet Series: Weeds Biocontrol,* No. 1.3. ARC-Plant Protection Research Institute, Pretoria, 1-4.

Klein, H. (2011). A catalogue of the insects, mites and pathogens that have been used or rejected, or are under consideration, for the biological control of invasive alien plants in South Africa. *African Entomology,* 19, 515-549. doi: 10.4001/003.019.0214

Klein, H., and Zimmermann, H.G. (2020). *Invasive cacti in South Africa: Their identification and control*. Plant Protection Research Handbook Series No. 20, Agricultural Research Council, Pretoria.

Klein, H., Zimmermann, H.G., and Xivuri, T. (2020). Exceptional biological control of two varieties of *Cylindropuntia fulgida* (Cactaceae) in South Africa using a recently identified different biotype of the cochineal insect, *Dactylopius tomentosus* (Dactylopiidae). *Biological Control,* 149. doi: 10.1016/j.biocontrol.2020.104314

Knöppel, A., Knopp, M., Albrecht, L.M., Lundin, E., Lustig, U., Näsvall, J., and Andersson, D.I. (2018). Genetic adaptation to growth under laboratory conditions in *Escherichia coli* and *Salmonella enterica*. *Frontiers in Microbiology*, 9, 756. doi: 10.3389/fmicb.2018.00756

Larsson, P. (2004). Introduced *Opuntia* spp. in Southern Madagascar: Problems and Opportunities. Minor Field Studies 285. Swedish University of Agricultural Sciences, Uppsala.

Le Houérou, H.N. (1996). Climate change, drought and desertification. *Journal of Arid Environments*, 34, 133-185.

Littlefield, J.L., Markin, G.P., Puliafico, K.P., and deMeij, A.E. (2008). The release and establishment of the tansy ragwort flea beetle in the Northern Rocky Mountains of Montana. In Julien, M.H., Sforza, R., Bon, M.C., Evans, H.C., Hatcher, P.E., Hinz, H.L., and Rector, B.G. (Eds.) Proceedings of the XII International Symposium on Biological Control of Weeds, CAB International, Wallingford, UK, 573-576.

Lloyd, S., and Reeves, A. (2014). *Situation statement on opuntioid cacti* (*Austrocylindropuntia* spp., *Cylindropuntia* spp. and *Opuntia* spp.) in Western Australia, Invasive Species Program, Department of Agriculture and Food, Western Australia.

Majure, L.C., Puente, R., Griffith, M.P., Judd, W.S., Soltis, P.S., and Soltis, D.E. (2012). Phylogeny of *Opuntia* s.s. (Cactaceae): Clade delineation, geographic origins and reticulate evolution. *American Journal of Botany*, 99(5), 847-864.

Mallet, J. (2005). Hybridisation as an invasion of the genome. *Trends in ecology and evolution*, 20(5), 229–237.

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

Martin, P. (2003). CRC for Australian Weed Management: A 2020 vision statement. Killing us softly - Australia's green stalkers. A call to action on invasive plants and a way forward. CRC for Australian Weed Management. Adelaide: Bowden.

Mathenge, C.W., Holford, P., Hoffmann, J.H., Spooner-Hart, R., Beattie, G.A.C., and Zimmermann, H.G. (2009b). The biology of *Dactylopius tomentosus* (Hemiptera: Dactylopiidae). *Bulletin of Entomological Research*, 99(6):551-559.

Mathenge, C.W., Holford, P., Hoffmann, J.H., Zimmermann, H.G., Spooner-Hart, R., and Beattie, G.A.C. (2009). Distinguishing suitable biotypes of *Dactylopius tomentosus* (Hemiptera: Dactylopiidae) for biological control 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.N., and Beattie, G.A.C. (2010). Hybridisation between *Dactylopius tomentosus* (Hemiptera:

Dactylopiidae) biotypes and its effects on host specificity. *Bulletin of Entomological Research*, 100, 331-338.

Mathenge, C.W., Riegler, M., Beattie, G.A.C., Spooner-Hart, R.N., and Holford, P. (2015). Genetic variation amongst biotypes of *Dactylopius tomentosus*. *Insect Science,* 22(3), 360-374.

Matos, T.K.B., Guedes, J.A.C., Filho, E.G.A., Luz, L.R., Lopes, G.S., do Nascimento, R.F., de Sousa, J.A., Cunato, K.M., Brito, E.S., Dias-Pini, N.S., and Zocolo, G.J. (2021). Integrated UPLC-HRMS, Chemometric tools, and metabolomics analysis of forage palm (*Opuntia* spp. and *Nopalea* spp.) to define biomarkers associated with non-specificity to carmine cochineal (*Dactylopius opuntiae*). *Journal of the Brazilian chemical society,* 32(8), 1617-1627.

Mauseth, J.D. (1983). Introduction to cactus anatomy. Part 16. Areoles and spines. *Cactus and Succulent Journal, 55*, 272-276.

McClay, A.S., and Balciunas, J.K. (2009). The role of pre-release efficacy assessment in selecting classical biological control agents for weeds - applying the Anna Karenina principle. *Biological Control,* 35, 197-207.

Moran, V.C., and Annecke, D.P. (1979). Critical reviews of biological pest control in South Africa. The jointed cactus, *Opuntia aurantiaca* Lindley. *Journal of the Entomological Society of Southern Africa*, 42, 299–329.

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

Moran, V.C., Gunn, B.H., and 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., and Zimmermann, H.G. (2013). 100 years of biological control of invasive alien plants in South Africa: History, practice and achievements. *South African Journal of Science*, 109(9/10), 1-6.

Moran, V.C., Zachariades, C., and Hoffmann, J.H. (2021). Implementing a system in South Africa for categorising the outcomes of weed biological control. *Biological Control*, 153, 104431. doi: 10.1016/j.biocontrol.2020.104431

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

Nelson-Rees, W.A. (1960). A study of sex-predetermination in the mealy bug *Planococcus citri* (Risso). *Journal of experimental zoology,* 144, 111-137.

Nobel, P.S. (Ed.) (2002). Cacti: Biology and Uses. University of California Press, Berkeley, California, USA.

Novoa, A., Brundu, G., Day, M., Delotoro, V., Essl, F., Foxcroft, L.C., Fried, G., Kaplan, H., Kumschick, S., Lloyd, S., Marchante, E., Marchante, H., Paterson, I.D., Pyšek, P., Richardson, D.M., Witt, A., Zimmermann, H.G., and Wilson, J.R.U. (2019). Global actions for managing cactus invasions. *Plants*, 8, 421

Novoa, A., Le Roux, J.J., Richardson, D.M., and Wilson, J.R. (2017). Level of environmental threat posed by horticultural trade in Cactaceae. *Conservation Biology, 31*(5), 1066-1075.

Novoa, A., Le Roux, J.J., Robertson, M.P., Wilson, J.R.U., and Richardson, D.M. (2015). Introduced and invasive cactus species: A global review. *AoB Plants 7*, plu078.

Obermeyer, A.A. (1976). Cactaceae. In Ross, J.H. (Ed.): *Flora of Southern Africa, 22*(4), 144-156.

Oulo, M.A., Yang, J., Dong, X., Wanga, V.O., Mkala, E.M., Munyao, J.N., Onjolo, V.O., Rono, P.C., Hu, G., and Wang, Q. (2020). Complete chloroplast genome of *Rhipsalis baccifera*, the only cactus with natural distribution in the Old World: Genome rearrangement, intron gain and loss, and implications for phylogenetic studies. *Plants*, 9, 979.

Osuna-Martínez, U., Reyes-Esparza, J., and Rodríguez-Fragoso, L. (2014). Cactus (*Opuntia ficus-indica):* a review on its antioxidants properties and potential pharmacological use in chronic diseases. *Natural Products Chemistry & Research*, 2(6), 153.

Palmer, W.A., Heard, T.A., and Sheppard, A.W. (2010). A review of Australian classical biological control of weeds programs and research activities over the past 12 years. *Biological Control,* 52, 271-287.

Parfitt, B.D., and Pinkava, D.J. (1988). Nomenclature and systematic reassessment of *Opuntia engelmannii* and *O. lindheimeri* (Cactaceae). *Madroño,* 35(4), 342-349.

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

Paterson, I.D., Klein, H., Muskett, P.C., Griffith, T.C., Mayonde, S.G., Mofokeng, K., Mnqeta, Z., and Venter, N.C. (2021). Biological control of Cactaceae in South Africa. *African Entomology*, 29(3), 713-734.

Paterson, I.D., Manheimmer, C.A., and Zimmermann, H.G. (2019). Prospects for biological control of cactus weeds in Namibia. *Biological Science and Technology, 29*(4), 393-399.

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

Paynter, Q., Paterson, I.D., and Kwong, R.M. (2020). Predicting non-target impacts. *Current opinion in insect science, 38*, 79-83.

Pelser, P.B., Nordenstam, B., Kadereit, J.W., Watson, L.E., and Watson, L.E. (2007). An ITS phylogeny of tribe Senecioneae (Asteraceae) and a new delimitation of *Senecio* L. Taxon, 56, 1077-1104.

Perez Guerra, G., and Kosztarab, M. (1992). Biosystematics of the family Dactylopiidae (Homoptera: Coccineae) with emphasis on the lifecycle 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.

Pettey, F.W. (1948). The biological control of prickly pears in South Africa. Scientific Bulletin of the Department of Agriculture and Forestry, Union of South Africa, 271, 1-163.

Potgieter, J.P., and Mashope, B.K. (2009). Cactus pear (*Opuntia* spp.) germplasm conservation in South Africa. *Acta Horticulturae*, 811, 47-54.

Rebman, J.P., and Pinkava, D.J. (2001). *Opuntia* cacti of North America: An overview. *Florida Entomologist, 84(4)*, 474–483.

Pimentel, D. (1963). Introducing parasites and predators to control native pests. *Canadian Entomologist,* 95(8), 785-792.

Realini, M.F., Gonzalez, G.E., Font, F., Picca, P.I., Poggio, L., and Gottlieb, A.M. (2015). Phylogenetic relationships in *Opuntia* (Cactaceae, Opuntioideae) from southern South America. *Plant systematics and evolution*, 301, 1123-1134.

Ross, L., Pen, I., and Shuker, D.M. (2010). Genomic conflict in scale insects: the causes and consequences of bizarre genetic systems. Biological Reviews of the Cambridge Philosophical Society, 85(4), 807-828.

Rule, N.F., and Hoffmann, J.H. (2018). The performance of *Dactylopius opuntiae* as a biological control agent on two *Opuntia* cactus species in South Africa. *Biological Control*, 119, 7-11.

Sáenz, C. (2013). Chemical composition and characteristics of *Opuntia* spp. In FAO. Agro-industrial utilization of cactus pear. Rome.

Sáenz, C., Sepulveda, E., and Matsuhiro, B. (2004). *Opuntia* spp. mucilage's: a functional component with industrial perspectives. *Journal of Arid Environments, 57*(3):275–290.

Schlesak, S., Hedenäs, L., Nebel, M., and Quandt, D. (2018). Cleaning a taxonomic dustbin: placing the European Hypnum species in a phylogenetic context. *Bryophyte Diversity and Evolution*, 40(2), 37-54.

Schulz, A.N., Lucardi, R.D., and Marsico, T.D. (2019). Successful invasions and failed biocontrol: the role of antagonistic species interactions. *BioScience*, 69, 711-724.

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

Shalabi, L.F. (2015). On the taxonomy of *Cactaceae* Juss by the evidence of seed micromorphology and SDS-page analysis. *European Journal of Botany, Plant Sciences and Phytology, 2*, 1-15.

Shetty, A.A., Rana, M.K., and Preetham, S P. (2011). Cactus: a medicinal food. *Journal of Food Science and Technology 49(5*), 530-536.

Simberloff, D., and Rejmánek, M. (2011). *Encyclopaedia of Biological Invasions*; University of California Press: Berkeley, CA, USA.

Simberloff, D., and Stiling, P. (1996). How risky is biological control? *Ecology,* 77(7), 1965-1974.

Simpson, G.G. (1951). The species concept. *Evolution*, 5(4), 285-298.

Southern African Plant Invaders Atlas (SAPIA) News. Henderson, L. (2019). October, 54. Agricultural Research Council Plant Health and Protection. https://www.arc.agric.za/

Srikanth, K., and Whang, S.S. (2015). Phylogeny of Korean *Opuntia* spp. based on multiple DNA regions. *Turkish Journal of Botany,* 39, 634-641.

Starmer, W.T., Aberdeen, V., and Lachance, M.A. (1988). The yeast community associated with decaying *Opuntia stricta* (Haworth) in Florida with regard to the moth, *Cactoblastis cactorum* (Berg). *Florida Scientist, 51*, 7-11.

Szűcs, M., Eigenbrode, S.D., Schwarzländer, M., and Schaffner, U. (2012). Hybrid vigour in the biological control agent, *Longitarsus jacobaeae*. *Evolutionary Applications*, 5, 489–497.

Tesoriere, T., Butera, D., Pintaudi, A.M., Allegra, M., and Livrea, M.A. (2004). Supplementation with cactus pear fruit decreases antioxidative stress in healthy humans: a comparative study with vitamin C. *American Journal of Clinical Nutrition 80*(2), 391–395.

Torres, L. G., Gumecindo, C., Bautista, E., and Gomez-y-Gomez, Y. (2018). *Opuntia ficus-indica* mucilage and edible chitosan biofilms including *Brassica olearacea* extract for extending the shelf-life of *Capsicum annum* var. *Serrano*. *Asian Research Journal of Agriculture, 8*(1), 1-9.

Tracy, J.L., and Robbins, T.O. (2009). Taxonomic revision and biogeography of the *Tamarix*-feeding *Diorhabda elongata* (Brullé, 1832) species group (Coleoptera: Chrysomelidae:

Galerucinae: Galerucini) and analysis of their potential in biological control of Tamarisk. *Zootaxa*, 1-152.

Tryon, H. (1910). The “wild cochineal insects”, with reference to its injurious action on prickly pear (*Opuntia* spp.) in India, etc. and to its availability for the subjugation of this plant in Queensland and elsewhere. *Queensland Agricultural Journal*, 25, 188-197.

Van Dam, A.R., and May, B. (2012). A new species of *Dactylopius* Costa (*Dactylopius gracilipilus* sp. nov.) (Hemiptera: Coccoidea: Dactylopiidae) from the Chihuahua Desert, Texas, U.S.A. *Zootaxa,* 3573, 33-39.

van Steenderen, C., Paterson, I.D., Edwards, S., and Day, M.D. (2021). Addressing the red flags in cochineal identification: the use of molecular techniques to identify cochineal insects that are used as biological control agents for invasive alien cacti. *Biological control. 152*, 104426.

van Wilgen, B.W., Forsyth, G.G., Le Maitre, D.C., Wannenburgh, A., Kotzé, J.D.F., van den Berg, E., and Henderson, L. (2012). An assessment of the effectiveness of a large, national-scale invasive alien plant control strategy in South Africa. *Biological Conservation*, 148, 28–38.

Van Zyl, K. (2016). Problem plant control compendium: A guide for the chemical control of certain declared weeds- invaders and other problem plants (2^{nd} ed.) AVCASA

Varone, L., Acosta, M.M., Logarzo, G.A., Briano, J.A., Hight, S.D., and Carpenter, J.E. (2012). Laboratory performance of *Cactoblastis cactorum* (Lepidoptera: Pyralidae) on South and North American *Opuntia* species and occurring in Argentina. Florida Entomologist 95(4), 1163-1173.

Varone, L., Logarzo, G.A., Briano, J.A., Hight, S.D., and Carpenter, J.E. (2014). *Cactoblastis cactorum* (Berg) (Lepidoptera: Pyralidae) use of *Opuntia* host species in Argentina. *Biological invasions*, 16, 2367–2380.

Volchansky, C.R., Hoffmann, J.H., and Zimmermann, H.G. (1999). Host-plant affinities of two biotypes of *Dactylopius opuntiae* (Homoptera: Dactylopiidae): enhanced prospects for biological control of *Opuntia stricta* (Cactaceae) in South Africa. *Journal of Applied Ecology*, 36, 85-91.

Waage, J.K., and Greathead, D.J. (1988). Biological control: challenge and opportunities. *Philosophical transactions of the Royal Society of London,* 318, 111-128.

Walters, M., Figueiredo, E., Crouch, N.R., Winter, P.J.D., Smith, G.F., Zimmermann, H.G., and Mashope, B.K. (Eds.) (2011). *Naturalised and invasive succulents of southern Africa. Abc Taxa and the Belgian Development Cooperation.* Brussels: Belgium, Volume 11.

Wells, M.J., Poynton, R.J., Balsinhas, A.A., Musil, K.J., Joffe, H., van Hoepen, E., and Abbot, S.K. (1986). The history of introduction of invasive alien plants in southern Africa. In: MacDonald, I.A.W., Kruger, F.J. and Ferrar, A.A. (Eds.) *The ecology and management of biological invasions in southern Africa*. Oxford University Press, Cape Town, 21-35.

Weniger, D. (1969). *Cacti of the Southwest: Texas, New Mexico, Oklahoma, Arkansas and Louisiana*. University of Texas Press.

Wilson, J.R.U., Dormontt, E.E., Prentis, P.J., Lowe, A.J., and Richardson, D.M. (2009). Something in the way you move: dispersal pathways affect invasion success. *Trends in ecology and evolution, 24,* 136-144.

Winston, R. L., Schwarzländer, M., Hinz, H. L., Day, M. D., Cock, M. J. W., and Julien, M. H. (2014). Biological control of weeds: a world catalogue of agents and their target weeds. *Biological control of weeds: a world catalogue of agents and their target weeds*, (5). USDA Forest Service, Forest Health Technology Enterprise Team Morgantown, West Virginia, 838.

Woodard, A.M., Ervin, G.N., and Marisco, T.D. (2012). Host plant defence signalling in response to a coevolved herbivore combats introduced herbivore attack. *Ecological Evolution,* 2(5), 1056-1064.

Zachariades, C., Paterson, I.D., Strathie, L.W., Hill, M.P., and van Wilgen, B. (2017). Assessing the status of biological control as a management tool for suppression of invasive alien plants in South Africa. Bothalia, 47(2), a2142. https://doi.org/10.4102/abc.v47i2.2142

Zimmermann, H.G. (2017). Global invasions of cacti: Control, management and conflicts of interest. In Inglese, P., Mondragon, C., Nefzaoui, A., Sáenz, C. (Eds.), *Crop ecology, cultivation and uses of cactus pear,* 171-185. The Food and Agriculture Organisation (FAO) of the United

Nations and the International Centre for Agricultural Research in the Dry Areas (ICARDA), Rome.

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

Zimmermann, H.G., Moran, V.C., and Hoffmann, J.H. (2004). Biological control in the management of invasive alien plants in South Africa, and the role of the Working for Water programme. *South African Journal of Science,* 100, 34–40.

Zimmermann, H.G., Moran, V.C., and Hoffmann, J.H. (2009). Invasive cactus species (Cactaceae). In Muniappan, R., Reddy, G.V.P. and Raman, A. (Eds.): *Biological control of tropical weeds using Arthropods*. CA: Cambridge University, 108-129.