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Hybridisation between two *Dactylopius opuntiae* (Homoptera: Dactylopiidae) lineages and implications for host plant affinities

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ABSTRACT

A population of *Dactylopius opuntiae* (referred to as the 'engelmannii' lineage) is a potential biocontrol agent against invasive *Opuntia megapotamica* in South Africa. The 'engelmannii' lineage and the already established 'ficus' and 'stricta' lineages of the same cochineal species will hybridise where they co-occur, possibly influencing the efficacy of the lineages on their respective hosts. This research focuses on the overlap of *Opuntia ficus-indica* and *O. megapotamica* populations in the field, and hybridisation of 'ficus' and 'engelmannii' lineages. Through laboratory hybridisation trials, the host affinity of F_1 and F_2 hybrids was compared with that of the purebred 'engelmannii' and 'ficus' lineages on *O. ficus-indica* and *O. megapotamica*. F_1 hybrids were not species-specific and some individual F_2 hybrids performed and developed better than the purebred lineages, but not better than the F_1 hybrids. Biological control of both *O. ficus-indica* and *O. megapotamica* in the Eastern Cape Province, South Africa, where both invasive alien plant species co-occur, could be enhanced by the less species-specific nature of the F_1 progeny, which developed equally well on both *O. megapotamica* and *O. ficus-indica*; and this benefit will be increased or reduced by the F_2 progeny. The success of biological control depends on whether the species-specific nymph encounters its target host, as the less species-specific nymphs will have substantial effect on controlling either weed. These outcomes propose that only purebred *D. opuntiae* lineages should be released in monoculture populations of their targeted weed and long-term consequences of hybridisation should be monitored in the field.

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Dactylopius opuntiae; host affinity; hybridisation; *Opuntia megapotamica*; *Opuntia ficus-indica*

Introduction

Globally, about 57 cactus species (Cactaceae) have been recorded to be invasive (Novoa et al., 2015; Zimmermann, 2017). Invasive alien cacti were the first plants to be targeted for classical biological control in 1795 in India (Tryon, 1910). In South Africa, *Opuntia*

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monacantha Haworth (Cactaceae) was the first plant targeted for biological control using the cochineal insect *Dactylopius ceylonicus* Green (Hemiptera: Dactylopiidae) in 1913 (Moran et al., 2013; Paterson et al., 2011; Zimmermann et al., 2004). Since then, many cactus species have been targeted for biological control and are under different levels of control (Klein, 2011; Paterson et al., 2011; Paterson et al., 2021; Zimmermann et al., 2009). *Dactylopius* (Homoptera: Dactylopiidae) cochineal is predominantly host specific to a few *Opuntia* Mill. and *Cylindropuntia* (Engelm.) F.M. Knuth species and *Dactylopius opuntiae* (Cockerell) and *Dactylopius tomentosus* Lamarck lineages generally exhibit differential host specificities (Jones et al., 2015; Jones et al., 2016; Mathenge et al., 2009; Mathenge et al., 2010; Rule & Hoffmann, 2018; Volchansky et al., 1999). Lineages (also referred to as biotypes) used in biological control in *Dactylopius opuntiae* are 'stricta' and 'ficus', while *D. tomentosus* consists of 'californica var. parkeri', 'cholla','bigelovii' 'cylindropuntia sp.', imbricata', and 'echinocarpa x acanthocarpa' (Jones et al., 2015; Jones et al., 2016; Paterson et al., 2011). *Dactylopius opuntiae* lineages are specific to *Opuntia* species (Githure et al., 1999; Hoffmann et al., 1999; Volchansky et al., 1999) and the 'stricta' lineage has been very effective in the biological control of *Opuntia stricta* Haworth, *Opuntia cespitosa* Raf. (formerly *Opuntia humifusa* (Raf.)), while the 'ficus' lineage controls *Opuntia ficus-indica* (L.) Mill. (Dodd, 1940; Hoffmann et al., 2002; Moran & Zimmermann, 1984; Paterson et al., 2021; Pettey, 1948). In the Eastern Cape Province in particular, *O. ficus-indica* sometimes grows in sympatry with *O. megapotamica* Arechav.. *Opuntia ficus-indica* remains sufficiently controlled by the *D. opuntiae* 'ficus' lineage cochineal, while the 'ficus' lineage does not cause enough damage to inhibit the spread of the *O. megapotamica* populations (Paterson et al., 2021). Only the correct lineage is effective in controlling its preferred target weed, so if the incorrect lineage is utilised then satisfactory control is not achieved (Githure et al., 1999).

Opuntia megapotamica is a serious problem in parts of the Eastern Cape Province, with most dense invasions restricted to areas around the towns of Cookhouse, Bedford, Adelaide and Fort Beaufort (Blue Crane Route Municipality and Raymond Mhlaba Municipality). No biological control agents have been released against *O. megapotamica* and although *D. opuntiae* lineages released against other invasive alien cacti do feed on *O. megapotamica*, the level of damage in the field is not considered sufficient to provide acceptable levels of control (Paterson et al., 2021).

In South Africa, *O. megapotamica* was, until recently, misidentified as a variety of the North American cactus species, *Opuntia engelmannii* Salm-Dyck ex Engelm. (Paterson et al., 2021). In Walters et al. (2011) it is stated *O. megapotamica* has had many identities, which included *Opuntia engelmannii* var. *lindheimeri* Engelm., *Opuntia lindheimeri* Engelm. and *Opuntia tardospina* Griffiths. *Opuntia tardospina* was then 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. As such, *O. megapotamica* in the Eastern Cape was mistakenly regarded as an unconfirmed variety of *O. engelmannii.* At least three varieties of *O. engelmannii* are recognised in South Africa and in the absence of reliable taxonomic identifications, have been grouped, as an interim measure, according to their morphological similarities and geographic abundance (Mbonani et al., 2022). *Opuntia megapotamica* was referred to as the 'Eastern Cape' variety of *O. engelmannii*, as it is most abundant within that province. The Northern Cape/Free State variety is abundant in

the Northern Cape, Free State and North West provinces, and is found in isolated but dense invasions in the Eastern Cape. Limpopo Province boasts the third variety which is subdivided into two variations of *O. engelmannii* that are differentiated by their spine colour i.e. yellow and red (Paterson et al., 2021; Witt, 2017).

While under the assumption that *O. megapotamica* was a variety of *O. engelmannii*, six lineages were collected on various varieties of *O. engelmannii* within Arizona and New Mexico. Of the six lineages, a *D. opuntiae* cochineal lineage which was collected from *O. engelmannii* var. *engelmannii* in Flagstaff, Arizona, United States of America was found to be the most damaging to the target weed under laboratory conditions. This cochineal collected off *O. engelmannii* var. *engelmannii* (referred to as 'engelmannii' henceforth) 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 (Paterson et al., 2021). 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 less capable to defend itself against this cochineal as *O. megapotamica* may be more palatable to the cochineal.

Two lineages of *Dactylopius opuntiae* ('stricta' and 'ficus' lineages) have already been introduced in 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). Volchansky et al. (1999), showed that the two 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 any of the *D. opuntiae* lineages can potentially interbreed freely when exposed to one another (Hoffmann et al., 2002). This may affect the host preference/affinity of the offspring and have an impact on the level of control the cochineal has on their target hosts (Hoffmann et al., 2002; Mathenge et al., 2010). Hybridisation between any new conspecific lineages of *Dactylopius* 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 efficacy of the cochineal could either increase, decrease or remain the same due to the hybridisation of these lineages (Hoffmann et al., 2002). If the hybrids develop on only one or neither of the target weeds, the efficacy of the hybrid progeny as biological control agents might be reduced. In the event the hybrids develop on only one of the targeted cacti, the species-specific hybrid nymphs would be ineffective in controlling the other host. Alternatively, if the hybrids do not exhibit any developmental restrictions on either target host, the level of biological control may be enhanced as there is a greater pool of suitable hosts available for the crawlers to utilise in areas where the two plants occur in proximity (Hoffmann et al., 2002).

The aim of the study was to determine the host affinity of the hybrids of the 'engelmannii' and 'ficus' lineages, and in so doing, attempt to understand the impact this would have on their efficacy on *O. megapotamica* and on the current level of control on *O. ficus-indica* in the field. Hybridisation experiments were conducted to determine the effect at least two generations of hybrids will have on the continued management of the target weeds. In this paper, we describe the results of the hybridisation

experiments, drawing similarities to the work done by Mathenge et al. (2010) on *D. tomentosus* 'imbricata' and 'cholla' lineages and especially Hoffmann et al. (2002), who detail the hybridisation of *D. opuntiae* 'stricta' and 'ficus' lineages.

Materials and methods

Opuntia megapotamica plants were collected around Bedford (Eastern Cape Province, South Africa). Spiny *Opuntia ficus-indica* plants were obtained from the University of Pretoria's Experimental Farm (25°44'57"S 28°15'15"E). These plants were maintained in the nursery at the Agricultural Research Council's Plant Health Protection (ARC-PHP) facility in Roodeplaat, Pretoria (25°36'52"S 28°21'51"E). *Dactylopius opuntiae* 'ficus' lineage cochineal was field-harvested from *O. ficus-indica* plants found around the PHP campus and reared on *O. ficus-indica* cladodes in netted cages. The 'engelmannii' lineage was reared on *O. megapotamica* cladodes in netted cages too, all contained in the air-conditioned quarantine facilities of the ARC-PHP, Pretoria where these experiments were undertaken. The two pure cultures were kept in separate rooms. The quarantine glasshouse compartment had natural lighting and was set at a maximum of 30 °C and minimum of 12 °C. Atmospheric humidity was set at 50% RH. Microscope observations of the development of the hybrids were made in the laboratory at a constant room temperature of 24 ± 2 °C and +50% RH.

To determine the developmental success and performance of the 'ficus' and 'engelmannii' lineages of *D. opuntiae* on *O. ficus-indica* and *O. megapotamica*, different familial lines were produced, including purebred lineages, F_1 hybrids and F_2 crosses of hybrids. As in Hoffmann et al. (2002), the following convention is used to represent the genotypes of the familial cochineal lineages: 'F' (for 'ficus' lineage) and 'E' (for 'engelmannii' lineage). The actual cross is represented by an 'x' between the two parents. For instance, when FF ('ficus' Female) is crossed (x) with Ee ('engelmannii' male) it is represented as FF x Ee. These couplets represent cochineal 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 pair representing the paternal lineage, resulting in the following combinations: FE-Fe, FE-Ef, EF-Fe, EF-Ef, where the first pair in each combination of couplets represents the maternal lineage and the second pair the paternal lineage (Table 1).

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

		Parent genotypes		Progeny genotypes			
Generation	Cross category	Female	Male	Female	Male	Progeny	Figure
F_0		FF	Ff	FF	Ff	'ficus'	1 (a)
F_0		EE	Ee	EE	Ee	'engelmannii'	1 (b)
F_1	i	FF	Ee	FE	Fe	Hybrid	2 (a)
F_1	i	EE	Ff	EF	Ef	Hybrid	2 (b)
F_2	ii	FE or EF	Ef	FE or EE	Fe or Ee	'engelmannii' and hybrid	3 (a)
F_2	ii	FE or EF	Fe	FF + EF	Ff + Ef	'ficus' and hybrid	3 (b)

Host affinities of the purebred 'ficus' and 'engelmannii' lineages of Dactylopius opuntiae

To determine the host plant affinities of the purebred 'engelmannii' and 'ficus' lineages, eight mature 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 rectangular plastic containers, i.e. one cladode per container. The containers had these dimensions: 7 litres, 35.5 cm long, 23.5 cm wide and 12 cm high with four 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 each infested with 30 newly hatched 'ficus' lineage crawlers and the other four with 30 newly hatched 'engelmannii' lineage crawlers. The same was done with each of the *O. megapotamica* cladodes, exposing them to both lineages. Each cladode was supported horizontally 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. Sexually mature females are those that have moulted twice (Perez Guerra & Kosztarab, 1992). Adult males are winged, can fly and only live for a few days, while the females are sessile with neotenous characteristics. The males and females mated freely. Each female was removed once she started laying eggs, unlike Hoffmann et al. (2002) who removed all females once one started producing eggs. The fertilised egg-laying female was then dewaxed (by winding the waxy threads that cover her around a pin), removed from the cladode, weighed, and placed in a separate container to allow her to continue laying eggs. Unfertilised females do not lay eggs. The number of offspring produced per female was counted, including the crawlers produced before the females were removed from the cladode. The number of females harvested from each cladode represented the percentage of individuals that survived to maturity from the 30 that were introduced to each cladode, and this is referred to as the sex ratio herein.

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 as the benchmark against which all hybrids were compared. This was calculated as follows: $R = (F \times P)/30$ where R is the net rate of increase, F is the number of sexually mature females, P is the average number of progeny produced per female and 30 represents the number of crawlers that were initially transferred onto each cladode (Begon & Mortimer, 1981; Hoffmann et al., 2002). This was used to determine and then compare the developmental success and performance of the different lineages on each host plant. 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*.

Hybridisation of lineages

In the natural environment, it is expected that the different cochineal lineages of *D. opuntiae* will interbreed due to them being the same species (Hoffmann et al., 2002; Mathenge et al., 2010; Volchansky et al., 1999). Paternally inherited traits of the species in the Dactylopiidae family are lost during spermatogenesis and males pass on only maternally inherited ones (Bull, 1979; de la Filia et al., 2015; Gavrilov, 2007;

Mathenge et al., 2010; Ross et al., 2010). Male *D. opuntiae* are haploid, possessing only a maternal genome, while the females are diploid, with both male and female genomes. Because of this Comstockiella chromosome 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. Host affinity not only depends on the specific cross, but also on the preference of the maternal species (Bitume et al., 2017). To ensure that all possible F_1 hybrids that could result from the hybridisation of the different lineages through two generations were assessed, the different levels of hybridisation were carefully set out in the experiments. While Mathenge et al. (2010) hybridised different lineages of *D. tomentosus* and investigated only the host affinity of the hybrid F_1 generation, this current study tested the host affinity for both the hybrid F_1 and F_2 generations, like Hoffmann et al. (2002) with *D. opuntiae* 'stricta' and 'ficus' lineages. Hoffmann et al. (2002), however, went a step further and included backcrosses between the F_1 hybrids and purebreds, which was not conducted in the current study.

Host affinity of F_1 hybrids

Thirty one day-old 'ficus' lineage nymphs were transferred onto each of four clean *O. ficus-indica* cladodes. Thirty nymphs of the 'engelmannii' lineage were transferred onto each of the four clean *O. megapotamica* cladodes, after which each cladode was individually placed in a separate plastic container on a polystyrene board. 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 are distinguishable by the cocoons they form. 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. Each *O. ficus-indica* cladode containing only females was then paired in a container with an *O. megapotamica* cladode containing only males, and each *O. ficus-indica* cladode with males was paired in a container 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 (female) and Fe (male), or EF (female) and Ef (male)).

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 net rate of increase (R) of both hybrid combinations, on each of the cactus hosts, was determined and analysed.

Host affinity of F_2 hybrids

The same method as described previously 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 or Fe and EF or 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 removed. The F_1 offspring were now hybridised in one of two ways: either with the inverse, or with the identical type of hybrid.

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, 30 newly hatched F_2 hybrid nymphs of each combination were transferred onto two fresh cladodes of both hosts, in insect-proof plastic containers, and were allowed to develop, mate and produce 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.

Analyses were conducted using Statistica Version 13.6 (© Stat Soft, Inc., USA.). All data were examined for outliers and entry errors and then checked for normality and were analysed by using t-tests to determine *P* and the significant differences between the variables, including the average mass of females (an indicator of individual size and may correlate with fecundity), number of progeny produced (a measure of reproductive output) and the development time it took from egg to adult female.

Results

Host affinity of the purebred 'ficus' and 'engelmannii' lineages of Dactylopius opuntiae

The 'engelmannii' lineage on *O. megapotamica* and the 'ficus' lineage on *O. ficus-indica* were statistically different to the 'ficus' lineage on *O. megapotamica* and to 'engelmannii' lineage on *O. ficus-indica* with regards to average female mass. The former represented the highest masses. There was a statistically significant difference between the 'engelmannii' lineage on *O. megapotamica* and the 'ficus' lineage on *O. ficus-indica* which yield the highest offspring numbers and engelmannii' lineage on *O. ficus-indica* which represented the lowest reproductive performance. The purebred lineages on their preferred hosts as well as the 'ficus' lineage on *O. megapotamica* showed a similar and relatively faster development rate while the 'engelmannii' lineage on *O. ficus-indica* took longer to reach maturity (Table 2). Generally, the 'ficus' lineage performed better, based on the parameters studied, on *O. megapotamica* than the 'engelmannii' lineage did on *O. ficus-indica*. Both purebred populations displayed a male-biased sex ratio as survival of the females (those harvested from each cladode) was variable. The highest performance in both female mass and progeny production occurred when each lineage was paired with its preferred host (i.e. 'engelmannii' lineage on *O. megapotamica* and 'ficus' lineage on *O. ficus-indica*) and the two lineages seemed to be host-specific, distinctly preferring one cactus species to the alternative.

The *R-values* clustered close to the x-axis (Figure 1(a)) because the 'ficus' lineage developed better on *O. ficus-indica* than on *O. megapotamica*. Figure 1(b) shows the points clustered close to the y-axis because the 'engelmannii' lineage developed better

Table 2. Summary of the means (±S.E.) of female mass, number of progeny produced and time to reach maturity of purebred lineages. 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 ♀ (female) mass (mg)	18.2 ± 0.8^a^	5.6 ± 0.9^c^	8.5 ± 0.7^b^	16.2 ± 0.8^a^	F (3, 12) = 32.77 $P <$ 0.00001
Average progeny	438.4 ± 26.7^a^	101.3 ± 26.7^c^	192.1 ± 16.9^b^	381.5 ± 22.5^a^	F (3, 12) = 28.46 $P =$ 0.00001
Maturity* (days)	44.5 ± 0.9^a^	48.2 ± 1.4^b^	44.1 ± 0.8^a^	41.9 ± 0.2^a^	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.

on *O. megapotamica* than on *O. ficus-indica*. This illustrated the affinity of the purebred offspring (Figure 1).

Host affinity of F_1 hybrids

No significant differences against the parameters were found among the four F_1 hybrids (Table 3). The hybrids developed uniformly regardless of which host plant they were on. This stability and consistency in the F_1 generation demonstrates hybrid vigour. The hybrids are less species-specific.

Both types of F_1 hybrids were able to develop and survive to maturity equally well on both host plant species. In both cases (i.e. FF x Ee and EE x 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 2 (a) and (b) respectively). These high *P* values indicate the performance of the crosses on the two plant species was not significant because of the crosses' lack of specificity. Figure 2 showed that the F_1 hybrid offspring of 'ficus' females (FF) crossed with 'engelmannii' males (Ee) fared

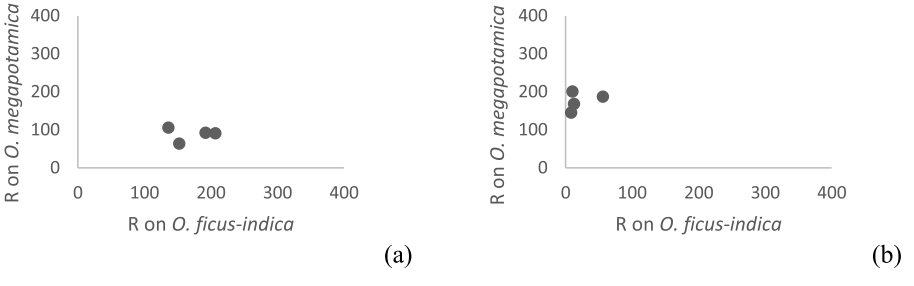

Figure 1. Net rate of increase (*R-values*) for purebred progeny of 'ficus' and 'engelmannii' lineages of *D. opuntiae*. (a) 'Ficus' females (FF) crossed with 'ficus' males (Ff); (b) 'engelmannii' females (EE) crossed with 'engelmannii' males (Ee).

Table 3. Summary of the means (±S.E.) of female mass, number of progeny produced and time to reach maturity of F_1 hybrids. ANOVA was used to calculate *P* and determine statistical significance.

	F_1 hybrids ($n = 4$)				
Aspect	EE x Ff on *O. megapotamica*	EE x Ff on *O. ficus-indica*	FF x Ee on *O. megapotamica*	FF x Ee on *O. ficus-indica*	ANOVA
Average ♀ mass (mg)	19.5 ± 0.8^a^	14.1 ± 1.0^a^	17.6 ± 1.0^a^	18.2 ± 1.0^a^	F (3, 12) = 0.83 P = 0.5
Average progeny	520.4 ± 29.3^a^	367.1 ± 24.7^a^	458.8 ± 27.8^a^	488.7 ± 31.7^a^	F (3, 12) = 1.25 P = 0.3
Maturity* (days)	53 ± 0.5^a^	56.7 ± 0.7^a^	56.6 ± 0.7^a^	55.9 ± 0.5^a^	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.

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.

Host affinity of 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(a) and Table 4), was significantly lower for female mass and progeny and produced offspring that favoured *O. megapotamica* over *O. ficus-indica* as host plants. The worst performance of inbred hybrids was that of EF-Ef on *O. ficus-indica* indicated by the lowest *R-values* of all the inbred hybrids. This was worse than the purebred 'ficus' lineage on *O. megapotamica*. It seemed to indicate that particular genetic combinations in the F_2 generation led to deleterious effects, possibly because of inbreeding depression which could then translate to hybrid breakdown. 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

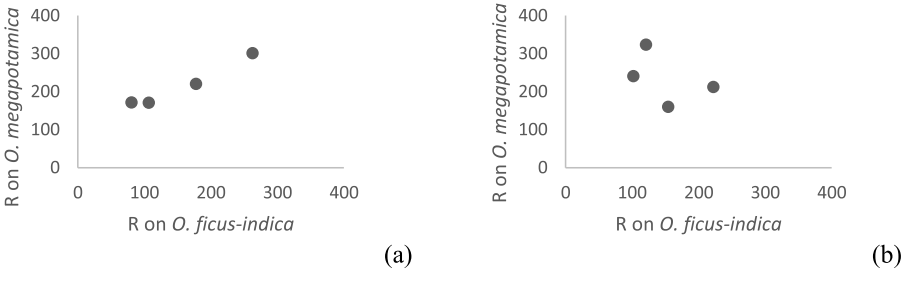

Figure 2. Net rate of increase (*R-values*) for F_1 progeny of crosses between 'ficus' and 'engelmannii' lineages of *D. opuntiae*. (a) 'Ficus' females (FF) crossed with 'engelmannii' males (Ee); (b) 'engelmannii' females (EE) crossed with 'ficus' males (Ff).

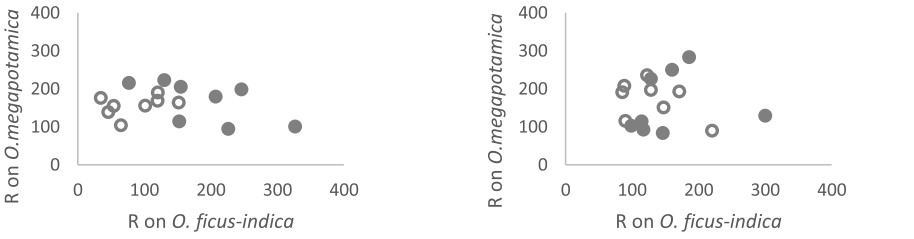

Figure 3. Net rate of increase (*R-values*) for F_2 progeny of crosses between hybrid females and hybrid males of *D. opuntiae*. (a) FE females (●) and EF females (○) crossed with Ef males; (b) FE females (○) and EF females (●) crossed with Fe males.

either host. All hybrid combinations survived well on either host, but FE (females) crossed with Ef (males) perform better on *O. ficus-indica* with higher *R-values* than the other hybrid crosses on either *Opuntia* host (Figure 3 and Table 4). 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*. There were no significant differences detected for maturity across all F_2 hybrids despite differences in female mass and progeny. The *R-values* of the F_2 hybrids show an increase in performance when compared with the purebred lineages, however, not better than the first generation of hybrids and this could be because of hybrid breakdown.

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). 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(a)), while many of the points fell more or less equidistantly between the two axes of the graph. Crossbred hybrids (male crossed with female with a different genotype) FE-Ef and EF-Fe developed well on *O. ficus-indica* but there was no significant difference statistically between the two.

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). Overall, the *R-values* of F_2 hybrids indicated that there was no significant difference between the siblings produced from the crosses. 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(a,b), but there was more hybrid than purebred individuals. There was an expectation for these crosses to produce a mixture of purebred and hybrid genotypes (Table 1).

Discussion

The aim of the study was to determine whether the introduction of another *D. opuntiae* lineage would, through hybridisation, influence the host affinity, and ultimately the efficacy, of the resultant hybrids.

The results have demonstrated that the two lineages of cochineal hybridise under controlled laboratory conditions and produce viable offspring. The reciprocal crosses were

Table 4. Summary of the means (±S.E.) of female mass, number of progeny produced and time to reach maturity of F_2 hybrids. 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
Average ♀ mass (mg)	19 ± 0.6^a^	22 ± 0.8^a^	17.5 ± 0.7^a^	10.5 ± 0.6^b^	17 ± 0.6^a^	16.1 ± 0.8^a^	18.5 ± 0.6^a^	20.2 ± 0.8^a^	F (7, 56) = 5.16 P = 0.0001
Average progeny	352.8 ± 13.3^a^	400.1 ± 15.8^a^	395.6 ± 17.2^a^	228 ± 18.4^b^	372.5 ± 14.5^a^	363.7 ± 18.6^a^	337.2 ± 13.2^a^	341.7 ± 14.5^a^	F (7, 57) = 3.60 P = 0.003
Maturity* (days)	62.7 ± 0.4^a^	62.5 ± 0.4^a^	53.1 ± 0.36^a^	61 ± 1.0^a^	53.4 ± 0.5^a^	54.8 ± 0.4^a^	63.8 ± 0.4^a^	63.1 ± 0.3^a^	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.

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 as the hybrids performed uniformly and consistently on either host and the parent females performed better on their preferred host than the purebred lineages, irrespective of the male they were crossed with. As a result, the first generation of hybrids will remain effective in controlling the targeted weed species of *O. ficus-indica* and *O. megapotamica* in the field, as is the case in the Eastern Cape Province, where *O. megapotamica* is most problematic. In the F_1 generation, genetic mixing may have reduced the host specificity observed in the purebreds. 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 proximity.

The second generation saw some individual hybrids performing better than the purebred lineages but not better than the F_1 hybrids, with evidence of some hybrid breakdown.

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. This study's 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 specific host and perform differently on various host plant species within the genus (Volchansky et al., 1999). The recommendation from this study is that in *O. megapotamica* monocultures that are already infested with the 'ficus' cochineal, the new 'engelmannii' cochineal lineage should be released as satisfactory control will still be achieved. The 'engelmannii' purebred lineage and the hybrids would still maintain control of the *O. megapotamica* infestation as they proved to be more virulent on *O. megapotamica* than on *O. ficus-indica*. Where both *O. ficus-indica* and *O. megapotamica* occur, it may be more difficult to predict the long-term effects the hybrids will have on the biological control of both *O. ficus-indica* and *O. megapotamica*.

From a hybridisation point of view, the 'engelmannii' lineage is therefore safe to release, and there is sufficient evidence to suggest that it will be damaging to *O. megapotamica*, 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*.

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