Application for the release of the cochineal *Dactylopius tomentosus* ('fulgida' biotype) for the biological control of *Cylindropuntia fulgida* var. *mamillata* (Cactaceae)

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Summary

Cylindropuntia (cholla cacti) is a genus of invasive cacti, originating in Mexico, southern USA and South America. There are eight species of *Cylindropuntia* recognised as being weedy in Australia, including *Cylindropuntia fulgida* var. *mamillata*, and their current distribution encompasses all mainland states and territories except the ACT. All members of the genus *Cylindropuntia* in Australia are declared weeds in most states and territories where their possession and cultivation is a prohibited activity. All species in the *Cylindropuntia* pose a threat to agricultural enterprises, biodiversity conservation and human and animal health in Australia.

There are few effective control methods for *Cylindropuntia* spp. The use of herbicides is made difficult by the types of terrain and vegetation in which infestations occur. Mechanical control is generally too expensive and is potentially dangerous to operators. An integrated control campaign against *Cylindropuntia* spp. would benefit from implementing biological control, particularly with the introduction of new biotypes of the cochineal insect, *Dactylopius tomentosus*.

A biotype of *D. tomentosus* specific to *Cylindropuntia imbricata* ('imbricata' biotype) was introduced into Australia as a biological control agent in 1925 and is now widespread and assisting in controlling *C. imbricata* throughout its distribution. This biotype also causes some damage to *C. leptocaulis* and there is minor feeding on some other *Cylindropuntia* species. However, no damage by *D. tomentosus* has been reported or observed on species outside the *Cylindropuntia* genus.

Studies in Australia and South Africa have shown that different biotypes of *D. tomentosus* prefer and develop better on particular species of *Cylindropuntia*. Therefore, new populations of *D. tomentosus* were imported to potentially control other species of *Cylindropuntia* not attacked by the biotype released on *C. imbricata*.

A biotype of *D. tomentosus* that was introduced into South Africa to control *C. fulgida* var. *fulgida* was found to be particularly damaging to this species and to *C. fulgida* var. *mamillata*. This biotype was introduced into quarantine at the Ecosciences Precinct, Brisbane for further host-specificity testing and to determine its efficacy on other *Cylindropuntia* species.

In nymphal no-choice trials, *D. tomentosus* ('fulgida' biotype) failed to develop on nine test species outside the genus *Cylindropuntia*. In efficacy trials, *D. tomentosus* ('fulgida' biotype) performed best on *C. fulgida* var. *mamillata,* killing the plant in 18 weeks. It also performed well on *C. imbricata* and *C. tunicata*. The studies have shown that *D. tomentosus* ('fulgida' biotype) is host specific to the *Cylindropuntia* and would not be a risk to other species outside this genus or the environment.

This document presents information supporting an application seeking the field release of *D. tomentosus* ('fulgida' biotype) to control *C. fulgida* var. *mamillata* in Australia.

1 Information on the target species

1.1 Taxonomy

Order: Caryophyllales
Family: Cactaceae
Tribe: Opuntioideae
Genus: *Cylindropuntia*
Species: *fulgida* var. *mamillata* (A.Schott) Backeb*., Cactaceae* **1**: 204. 1958.

Synonyms: *Opuntia mamillata* A.Schott., *Proc. Amer. Acad. Arts* **3**: 308 (1857).
Opuntia fulgida var. *mamillata* (A.Schott) J.M.Coult., *Contr. U.S. Natl. Herb.* **3**: 449 (1896).
Cylindropuntia fulgida var. *mamillata f. monstruosa* P.V.Heath, *Calyx* **4(4)**: 142 (1994).

Common Name: Coral cactus, boxing glove cactus (cholla)

Close Relatives in the Australian Region:

There are no Australian native species of Cactaceae (Telford 1984) and most species in the family are regarded as weeds. Within the Cactaceae, there are eight species in the genus *Cylindropuntia* present in Australia (Bob Chinnock, South Australian Herbarium, pers. comm. 2012), all of which are regarded as weedy. Two other species of Cactaceae, *Hylocereus undatus* (night-blooming cereus, Dragon fruit) and *Opuntia ficus-indica* (Indian fig) are sometimes cultivated for their edible fruits. The phylogeny of the Cactaceae is shown in Figure 1.

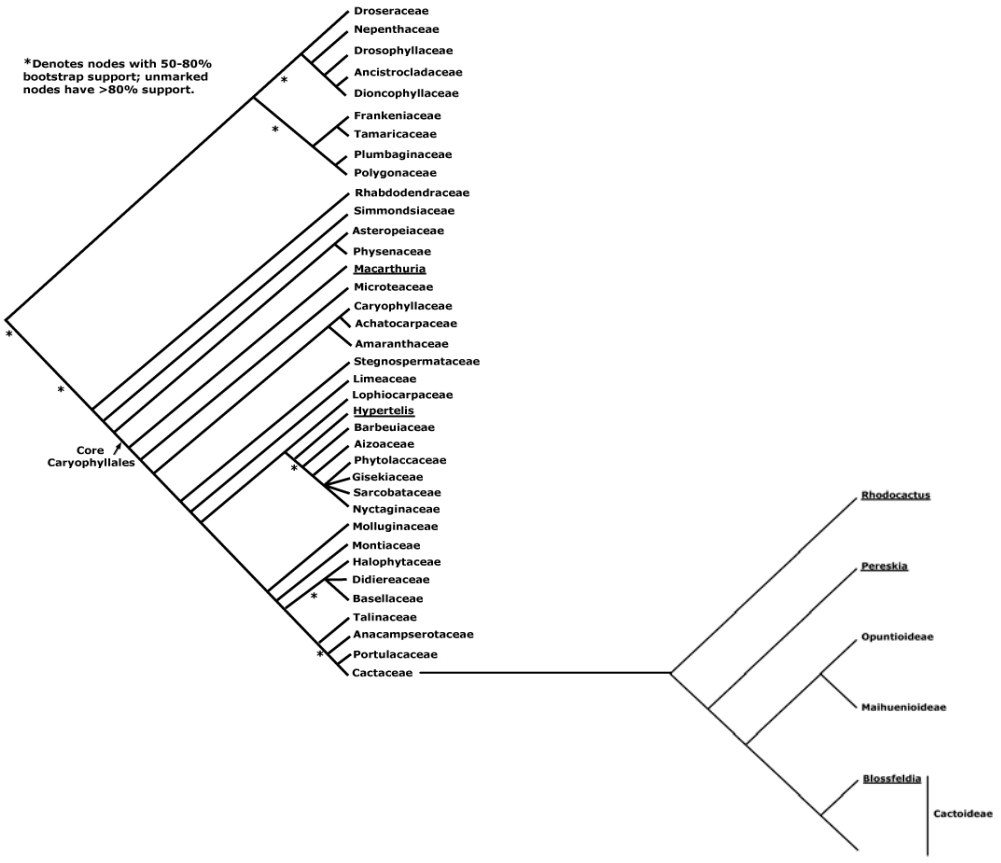

Figure 1 – Phylogeny of the Caryophyllales (Stevens 2001 onwards).

1.2 Description

Cylindropuntia fulgida var. *mamillata* is an erect sprawling shrub, growing to approx. 1m in height. Australian populations of this species rarely produce flowers and the species spreads primarily by movement of vegetative material. It is capable of producing dense thickets which hinder agricultural operations and may impact on biodiversity.

The biology and ecology of *C. fulgida* var. *mamillata* and some other members of the genus *Cylindropuntia* in Australia have been discussed in more detail by Potter (2011).

1.3 Native range and centre of origin

Cylindropuntia fulgida var. *mamillata* occurs naturally in the Sonoran Desert of Arizona, United States of America and Sonora, Sinaloa and Baja California states of Mexico (Anderson 2001).

1.4 Australian and overseas distribution

The current Australian distribution of *C. fulgida* var. *mamillata* encompasses all mainland states and territories except Victoria and ACT (Figure 2). Infestations are largely restricted to arid, rangeland habitats. Significant infestations occur in the goldfield areas of Western Australia (Figure 3), western Queensland and northern New South Wales. The exact locations and areas of infestation are unknown due in part, to the difficulty in locating patchily-distributed plants in remote areas.

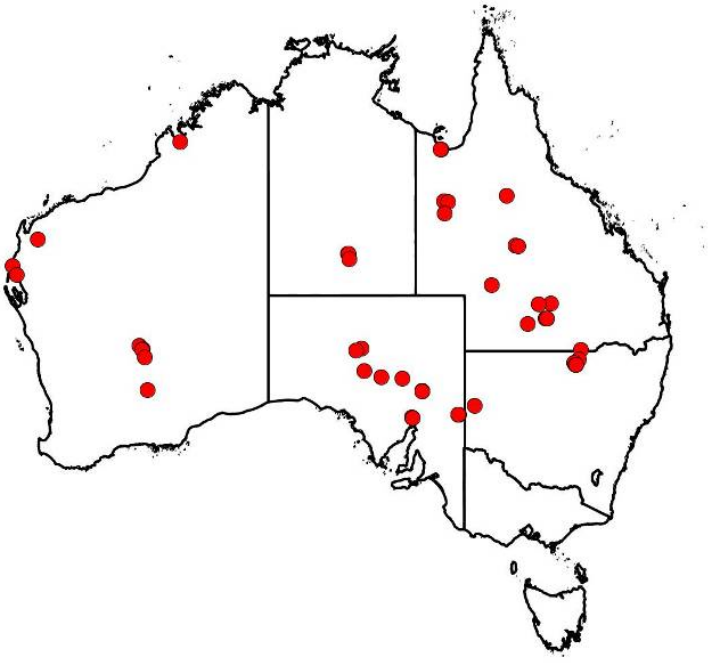

Figure 2 – Australia showing current distribution of *Cylindropuntia fulgida* var. *mamillata*. (Data from Australia's Virtual Herbarium (AVH 2013) plus some other known infestations.)

Figure 3 – *Cylindropuntia fulgida* var. *mamillata* near Gwalia, Western Australia (Photo: Sandy Lloyd).

Another variety of *C. fulgida*, *C. fulgida* var. *fulgida* (Engelm.) F.M. Knuth occurs in South Africa and Zimbabwe and is regarded by many researchers as the most invasive cactus species in those two countries (Walters *et al.* 2011). There is evidence from some Australian and South African sites that *C. fulgida* var. *mamillata* is reverting to what is believed to be this original *C. fulgida* var. *fulgida* type (Figure 4), which is responsible for many wildlife deaths in South Africa (Figure 5).

Figure 4 – *Cylindropuntia fulgida* var. *mamillata* reverting to var. *fulgida* type. (Photo: Helmuth Zimmermann)

Figure 5 – Kingfisher impaled on *Cylindropuntia fulgida* var. *fulgida* in South Africa. (Photo: Helmuth Zimmermann)

1.5 Importance of the plant

There are eight species of *Cylindropuntia* in Australia (Bob Chinnock, South Australian Herbarium, pers. comm. 2012). Collectively, they occur in every mainland state and territory except the ACT.

1.5.1 Detrimental aspects

Cylindropuntia fulgida var. *mamillata* presents a threat to grazing industries through its ability to form dense infestations that can reduce access to feed and hinder mustering activities. Depending on the location and density of an infestation, the cost of control may outweigh the economic value of the land (Chuk 2010). This can influence people's motivation to manage these plants, even if their impacts are known and understood.

The spines are capable of causing serious injury to humans, livestock and working animals, such as horses and dogs, damage fleece and hides and affect the safe handling of affected animals for shearing purposes. Due to the spines, stock do not generally feed on cacti. *Cylindropuntia fulgida* var. *mamillata* have spines which are capable of penetrating footwear and even vehicle tyres. Spines of all *Cylindropuntia* species are encased in a detachable sheath which may remain embedded in a wound, even after the body of the spine is removed.

Infestations of *Cylindropuntia fulgida* var. *mamillata* may pose a threat to native fauna and may also displace native flora, with a consequential impact on biodiversity (Anonymous 2012). The risk of injury from spines also applies to native wildlife, either through impalement or the lodgement of spiny segments in limbs, hides and mouths, leading to immobilisation and a painful death. Dense infestations of cacti can also impede the movement of native wildlife through corridors and limit access to refuges such as rock shelters and caves. Competition from cacti can limit the growth of native vegetation, including small shrubs and groundcovers.

1.5.2 Legislative status

The Opuntioid cacti, including *Cylindropuntia* spp. were collectively declared as Weeds of National Significance in 2012, resulting in the production of a strategic plan (Anonymous 2012).

At various times, most *Opuntia* species (and this covered *Cylindropuntia* spp. at the time) were proclaimed over much of Australia (Parsons and Cuthbertson 1992). All Australian states and territories except Tasmania and the ACT, list *Opuntia* spp. as declared weeds and current legislation in some other states may not yet reflect the change where some *Opuntia* species are now considered to be in the segregate genus *Cylindropuntia*.

1.5.3 Threat potential

Soil types and climatic zones vary across the range of *Cylindropuntia*, but do not appear to limit distribution to any great extent. Climate modelling of the genus indicates there is still potential for large range expansion throughout much of Australia, especially the arid, rangelands areas which represent 80% of Australia's land mass (Chuk 2010).

1.5.4 Beneficial aspects

Cylindropuntia spp., have in the past, been grown as ornamental plants but this practice was uncommon, largely due to the ferocity of spines possessed by members of this genus. All *Cylindropuntia* spp. are now declared weeds across most of Australia making this practice a prohibited activity.

1.6 Control methods

Current control strategies for all *Cylindropuntia* spp. are largely based on the use of herbicides. Australian Pesticides and Veterinary Medicines Authority permits PER13812 for the use of Access in Queensland and PER14442 for the use of Grazon DS, Grazon Extra and Garlon 600 in NSW are in force until June 2017 and June 2018, respectively. The use of herbicides is made difficult by the types of terrain and vegetation in which *Cylindropuntia* spp. infestations are often found. Plants occur over extremely large areas and there is little possibility of successfully locating and destroying all of the potential propagules in an area. Herbicide control is thus on-going.

The use of herbicides over the large areas infested with *Cylindropuntia* spp. may incur considerable costs for landholders and may also result in off-target damage to native species. However, costs of control are extremely difficult to estimate, as there is no single coordinated control campaign across either Australia or individual states. Rather, there are many groups and individuals scattered throughout the states performing control activities on land which they either manage or in which they have an interest. In addition, much of the labour used in control programs is voluntary or 'in-kind' and, as such, is uncosted.

It is important to note that, depending on the location and density of an infestation, the cost of control may outweigh the economic value of the land currently infested (Chuk 2010). However, many of these control programs are conducted in an effort to limit ecological damage to areas of biodiversity conservation or to prevent spread to areas of higher land values or agricultural significance.

Physical removal, while successful on isolated plants, is not recommended because of the risk of serious injury occurring during the process of removal. Physical removal also necessitates correct disposal of weed material to avoid creating new infestations. Techniques commonly used include burying and burning. However, no adequate depth for

burying has been determined. Burnt material also requires re-checking for any regeneration. Physical removal of larger infestations is not viable because it would be extremely labour intensive and any missed plants or plant parts have the capacity to form new infestations if they come into contact with the ground and form roots.

Biological control of *Cylindropuntia imbricata* (Haw.) F.M. Knuth using a biotype of *Dactylopius tomentosus* ('imbricata' biotype) released in Australia in 1925 has proven extremely successful. Unfortunately, other than *C. imbricata*, this insect biotype only causes damage to *C. leptocaulis* (DC.) F.M. Knuth and some minor feeding on a few other *Cylindropuntia* species. The 'imbricata' biotype does not have a significant impact on *C. fulgida* var. *mamillata.*

1.7 Stakeholders

The major groups with an interest in *C. fulgida* var*. mamillata* are land managers and others concerned about its impact on land used for amenity, biodiversity conservation and agriculture. Growers of cacti for domestic and horticultural purposes may also have an interest, but as this species, like all *Cylindropuntia* spp*.*, is a declared weed across most of Australia, this practice is a prohibited activity.

Consultation with State Government Departments in states affected by *C. fulgida* var*. mamillata* has indicated unanimous support for the introduction of a biological control agent to assist in the management of this species.

1.8 Approval as target species for biological control

Nomination of all *Cylindropuntia* spp. as a target for biological control was approved by the Australian Weeds Committee on 13 June 2013 on behalf of the Natural Resource Management and Primary Industries Standing Committees.

2 Information on the agent, *Dactylopius tomentosus* (*Cylindropuntia fulgida* var. *fulgida* biotype)

2.1 Taxonomy

Class:	Insecta
Order:	Hemiptera
Suborder:	Sternorrhyncha
Superfamily:	Coccoidea
Family:	Dactylopiidae
Scientific name:	*Dactylopius tomentosus* (Lamarck)

The family Dactylopiidae contains only one genus *Dactylopius* Costa which has nine species (De Lotto 1974), all of which are restricted to hosts in the family Cactaceae (Mann 1970; De Lotto 1974; ABRS 2009).

2.2 Biology

The following biological data on *D. tomentosus* ('fulgida' biotype) were collated from studies of the biotype maintained on the control plant (*C. fulgida* var. *mamillata*) during host specificity trials. These trials were conducted in the quarantine facility at the Ecosciences Precinct, Brisbane, Queensland. A more detailed study of the biology of *D. tomentosus* by Mathenge *et al*. (2009a) is presented in Appendix 1.

Eggs are red in colour, laid individually but held together in a mesh of waxy threads forming a large waxy egg mass. Red-coloured first instar crawlers emerged after approximately 17 days and dispersed from the waxy threads to search for suitable feeding sites, usually at the base of spines. The mean duration of first instar crawlers was 18 days.

The mean duration of the second instar for males was 7 days and 11 days for females. The biology of males and females differed at the end of the second instar, females being sessile and remaining at the original site, whereas males actively searched for suitable pupation sites. At maturity, males do not feed and are very weak fliers. Therefore, mobility was usually restricted to walking, while searching for females. As a consequence, males quite often pupated adjacent to a developing female. Eclosion of an adult male took approximately 14 days.

The change of instar to the preoviposition stage for females occurred at the original feeding site, underneath a waxy covering. The preovipositional period was 14 days. The mean development time for females from egg to oviposition was approximately 59 days.

2.3 Native range

The native distribution of *D. tomentosus* appears to be California, Arizona, New Mexico and Texas in the USA and Mexico (Rodriguez *et al*. 2001).

2.4 Related species and summary of their host range

Nine species are recognised in the genus *Dactylopius* Costa (De Lotto 1974), all of which are restricted to hosts in the family Cactaceae (Mann 1970; De Lotto 1974; ABRS 2009). Five *Dactylopius* species, including *D. tomentosus* have been utilised as biocontrol agents and are listed in Table 1 below. Particular biotypes of *D. tomentosus* have been introduced to control *C. fulgida* var. *fulgida* and *C. fulgida* var. *mamillata* in South Africa, *C. imbricata* in Australia and South Africa and *C. leptocaulis* in South Africa (Mann 1970; Moran & Zimmermann 1991), as each biotype performs significantly better on particular species or varieties of *Cylindropuntia*. Different biotypes of *D. opuntiae* were also utilised as biocontrol agents for particular *Opuntia* species, with each biotype specific to one or two cactus species.

Table 1. *Dactylopius* species that have been utilised as biocontrol agents and their target weed species.

Biocontrol agent	Host plant	Country of introduction
D. austrinus	*Opuntia aurantiaca*	Australia, South Africa
D. ceylonicus	*O. monacantha*	Australia, India, Kenya, Mauritius, South Africa, Sri Lanka, Tanzania
D. confusus	*O. dillenii*	Australia
*D. opuntiae**	*O. elatior*	India, Indonesia
	O. engelmannii	South Africa
	O. ficus-indica	USA (Hawaii), South Africa
	O. humifusa	South Africa
	O. littoralis	USA (mainland)
	O. monacantha	Mauritius
	O. oricola	USA (mainland)
	O. streptacantha	Australia
	O. stricta	Australia, India, South Africa, Sri Lanka
	O. tomentosa	Australia
	O. tuna	Mauritius
*D. tomentosus**	*C. fulgida*	South Africa
	C. imbricata	Australia, South Africa
	C. leptocaulis	South Africa

* Different biotypes of these species were introduced to control the different cactus species.

2.5 Proposed source of the agent

Dactylopius tomentosus ('fulgida' biotype) was originally collected from *Cylindropuntia cholla* (F.A.C. Weber) F.M. Knuth, in its native range at Loretto (23°44'N 110°06'W) and La Paz (24°10'N 110°17'W), Baja California Sur, Mexico. This biotype was released and has established in South Africa as a biological control agent against *C. fulgida* var. *fulgida* (Paterson *et. al.* 2011). The culture imported into the quarantine facilities at the Ecosciences Precinct (ESP), Brisbane, was collected from cladodes of *C. fulgida* var. *fulgida* at Musina, Limpopo Province, South Africa, on 26 August 2011. However, the laboratory colony at the ESP was maintained on potted plants of *C. fulgida* var. *mamillata*. It is the progeny of this culture which will be released into the field in Australia if approval is granted.

2.6 Mode of action

Adult males of *D. tomentosus* ('fulgida' biotype) are small, weak-flying insects that do not feed, whereas adult females are sessile and remain attached to cladodes of their host plant. Toxins present in cochineal saliva are injected into the plant during the feeding process and it is suggested that these toxins are responsible for the death of plant tissue at the feeding site (Moran 1981). The multiple overlapping generations, combined with the high establishment rate of the crawlers, enable *D. tomentosus* ('fulgida' biotype) to build up into large populations quickly. The sustained feeding by large numbers of crawlers and female adults, ultimately leads to the death of the host plant. The impact of the cochineal appears to increase when conditions are dry. There is no evidence of the involvement of any plant pathogens in the death of infested cactus species.

2.7 Potential for control

The release of *D. tomentosus* ('fulgida' biotype) for the biological control of *C. fulgida* var. *fulgida* in South Africa proved to be highly successful. Four months after the release of this biotype, all the inoculated plants were heavily infested and small plants were dying. Within one year, most plants were dead and within two years after release, 87 ha of *C. fulgida* var. *fulgida* had been colonised by *D. tomentosus* ('fulgida' biotype). Most of the small plants had died and only a few plants with woody stems survived (Paterson *et. al.* 2011) and the plant is now considered to be under complete control (Klein 2011). *Dactylopius tomentosus* ('fulgida' biotype) has been reported to also attack *C. fulgida* var. *mamillata* in South Africa where the insect is aiding its control.

Since its introduction and establishment, *D. tomentosus* ('fulgida' biotype) has spread to neighbouring Zimbabwe, where it is aiding the control of both *C. fulgida* var. *fulgida* and *C. fulgida* var. *mamillata*. It is anticipated that this biotype will achieve similar results if released on *C. fulgida* var. *mamillata* in Australia.

2.8 Possible interactions with existing biological control agents

Of the *Cylindropuntia*, only *C. imbricata* has been a target for biological control in Australia. Another biotype of *D. tomentosus* ('imbricata' biotype) was released to control this species in 1925 and is now widespread throughout areas where *C. imbricata* is present. There is a shared range among species within the genus *Cylindropuntia* and hence an overlap between *D. tomentosus* ('imbricata' biotype) and *D. tomentosus* ('fulgida' biotype) is likely. However, the latter biotype is highly virulent and more damaging than the already-released 'imbricata' biotype on all *Cylindropuntia* spp. tested. Hybridization trials conducted in South Africa between *D. tomentosus* ('fulgida' biotype) and *D. tomentosus* ('imbricata' biotype) revealed that the two biotypes were reproductively compatible and that the offspring had a wider host range within the *Cylindropuntia* than each of the parent biotypes (Paterson *et. al.* 2011).

2.9 Non-target organisms at risk

Species within the genus *Dactylopius* are highly specific and several species have already been utilised as biocontrol agents (De Lotto 1974; Moran 1980; Moran & Zimmermann 1984 a & b; Volchansky *et al*. 1999; ABRS 2009). Indeed, another biotype of the same species has already been introduced into Australia to control *C. imbricata*. Host range studies in Australia demonstrate that *D. tomentosus* ('fulgida' biotype) is restricted to the genus *Cylindropuntia.* These results support findings of earlier studies by Mathenge *et al.* (2009a) and Zimmerman and Granata (2002) in South Africa, where the agent has already been released and is controlling *C. fulgida* var. *fulgida*. Therefore, there is a very low risk of attack to non-target organisms in Australia.

2.10 Host-specificity studies

2.10.1 General summary

Comprehensive host-specificity testing was conducted in quarantine facilities at the Ecosciences Precinct, Brisbane, Queensland. Host specificity was determined using no-choice larval survival studies on 17 plant species selected by the centrifugal phylogenetic method (Wapshere 1975).

Dactylopius tomentosus ('fulgida' biotype) was restricted to the genus *Cylindropuntia*. No crawlers were able to develop past the first instar when placed on plant species outside of this genus. *Dactylopius tomentosus* ('fulgida' biotype) was highly damaging on four of the

eight naturalised *Cylindropuntia* species; namely *C. fulgida* var. *mamillata*, *C. imbricata*, *C. tunicata* (Lehm.) F.M. Knuth and *C. kleiniae* in Australia.

2.10.2 Proposed Test List

The species *D. tomentosus*, albeit another biotype ('imbricata' biotype), has already been thoroughly tested and subsequently released in Australia. In addition, *D. tomentosus* ('fulgida' biotype) has been comprehensively tested in South Africa prior to its release there. Consequently, a test plant list with a reduced number of species than would normally be tested was compiled. The proposed test list was compiled using the centrifugal phylogenetic method (Wapshere 1975). This method proposes that taxa closely related to the target weed should be well represented in the test list while those more distantly related should have fewer representatives (Table 2).

The 17 species included in the test list are all members of the order Caryophyllales. There are 13 species representing the family Cactaceae, which contains no native Australian species. Eight of these species belong to the genus *Cylindropuntia*, all of which are declared as targets for biocontrol. Three species belong to the genus *Opuntia*. *Opuntia ficus-indica* (L.) Jacq. is the only member of either the *Cylindropuntia* or the *Opuntia* which is allowed to be grown legally in New South Wales. In most states of Australia, *O. ficus-indica* is commonly grown in gardens owned by people of Mediterranean extraction. Due to its popularity as an edible fruit, *O. ficus-indica* has also been included on the proposed test list.

Hylocereus undatus (Haw.) Britton & Rose (dragon fruit) is included in the test list as it is an exotic tropical/sub-tropical species grown in the Northern Territory, Queensland and northern New South Wales for its edible fruit. Its popularity has increased in recent years and an industry has now been established which produces fruit for the domestic market and export to Asia.

Three species in the family Portulacaceae are included due to their close phylogenetic relationship to the Cactaceae. *Portulacaria afra* (L.) Jacq. is a popular ornamental plant which is widely cultivated and sold through nurseries. Although *Portulaca oleracea* L. is a widespread weed species, it is included on the host test list because its distribution overlaps that of *C. rosea.* The origins of *P. oleracea* are subject to conjecture with some Australian states, including New South Wales, regarding it as native while other states consider it to be exotic. *Anredera cordifolia* (Ten.) Stennis is a member of the Basellaceae which also does not contain any Australian native representatives. It is included on the host test list because of the phylogenetic proximity of Basellaceae to Cactaceae, although its largely tropical/sub-tropical coastal distribution does not overlap with either the current or potential distribution of any of the *Cylindropuntia* species.

Table 2. Plant species tested to determine the host range of *D. tomentosus* ('fulgida' biotype)

Family	Genus/Species	Common name
Cactaceae	*Cylindropuntia fulgida* var. *mamillata* (DC.) Backeb	boxing glove cactus, coral cactus
	Cylindropuntia imbricata (Haw.) F.M. Knuth	rope pear, Devil's rope
	Cylindropuntia kleiniae (DC.) F.M. Knuth	candle cholla
	Cylindropuntia leptocaulis (DC.) F.M. Knuth	pencil cactus
	Cylindropuntia prolifera (Engelm.) F.M. Knuth	jumping cholla
	Cylindropuntia rosea (DC.) Backeb (Mexico)	Hudson pear
	Cylindropuntia rosea (DC.) Backeb (Spain)	
	Cylindropuntia rosea (DC.) Backeb (Grawin, NSW)	
	Cylindropuntia rosea (DC.) Backeb (Lorne Station, NSW)	
	Cylindropuntia spinosior (Engelm.) F.M. Knuth	snake cactus
	Cylindropuntia tunicata (Lehm.) F.M. Knuth (Cracow, Qld)	Hudson pear
	Cylindropuntia tunicata (Lehm.) F.M. Knuth (Grawin, NSW)	
	Hylocereus undatus (Haw.) Britton & Rose	dragon fruit
	Mammillaria elongata DC.	ladyfinger cactus
	Opuntia aurantiaca Lindl.	tiger pear
	Opuntia ficus-indica (L.) Jacq.	sweet prickly pear, Indian fig
	Opuntia stricta (Haw.) Haw	common pear
Basellaceae	*Anredera cordifolia* (Ten.) Stennis	Madeira vine
Portulacaceae	*Calandrinia eremaea* Ewart	purslane
	Portulaca oleracea L.	pigweed
	Portulacaria afra (L.) Jacq.	jade plant

2.10.3 No-choice tests using larvae

Host specificity was determined using no-choice larval survival and development trials. All host testing followed the protocols outlined by Mathenge *et al*. (2009a & b). Twenty neonate crawlers, less than 24 hours old, were transferred directly to a cladode of the host test plant. Each inoculated cladode was then placed in a separate sealed plastic container and stored in a constant temperature room set at 27^{o}C and 65% relative humidity. Each trial was accompanied by a control where 20 neonate crawlers were transferred to a cladode of *C. fulgida* var. *mamillata*. Each test species was tested five times (i.e. five replicates), using a cladode from a different plant of the same species.

Crawler survival was assessed three times a week and the number of days for crawlers to settle at a feeding spot after transfer was recorded. The biology of males and females differ at the end of the second instar, with females being sessile and remaining at the original feeding location, whereas males actively search for suitable pupation sites (Mathenge *et al*. 2009a). Survival and time to each life stage was therefore recorded as the time to first and second instar, and pupal case construction (for the males) and third instar (pre-oviposition for the females). Individuals were allowed to develop to the adult stage, upon which male emergence and date of female oviposition were recorded.

The host range of *D. tomentosus* ('fulgida' biotype) was confined to the genus *Cylindropuntia,* as no crawlers were able to develop past the first instar when reared on plant species outside

of this genus. This biotype generally had a high development success rate on most *Cylindropuntia* species tested, but the most susceptible hosts were *C. fulgida* var. *mamillata*, *C. imbricata*, *C. tunicata* and *C. kleiniae*. *Cylindropuntia rosea* (DC.) Backeb collected from two sites in Australia were the only *Cylindropuntia* species that did not support development to the adult stage in all trials (Table 3).

Table 3. The developmental success of 20 *D. tomentosus* ('fulgida' biotype) neonate crawlers when placed on each of eight *Cylindropuntia* species naturalised in Australia.

***Cylindropuntia* species**	**Development success (mean)**
C. fulgida var*. mamillata*[t]	13.5±0.6
C. imbricata	9.2±1.9
C. kleiniae	7.4±1.0
C. leptocaulis	2.6±1.3
C. prolifera	0.6±0.6
C. rosea (Grawin)	0.0±0.0
C. rosea (Lorne Station)	0.0±0.0
C. rosea (Mexico)	8.6±1.6
C. rosea (Spain)	n/a
C. spinosior	2.2±0.9
C. tunicata (Cracow)	8.4±0.6
C. tunicata (Grawin)	7.6±2.0

[t] Control plant species

2.10.4 Efficacy trials

Efficacy trials were conducted to determine whether *D. tomentosus* ('fulgida' biotype) can reduce the vigour and/or kill those test species that supported an average of four or more individuals developing to maturity in the host specificity trials.

One fecund female and her associated egg mass were transferred to a growing plant of each of the test species. The eggs were left to incubate and the emerging first generation crawlers were allowed to develop to maturity. Each plant was monitored every fortnight and an estimate of the number of crawlers emerging, attaching to a feeding spot and their development over time were recorded. A photographic analysis of crawler development and subsequent effect on the plant were also conducted every fortnight until the plant died from the crawler infestation or when the insect colony died, due to the plant being an unsuitable host. A colony was classified as established when 10 or more fecund first generation females were present or 50 or more second generation crawlers were settled at feeding sites. This classification is based on the results of Zimmerman (2007) and Mathenge et al. (2009a) who state that an individual ovipositing female can produce between 72 – 338 progeny and that development success can be as high as 80% for D. tomentosus crawlers when reared on its natural or a suitable host. Once these baseline numbers have been reached for either of the two indicators, the resulting population increase can be dramatic.

Four plant species were screened using *D. tomentosus* ('fulgida' biotype): *C. kleiniae*, *C. imbricata*, *C. fulgida* var. *mamillata*, *C. tunicata* (Grawin) and *C. tunicata* (Cracow) (Table 3).

D. tomentosus ('fulgida' biotype) displayed very high settling rates (>50 crawlers) on all *Cylindropuntia* species tested except *C. tunicata* (Cracow) which had a settling rate of 20 crawlers. There was also a high establishment rate, with colonies recorded as established by week six on every plant species except for *C. tunicata* (Cracow) (Table 4). However, by week 14, the population on *C. tunicata* (Cracow) was also classified as established.

Table 4.Summary of the key development indicators measured during the efficacy trials for *D. tomentosus* ('fulgida' biotype) on selected *Cylindropuntia* species.

Development indicator	***Cylindropuntia* species**				
	C. fulgida	***C. kleiniae***	***C. imbricata***	***C. tunicata* (Cracow)**	***C. tunicata* (Grawin)**
No. of crawlers settled after two weeks	>50	>50	>50	20	>50
Development time (crawler to oviposition) (weeks)	6	6	8	8	6
Time to colony establishment (weeks)	6	6	6	14	6
Time for 2nd generation to emerge (weeks)	8	8	10	10	8
No. of crawlers settled in 2nd generation	1000	1000	200	50	50
Death of plant due to feeding (weeks)	18	52	18	52	52

Populations of *D. tomentosus* ('fulgida' biotype) on each of the *Cylindropuntia* species tested continued to increase rapidly and the *C. imbricata* and *C. fulgida* var. *mamillata* plants died in week 18 due to sustained feeding activity. Populations on the remaining plant species continued to spread over the entire plant. Over the course of the trials, there was very little plant growth, probably due to sustained feeding activity of the crawlers. These plants eventually died or were heavily infested within one year (Figures 6 & 7).

C. fulgida week 0

C. fulgida week 18

C. imbricata week 0

C. imbricata week 18

Figure 6. The health of *C. fulgida* var. *mamillata* and *C. imbricata* at day 0 and death of plant at week 18, during the efficacy trials for *D. tomentosus* ('fulgida' biotype).

C. kleiniae week 0

C. kleiniae week 52

C. tunicata week 0

C. tunicata week 52

Figure 7. The health of *C. kleiniae* and *C. tunicata* (Grawin) at day 0 and death of plant at week 52, during efficacy trials for *D. tomentosus* ('fulgida' biotype).

2.11 Proposed field release procedure

2.11.1 Release from quarantine

Dactylopius tomentosus ('fulgida' biotype) remains in culture within the quarantine facilities at the Ecosciences Precinct, Brisbane. Upon approval, voucher specimens of this culture will be deposited with AQIS. Newly-emerged nymphs from this culture will be removed from the quarantine, without plant material, after careful inspection to confirm identity and to ensure that no pathogen or other arthropod is taken from the quarantine. Two issues have determined the decision to use newly-emerged nymphs as starting material for a colony outside of quarantine. First, mated females would need to be de-waxed to ensure that there are no contaminants on the females being removed. This is both time consuming and this process removes the associated egg batch held together by the waxy covering. Second, if the de-waxed females are used, a lag period of 16 days (incubation period) occurs before any crawlers will emerge. By using newly emerged crawlers, an immediate colony can be established on individual plants, with an accurate record of numbers initiating each colony. Once removed from quarantine, the insects will be placed on *C. fulgida* var. *mamillata* plants in non-quarantine glasshouses for mass rearing.

2.11.2 Field releases

Dactylopius tomentosus ('fulgida' biotype) will be released on *C. fulgida* var. *mamillata* at selected sites throughout the weed's range in Australia. Release sites will be recorded with their GPS coordinates. It is hoped that community groups such as Landcare and Bushcare, as well as schools, may contribute to the distribution of the insect.

2.11.3 Establishment and evaluation

Release sites will be monitored for some years after releases, to determine whether the insect has established. Once the insect has established, assessments will be conducted to determine its effect on *C. fulgida* var. *mamillata* and if there are any non-target impacts, although the latter are highly unlikely.

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The biology of *Dactylopius tomentosus* (Hemiptera: Dactylopiidae)

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Abstract

Dactylopius tomentosus (Lamarck) (Hemiptera: Dactylopiidae) is a cochineal insect whose host range is restricted to *Cylindropuntia* species (Caryophyllales: Cactaceae). This insect has been utilized successfully for biological control of *Cylindropuntia imbricata* (Haw.) F.M. Knuth in Australia and South Africa. Despite this, its biology has not been studied previously, probably due to the widely held belief that the biology of all *Dactylopius* species is similar. This study investigated the life cycle and the morphological and reproductive characteristics of *D. tomentosus*. Results revealed some unique characteristics of *D. tomentosus*: (i) eggs undergo a much longer incubation period, an average of 17 days compared to <1 day in its congeners; (ii) eggs are laid singly but are retained as an egg mass secured in a mesh of waxy threads attached to the female; (iii) the developmental times of males and females are longer compared to other *Dactylopius* spp. due to a longer egg incubation period; (iv) *D. tomentosus* does not undergo parthenogenesis; (v) *D. tomentosus* is smaller in size than its congeners; and (vi) male mating capacity and reproductive potential were both high and variable between males. There was a significant, strong, positive relationship ($r = 0.93$) between female mass and fecundity, whereas the relationship between the number of females mated per male that became gravid and their fecundity was negative ($r = -0.68$). Besides contributing to our knowledge of this economically important species, the finding of unique characteristics of *D. tomentosus* biology underlines the need to study each species in this genus.

Keywords: *Dactylopius*, cochineal insects, life cycle, parthenogenesis, male mating capacity, fecundity, *Cylindropuntia*

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Introduction

Dactylopius tomentosus (Lamarck) is one of nine species belonging to the monogeneric family, Dactylopiidae (De Lotto, 1974) which is part of the superfamily Coccoidea, within the Hemiptera. The coccoids are commonly known as scale insects and are characterized by distinct sexual dimorphism and a high degree of specialization to their

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parasitic lifestyle (Miller & Kosztarab, 1979; Gullan & Kosztarab, 1997; Gullan & Martin, 2003). Scale insects are economically important in that some are serious pests of crops, whereas others are beneficial and are utilized for the commercial production of natural dyes and resins, or for the biological control of weeds (Claps & de Haro, 2001). For example, carminic acid, naturally used for protection by cochineal insects (Eisner *et al*., 1980, 1994), has been exploited for carmine dye production (Baranyovits, 1978; Guerra & Kosztarab, 1992). In addition, host specialization of *Dactylopius* species on cacti (Moran, 1980) has rendered them useful in the biological control of invasive cactus weeds in many parts of the world (Moran & Zimmermann, 1984; Julien & Griffiths, 1998).

The biology of several *Dactylopius* species has been studied, namely, *D. austrinus* De Lotto (Gunn, 1978; Moran & Cobby, 1979; Hosking, 1984), *D. ceylonicus* Green (Sullivan, 1990), *D. coccus* Costa (Guerra & Kosztarab, 1992), *D. confusus* (Cockerell) (Gilreath & Smith, 1987) and *D. opuntiae* Cockerell (Karny, 1972; Flores-Hernández *et al*., 2006). The biologies of all these *Dactylopius* species, particularly their life history characteristics and general morphology, are similar. For these species, eggs are laid singly and hatch within a day into mobile, first instar crawlers. The crawler is the dispersal stage and has behavioural and morphological adaptations for dispersal (Gunn, 1978; Moran *et al*., 1982; Washburn & Washburn, 1984). Sexual dimorphism is exhibited from the late first-instar stage onwards, resulting in distinct morphological differences between males and females. Females undergo hemimetabolous metamorphosis in which the eggs hatch into crawlers and, after the first moult, they retain their nymphal characteristics, only growing in size into a globular, sedentary stage. The males, however, undergo complete metamorphosis during which the second instar crawlers spin a white, silky cocoon inside which they undergo their subsequent three moults and develop into white-winged adults. The adult male is short-lived and a weak flier that moves predominantly by walking. In addition, the numerical paucity of adult males, relative to females and crawlers in a colony, led to the conclusion by Mann (1969) that, like many scale insects (Miller & Kosztarab, 1979; Gullan & Kosztarab, 1997), cochineal insects are parthenogenetic. However, no parthenogenesis was found in *D. austrinus* (Moran & Cobby, 1979), *D. ceylonicus* (Sullivan, 1990) and *D. coccus* (Guerra & Kosztarab, 1992), and there are conflicting reports of its occurrence in *D. opuntiae* (Karny, 1972; Flores-Hernández *et al*., 2006). Therefore, the occurrence of parthenogenesis needs to be examined for each cochineal species.

Of the little information available on *D. tomentosus*, none has addressed its life history. Its taxonomy, based on the external morphology of female cuticles, is described by De Lotto (1974), Guerra & Kosztarab (1992), Gill (1993) and Ben-Dov (2006). This species is only briefly mentioned in articles reporting the occurrence of *Dactylopius* species in various regions or countries (Denoth *et al*., 2002; Claps *et al*., 2006; Portillos & Vigueras, 2006). The native distribution of *Dactylopius tomentosus* appears to be within parts of North America, namely, Arizona, New Mexico, Texas and Mexico (Rodríguez *et al*., 2001), and it has been introduced into Australia (Dodd, 1940; Mann, 1969) and South Africa (De Lotto, 1974; Moran & Zimmermann, 1991). *D. tomentosus* has a narrow host range and is only associated with *Cylindropuntia* species (Zimmermann & Granata, 2002); as such, it has been used successfully as a biological control agent against *C. imbricata* in Australia and South Africa (Moran & Zimmermann, 1991). As this species is a significant biological control agent, it is important to elucidate its reproduction and development to determine whether its biology is similar, or otherwise, to other *Dactylopius* species.

Materials and methods

Species identification, morphology and behaviour

This study used *D. tomentosus* collected from plants of *Cylindropuntia imbricata* (Haw.) F.M. Knuth (Caryophyllales: Cactaceae) from Gauteng Province, South Africa. *Cylindropuntia imbricata* was identified by taxonomists from the National Botanical Institute, Pretoria, and this material was used in all the experiments described below. Prior to the study of its biology, females from cultures were prepared and mounted on slides using the methods described by Cox (1987), Williams & Granara de Willink (1992) and Watson & Chandler (1999) to facilitate their identification. The species was identified by the characteristic arrangement of the medial and sub-medial rows of setae on the dorsal surface of the insect: the patterns observed were compared with the identification key of Guerra & Kosztarab (1992). Insects were identified as *Dactylopius tomentosus* and voucher specimens deposited in the insect collection of the Biosystematics Division of the Plant Protection Research Institute (PPRI), Agricultural Research Council (ARC), Pretoria, South Africa.

Cultures of *D. tomentosus* were maintained on mature cladodes of *C. imbricata* in glasshouses at the Weeds Research Division, PPRI, ARC, Rietondale, Pretoria, South Africa. Unless otherwise stated, all experiments were initiated as follows. Adult females were collected from cultures and placed in glass Petri dishes (90 mm Ø) in which they laid eggs. Hatching crawlers were then transferred onto fresh, clean cladodes of *C. imbricata*. The cladodes were pinned to polystyrene blocks (120 × 160 mm) and placed in cages measuring 250 mm in diameter and 150 mm in height. All experiments were conducted in a quarantine laboratory at 26°C and 60% RH.

The morphology of the different life cycle stages and the behaviour of the insects were examined using a stereo microscope in the laboratory or a large portable magnifying lens mounted on an adjustable stand for cultures in glasshouses. These observations included: oviposition and wax secretion during oviposition; shape, colour and hatching of eggs; settling of crawlers and their dispersal behaviour; colour, shape and wax secretion of crawlers, immature and mature gravid females; male pupation and the shape and colour of the cocoon. Pupal cases were selected at random and opened to expose the developing male in order to describe its morphology.

The size of individuals of each instar was determined by measuring the length and widest part of each individual using an ocular micrometer at 25× magnification. Insects were measured to the nearest ocular unit with one ocular unit being equivalent to 0.4 mm. In addition, the length of the adult males was measured from the head to the posterior end of the abdomen and also from the head to the tip of the wings at rest alongside the body.

Developmental and reproductive biology

Different aspects of developmental and reproductive biology of the *D. tomentosus* were studied in separate experiments designed either to minimize disturbance of the various stages of the insects or ensure confinement of the crawlers and males within cages. Independent investigations were required to study male life stages, which allowed males to be removed from cages at the pupal stage and placed into vials until emergence of adult males for subsequent longevity studies. Separate experiments were conducted that involved destructive sampling. These experiments included the study of the male pupal stage (obscured by the cocoon) and the pre-ovipositing adult females (concealed under copious amounts of waxy secretions). For the latter study, to observe and measure the females, it was necessary to remove their wax covering. This was done by winding the wax onto a stainless steel pin, which disconnected the females from their feeding spot, and they were then placed on a microscope slide for observation.

Egg biology

Ten gravid females of equal age were confined in separate Petri dishes. For each female, the eggs were removed daily and placed in separate Petri dishes, corresponding to the females. The dates that eggs were laid were noted and, from the commencement of hatching, the eggs were observed daily and the date that hatching occurred was recorded to determine the incubation period.

Crawler biology

To determine the duration of crawler survival without feeding, ten newly-emerged crawlers from each of ten females were confined in Petri dishes, without host plant cladodes, and the time to the death of the insects recorded. Crawler developmental time was determined in a separate experiment commencing with one-day-old crawlers and terminating at male pupation. In this experiment, 30 crawlers were placed on each of five cladodes and allowed to develop. The number of instars for each sex was noted, and the developmental time from hatching to the first moult was recorded.

Female biology

To study reproduction, insects were allowed to develop to maturity. At the onset of oviposition, females were individually removed from cladodes, dewaxed by rolling their wax onto a pin, weighed and transferred into separate vials to determine the total number of progeny per female. Female developmental time from crawler to maturity was also determined.

Assessment of parthenogenesis

To investigate whether *D. tomentosus* reproduces parthenogenetically, 30 crawlers were introduced onto each of four cladodes of *C. imbricata* with each cladode being placed in a separate cage where the crawlers were allowed to develop. All males were removed as soon as they pupated and the females were allowed to develop to maturity. A control treatment was set up as above but, after male pupation, the cladodes were paired up to ensure that each cage had sufficient numbers of males to mate with the females. Changes in female size were assessed visually in both treatments, and the females were examined daily for oviposition. The number of females that oviposited and the date that oviposition commenced were also recorded.

Male biology

Separate studies of the male life cycle were conducted. In the first experiment, male developmental time from hatching to pupation was recorded. Subsequent experiments commenced at the pupal stage. The duration of the pupal stage was determined by recording the day that pupation took place and time of emergence of adult males from cocoons. The male life span was studied by collecting equal-aged male pupae that were confined in vials until the emergence of the adults. The date of emergence was recorded, and newly emerged males were transferred into new vials; the vials were observed daily until the death of the male. The male life span could be determined in the vials as adult males do not feed (Karny, 1972).

Male mating capacity and reproductive potential

Male mating capacity (the number of females with which a single male can mate) and male reproductive potential (the mean number of progeny per female mated by each male) were determined as follows. Thirty, one-day-old, first instar crawlers were seeded onto each of ten cladodes and placed separately in cages. All crawlers were allowed to develop to pupation of the males after which all cocoons except one were removed from the cladodes, leaving one male and a varying number of females (10–29) in each cage. This was aimed at ensuring an adequate number of females were available to mate with each male. Cages were covered with fine gauze and left undisturbed for up to three weeks during which time the males emerged and mated with females. After this period, the females were examined daily for the onset of oviposition. Based on the results previously obtained on the absence of parthenogenesis in *D. tomentosus* and reported in this paper; oviposition was considered to be an indication of successful mating and, therefore, the number of females that oviposited represented the male mating capacity. As soon as oviposition commenced, females were removed individually from the cladodes, dewaxed, weighed and transferred into separate vials to determine the number of eggs laid. Females that did not lay eggs were allowed to remain in the vials for an extra 60 days, after which they were removed and weighed.

Data analysis

Statistica (Ver. 7; StatSoft, Inc., 2007) was used for analyses of variance, *t*-tests, Tukey HSD tests and to determine correlations between the number of gravid females per male, mean female mass and mean progeny numbers. A contingency χ^2 test (Snee, 1974) was used to determine if differences existed in male mating capacity.

Results

This study has examined the reproduction and development of *D. tomentosus* to determine whether its biology is similar, or otherwise, to other *Dactylopius* species. The life stages of *D. tomentosus*, including eggs, crawlers, male pupae, adult male and adult female, are presented in fig. 1; and a description of the unique features of these life stages is given below.

Egg biology

Newly laid eggs of *D. tomentosus* were red (crimson) in colour, had a smooth shiny texture and were oval in shape. A number of characteristics that had not been observed in eggs of other *Dactylopius* species were seen. Eggs were laid singly but remained held together in a ball-shaped mesh of waxy threads anchored to the posterior end of the female's abdomen. As more eggs were laid, and depending on the position of female on the cladode, the increasingly heavier egg mass sometimes sagged away from its original position but remained attached to the female by the threads. The eggs underwent a mean incubation period of 17.0 days (table 1).

Crawler biology

Newly hatched first-instar crawlers were small (table 2) and bright red in colour. After eclosion, the first-instar crawlers remained within the mesh of waxy threads of the egg mass for a few minutes to a few hours before becoming mobile and leaving the threads to search for suitable settling and feeding sites. Most crawlers settled at the base of spines and away from light. The mean duration of the first instar (i.e. the time between hatching and the first moult) was 18.0 days (table 1); more than 90% of crawlers moulted between 12 and 18 days and only a few individuals (< 4%) had a longer developmental time. Sexual dimorphism became apparent during the second instar stage, after which the developmental cycle and morphology differed for males and females.

Female biology

The first wax secretions produced by second-instar female crawlers initially appeared as a white dust that gradually elongated into white coils. The wax increased in quantity, forming a thick, white, cottony cushion that eventually concealed the developing female insect. The second moult took place underneath this covering, and the white exuviate was displaced to the edge of the covering. Pre-ovipositing females were smaller than gravid females (table 2), and their bodies tapered slightly towards the tip of the abdomen. The bodies of fertilized females became distended, gradually acquiring a sub-globular shape. The mean duration of the female life cycle from egg to the commencement of oviposition was 63.3 days (table 1).

Parthenogenesis

All females ($n = 110$) in the presence of males produced eggs and offspring. No females ($n = 87$) in the absence of males produced eggs, despite being allowed to remain on the cladodes for at least an additional 60 days after commencement of oviposition by mated females. While gravid females gradually increased in size with their bodies becoming more globular in shape, the size and shape of the non-gravid adult females remained unchanged throughout the experimental period.

Male biology

In contrast to females, which became sedentary, second-instar male crawlers frequently shifted their feeding positions before moving about in search of a favourable site for pupation. The duration of the second instar from the first moult to the commencement of pupation averaged three days. The spinning of a white cocoon commenced soon after the second instar nymph attached to a suitable substrate and was completed within 2–3 days. The pupal period lasted approximately 13 days, at the end of which emerged a red, white-winged male. The male was half the length of the mature female (table 2) and measured 1.55 (± 0.02) mm from head to tip of wings, with wings at rest. The male was short-lived and died within five days of emergence. Mean male developmental time from egg to death was 57.5 days.

Male mating and reproductive potential

Table 3 summarizes the data on the proportion and mean fecundity of females that became gravid, as well as the mean mass of females that did not become gravid. Male mating capacity varied widely (range: 1–23 females) and the proportions of gravid and non-gravid females per male differed significantly ($\chi^2_9 = 120.27$; $P < 0.05$). For example, for half of the males, more than 80% of the females provided were gravid, compared to 7–12% for four males.

Male reproductive potential was high and varied between males (table 3). For this analysis (and for the analysis of female mass shown below), data for two males (M6 and M9) were excluded from the analysis of variance as only one female in each cage produced offspring. The mean reproductive potential of the other eight males that produced two or more gravid females was significantly different ($F = 7.42$; $df = 7, 8$; $P < 0.05$). Mean mass of inseminated females per male also differed significantly ($F = 2.76$, $df = 7, 8$; $P = 0.01$), varying from 3–6 mg. In addition, the mean mass (4.4 ± 0.1 mg) of gravid females was significantly greater ($F = 108.9$; $df = 1, 171$; $P < 0.05$) than that of non-gravid females (1.9 ± 0.9 mg). The mean mass of the non-gravid females in each cage did not differ significantly ($F = 1.14$; $df = 6, 78$; $P = 0.35$).

Pearson correlation analysis revealed a highly significant, positive relationship between mean female mass and the mean number of progeny ($r = 0.93$; $P < 0.001$). In addition, the relationship between the number of gravid females per male and mean mass of the gravid females was negative and significant ($r = -0.79$; $P = 0.02$). There was also a negative relationship between the number of females inseminated by each male and mean number of progeny per female that just failed to be statistically significant ($r = -0.68$; $P = 0.07$).

Discussion

Contrary to the widespread misconception concerning the high degree of uniformity of the biology and morphology of *Dactylopius* species (Karny, 1972; De Lotto, 1974; Guerra & Kosztarab, 1992), the results of this study revealed that *D. tomentosus* has unique biological and morphological

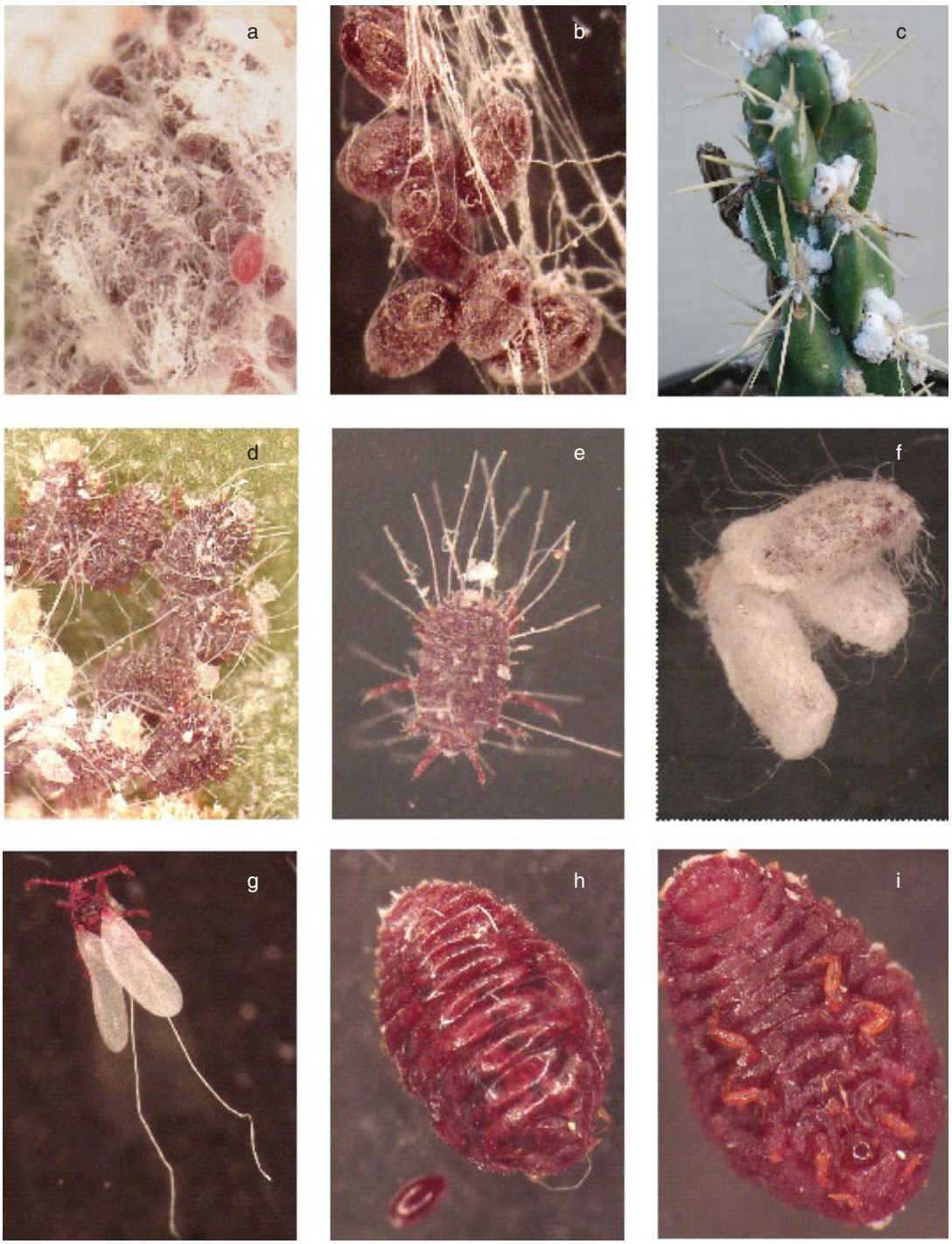

Fig. 1. (a) eggs in a meshwork of waxy threads forming a white, spherical egg mass (mag. ~14×); (b) eggs held together by waxy threads (mag. ~36×); (c) colonies of sessile, mature females (including egg masses) covered in copious cottony wax; many females may settle close together forming clusters of colonies (mag. ~1×); (d) crawlers aggregating at a feeding spot (mag. ~30×); (e) crawler with long filaments on the posterior part of abdomen (mag. ~50×); (f) two cocoons containing developing males and a cocoon under construction (upper cocoon) (mag. ~23×); (g) male with two long, waxy filaments attached to posterior end of the abdomen (mag. ~17×); (h) dorsal view of a mature female with an egg (mag. ~19×); (i) ventral view of a mature female (mag. ~23×).

characteristics that differ considerably from other species. Although every developmental stage was studied, only those characteristics that are unique to this species are discussed in detail, and other features of the insect's biology are only included for comparison with its congeners where necessary.

Table 1. Mean developmental time of each life stage of *D. tomentosus*.

Life Stage	Mean Duration (days)	Range
Egg incubation period	17.0	15–20
1st–2nd instar	18.0	12–25
1st instar–pupation ♂	21.7	14–25
Pupation–emergence ♂	13.7	10–17
Adult male lifespan[a]	5.1	3–7
1st instar–mature female[b]	46.3	32–56
Egg–mature female[c]	63.3	47–76
Male life cycle[d]	57.5	42–67

[a] the time between emergence and death of male;
[b] the time from hatching of eggs to first egg production by a female;
[c] the estimated developmental time for females from egg to their first egg production;
[d] the estimated developmental time from egg to death.

Table 2. Mean (±SE) length and width of *D. tomentosus* life stages; width was measured across the widest part of the body.

Life stage	Mean length (mm)	Mean Width (mm)
Egg	0.5 (±0.1)	0.3 (±0.1)
Crawler	0.5 (±0.1)	0.3 (±0.1)
Cocoon	1.5 (±0.1)	0.7 (±0.1)
Adult male	1.0 (±0.1)	0.4 (±0.1)
Females (pre-oviposition)	2.0 (±0.1)	1.3 (±0.1)
Females (ovipositing)	2.7 (±0.1)	2.2 (±0.1)

Egg biology

The incubation period (17 days) of *D. tomentosus* eggs was long compared to other species in this genus. This contrasts with *D. coccus* whose eggs hatched within 30 min of laying (Guerra & Kosztarab, 1992) at 25–26°C, *D. austrinus* where hatching took place within 67 min (Moran & Cobby, 1979) at 26°C, and other *Dactylopius* spp. where hatching occurred within 3–5 h at 26°C (Karny, 1972; Sullivan, 1990). The hatching process itself, however, was similar to that described by Karny (1972) and Guerra & Kosztarab (1992).

Another unique feature is that, although laid singly, the eggs of *D. tomentosus* are held together in a mesh of waxy threads for the entire incubation period. In contrast, eggs of other *Dactylopius* species are not enclosed in a mesh and continue to hatch as more are laid, and first instar crawlers disperse away from the female upon hatching (Karny, 1972; Moran & Cobby, 1979; Guerra & Kosztarab, 1992). The waxy mesh that encloses the eggs may play a protective role in that eggs are less accessible to natural enemies, such as predators, which could become entangled in the waxy threads; additionally, predators may not recognize the egg masses as food. Keeping eggs clustered and in close proximity to females or the colony may also protect them from desiccation during their long incubation period in the hot, dry habitat in which cactus and cochineal insects are found. The wax may also act as a means of attachment of eggs to the host plant, ensuring that the crawlers eclose on a suitable host.

Developmental biology of crawler, female and male

Developmental studies revealed that the life cycle of *D. tomentosus*, including the incubation period, was much longer than that of other *Dactylopius* species. The average developmental time for *D. tomentosus* crawler from egg to first moult was 35 days compared to 15, 17 and 18 days for *D. ceylonicus*, *D. austrinus* and *D. opuntiae*, respectively, at 26°C (Moran & Cobby, 1979; Sullivan, 1990; Volchansky *et al.*, 1999). Female maturity of *D. tomentosus* averaged 63 days in contrast to 40–50 days for *D. austrinus* at 25 and 26°C (Moran & Cobby, 1979; Hosking, 1984), *D. opuntiae* at 26°C (Githure *et al.*, 1999; Volchansky *et al.*, 1999; Flores-Hernández *et al.*, 2006) and *D. coccus* (Guerra & Kosztarab, 1992). The male developmental time from egg to adult emergence was 57 days compared to 43 days for *D. opuntiae* (Flores-Hernández *et al.*, 2006), 37.1 days for *D. austrinus* at 25°C (Hosking, 1984) and 31.3 days for *D. ceylonicus* (Sullivan, 1990). However, developmental times for males and females of *D. tomentosus*, excluding the egg incubation period, fell within the ranges for *D. ceylonicus* (Sullivan, 1990), *D. opuntiae* (Karny, 1972; Flores-Hernández *et al.*, 2006) and *D. austrinus* (Moran & Cobby, 1979; Hosking, 1984) and, though shorter, overlapped with that of *D. coccus* (Guerra & Kosztarab, 1992). Consequently, it is the prolonged egg incubation period that accounts for the difference in total developmental time. The range of developmental times of the first instar *D. tomentosus* crawlers (from egg hatch to first moult) was notably wide (12–25 days). Similar, wide ranges were reported for other cochineal insects, namely, *D. austrinus* (Moran & Cobby, 1979; Hosking, 1984), *D. opuntiae* (Karny, 1972) and *D. ceylonicus* (Sullivan, 1990) but not others, e.g. *D. coccus* (Guerra & Kosztarab, 1992); the cause of this variation is unknown.

Female morphology and reproductive characteristics

The size and shape of *D. tomentosus* adult females differed from those recorded for other cochineals. For example, the females of *D. coccus* are 4–6 mm long and 3.0–4.5 mm wide (Guerra & Kosztarab, 1992), which is twice as large as *D. tomentosus* females, with the latter averaging 2.72 mm long and 2.15 mm wide. In addition, the females of *D. coccus* are globular in shape, whereas the bodies of *D. tomentosus* females are tapered slightly towards the tip of the abdomen, giving them a sub-globular shape. However, *D. tomentosus* is similar in size to *D. confusus*, which measures 2.5–3.0 mm long and 1.5–2.0 mm wide. The mean mass of *D. tomentosus* females was much smaller (4.4 mg) than that of a number of other *Dactylopius* species, such as *D. opuntiae* (16 mg) (Githure *et al.*, 1999) and *D. coccus* (40–47 mg) (Guerra & Kosztarab, 1992). The white waxy covering of *D. tomentosus* females is cottony and similar to *D. opuntiae*, but different from that of *D. coccus*, which is powdery and *D. salmianus* De Lotto, which is composed of thin, brittle threads (Guerra & Kosztarab, 1992).

Parthenogenesis

The few males relative to the large number of females and crawlers at any given time in a colony have led to the earlier erroneous reports that all cochineal insects are parthenogenetic (Mann, 1969). Contrary to these reports, the lack of

Table 3. Male mating potential of ten individual *D. tomentosus* males (as defined by the number of females becoming gravid), the weights of gravid and non-gravid females at maturity and the number of progeny produced by the graved females. Means within columns followed by the same letter do not differ significantly according to Tukey HSD test at $P < 0.05$.

Male	No of females becoming gravid	% of females becoming gravid	Mean number of progeny (±SE) from gravid females	Mean weight (±SE) of gravid females (mg)	Mean weight (±SE) of non-gravid females (mg)
M1	2	12	245.0 (±48.0)ab	6.0 (±0.1)a	2.2 (±0.3)
M2	2	11	338.0 (±94.0)a	6.0 (±0.1)a	1.9 (±0.2)
M3	8	33	72.0 (±7.4)c	4.3 (±0.4)ab	1.5 (±0.2)
M4	16	100	122.0 (±15.6)bc	4.5 (±0.3)ab	
M5	18	100	143.7 (±19.2)bc	4.8 (±0.2)ab	
M6	1	5	139.0 (−)	3.0 (−)	1.8 (±0.2)
M7	23	83	86.9 (±14.1)c	4.0 (±0.2)b	2.4 (±0.4)
M8	10	100	82.9 (±9.4)c	4.5 (±0.5)ab	
M9	1	7	115.0 (−)	5.0 (−)	1.9 (±0.3)
M10	16	100	86.1 (±12.1)c	3.9 (±0.2)b	
Means	9.8	52	110.7 (±7.7)	4.4 (±0.1)	1.8 (±0.9)

egg production by unmated females in this study confirmed that *D. tomentosus* does not reproduce parthenogenetically. Lack of parthenogenesis in *D. tomentosus* is similar to the finding that *D. austrinus* (Moran & Cobby, 1979) and *D. ceylonicus* (Sullivan, 1990) do not reproduce parthenogenetically. According to Moran & Cobby (1979), *D. austrinus* females had to be mated at least once to reproduce, and this appears to be the case for *D. tomentosus* females. However, *D. opuntiae* has recently been reported to sometimes reproduce parthenogenetically, but predominantly sexually (Flores-Hernández *et al*., 2006); therefore, the use of parthenogenesis as a means of reproduction is variable amongst *Dactylopius* species.

Parthenogenetic reproduction is prevalent among many insect groups and is associated with greater genetic stability (Suomalainen, 1962). Among coccoids, parthenogenesis has the advantage that sedentary females are able to reproduce without the presence of the short-lived and scarce males that may not always be available for mating (Gullan & Kosztarab, 1997). However, as has been shown during this study, *D. tomentosus* males are able to fertilize many females, thus compensating for their short life span. In addition, unmated females continued to survive for up to 60 days after commencement of egg production by mated females of the same age, enabling them to overlap with the next generation. This has the advantage that if a shortage of males is experienced in one generation, unmated females can survive until maturity of males in the next generation. Given these strategies, male shortage does not appear to be a limiting factor to population growth for *D. tomentosus*. This may explain why *D. tomentosus* and other cochineal insects may not have evolved parthenogenetic reproduction.

Male mating capacity and reproductive potential

The maximum mating capacity of male *D. tomentosus* appears to be substantial as evidenced by the high numbers of gravid females recorded for some of the males. The current study showed that male mating capacity in *D. tomentosus* was approximately double that reported for *D. coccus* (Guerra & Kosztarab, 1992). The results of our study may more accurately represent male mating capacity than the latter, as one male was used in each replicate in the current study, whereas in the previous study two males were used in some of the cages making it difficult to accurately determine mating capacity per male. Indeed, our study may have underestimated the mating capacity of some males as, in some cages, the males mated with all females and may have been able to mate with more had they been available. However, individual male mating capacity varied greatly in both studies; the range in the current study was larger than in the study of Guerra & Kosztarab (1992), range 1–23 and 3–11, respectively. The large variation between individual male mating capacity in the two studies remains unexplained. However, cochineal males have been observed to mate with the same female more than once (Karny, 1972; Guerra & Kosztarab, 1992); therefore, multiple matings with a single female may have obscured the actual mating capacity of an individual male. This, in turn, may be responsible for the variation in male mating capacity observed in the current and previous studies.

As with male mating capacity, male reproductive potential was also high and variable. The high reproductive potential of *D. tomentosus* males can counteract both their short lifespan and any paucity of males within a population of the insect; this supports our contention that numbers of males need not be a limiting factor to population growth. The variation in male reproductive potential may be due to a number of factors. Firstly, this study has shown that the weight and fecundity of gravid females is inversely related to the number that became gravid. This may be due to either fewer fertilized eggs per female or lower egg viability in cases where a high proportion of females become gravid. Secondly, the variability may also relate to selective matings, where a small number of females may have been multiply mated. Multiple-mated females tend to have higher life-time fecundity (Arnqvist & Nilsson, 2000). Thirdly, the variation may also be due to individual differences in female characteristics, such as weight, as this study showed that female fecundity correlated positively with female weight. Fecundity in many insect groups varies with female size (Honek, 1993; Zanuncio *et al*., 2002), and this phenomenon has been reported in other cochineal species (Moran & Cobby, 1979; Volchansky *et al*., 1999). Fourthly, fecundity may also be influenced by other factors, such as egg size (Fox *et al*., 1997), larval developmental conditions (Tamaru *et al*., 1996) and food quality for adults (Leather, 1988; Awmack & Leather, 2002). However, these latter factors could not have

contributed to the observed relationships in this study because: (i) crawlers that were used to initiate experiments were randomly collected from females who themselves were obtained from cultures at random and were all reared under uniform laboratory conditions; (ii) as revealed by results of the current study, the eggs of *D. tomentosus* do not vary widely in size; and (iii) the cladodes that were used in this study were equal in size and age, making food supply uniform. Besides these factors, variation amongst males in their potential to produce offspring may also be caused by individual differences in male genetic and phenotypic characteristics, such as male size, and quantity and quality of reproductive substances transferred by males to females during mating that are known to influence fecundity (Fox & Czesak, 2000).

In conclusion, this paper reports on aspects of the biology of *D. tomentosus* and provides data that can be used for studies on the ecology of this species and for its use in biological control. This study has detailed unique life history, reproductive and morphological characteristics of *D. tomentosus* that differ from its congeners, implying that the biologies of *Dactylopius* spp. are not as similar as has been suggested. It is possible that the remaining, unstudied species may also have other unique biological features, which if studied and documented would enhance the overall understanding of this economically important group of insects.

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