RESEARCH ARTICLE

The host range of four new biotypes of *Dactylopius tomentosus* (Hemiptera: Dactylopiidae) from southern USA and their potential as biological control agents of *Cylindropuntia* spp. (Cactaceae) in Australia: Part II

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ABSTRACT

Eight *Cylindropuntia* species have naturalised in Australia and pose serious economic, environmental and social impacts. The host range of four additional biotypes of *D. tomentosus* from southern USA was investigated. Feeding and development were restricted to the genus *Cylindropuntia*. However, they showed differences in specificity within this genus and some biotypes discriminated between the provenances of *Cylindropuntia rosea* and *Cylindropuntia tunicata*. Efficacy trials were conducted to determine whether populations of each biotype could be sustained on the naturalised *Cylindropuntia* species and if these populations could retard the growth or kill these plants. The ‘acanthocarpa’ biotype offers potential control of *C. rosea* (Lorne Station), while the ‘cylindropuntia sp.’ biotype shows great potential to control *C. rosea* (Grawin). The ‘cylindropuntia sp.’ biotype also had a high impact on *Cylindropuntia kleiniae* and *Cylindropuntia imbricata*, and a moderate impact on *Cylindropuntia leptocaulis* and *Cylindropuntia prolifera*. The ‘acanthocarpa × echinocarpa’ biotype had its greatest impact on *C. tunicata* (Grawin), killing this plant in 18 weeks. A fourth biotype, ‘leptocaulis’, was damaging to some species, but was less effective than the other biotypes. *Cylindropuntia spinosior* is the only naturalised species in Australia where no effective biocontrol agent has been found.

Introduction

The genus *Cylindropuntia* (Engelman) Knuth is native to regions of southwestern and southern USA and Mexico. It comprises 33 species (Anderson, 2001; Benson, 1982; Rebman & Pinkava, 2001), of which 8: *Cylindropuntia fulgida* var. *manillata* (DC.) Backeb.; *Cylindropuntia imbricata* (Haw.) F.M. Knuth; *Cylindropuntia kleiniae* (DC.) F.M. Knuth; *Cylindropuntia leptocaulis* (DC.) F.M. Knuth; *Cylindropuntia prolifera* (Engelm.) F.M. Knuth; *Cylindropuntia rosea* (DC.) Backeb.; *Cylindropuntia spinosior* (Engelm.) F.M. Knuth and *Cylindropuntia tunicata* (Lehm.) F.M. Knuth (Botanic Gardens Trust, 2013; Holtkamp, 2012b) are found...
naturalised in Australia. These eight species are shrubs, ranging in height from 60 cm to 3 m, with succulent segments growing end-to-end and flowers that vary in shades of yellow, pink and purple, forming within or near areoles.

The *Cylindropuntia* species naturalised in Australia pose environmental, agricultural and recreational problems by forming large monospecific stands threatening biodiversity, agricultural productivity and injury to animals due to their spines (Chuk, 2010; Potter, 2011). Reproduction and dispersal in Australia is mainly by vegetative means as the easily detachable segments attach to animals, humans and vehicles. Only some *Cylindropuntia* species can reproduce by seed (Holtkamp, 2012b; Potter, 2011). Current distribution and climate modelling indicates that *Cylindropuntia* spp. have the potential to spread through much of arid and semi-arid Australia (Chuk, 2010; Holtkamp, 2012b).

Biological control of some *Cylindropuntia* spp. using biotypes of *Dactylopius tomentosus* has been successful in Australia (Hosking, McFadyen, & Murray, 1988) and the Republic of South Africa (RSA) (Klein, 2011). *C. imbricata* has been successfully controlled by the ‘imbricata’ biotype while in South Africa, the ‘cholla’ biotype of *D. tomentosus* is controlling both *C. fulgida* var. *fulgida* and *C. fulgida* var. *mamillata* (Sheppard, Day, Grice, & Neser, 2012).

Zimmermann and Granata (2002) and Jones, Holtkamp, Palmer, and Day (2015) found that the host range of a number of biotypes of *D. tomentosus* is restricted to the genus *Cylindropuntia*. Furthermore, Mathenge, Holford, Hoffmann, Zimmermann, et al. (2009), Mathenge et al. (2010a) and Jones et al. (2015) showed that populations of *D. tomentosus* displayed distinct host preferences when collected from different *Cylindropuntia* species within their native ranges. These host-adapted populations are referred to as biotypes and are unable to be recognised as being morphologically different. However, they can be distinguished by feeding preferences and by biological indicators such as development time, fecundity and their feeding impact on different *Cylindropuntia* species (Diehl & Bush, 1984; Jones et al., 2015; Mathenge, Holford, Hoffmann, Zimmermann, et al., 2009; Mathenge et al., 2010a).

Mathenge, Holford, Hoffmann, Zimmermann, et al. (2009), Mathenge et al. (2010a) and Jones et al. (2015) showed that the naturalised *Cylindropuntia* species in Australia and RSA could potentially be controlled by these specialised biotypes of *D. tomentosus*. A biological control program in Australia commenced in 2009 in a concerted effort to control the remaining seven *Cylindropuntia* species in Australia not attacked by the ‘imbricata’ biotype. Recently, the ‘cholla’ biotype was tested (Jones et al., 2015) and was approved for release against *C. fulgida* var *mamillata*. Another four distinct populations of *D. tomentosus* collected from four different *Cylindropuntia* species in southern USA were imported for screening. This paper reports on the host range of these four *D. tomentosus* biotypes collected from southern USA and their potential to control *Cylindropuntia* species in Australia.

**Materials and methods**

**Biotypes**

Surveys were conducted in the USA in November 2012, which coincided late in the season where the presence of natural enemies such as dipteran and coccinellid predators were prevalent. As a result, only four populations of *D. tomentosus* were sourced:
C. leptocaulis (referred to as ‘leptocaulis’ population as it has not yet been confirmed as a distinct biotype) at Search Light Mountains, California (34°49.50’ N, 114°58.20’ W), Cylindropuntia acanthocarpa (‘acanthocarpa’ population) at Windwill Ranch, California (35°08.70’ N, 113°04.00’ W), possible C. acanthocarpa × Cylindropuntia echinocarpa hybrid at Ajo, Arizona (32°24.31’ N, 112°52.18’ W) (‘acanthocarpa × echinocarpa’ population) and from an undetermined Cylindropuntia sp. at Alamagordo, Arizona (32°53.20’ N, 108°58.20’ W) (‘cylindropuntia sp.’ population).

Colony rearing

These four populations of D. tomentosus were imported on field collected cladodes into a quarantine facility at the Ecosciences Precinct, Brisbane, Australia. As the shipment did not contain sufficient clean host plant material to maintain colonies, eggs and first instar nymphs were harvested from the infested cladodes and placed on fresh cladodes of a range of naturalised Cylindropuntia species to establish colonies. To prevent cross contamination between D. tomentosus populations, all newly infested cladodes were placed in separate, sealed, individual plastic food containers and maintained as per Jones et al. (2015). These colonies were held in a constant environment room at 27°C day, 24°C night, 12:12 day:night cycle and 65% RH.

Preliminary studies using the naturalised Cylindropuntia species in Australia were conducted on each of the four D. tomentosus populations and host plants were selected based on the development success during these trials. The ‘leptocaulis’ and ‘cylindropuntia sp.’ populations were reared on C. kleiniae, while the ‘acanthocarpa’ and ‘acanthocarpa × echinocarpa’ populations were reared on C. imbricata.

Individual D. tomentosus cultures were maintained every fortnight by transferring one fecund female and her associated egg mass to a fresh host cladode of the respective host and placing in a sealed individual plastic food container. Each container was labelled with the biotype and date of exposure.

Host-specificity testing

As the testing procedure of the earlier biotypes of D. tomentosus had already been developed, the host specificity of these new biotypes was determined using the same test list (Table 1) and protocols as in Jones et al. (2015). As in Jones et al. (2015), host specificity of the four populations of D. tomentosus (‘leptocaulis’, ‘acanthocarpa’, ‘acanthocarpa × echinocarpa’ and ‘cylindropuntia sp.’) was determined using no-choice nympha survival studies. Twenty neonate nymphs, less than 24 hours old, were transferred onto cladodes of each of the test plants and kept in separate sealed plastic containers as for the culture maintenance. The four test plant species not within the Cactaceae family were tested as whole plants, as the morphology of these plants was not appropriate for this testing regime. Each batch of plants tested was accompanied by a control, where 20 neonate nymphs were placed onto a cladode of the same species on which the population was maintained. Each test plant species was tested five times (not concurrently) for each of the four D. tomentosus populations, using a fresh cladode or plant on each occasion.
Cladodes were examined three times per week. The number of nymphs and the number of days after transfer for them to settle at a feeding site was recorded. Nymphal survival, the time to each change of instar (first, second, pupal case construction for males) and third instar change (pre-oviposition for females) (Jones et al., 2015) were also recorded. Development to adult stage, date of male emergence and the date first egg was laid by each female were recorded. Female maturity was defined at the pre-ovipositional stage, as D. tomentosus is not parthenogenic (Mathenge, Holford, Hoffmann, Spooner-Hart, et al., 2009) and some host tests did not produce males. Therefore, development success was defined as the number of individuals reaching maturity (male emergence and pre-oviposition stage for females). The differences of each of the four populations of D. tomentosus in the mean settling rate, mean number of individuals changing to second instar and mean development success between test species and their control were assessed by a one-way ANOVA.

**Efficacy trials**

The efficacy trials were basic time-efficient trials conducted as a complementary test to the host-specificity trials and followed the same procedure outlined by Jones et al. (2015). The trials had two aims: (a) determine which of the four D. tomentosus populations could be sustained as colonies on the screened Cylindropuntia species and (b) determine which of these Cylindropuntia species were the most susceptible and if the sustained feeding activity of a D. tomentosus colony can reduce the vigour or kill any of the target Cylindropuntia species.

Efficacy trials were set up on the Cylindropuntia species which supported the development to maturity of an average of four or more individuals in the host-specificity trials. The ‘leptocaulis’ and ‘cylindropuntia sp.’ populations were each tested against seven species while the ‘acanthocarpa’ and the ‘acanthocarpa × echinocarpa’ populations were

| Table 1. Plants tested to determine host specificity of the four D. tomentosus biotypes |
|-----------------------------------------------|-----------------------------------------------|
| Family | Genus/species | Common name |
| Cactaceae | C. fulgida var. mamillata (DC.) Backeb. | boxing glove cactus |
| | C. imbricata (Haw.) F.M. Knuth | rope pear |
| | C. kleiniae (DC.) F.M. Knuth | candle cactus |
| | C. leptocaulis (DC.) F.M. Knuth | pencil cactus |
| | C. prolifera (Englem.) F.M. Knuth | jumping cactus |
| | C. rosea (DC.) Backeb. | Hudson pear |
| | Mexico | |
| | Spain | |
| | Grawin (NSW, Aus) | |
| | Lorne Station (NSW, Aus) | |
| | C. spinosior (Englem.) F.M. Knuth | snake cactus |
| | C. tunicata (Lehm.) F.M. Knuth | Hudson pear |
| | Cracow (Qld, Aus) | |
| | Grawin (NSW, Aus) | |
| | Hylocereus undatus (Haw.) Britton & Rose | dragon fruit |
| | Mammillaria elongata DC. | ladyfinger cactus |
| | Opuntia aurantiaca Lindl. | tiger pear |
| | Opuntia ficus-indica (L.) Jacq. | 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 |
screened against six plant species. Table 2 lists the species tested against each of the 
*D. tomentosus* populations in these trials.

One fecund female and her associated egg mass (up to 17 d in age) was collected from 
the maintenance colony and placed on a well-established healthy potted test plant, free of 
pests and diseases, for each of the test species and placed in an organza-mesh screened 
cage. Eggs were left to incubate and the nymphs were allowed to settle at feeding sites 
and develop to maturity.

The number of nymphs emerging, attaching to a feeding site (settling rate) and their 
developmental stage was monitored every fortnight for each test plant. Photographic ana-
lyses of nymph development and subsequent effect on the plants were also conducted 
every fortnight until the plant died from the infestation or until the insect colony died; 
the latter suggesting the plant was an unsuitable host. Plant death was defined as the 
point when the main trunk and root system could not support any further growth.

Colony establishment was defined when 50 or more second generation nymphs were 
settled at feeding sites, as the colony is characterised by fecund first generation females 
producing viable nymphs that have found suitable feeding sites and have commenced 
their own progression towards maturity. This definition was based on studies by Zimmer-
mann (2007) and Mathenge, Holford, Hoffmann, Spooner-Hart, et al. (2009) stating that 
an individual ovipositing female can produce between 72 and 338 progeny and that devel-
opment success can be as high as 80% for *D. tomentosus* nymphs when reared on its 
natural or a suitable host. Once this baseline number had been reached, the population 
increased rapidly.

**Results**

**Host-specificity testing**

All four *D. tomentosus* populations had a host range restricted to the genus *Cylindropuntia*. No nymphs of any of the four populations survived past the first instar when placed on 
species outside this genus. However, the specificity and development success of the four 
populations varied within the *Cylindropuntia*.

Nymphs of the four populations settled at feeding sites (Figure 1) and progressed to the 
second instar on all the naturalised *Cylindropuntia* species in Australia. However, the
number of nymphs that progressed to second instar was markedly lower on C. leptocaulis and C. spinosior (Figure 2). The nymphs of the ‘leptocaulis’ and ‘acanthocarpa × echinocarpa’ populations failed to reach maturity on C. leptocaulis (Figure 3). All four D. tomentosus populations displayed very poor development success on C. spinosior.

The ‘acanthocarpa’ population had its highest mean number of individuals reaching maturity on C. rosea (Lorne Station) (11.6 ± 1.6), which was significantly greater than the control, C. imbricata (7.7 ± 0.6) ($F_{10,70} = 4.09, P < .001, n = 81$). It also performed well on C. rosea (Grawin) (9.4 ± 1.1), C. tunicata (Cracow) (9.2 ± 1.6) and C. tunicata (Grawin) (7.2 ± 1.7), which were also deemed suitable hosts as development success was no different to the control plant, C. imbricata (Figure 3).

The ‘acanthocarpa × echinocarpa’ population had its highest mean developmental success on C. fulgida (10.4 ± 1.0) and C. imbricata (9.2 ± 0.6), which was significantly greater than its development on the remaining species ($F_{10,66} = 8.38, P < .001, n = 77$). There was a low proportion of nymphs reaching the second instar on C. leptocaulis and C. spinosior and consequently low development success rate to maturity (Figure 2).

The ‘cylindropuntia sp.’ population had a relatively lower development success on all Cylindropuntia species. The preferred hosts for the ‘cylindropuntia sp.’ population were C. leptocaulis (6.6 ± 2.1) and C. kleiniae (6.6 ± 0.9), which were significantly greater than C. prolifera (1.6 ± 0.8), C. rosea (Mexico) (0.8 ± 0.4) and C. spinosior (0.2 ± 0.2) ($F_{10,67} = 2.38, P < .018, n = 78$) (Figure 3).

The ‘leptocaulis’ population development success was significantly greater on C. kleiniae (6.7 ± 0.6) and C. prolifera (6.2 ± 2.0) than the other species of Cylindropuntia ($F_{10,70} = 4.72, P < .001, n = 81$). The ‘leptocaulis’ population on C. leptocaulis and
Figure 2. Percentage of individuals of each of the four *D. tomentosus* biotypes reaching the second instar during the host-specificity trials. *C. kleiniae* is the control for the ‘leptocaulis’ and ‘cylindropuntia sp.’ biotypes, while *C. imbricata* is the control for the ‘acanthocarpa’ and ‘acanthocarpa × echinocarpa’ biotypes.

Figure 3. Percentage development success of 20 neonate nymphs of each of the four *D. tomentosus* biotypes placed on eight naturalised *Cylindropuntia* plant species in Australia during the host-specificity trials. *C. kleiniae* is the control for the ‘leptocaulis’ and ‘cylindropuntia sp.’ biotypes, while *C. imbricata* is the control for the ‘acanthocarpa’ and ‘acanthocarpa × echinocarpa’ biotypes.
C. spinosior had a significantly lower proportion of nymphs reaching the second instar \( (F_{10,70} = 7.86, P < .001, n = 81) \) (Figure 2).

When reared on their respective control species, the proportion of pre-ovipositional females mated to become fecund was significantly lower for the ‘acanthocarpa’ population \( (F_{3,114} = 6.85, P < .001, n = 81) \) (Figure 2) and ‘leptocaulis’ population \( (F_{3,114} = 5.52, P < .002, n = 118) \). In contrast, there was no difference in the number of males to emerge between the D. tomentosus populations \( (F_{3,114} = 0.57, P < .634, n = 118) \).

When results for all the Cylindropuntia species were pooled, the settling rates of nymphs at suitable feeding sites was not significantly different between the populations of D. tomentosus \( (F_{3,313} = 1.49, P < .218, n = 317) \) (Figure 1 and Table 3). However, the number of nymphs of the ‘leptocaulis’ population reaching the second instar was significantly less than the remaining D. tomentosus populations \( (F_{3,313} = 4.14, P < .007, n = 317) \) (Figure 2 and Table 3). The number of nymphs developing to maturity was also significantly different between the D. tomentosus populations \( (F_{3,313} = 8.07, P < .001, n = 317) \) (Figure 3 and Table 3). The ‘acanthocarpa’ population and the ‘acanthocarpa × echinocarpa’ population showed higher mean development success than the ‘cylindropuntia sp.’ and ‘leptocaulis’ populations (Table 3).

**Efficacy trials**

Overall, the ‘acanthocarpa × echinocarpa’ population colonies performed better in respect to the demographic performance indicators than the other three D. tomentosus populations. The ‘acanthocarpa × echinocarpa’ population had high settling rates (>50 nymphs) of first generation nymphs and second generation nymphs (≥500) on more Cylindropuntia species than any other population (Tables 4 and 5). Time to colony establishment was also much faster for the ‘acanthocarpa × echinocarpa’ population than the other three D. tomentosus populations (Table 6). Although the ‘acanthocarpa × echinocarpa’ population rated higher on demographic performance indicators than all other D. tomentosus populations, the ‘cylindropuntia sp.’ population had the greatest impact of the four biotypes on the Cylindropuntia species tested (Tables 7 and 8).

**‘Acanthocarpa’ biotype**

The ‘acanthocarpa’ population had moderate to high settling rates of first generation nymphs after two weeks on all Cylindropuntia species tested (Table 4). Most colonies were characterised by a moderate number (100–200) of second generation nymphs.

**Table 3.** Mean number of nymphs to reach maturity in the host-specificity trials for each of the four D. tomentosus biotypes when all Cylindropuntia species were pooled.

| Development stage | 'leptocaulis' | 'acanthocarpa' | 'acanthocarpa × echinocarpa' | 'cylindropuntia spp.' |
|-------------------|--------------|---------------|----------------------------|-----------------------|
| Settling rate     | 11.3 ± 0.41\(^a\) | 12.1 ± 0.37\(^a\) | 12.4 ± 0.37\(^a\) | 12.0 ± 0.39\(^a\) |
| Second instar     | 7.6 ± 0.47\(^b\) | 9.7 ± 0.45\(^a\) | 9.2 ± 0.48\(^a\) | 9.3 ± 0.48\(^a\) |
| Adult stage       | 4.6 ± 0.43\(^b\) | 7.2 ± 0.46\(^a\) | 6.7 ± 0.48\(^a\) | 4.8 ± 0.47\(^b\) |

Note: Different superscripts signify that the means are significantly different.
settled at feeding sites and all were established by week 18 (Tables 5 and 6). The ‘acanthocarpa’ population did not establish on \( C. \) kleiniae. The ‘acanthocarpa’ population established colonies of second generation nymphs on \( C. \) imbricata (500), \( C. \) leptocaulis (200), \( C. \) rosea (Grawin) (200) and \( C. \) rosea (Lorne Station) (200). This \( D. \) tomentosus population caused the death of \( C. \) imbricata, \( C. \) rosea (Grawin) and \( C. \) rosea (Lorne Station) within 28 weeks (Table 7, Supplement Figure 5). \( C. \) tunicata (Cracow) and \( C. \) tunicata (Grawin) plants were killed within 52 weeks. The ‘acanthocarpa’ population colonies that established on \( C. \) fulgida and \( C. \) leptocaulis had limited impact on the plants, which subsequently survived the prolonged feeding activity.

‘Acanthocarpa × echinocarpa’ biotype

The ‘acanthocarpa × echinocarpa’ population displayed very high settling rates of greater than 50 nymphs on most of the \( Cylindropuntia \) species tested except for the two Australian \( C. \) rosea provenances and \( C. \) prolifera (Table 4). The ‘acanthocarpa × echinocarpa’ population had the shortest time to colony establishment, taking only 8 weeks to establish on four species and 10 weeks on the other two species tested (Table 6). On \( C. \) prolifera, no nymphs settled at feeding sites and a population did not establish. The number of second generation nymphs settled at feeding sites was high (500–1000 nymphs) for

Table 4. Number of first generation crawlers of each of the four \( D. \) tomentosus biotypes that settled at feeding sites two weeks after inoculation on the screened \( Cylindropuntia \) species during the efficacy trials.

| \( Cylindropuntia \) spp. | \( D. \) tomentosus biotype | \( Cylindropuntia \) spp. |
|---------------------------|-----------------------------|---------------------------|
|                           | leptoacaulis | acanthocarpa | acanthocarpa × echinocarpa | cylindropuntia spp. |
| \( C. \) fulgida           | 29           | 36           | >50                       | 40 |
| \( C. \) imbricata         | 16           | 53           | >50                       | >50 |
| \( C. \) kleiniae          | >50          | 49           | >50                       | 40 |
| \( C. \) leptocaulis       | –            | 33           | –                         | 19 |
| \( C. \) prolifera         | 12           | –            | 0                         | 30 |
| \( C. \) rosea (Grawin)    | >50          | 38           | 25                        | 40 |
| \( C. \) rosea (Lorne Station) | 12         | 58           | 7                         | 21 |
| \( C. \) spinosior         | –            | –            | –                         | – |
| \( C. \) tunicata (Cracow) | 14           | 44           | >50                       | 50 |
| \( C. \) tunicata (Grawin) | 9            | 62           | >50                       | >50 |

–, not tested.

Table 5. Number of second generation crawlers of each of the four \( D. \) tomentosus biotypes that emerged and settled at feeding sites on the screened \( Cylindropuntia \) spp. during the efficacy trials.

| \( Cylindropuntia \) spp. | \( D. \) tomentosus biotype | \( Cylindropuntia \) spp. |
|---------------------------|-----------------------------|---------------------------|
|                           | leptoacaulis | acanthocarpa | acanthocarpa × echinocarpa | cylindropuntia spp. |
| \( C. \) fulgida           | 8            | <50          | 1000                      | DNE |
| \( C. \) imbricata         | 500          | 500          | 1000                      | 1000 |
| \( C. \) kleiniae          | 100          | <50          | 200                       | 200 |
| \( C. \) leptocaulis       | –            | 200          | –                         | 100 |
| \( C. \) prolifera         | 50           | –            | 0                         | 100 |
| \( C. \) rosea (Grawin)    | 100          | 200          | 1000                      | 200 |
| \( C. \) rosea (Lorne Station) | 20        | 200          | 100                       | 50 |
| \( C. \) spinosior         | –            | –            | –                         | – |
| \( C. \) tunicata (Cracow) | 0            | 100          | 500                       | 100 |
| \( C. \) tunicata (Grawin) | 100          | 100          | 200                       | 100 |

–, not tested.
four of the six Cylindropuntia species tested (Table 5). The colonies that established on C. fulgida, C. imbricata and C. rosea (Grawin) had up to 1000 second generation nymphs feeding and developing (Table 5).

The ‘acanthocarpa × echinocarpa’ population had a high impact on all the plant species tested except for C. prolifera (Table 7). Plant death was recorded within 24 weeks for C. fulgida, C. imbricata and C. tunicata (Grawin) due to the sustained feeding of the colonies. The colonies of the ‘acanthocarpa × echinocarpa’ established on C. kleiniae, C. rosea (Lorne Station), C. rosea (Grawin) and C. tunicata (Cracow) killed the plants within 40 weeks (Supplement Figure 4).

'Cylindropuntia sp.' biotype
The ‘cylindropuntia sp.’ population displayed moderate to high settling rates for all the Cylindropuntia species tested except for C. leptocaulis and C. rosea (Lorne Station) (Table 4). Time to colony establishment for the ‘cylindropuntia sp.’ population was 14 weeks for most species. The exceptions were C. imbricata and C. rosea (Grawin), which took only eight weeks for colony establishment (Table 6). The ‘cylindropuntia sp.’ population had established colonies that were characterised by a moderate number (100–200) of second generation nymphs settled at feeding sites (Table 5). The exception was the colony on C. imbricata (1000 nymphs). The ‘cylindropuntia sp.’ population had a high

Table 6. Time taken for colony establishment of each of the four D. tomentosus biotypes on the screened Cylindropuntia species during the efficacy trials.

| Cylindropuntia spp. | D. tomentosus biotype | Cylindropuntia spp. |
|---------------------|-----------------------|---------------------|
|                      | leptocaulis | acanthocarpa | acanthocarpa × echinocarpa | Cylindropuntia spp. |
| C. fulgida          | DNE        | 24           | 8                         | DNE                  |
| C. imbricata        | 16         | 14           | 10                        | 8                    |
| C. kleiniae         | 18         | DNE          | 8                         | 14                   |
| C. leptocaulis      | 16         | 16           | DNE                       | 14                   |
| C. prolifera        | 16         | –            | DNE                       | 14                   |
| C. rosea (Grawin)   | 16         | 18           | 8                         | 8                    |
| C. rosea (Lorne Station) | DNE | 16         | 10                        | 14                   |
| C. spinosior        | –          | –            | –                         | –                    |
| C. tunicata (Cracow)| 36         | 18           | 8                         | 14                   |
| C. tunicata (Grawin)| 16         | 16           | 8                         | 14                   |

–, not tested; DNE, did not establish.

Table 7. The fate of the biotype colony and the test plant and the time taken in weeks during efficacy trial.

| Cylindropuntia spp. | D. tomentosus biotype | Cylindropuntia spp. |
|---------------------|-----------------------|---------------------|
|                      | leptocaulis | acanthocarpa | acanthocarpa × echinocarpa | Cylindropuntia spp. |
| C. fulgida          | Colony dead 22 | Plant dead 60 | Plant dead 24 | Colony dead 12 |
| C. imbricata        | Plant dead 26 | Plant dead 26 | Plant dead 22 | Plant dead 14 |
| C. kleiniae         | Plant survive | Colony dead 24 | Plant dead 32 | Plant dead 18 |
| C. leptocaulis      | –          | Plant dead 60 | –              | Plant dead 28 |
| C. prolifera        | Plant survive | –              | Colony dead 6  | Plant dead 36 |
| C. rosea (Grawin)   | Plant dead 24 | Plant dead 28 | Plant dead 40 | Plant dead 12 |
| C. rosea (Lorne Station) | Colony dead 32 | Plant dead 28 | Plant dead 40 | Plant survive |
| C. spinosior        | –          | –              | –              | –                    |
| C. tunicata (Cracow)| Plant survive | Plant dead 32 | Plant dead 32 | Plant dead 42 |
| C. tunicata (Grawin)| Plant dead 50 | Plant dead 34 | Plant dead 18 | Plant dead 20 |

–, not tested.
impact on four of the seven plant species tested. These included *C. rosea* (Grawin) which died within 12 weeks, *C. imbricata* (14 weeks), *C. kleiniae* (18 weeks) and *C. tunicata* (Grawin) (20 weeks). The impact on the other three *Cylindropuntia* species, *C. leptocaulis*, *C. prolifera* and *C. tunicata* Cracow, was slightly protracted, but all these plants died within 52 weeks (Table 7, Supplement Figure 6).

### ‘Leptocaulis’ biotype

The settling rates of first generation nymphs for the ‘leptocaulis’ population was generally low, except on *C. kleiniae* and *C. rosea* (Grawin) which had settling rates greater than 50 nymphs (Table 4). The time to colony establishment was 16–18 weeks (Table 6) and these colonies were characterised by low settling rates of second generation nymphs (Table 5). The sustained feeding of the ‘leptocaulis’ population colonies killed *C. rosea* (Grawin) within 24 weeks, *C. imbricata* (26 weeks) and *C. tunicata* (Grawin) (50 weeks) (Table 7, Supplement Figure 7). *C. fulgida*, *C. kleiniae* and *C. rosea* (Lorne Station) plants survived when inoculated with the ‘leptocaulis’ population.

An index of the impact of each biotype on the *Cylindropuntia* species tested in the efficacy trials is summarised in Table 8. The ‘cylindropuntia sp.’ population caused substantial impacts in the efficacy trials (where the plant died within 20 weeks of inoculation) on *C. imbricata*, *C. kleiniae*, *C. rosea* and *C. tunicata* (Grawin). The ‘acanthocarpa × echinocarpa’ population also caused a substantial impact on *C. tunicata* (Grawin). A moderate to high impact (plants died after 20 weeks) was recorded by at least one of the four *D. tomentosus* populations on *C. fulgida*, *C. leptocaulis*, *C. prolifera*, *C. rosea* (Lorne Station) and *C. tunicata* (Cracow). Of the eight naturalised *Cylindropuntia* spp. in Australia, *C. spinosior* is the only species that was not impacted by any of the four *D. tomentosus* populations.

### Discussion

The host range of all four populations of *D. tomentosus* was found to be restricted to the genus *Cylindropuntia* and supports earlier findings (Jones et al., 2015; Mathenge, Holford, Hoffmann, Spooner-Hart, et al., 2009; Mathenge, Holford, Hoffmann,
Zimmermann, et al., 2009; Zimmermann & Granata, 2002) that the species is host specific to the genus *Cylindropuntia*. The four populations of *D. tomentosus* showed significant differences in their development success and host impact, which are also consistent with previous studies (Jones et al., 2015; Mathenge, Holford, Hoffmann, Zimmermann, et al., 2009; Mathenge et al., 2010a; Zimmermann & Granata, 2002). The results support the recognition of biotypes within *D. tomentosus*, including these four populations.

The efficacy trials were simple, time-efficient evaluations of the impact of the four biotypes of *D. tomentosus* and were complementary to the host-specificity trials. Both the time taken for second generation nymphs to emerge and the proportion of these nymphs to settle at feeding sites were important indicators of effectiveness. Their effect on the health of the plant was also a sensitive measure of efficacy. This was most marked for the ‘cylindropuntia sp.’ biotype colonies on *C. kleiniae* and *C. rosea* (Grawin), where the host plant died only four weeks after the colony was established, while the *C. tunicata* (Grawin) and *C. imbricata* host plants both died only six weeks after colony establishment. These colonies were characterised by hundreds of second generation nymphs feeding on the host plant, with 1000 second generation nymphs on *C. imbricata*. Their feeding activity caused plant chlorosis and plant tissue necrosis until the plant could not support any further growth.

Surprisingly, this was not the outcome for the ‘acanthocarpa × echinocarpa’ biotype colonies on *C. fulgida*, *C. imbricata* and *C. rosea* (Grawin). These colonies were fast to establish (8–10 weeks) and were characterised by a population of up to 1000 second generation nymphs. Rather than a very quick and major impact on the health of the plant, it took another 12 weeks for the *C. imbricata* plant, 16 weeks for the *C. fulgida* plant and 32 weeks for the *C. rosea* (Grawin) plant to die.

Compared to the ‘acanthocarpa × echinocarpa’ biotype, the ‘cylindropuntia sp.’ biotype had a greater impact on their host plants relative to the number of second generation nymphs developing and feeding on the plant. These results and those of Mathenge, Holford, Hoffmann, Zimmermann, et al. (2009) give credence to the ‘new association’ hypothesis (Hokkanen & Pimentel, 1984), which states that a plant species that has long been associated with an insect will have developed effective defence mechanisms, but these mechanisms may not be as effective against a new introduced insect, such as a different biotype.

A surprising result from the efficacy trials was the fate of the biotype colonies and plants used in the trials for the two *C. rosea* and the two *C. tunicata* provenances (Table 7). The impact on the plants by the sustained feeding of the ‘leptocaulis’ and ‘cylindropuntia sp.’ biotypes differed between the two *C. rosea* provenances. The ‘leptocaulis’ biotype colony killed the *C. rosea* (Grawin) plant by week 24, whereas the *C. rosea* (Lorne Station) plant did not support a colony of the ‘leptocaulis’ biotype and this colony died in week 32. The same occurred for the ‘cylindropuntia sp.’ colonies: the *C. rosea* (Grawin) plant died in week 12, but the *C. rosea* (Lorne Station) plant survived more than 52 weeks, with a persisting ‘cylindropuntia sp.’ colony. These two *C. rosea* infestations are separated by only 35 km and have been identified as *C. rosea* by recognised taxonomists. The disparity of the biotypes’ impact on the *C. rosea* provenances will have critical implications for the success of *C. rosea* control in the field.
The impact of sustained feeding by the ‘acanthocarpa × echinocarpa’ biotypes on C. tunicata also differed between the two provenances. The ‘acanthocarpa × echinocarpa’ biotype killed the Grawin provenance of C. tunicata in 18 weeks, but it took 32 weeks to kill the Cracow provenance. Similarly, sustained feeding by the ‘cylindropuntia sp.’ biotype killed the Grawin provenance in 20 weeks and the Cracow provenance in 40 weeks. Cactus species are known to display high levels of variability (Rebman & Pinkava, 2001) and the slight differences in these provenances may have been enough to influence the performance of these biotypes.

The population response of D. tomentosus biotypes on their Cylindropuntia hosts is summarised in three categories by Mathenge, Holford, Hoffmann, Zimmermann, et al. (2009): (i) thrive on their host, that is, the insects develop quickly and are highly fecund, (ii) survive on their host, that is, insects are slower to develop and less fecund and (iii) those dying on their host, that is, no insects develop to maturity. Results from the efficacy trials, in particular the time for colony establishment and the abundance of second generation nymphs settling at a feeding site, can be used to classify each of the D. tomentosus biotypes on each of the Cylindropuntia species screened into one of the three population response categories.

The ‘acanthocarpa’ biotype thrived on three species; C. imbricata, C. rosea and C. tunicata plants were killed within a year and appears to be a suitable candidate to control C. rosea in Australia. The impact of this biotype on these three species supports the findings in the host-specificity trials where the developmental success was highest for the same three species. The biotype would be categorised as surviving on C. fulgida and C. leptocaulis and dying on C. kleiniae, C. prolifera and C. spinosior.

The ‘acanthocarpa × echinocarpa’ biotype thrived on C. fulgida, C. imbricata, C. kleiniae, C. rosea and C. tunicata, while the biotype died out on C. leptocaulis, C. prolifera and C. spinosior. The development success of the ‘acanthocarpa × echinocarpa’ biotype in host-specificity trials was greatest on C. fulgida, C. imbricata, C. kleiniae and C. tunicata. These results indicated the anticipated impacts of an established colony of this biotype on these Cylindropuntia species during the efficacy trials. This biotype had a very high impact on C. tunicata, particularly the Grawin provenance (Tables 7 and 8), indicating it has great potential to control this species.

The ‘cylindropuntia sp.’ biotype had a substantial impact on C. imbricata, C. kleiniae, C. rosea (Grawin) and C. tunicata (Grawin) and shows great potential to control these species in the field, as it killed these plants in under 20 weeks in the efficacy trials. However, the development success of the ‘cylindropuntia sp.’ biotype in the host-specificity trials was relatively low on all Cylindropuntia species and showed little difference between species except for C. spinosior and C. prolifera. This biotype also thrived on two other species; C. leptocaulis and C. prolifera but the impact was not as effective.

Populations of the ‘leptocaulis’ biotype thrived on three species, C. imbricata, C. rosea (Grawin) and C. tunicata (Grawin), killing plants within a year (Tables 7 and 8). The remaining two Cylindropuntia species, C. kleiniae and C. prolifera supported colonies for one year but the plants survived. However, the development success of the ‘leptocaulis’ biotype was highest on C. kleiniae and C. prolifera in the host-specificity trials. These results were not reflected by the impact of established colonies on these species in the efficacy trials, as plants were able to survive for greater than a year.
The variability in performance of the biotypes on each of the *Cylindropuntia* species shows that there is a need to match the most effective *D. tomentosus* biotype to each of the naturalised *Cylindropuntia* species in Australia. However, for some biotypes, it is not apparently clear which is the most effective *D. tomentosus* biotype for each of the *Cylindropuntia* species, particularly for the provenances of *C. rosea* and *C. tunicata*. Therefore, additional tests similar to the fitness index trials conducted by Mathenge, Holford, Hoffmann, Zimmermann, et al. (2009) may be required to distinguish the effectiveness of the biotypes on each of the *Cylindropuntia* species.

Cactus infestations in Australia are not monocultures of one *Cylindropuntia* species but a variety of *Cylindropuntia* species within close proximity of each other. Therefore, more than one *D. tomentosus* biotype may be needed to control such infestations. One issue that needs to be addressed in the future is the effect interbreeding between the biotypes will have on the host specificity and virulence of any hybrids. Mathenge et al. (2010b) conducted a hybridisation study between two biotypes in RSA; one that controls *C. imbricata* and the other that controls *C. fulgida*. Their results showed both hybrids clearly outperformed the parents and concluded that hybridisation between the biotypes will not have any detrimental effect on the control of the weeds.

This study confirms that *D. tomentosus* is host specific to the *Cylindropuntia* and that different biotypes can be used to effectively control different species within the *Cylindropuntia* in Australia. Most *Cylindropuntia* species in Australia appear to be attacked by at least one of the four biotypes. However, a suitable biotype to control *C. spinosior* has not yet been found.

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