Targeting biotypes of *Dactylopius tomentosus* to improve effective biocontrol of *Cylindropuntia* spp. in Australia

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Summary Seven *Dactylopius tomentosus* (Lamarck) biotypes were collected from a range of *Cylindropuntia* spp. in Mexico, South Africa and United States of America (USA) and imported into quarantine facilities at the Ecosciences Precinct. Host range trials were conducted for each biotype and further assessed against the *Cylindropuntia* species that are naturalised in Australia to determine the most effective biotype for each species. Host range was confined to the *Cylindropuntia* for all seven biotypes. In the efficacy trials, *C. imbricata* (Haw.) F.M.Knuth was killed by the 'imbricata' biotype within 16 weeks and *C. kleiniae* (DC.) F.M.Knuth died within 26 weeks. *Cylindropuntia fulgida* var. *mamillata* (DC.) Backeb. and *C. imbricata* were killed by the 'fulgida' biotype within 18 weeks. On-going trials suggest that *C. rosea* (DC.) Backeb. could be controlled by either the 'acanthocarpa' or the 'acanthocarpa × echinocarpa' biotypes. *Cylindropuntia spinosior* (Englem.) F.M.Knuth was not susceptible to any of the *D. tomentosus* biotypes assessed. A clear designation of which *D. tomentosus* biotype is most suited for each *Cylindropuntia* species will improve and increase the effectiveness of biological control of these weed species.

Keywords Biotype, efficacy trial, biological control.

INTRODUCTION

The genus *Cylindropuntia* (Cactaceae) occurs naturally from south-western and southern USA to Mexico. The genus comprises 33 species, including six varieties and nine known hybrids (Rebman and Pinkava 2001). Eight *Cylindropuntia* spp. (Table 1) are naturalised in Australia (Holtkamp 2012).

All species have serious agricultural, environmental and recreational impacts. Spines of some species are capable of penetrating car tyres and can cause serious injury to stock, contaminate wool and hides and hinder mustering activities. The Australian rangelands are particularly vulnerable to cacti infestations and in some cases the cost of control is greater than the value of the infested land (Chuk 2010).

Control strategies are mainly by herbicide application and physical removal. The latter is successful with small infestations and isolated plants. However, these removed plants also require correct disposal by burning or burial to ensure new infestations do not occur.

Biological control of *Cylindropuntia* spp. is a cost effective and successful control strategy used in the Republic of South Africa and Australia. *Dactylopius tomentosus* ('imbricata' biotype) was imported into Australia in 1925, as a biocontrol agent for *C. imbricata*. It is now widespread throughout areas where *C. imbricata* is present and is assisting in its control (Hosking *et al*. 1988).

Recent studies (Mathenge *et al*. 2009) have shown that host specialisation occurs within *D. tomentosus* to form biotypes. These host-adapted biotypes show clear differences in their impact and life strategies on different *Cylindropuntia* hosts and could potentially be used to target other *Cylindropuntia* species in Australia.

This paper reports on the efficacy and host specificity of seven biotypes of *D. tomentosus* in controlling the eight *Cylindropuntia* spp. naturalised in Australia and attempts to match the most suitable biotype with each *Cylindropuntia* species.

MATERIALS AND METHODS

Seven biotypes of *D. tomentosus* (biotype in brackets) were collected from a range of *Cylindropuntia* spp. including: *C. imbricata* ('imbricata'), *C. fulgida* ('fulgida'), *C. rosea* ('rosea'), *C. leptocaulis* (DC.) F.M.Knuth ('leptocaulis'), *C. acanthocarpa* (Engelm. & Bigelow) F.M.Knuth ('acanthocarpa'), *C. acanthocarpa* × *echinocarpa* (Engelm. & J.M.Bigelow) ('acanthocarpa × echinocarpa') and *Cylindropuntia* spp. ('cylindropuntia spp.') from sites in northern Mexico, south-western USA and South Africa. These biotypes of *D. tomentosus* were imported into a quarantine facility at the Ecosciences Precinct, Brisbane, and cultured in a controlled environmental room at 26°C and 65% RH. A two-phase evaluation regime (larval host specificity trials and efficacy trials) was employed to determine the ideal biotype for each of the eight *Cylindropuntia* species.

Host-specificity trials A shortened host test list was adopted as *D. tomentosus* 'imbricata' biotype has already been approved for release in 1925. Host specificity was determined by testing 17 plant species from three families, all within the order Caryophylles using no-choice larval survival studies. For each of the seven biotypes of *D. tomentosus*, 20 neonate crawlers were transferred directly to a cladode of each of the test plants. The inoculated cladodes were then contained in separate sealed plastic containers and held in a constant temperature room. Each replicate was accompanied by a control test, where 20 neonate crawlers were transferred to a cladode on which the biotype was cultured. Each test species was tested five times against each of the seven biotypes, using a fresh cladode on each occasion.

Crawlers were allowed to develop to the adult stage. Percent survival, date of male emergence and date of first egg lay by each female were recorded.

Efficacy trials Efficacy trials were conducted to determine the extent to which biotypes of *D. tomentosus* reduced the vigour of the host plants and if the plant was killed. Only those plant species that supported the development of at least an average of four or more *D. tomentosus* individuals to maturity in the host specificity trials were used in these trials. All *Cylindropuntia* spp. were represented in the efficacy trials with at least one *D. tomentosus* biotype.

One fecund female and her associated egg mass were transferred from the host plant rearing colony to a growing plant of each of the test species. Eggs were left to incubate and the emerging 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 (settling rate) and their development over time were recorded. The subsequent feeding damage to the plant was also recorded.

The progression towards colony establishment on each host plant was assessed by monitoring the number of individuals and their developmental stage over time. The definition of colony establishment was an arbitrary classification based on two indicators; number of fecund females present and the number of first generation crawlers settled at suitable feeding sites. Colony establishment was proposed when 10 or more fecund females were present and/or 50 or more first generation crawlers were settled at feeding sites. This classification is based on the results of Zimmerman (2007) and Mathenge *et al.* (2009a) who reported 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.

RESULTS

Host specificity trials Host range was confined to the *Cylindropuntia* for all seven biotypes of *D. tomentosus* as no crawlers advanced to the second instar when reared on plant species outside of this genus. However, these host-adapted biotypes displayed clear differences in their impact on the range of *Cylindropuntia* spp. tested (Table 1).

Table 1. Percentage development success of 20 neonate crawlers of each of the seven *D. tomentosus* biotypes placed on to eight naturalised *Cylindropuntia* species in Australia.

	Dactylopius tomentosus biotypes tested						
Cylindropuntia species	imb	ros	ful	lept	acan	acan × echin	cyl
C. fulgida var. *mamillata*	12	22	68	9	44	52	29
C. imbricata	21	30	46	34	41	51	25
C. kleiniae	35	47	37	29	35	38	25
C. leptocaulis	9	0	13	0	24	0	25
C. prolifera	0	0	3	31	14	27	9
C. rosea (Grawin)	30	0	0	19	47	24	22
C. rosea (Lorne Station)	27	0	0	37	58	20	26
C. rosea (Mexico)	23	32	43	7	40	27	5
C. rosea (Spain)*	34	0	na	na	na	na	na
C. spinosior	1	8	11	1	5	1	2
C. tunicata (Cracow)	4	0	42	15	46	37	26
C. tunicata (Grawin)	2	0	38	23	36	40	25

imb = 'imbricata', ros = 'rosea', ful = 'fulgida', lept = 'leptocaulis', acan = 'acanthocarpa', acan × echin = 'acanthocarpa × echinocarpa, cyl = 'cylindropuntia spp.'

*An order to destroy the Spanish *C. rosea* plants by AQIS resulted in only partial testing of this provenance.

All *Cylindropuntia* spp. in Australia were hosts to at least one of the seven biotypes tested. *Cylindropuntia fulgida*, *C. imbricata*, *C. kleiniae* and *C. rosea* (Lorne Station) could each support several biotypes and had a strong host association with at least one biotype, displaying development success greater than 50%. In contrast, *C. leptocaulis* and *C. prolifera* (Englem.) F.M.Knuth were only able to support moderate development success for one or two biotypes (Table 1).

Cylindropuntia spinosior was the poorest host for all biotypes, with development success never higher than 11%, while *C. leptocaulis* also displayed low (25%) developmental success. The remaining *Cylindropuntia* spp. all supported medium levels of development, ranging from 31% to 47%.

Efficacy trials The 'fulgida' biotype displayed very high settling rates of >50 crawlers on all of the *Cylindropuntia* spp. tested except for *C. tunicata* (Lehm.) F.M.Knuth (Cracow). Colonies established on all species by week six except for *C. tunicata* (Cracow). Infestations of the 'fulgida' biotype on *C. imbricata* and *C. fulgida* caused the death of these plants by week 18. Infestations on the remaining plant species, *C. kleiniae*, *C. tunicata* (Grawin) and *C. tunicata* (Cracow) reduced plant growth and killed plants within a year.

The 'imbricata' biotype had very high settling rates on *C. imbricata* and *C. kleiniae* and killed the plants by week 16 and 26 respectively. This biotype failed to establish on *C. rosea* (Grawin), *C. rosea* (Lorne Station) and *C. fulgida.* However, sustained feeding over time on *C. rosea* (Grawin) may have contributed to the death of this plant.

The 'leptocaulis' biotype had very high settling rates on *C. kleiniae* and *C. rosea* (Grawin) and a moderate settling rate on *C. fulgida* (>20 crawlers). This biotype is characterised by a prolonged development time with most crawlers still at second instar ten weeks after initiation.

The 'acanthocarpa' biotype had high settling rates (>50 crawlers) on *C. imbricata, C. rosea* (Lorne) and *C. tunicata* (Grawin). The remaining species, *C. fulgida*, *C. kleiniae*, *C. leptocaulis*, *C. rosea* (Grawin) and *C. tunicata* (Cracow) all had settling rates >30 crawlers. Colonies on *C. imbricata* and *C. rosea* (Lorne) established by week 12, *C. leptocaulis* and *C. tunicata* (Grawin) by week 14 and on the remaining *Cylindropuntia* spp. by week 16. Trials are continuing.

The 'acanthocarpa × echinocarpa' biotype displayed high settling rates (>50) on all the *Cylindropuntia* spp. except for *C. prolifera* and *C. rosea* (Lorne) Infestations caused the death of *C. tunicata* (Grawin) by week 18 and *C. imbricata* by week 22. Severe plant damage, chlorosis and main trunk dieback, was evident by week 20 on most of the remaining test plants. Crawlers never settled on *C. prolifera* and were confirmed dead by week 6.

There was medium to high settling rates of the 'cylindropuntia spp.' biotype on all plants tested except for *C. leptocaulis* and *C. rosea* (Lorne). By week six, feeding by first generation nymphs had caused severe damage to the main stem of the *C. rosea* (Grawin) plant. Establishment of the colony was also confirmed on *C. imbricata* at week six and plant death at week eight. By week 10, the main trunk of the *C. rosea* (Grawin) plant was dead and unable to support emerging nymphs.

The 'rosea' biotype showed low settling rates on all *Cylindropuntia* spp. except for *C. kleiniae.* The colony on *C. imbricata* and *C. spinosior* (one pre-ovipositing female) survived for 22 weeks but never established. The trial for the 'rosea' biotype was concluded at this stage as a decision was made to destroy this culture due to its low potential as a biological control agent.

DISCUSSION

Conducting efficacy trials as well as normal host-specificity trials, is useful in identifying the most appropriate biotype for each *Cylindropuntia* spp. The efficacy trials provide evidence of the potential impact of biotypes on plants in the field, but other biotic and abiotic factors will influence the realised impact.

In these trials, short development times, high fecundity and high settling rates of first generation nymphs were the traits that determined the more effective biotypes. The multiple, overlapping generations, combined with high survival rates of the crawlers, enabled infestations of the biotypes to build large and damaging populations quickly on their more suitable hosts.

Together, the seven biotypes heavily damaged or killed seven of the eight *Cylindropuntia* species, with *C. spinosior* the only species not impacted by any biotype.

The 'fulgida' biotype was by far the most promising agent, heavily damaging and killing four species: *C. imbricata*, *C. fulgida*, *C. kleiniae* and both provenances of *C. tunicata*, while the 'imbricata' biotype was also promising, killing three species: *C. imbricata*, *C. kleiniae* and *C. rosea* (Grawin).

The 'rosea' biotype displayed a narrow host range within the *Cylindropuntia* and was by far the least virulent tested. Consequently, the colony was terminated.

Trials on the remaining biotypes are still in progress. All biotypes except for the 'leptocaulis' biotype are characterised by quick development and high fecundity. The 'acanthocarpa' biotype shows the greatest potential in controlling *C. rosea* while the

'cylindropuntia spp.' biotype has potential in controlling *C. prolifera* and *C. leptocaulis*.

The efficacy of each biotype varies among the different Australian *Cylindropuntia* species and so care will need to be taken to ensure the most appropriate biotypes are released on each species.

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