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Cat's claw creeper leaf-mining jewel beetle *Hylaeogena jureceki* Obenberger (Coleoptera: Buprestidae), a host-specific biological control agent for *Dolichandra unguis-cati* (Bignoniaceae) in Australia

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Abstract Cat's claw creeper, *Dolichandra unguis-cati* (L.) L.G. Lohman (syn: *Macfadyena unguis-cati* (L.) A.H. Gentry) (Bignoniaceae), a major environmental weed in Queensland and New South Wales, is a Weed of National Significance and an approved target for biological control. A leaf-mining jewel beetle, *Hylaeogena jureceki* Obenberger (Coleoptera: Buprestidae), first collected in 2002 from *D. unguis-cati* in Brazil and Argentina, was imported from South Africa into a quarantine facility in Brisbane in 2009 for host-specificity testing. *H. jureceki* adults chew holes in leaves and lay eggs on leaf margins and the emerging larvae mine within the leaves of *D. unguis-cati*. The generation time (egg to adult) of *H. jureceki* under quarantine conditions was 55.4 ± 0.2 days. Host-specificity trials conducted in Australia on 38 plant species from 11 families supplement and support South African studies which indicated that *H. jureceki* is highly host-specific and does not pose a risk to any non-target plant species in Australia. In no-choice tests, adults survived significantly longer (>32 weeks) on *D. unguis-cati* than on non-target test plant species (<3 weeks). Oviposition occurred on *D. unguis-cati* and one non-target test plant species, *Citharexylum spinosum* (Verbenaceae), but no larval development occurred on the latter species. In choice tests involving *D. unguis-cati*, *C. spinosum* and *Avicennia marina* (Avicenniaceae), feeding and oviposition were evident only on *D. unguis-cati*. The insect was approved for field release in Australia in May 2012.

Key words cat's claw creeper, *Dolichandra unguis-cati*, host-specificity, *Hylaeogena jureceki*, *Macfadyena unguis-cati*.

INTRODUCTION

Cat's claw creeper *Dolichandra unguis-cati* (L.) L.G. Lohmann (syn: *Macfadyena unguis-cati* (L.) A.H. Gentry) (Bignoniaceae), a climbing woody vine, has a native range extending from Mexico through Central America to tropical South America (Rafter *et al*. 2008). In Australia, *D. unguis-cati* is a major environmental weed in coastal Queensland and New South Wales (Batianoff & Butler 2003; Dhileepan 2012), where it poses a significant threat to biodiversity in riparian and rainforest communities. It has been declared recently as a Weed of National Significance (http://www.weeds.org.au/WoNS/catsclawcreeper). Cat's claw creeper is also recorded as invasive in South Africa, India, Mauritius, China, Hawaii and Florida in the USA, and New Caledonia (Downey & Turnbull 2007; Starr & Starr 2008).

Dolichandra unguis-cati is a structural parasite that produces stolons and subterranean root tubers. Cat's claw creeper does not have a persistent seed bank, suggesting that while its mechanism for spread is through seeds, its mechanism for persistence is through the tuber bank (Vivian-Smith & Panetta 2004). The inaccessibility of root tubers and their ability to regenerate are major barriers to the control of this weed. Whilst above-ground growth can be effectively treated, regeneration continues over the long term from subterranean tubers. The management objectives for this weed are therefore focused on reducing the rate of shoot growth to limit its ability to climb and smother native vegetation, and reducing tuber biomass to minimise the soil tuber bank (Raghu *et al*. 2006). Chemical and mechanical control options for *D. unguis-cati* are available, but are often not used due to the sensitive ecosystems (riparian vegetation and rainforest) where it occurs (Dhileepan 2012). The need to apply these controls repeatedly over a number of years severely limits the size of infestations that can be treated. Hence, biological control has become the most desirable option to manage this invasive species.

Surveys on *D. unguis-cati* in Brazil, Argentina, Paraguay, Venezuela and Trinidad have identified nine insect species as potential biological control agents (Sparks 1999; King *et al*. 2011). Simulated herbivory studies in Australia have suggested that specialist leaf-feeding herbivores are desirable as biocontrol agents for *D. unguis-cati* (Raghu & Dhileepan 2005; Raghu *et al*. 2006). A leaf-feeding beetle, *Charidotis auroguttata* (Boheman) (Chrysomelidae: Coleoptera) was the first agent to be introduced to South Africa (Sparks 1999; King

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et al. 2011), but was not approved for release in Australia due to its perceived non-target risk to a native plant (Dhileepan *et al*. 2005). Two species of leaf-sucking tingid bugs, *Carvalhotingis visenda* Drake & Hambleton and *Carvalhotingis hollandi* Drake (Hemiptera: Tingidae), a leaf-mining jewel beetle, *Hylaeogena jureceki* Obenberger (Coleoptera: Buprestidae) and a leaf-tying moth, *Hypocosmia pyrochroma* Jones (Lepidoptera: Pyralidae), all native to tropical South America (Argentina, Paraguay and Brazil), were later released in South Africa (Williams *et al*. 2008; King *et al*. 2011). The leaf-sucking tingid bug *C. visenda* and the leaf-tying moth *H. pyrochroma* were subsequently released in Australia (Dhileepan *et al*. 2007a,b). The tingid bug (*C. visenda*) became widely established from all release sites in Australia (Dhileepan *et al*. 2010), but so far, there is no evidence of field establishment of the leaf-tying moth.

To supplement the leaf-sucking tingid bug, the leaf-mining jewel beetle (*H. jureceki*) was imported for host-specificity testing in Australia because of its wide native range distribution, host-specificity and damage potential. Host-specificity tests in South Africa indicated that *H. jureceki* is specific to *D. unguis-cati* (Williams 2003). The insect was approved for field release in South Africa in 2007 and there is evidence of its field establishment at some release sites (King *et al*. 2011). *Hylaeogena jureceki* was imported into Australia in 2009 for host-specificity testing. Here we report the host-specificity and non-target risks of *H. jureceki* as a biological control agent for *D. unguis-cati* in Australia.

MATERIALS AND METHODS

Insect source

Adults of *H. jureceki* are small and metallic black in colour. They feed on leaves, preferring new leaflets. Females oviposit most eggs singly along outer edges of larger basal leaves. Newly laid eggs are shiny, flat, transparent disks that darken to black as they mature. Neonates mine directly into the leaf after hatching and develop through three instars (Williams 2003). Fully developed third instars form sealed pupal cells by chewing out circular disks (Williams 2003) which may or may not remain attached to the leaf. Adults emerge after 2 weeks and live for more than 5 months.

Hylaeogena jureceki adults were sourced from a laboratory colony maintained at the Agricultural Research Council-Plant Protection Research Institute (ARC-PPRI), Pretoria, South Africa. The colony was established from material collected from *D. unguis-cati* by S. Neser and C.J. Cilliers in Argentina and Brazil in April 2002 (King *et al*. 2011). *Hylaeogena jureceki* was imported into Australia in mid 2009. Dr C.L. Bellamy from the Plant Pest Diagnostics Laboratory, California Department of Food and Agriculture, Sacramento, California, USA, confirmed its identification. Voucher specimens have been lodged with the Department of Agriculture, Fisheries and Forestry (DAFF Biosecurity) in Brisbane.

Life cycle

The life cycle of *H. jureceki* was studied using potted *D. unguis-cati* plants in a quarantine glasshouse under controlled climatic conditions (night temperature: 20°C, day temperature: 27°C; RH 65%; photoperiod: 12 h dark: 12 h light). A pair of newly emerged and mating adults ($n = 10$) was transferred on to a potted plant enclosed in a transparent cylindrical Perspex tube (34 cm high and 12 cm diameter) with a gauze cap, as used in host-specificity testing other cat's claw creeper biological control agents (Dhileepan *et al*. 2005, 2007a,b). The adults were transferred on to a fresh plant each week, and their longevity, the pre-oviposition period and the number of eggs laid per female per week were recorded.

Test plants

A test plant list comprising 38 plant species (Table 1), similar to the one used for previously tested biological control agents for cat's claw creeper in Australia (Dhileepan *et al*. 2005, 2007a,b), was developed in consultation with experts representing all of the appropriate Australian jurisdictions. The test plant species were selected using the centrifugal phylogenetic method (Wapshere 1974; Briese 2003), starting with the nearest relatives of the target within the family Bignoniaceae in Australia and proceeding to plants in other families in the order Lamiales (Spangler & Olmstead 1999; Schwarzbach & McDade 2002; Angiosperm Phylogeny Group 2003).

The phylogenetic relationships within Bignoniaceae and particularly Lamiales have yet to be fully resolved. On the test plant list, the Bignoniaceae is represented by 12 species, with at least one native species from each of four Australian genera, as well as five introduced species that are available in commercial nurseries (Table 1). There are no Australian natives that are closely related to the cat's claw creeper tribe (Bignoneae) according to the phylogenetic tree constructed by Lohmann (2006). The genera *Deplanchea* Vieill., *Neosepicaea* Diels., *Tecomanthe* Baill. and *Pandorea* Spach have been tentatively assigned to the tribe Tecomeae. Other families within the Lamiales have not been placed in any significant order due to the unresolved nature of relationships. Relatively more plant species representing the family Myoporaceae were included on the test list in view of the adult and larval feeding of *C. auroguttata* on the native *Myoporum boninense* ssp. *australe* Chinnock in quarantine (Dhileepan *et al*. 2005). *Myoporum parvifolium* R.Br. was added because it is a popular native ornamental and is readily available. *Pandorea floribunda* (A.Cunn. ex DC.) Guymer was also added as it is a recently described species from south-eastern Queensland, previously known as *Pandorea* sp. Ipswich (Guymer 2008).

The families Acanthaceae, Gesneriaceae, Avicenniaceae, Myoporaceae, Oleaceae, Pedeliaceae, Scrophulariaceae, Plantaginaceae and Verbenaceae were also represented on the list. *Uncarina grandidieri* (Baill.) Stapf (Pedaliaceae) is an introduced ornamental shrub native to Madagascar. It is not commonly cultivated and as we were unable to source the species locally, *Uncarina roeoesliana* W. Rauh. was used instead. Lentibulariaceae (bladderwort family) are mainly submerged

Table 1 Adult survival, oviposition and larval development to adult in no-choice test trials of *Hylaeogena jureceki*. Values are means ± SE

Order/family	Test species	Plant habit†	Replications	Adult feeding‡	Adult longevity (days)	No. of eggs§	% egg to adult§	Adult emergence (No. of adults)
Lamiales								
Bignoniaceae								
	Dolichandra unguis-cati (L.) L.G.Lohmann	Vine[1]	38	+++++	57.9 ± 4.5	72.3 ± 19.5	79.1 ± 5.5	57.9 ± 1.4
	Pyrostegia venusta (Ker Gawl.) Miers	Vine[2]	5	–	6.2 ± 0.8	0	0	0
	Tabebuia palmeri Rose	Vine[2]	6	–	5.5 ± 0.6	0	0	0
	Spathodea campanulata P.Beauv.	Tree[1, 2]	5	–	6.0 ± 0.1	0	0	0
	Deplanchea tetraphylla (R.Br.) F.Muell.	Tree[3]	6	++ (1/6)	11.1 ± 3.9	0	0	0
	Dolichandrone heterophylla (R.Br.) F.Muell.	Tree/shrub[4]	3	+ (1/3)	5.0 ± 0.1	0	0	0
	Neosepicaea jucunda (F.Muell.) Steenis	Vine[3]	5	–	4.3 ± 0.6	0	0	0
	Pandorea jasminoides (L.) K.Schum.	Vine[2, 3]	5	–	5.6 ± 0.2	0	0	0
	Pandorea floribunda (A.Cunn. ex DC.) Guymer	Vine[2, 3]	5	–	5.8 ± 0.3	0	0	0
	Pandorea pandorana (Andrews) Steenis	Vine[3]	5	–	8.4 ± 1.7	0	0	0
	Tecomanthe hillii (F.Muell.) Steenis	Vine[3]	5	–	6.5 ± 0.4	0	0	0
	Tecoma stans (L.)	Shrub[1, 2]	5	–	8.1 ± 1.2	0	0	0
	Jacaranda mimosifolia D.Don	Tree[2]	5	–	6.8 ± 1.8	0	0	0
Acanthaceae								
	Graptophyllum excelsum (F.Muell.) Druce	Shrub[3]	5	–	5.4 ± 0.7	0	0	0
	Hypoestes floribunda R.Br.	Shrub[3]	5	–	6.2 ± 0.5	0	0	0
	Hypoestes phyllostachya Baker.	Shrub[3]	5	–	6.8 ± 0.5	0	0	0
	Thunbergia grandifolia (Roxb.) ex.(Rottler) Roxb.	Vine[1, 2]	6	+ (2/6)	7.8 ± 0.5	0	0	0
Avicenniaceae								
	Avicennia marina (Forssk.) Vierh	Tree[4]	6	+ (4/6)	5.7 ± 0.5	0	0	0
Gesneriaceae								
	Boea hygroscopica F.Muell.	Ground cover[3]	5	–	5.7 ± 0.3	0	0	0
	Saintpaulia ionantha Wendl.	Ground cover[2]	5	–	5.8 ± 0.4	0	0	0
Myporaceae								
	Myoporum acuminatum R.Br.	Shrub[2, 3]	6	–	6.0 ± 0.5	0	0	0
	Myoporum boninense ssp. *australe* Chinnock	Shrub[2, 3]	5	–	5.7 ± 0.7	0	0	0
	Myoporum montanum R.Br.	Shrub[2, 3]	5	–	5.7 ± 0.6	0	0	0
	Myoporum parvifolium R.Br.	Shrub[2, 3]	5	–	4.7 ± 0.4	0	0	0
	Eremophila maculata (Ker Gawl.) F.Muell.	Shrub[2, 3]	5	–	6.6 ± 1.0	0	0	0
	Eremophila bignoniiflora x polyclada	Shrub[2, 3]	5	–	7.4 ± 1.0	0	0	0
Oleaceae								
	Olea europaea L.	Tree[4]	5	–	6.7 ± 0.7	0	0	0
	Olea paniculata R.Br.	Tree[3]	5	–	5.7 ± 0.9	0	0	0
	Jasminum suavissimum Lindl.	Vine[3]	5	–	4.9 ± 0.2	0	0	0
Pedaliaceae								
	Uncarina roeoesliana W. Rauh.	Shrub[2]	5	–	2.8 ± 0.1	0	0	0
	Sesamum indicum L.	Shrub[4]	5	–	4.8 ± 0.2	0	0	0
Scrophulariaceae								
	Antirrhium sp.	Herb[2]	5	–	2.8 ± 0.14	0	0	0
	Artanema fimbriatum (Graham) D.Don	Herb[3]	6	+ (2/6)	6.8 ± 1.2	0	0	0
	Paulownia tomentosa (thumb.) Sieb. & Zucc.	Tree[1, 4]	5	+ (1/5)	7.2 ± 1.9	0	0	0
Verbenaceae								
	Citharexylum spinosum L.	Tree[2]	5	+ (2/5)	11.9 ± 1.9	0.8 ± 0.4	0	0
Lamiaceae								
	Vitex lignum-vitae Schauer.	Tree[3]	5	–	5.7 ± 0.2	0	0	0
	Ocimum basilium L.	Herb[4]	5	–	5.9 ± 0.4	0	0	0
Plantaginaceae								
	Plantago lanceolata L.	Herb[3]	6	+ (2/6)	8.9 ± 1.4	0	0	0
Solanales								
Solanaceae								
	Lycopersicon esculentum Mill.	Herb[4]	5	–	4.5 ± 2.3	0	0	0
Water (negative control)		–	6	–	6.3 ± 0.6	0	0	0

†1 = invasive; 2 = ornamental; 3 = native; 4 = crop. ‡–, no feeding; +, nibbling/exploratory feeding; ++, minor feeding; +++, moderate feeding; ++++, significant feeding; +++++, extensive feeding. Figures in parentheses refer to number of replications with feeding damage. §Oviposition and percentage eggs to adult are for the first 10 replicates only. Eggs not counted on the target weed after this point.
–, no feeding.

plants and are unlikely to be exposed to terrestrial insects. Hence, these were not included on the test list. Members of Orobanchaceae (broomrape family) are parasitic succulent plants with much reduced leaves and hence were not included in the test. Test plants representing various out-groups, including crops that were tested in South Africa (Williams 2003), were not included in the tests. However, one representative species from the order Solanales (*Lycopersicon esculentum* Mill.; Solanaceae), the most closely related order to Lamiales, was included.

Host-specificity trials

Detailed biological studies and host-specificity testing were conducted in a temperature-controlled (22–27°C) quarantine insectary at the Alan Fletcher Research Station, Sherwood, Brisbane, until January 2012, when the host-specificity testing was shifted to a new quarantine facility (temperature 22–27°C, humidity 65% and natural photoperiod) at the Ecoscience Precinct, Dutton Park, Brisbane. All tests were done on potted plants. Adult longevity and fecundity, together with pre-oviposition, larval, prepupal and pupal periods were determined on *D. unguis-cati*.

The potential host range of *H. jureceki* was evaluated on the basis of no-choice adult feeding, survival and oviposition preference, and choice adult feeding and oviposition preference, involving 38 plant species in 10 families (Table 1). In all tests, unsexed adults were used, due to difficulties in sexing adults visually.

No-choice tests

Batches of test plants, each with one *D. unguis-cati* as a control, were tested as they became available. A minimum of five replications were made with each test plant species, with the exception of *Dolichandrone heterophylla* (R.Br.) F.Muell., where only three replicates were done due to difficulties in procuring the plants from North Queensland. Ten unsexed newly emerged adults were enclosed with a plant in a cylindrical transparent Perspex cylinder (34 cm tall and 12 cm diameter) with a gauze cap. For larger test plants, the adults were enclosed in a fine nylon gauze bag secured around a branch of the potted test plant. Plants were checked three times a week (Monday, Wednesday and Friday), and the surviving adults and eggs were counted. Test plants with feeding and/or oviposition were replaced weekly, and egg hatching and larval development were monitored until adult emergence or death of immatures.

Choice tests

Test plant species on which adult feeding or oviposition were observed in no-choice tests were subjected to multiple-choice oviposition and feeding tests. One plant each of the native grey mangrove *Avicennia marina* (Forssk.) Vierh., the exotic fiddlewood *Citharexylum spinosum* L and *D. unguis-cati* were placed randomly in a cage with 10 newly emerged adults. All plants were sampled on days 1, 2, 5, 6, 7, 8, 9, 13, 14 and 15 and the number of adults on each plant was recorded. Plants were also examined for any evidence of feeding and oviposition, and the number of leaves with feeding damage, the leaf area of feeding and the number of eggs on each test plant were recorded. The test was repeated five times.

Data analysis

The duration of adult survival on various test plants in no-choice tests, together with feeding and oviposition preferences of adults, was compared using one-way ANOVA and the means compared using Tukey's HSD test. The risk of non-target feeding and oviposition by adult *H. jureceki* in choice tests was evaluated by regressing the proportion of adults present on target and non-target plants over time in the test arena. All analyses were performed using SigmaStat 3.5. All results in the text are presented as means ± standard error.

RESULTS

Life cycle

Newly emerged adults started laying eggs after 13.2 days (range: 11–20 days). Adults lived for 51.6 ± 4.6 days (range: 7–314 days) and the females laid 57.9 ± 7.5 eggs (range: 2–161 eggs) in their lifetime. The eggs hatched in 14.6 ± 0.12 days (range: 12–17 days). The hatched larvae mined through the leaves, and when mature (mean: 17.3 ± 0.2 days; range: 14–24 days), mined out a circular pupal cell. The prepupal (mean: 7.1 ± 0.08 days; range: 6–9 days) and pupal (mean: 16.3 ± 0.2 days; range: 11–24 days) stages were spent within the pupal cell in the same leaf where the larval development was completed. The average duration of development (egg to adult) was 55.4 ± 0.2 days (range: 52–63 days).

No-choice tests

Adult feeding

Feeding by *H. jureceki* adults (10 per plant) caused almost complete defoliation of potted *D. unguis-cati* plants in 1 to 2 weeks, depending on plant size. In contrast, only minor exploratory feeding/nibbling by adults, resulting in very small feeding holes along the margins of young leaves, was observed on eight test plant species: *Dolichandrone heterophylla* (Bignoniaceae), *Deplanchea tetraphylla* (R.Br.) F.Muell. (Bignoniaceae), *C. spinosum* (Verbenaceae), *A. marina* (Avicenniaceae), *Paulownia tomentosa*, *Artanema frimbriatum* (Scrophulariaceae), *Plantago lanceolata* (Plantaginaceae) and *Thunbergia grandifolia* (Acanthaceae) (Table 1). All adults on these non-target plants died within 3 weeks of the trial commencing (Fig. 1), except for one of the 60 adults on *D. tetraphylla* (Bignoniaceae) that survived for 33 days.

The duration of adult survival varied significantly on the different test plant species (Table 1; Fig. 1; $F_{39,\ 192} = 13.65$, $P < 0.001$). Adults survived significantly longer on *D. unguis-cati* (up to 32 weeks) than on any other test plant species

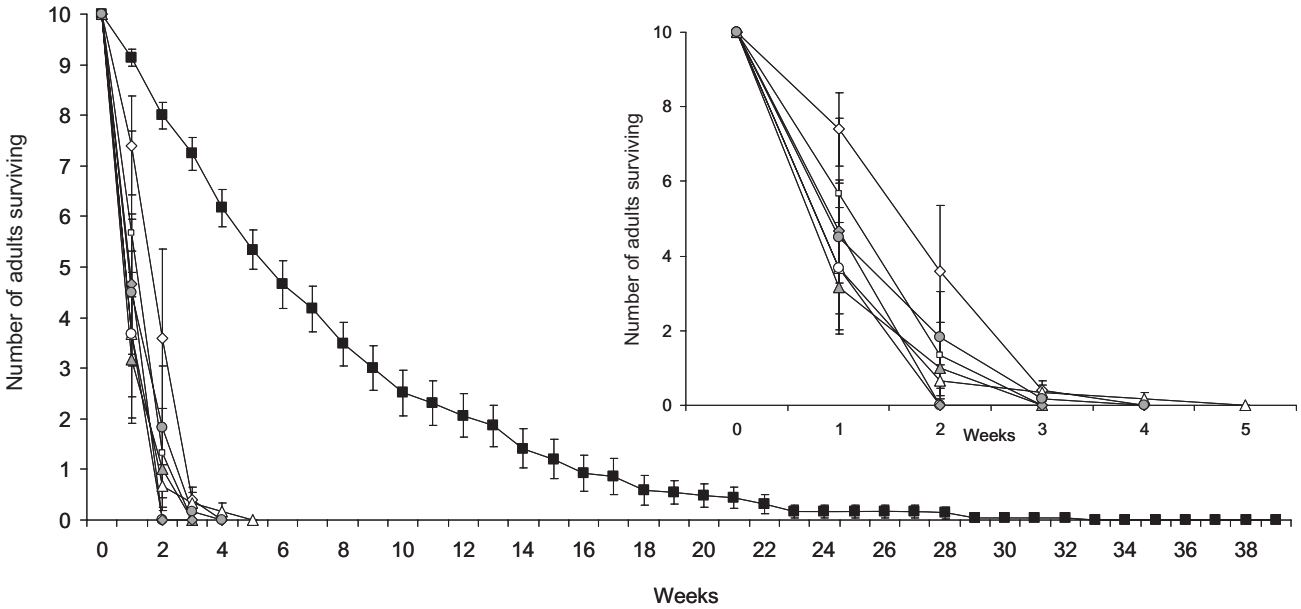

Fig. 1. Number of Hylaeogena jureceki adults surviving (mean ± standard error) on target weed and non-target test plants (expanded in insert) over time in no-choice tests. –■–, *Dolichandra unguis-cati*; –◇–, *Citharexylum spinosum*; –△–, *Deplanchea tetraphylla*; –○–, *Avicennia marina*; –▲–, *Artanema fimbriatum*; –□–, *Paulownia tomentosa*; –◆–, *Thunbergia grandiflora*; –●–, *Plantago lanceolata*.

(Tukey test, $P < 0.05$) (Fig. 1). Adults exposed to water alone survived for an average of 6 days (Table 1). The duration of survival on non-target tests plants, including those test plant species on which minor feeding occurred, was not statistically different from that of adults exposed to water alone (Tukey test, $P > 0.05$; Table 1; Fig. 1).

Oviposition and larval development

Beetles oviposited only on *D. unguis-cati* and *C. spinosum* (Table 1). Four eggs were laid on *C. spinosum* across five replicates, an average of less than one egg per replicate. In contrast, an average of 72 eggs was laid per cat's claw creeper replicate, with up to 160 eggs recorded in a single replicate. Development of larvae occurred only on *D. unguis-cati* as no larva emerged from the four eggs laid on *C. spinosum* (Table 1). Nearly 80% of eggs laid on *D. unguis-cati* completed development to adults, and on average, 58 adults emerged per *D. unguis-cati* replicate (Table 1).

Choice tests

The proportion of adults found on *D. unguis-cati* (0.73 ± 0.06) was significantly higher than on *A. marina* (0.06 ± 0.03), *C. spinosum* (0.01 ± 0.01) and cage walls (0.21 ± 0.04) ($F_{3,\ 16} = 76.2$, $P < 0.001$). The proportion of adults on the cage walls was significantly higher ($P > 0.05$) than on *A. marina* and *C. spinosum*. The proportion of adults on *D. unguis-cati* increased with time ($y = 0.3746x^{0.3443}$; $R^2 = 0.845$), and correspondingly, the proportion of adults on the cage walls declined (Fig. 2). There was also a significant test plant × time interaction for the proportion of adults on test plants in the choice-test arena ($F_{27,\ 160} = 4.33$, $P < 0.001$), with no adults being found on any of the non-target plants 14 days after commencement of the test (Fig. 2). In contrast, the proportion of adults present on the walls of the cages always remained higher than on the non-target plants (Fig. 2). At the end of the trial, oviposition was evident only on *D. unguis-cati* (24 ± 8 eggs), with no eggs laid on *A. marina* and *C. spinosum* (Fig. 3). The proportion of leaves with adult feeding damage was significantly higher on *D. unguis-cati* (52.2 ± 12.9%) than on *A. marina* (1.26 ± 1.26%), and there was no feeding damage on *A. marina* ($F_{2,\ 12} = 15.7$, $P < 0.001$) (Fig. 3).

DISCUSSION

The genus *Hylaeogena* consists of 110 valid species (Bellamy 2009), nearly all of which are specialised as leaf-mining larvae on vines of the family Bignoniaceae (Hespenheide 1974). During surveys by Stefan Neser (ARC-PPRI, South Africa), *H. jureceki* was collected only from *D. unguis-cati* in Argentina, Paraguay and Brazil. In South Africa, host-specificity tests involving 21 test plant species from 10 families confirmed that *H. jureceki* is specific to *D. unguis-cati* (Williams 2003), and the insect was approved for release in 2007 (King *et al*. 2011). Field establishment of *H. jureceki* has been confirmed at release sites in South Africa (King *et al*. 2011).

Hylaeogena jureceki adults chew holes in leaves and feed preferably on the smaller leaflets. In quarantine, high adult populations can completely defoliate *D. unguis-cati* plants in a short time. One larva was able to destroy an entire leaflet, as the mined leaflet drops off prematurely (Williams 2003). *Hylaeogena jureceki* has several biological attributes (e.g.

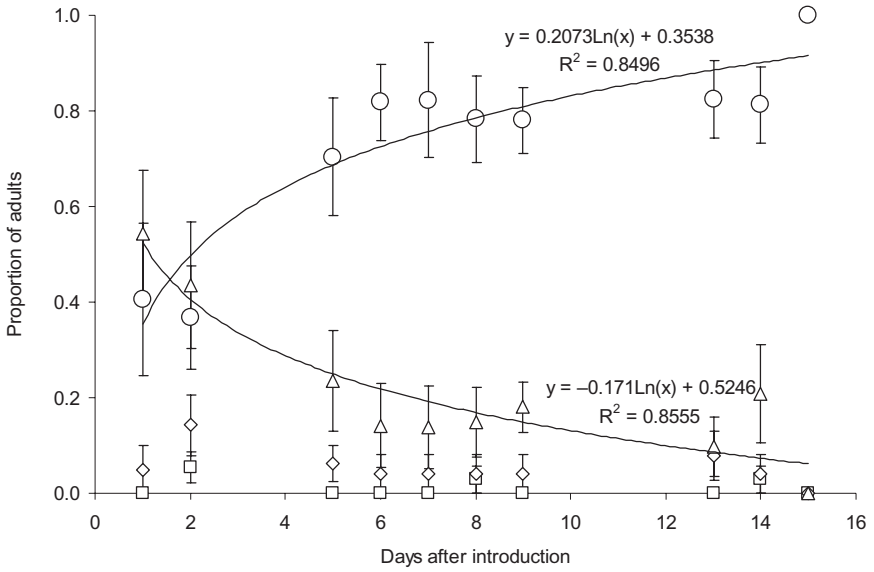

Fig. 2. Proportion of *Hylaeogena jureceki* adults (mean ± standard error) on cage walls, target (*Dolichandra unguis-cati*) and non-target (*Avicennia marina* and *Citharexylum spinosum*) test plants in choice tests. Regression was not significant for both non-target plants. ○, *Dolichandra unguis-cati*; □, *Citharexylum spinosum*; ◇, *Avicennia marina*; △, Cage.

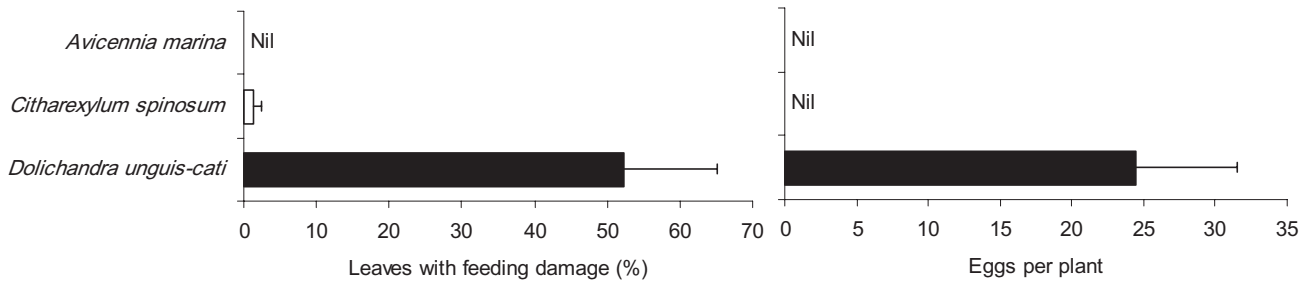

Fig. 3. Proportion of leaves with feeding damage (mean ± standard error (SE)) and number of eggs per plant (mean ± SE) on target (*Dolichandra unguis-cati*) and non-target (*Avicennia marina* and *Citharexylum spinosum*) test plants in choice tests. Nil, no feeding/no eggs.

short generation time, long-lived adults, high reproductive output) that could contribute to its success as a biological control agent (Williams 2003). *Hylaeogena jureceki* also has a number of characteristics that help it evade predation (adults that drop from the plant when disturbed and leaf-mining larvae that are protected from ants and spiders) which is anticipated to further enhance its survival in the field (Williams 2003).

In no-choice trials in Australia, minor exploratory feeding by adults was observed on eight non-target test species, but the duration of adult survival on these species was significantly less than on *D. unguis-cati*. Feeding damage on non-target test plant species was generally restricted to the margins of young leaves and occurred over a 2 to 3 week period, depending on how long adults survived. During the same period, the same number of adults (10 per plant) almost completely defoliated *D. unguis-cati* once or twice, depending on plant size. The duration of survival on non-target plants, on which minor feeding damage was evident, did not differ significantly from the duration of survival of adults exposed to water alone, suggesting that no nutritional benefit was gained from any of the non-target species.

For *H. jureceki* to develop successfully on a plant, the adults must lay eggs on the plant and the emerging larvae must mine directly into the leaf immediately after hatching. Larvae complete their development within the leaf on which the egg was laid and never move between leaves. Transfer of developing larvae from mined leaves to healthy leaves always resulted in the death of the larvae. Hence, it was not possible to conduct no-choice larval development tests on non-target test plants. The lack of oviposition on non-target test plants other than *C. spinosum* and the failure of egg/larval development on *C. spinosum* both provide evidence that only *D. unguis-cati* can sustain larval development of *H. jureceki*.

In choice tests, *H. jureceki* adults showed distinct preference for *D. unguis-cati* over the two non-target plant species, and the preference level increased over time as the adults moved away from the non-target plants. At the end of the trial, no adults were evident on any of the non-target plants. In these tests, significantly more adults were recorded on the cage-walls than on the non-target test plants. Lack of oviposition and significantly fewer feeding marks on *C. spinosum* than on *D. unguis-cati* confirm that *C. spinosum* is unsustainable as a host for *H. jureceki*. No-choice and choice tests conducted in Australia support the findings of host-specificity testing conducted in South Africa, and confirm that *H. jureceki* is specific to *D. unguis-cati* and does not pose risk to any non-target plants. *Hylaeogena jureceki* was approved for field release in Australia in May 2012.

Hylaeogena jureceki is the third agent to be approved for release against *D. unguis-cati* in Australia. It is unlikely that the release of *H. jureceki* will have any negative impacts on the widely established *C. visenda*, as the agents occupy different niches, with *H. jureceki* preferring younger leaves on plants growing on tree trunks and canopies, while *C. visenda* prefers older leaves on plants growing along the ground. In South Africa, where *H. jureceki* has been released, *C. visenda* and *H. jureceki* appear to co-occur (A. King, pers. comm. 2012). Observations in the native range also suggest that *H. jureceki* and *C. visenda* do not negatively impact each other (K. Dhileepan pers. obs. 2012).

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