Queensland Government
Natural Resources, Mines and Water

Application to release the leaf-sucking bug *Carvalhotingis visenda* (Hemiptera: Tingidae), a potential biological control agent for cat's claw creeper *Macfadyena unguis-cati* (Bignoniaceae)

April 2006

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SUMMARY

Cat's claw creeper, *Macfadyena unguis-cati* (L.) Gentry is a major environmental weed in coastal and sub-coastal areas of Queensland and NSW. Control of this weed by non-biological means is both difficult and expensive, and biocontrol appears the most suitable method. The host specificity trials conducted in Australia supplement and support South African studies, which indicate that the tingid *Carvalhotingis visenda* (Drake & Hambleton) is a highly host specific biocontrol agent, which does not pose risk to any non-target plants in Australia. The tingid can lay eggs and complete nymphal development only on the target weed *M. unguis-cati*. We therefore recommend that *C. visenda* be released in Australia, as it has the potential to play an important role in the control of *M. unguis-cati* while posing no threat to any non-target plants.

INFORMATION ON THE TARGET WEED

1. 1 Taxonomy

Scientific name:	*Macfadyena unguis-cati* (L.) Gentry
Common name:	Cat's claw creeper
Tribe	Bignonieae
Family	Bignoniaceae
Order	Lamiales

1.2. Close relatives in the Australian region

1.2.1. Native plants

The family Bignoniaceae is represented in the native Australian flora by the following species in 5 genera (Henderson, 1997; Hnatiuk, R. J. 1990; Quirico, A. L., 1992; Wheeler, 1992): *Deplanchea tetraphylla* (R.Br.) F.Muell., *Dolichandrone alternifolia* (R.Br.) Seem, *Dolichandrone filiformis* (Fenzl.) F.Muell., *Dolichandrone heterophylla* (R.Br.) F.Muell., *Dolichandrone spathacea* (L.f.) K.Schum., *Neosepicaea jucunda* (F.Muell.) Steenis, *Neosepicaea viticoides* Diels, *Pandorea baileyana* (Maiden and R.T.Baker) Steenis, *Pandorea jasminoides* (Lindl.) K.Schum., *Pandorea nervosa* Steenis, *Pandorea* sp. (Mt. Maroon, P.I. Forster PIF7111), *Pandorea* sp. (Ipswich, K Williams 86020), *Pandorea pandorana* (Andrews) Steenis, *Tecomanthe hillii* (F.Muell.) Steenis, and *Tecomanthe* sp. Roaring Meg, L.J. Brass 20326).

1.2.2. Introduced Plants

Introduced ornamentals *Jacaranda mimosifolia* D. Don, *Parmentiera aculeata* Seem., *Parmentiera cerifera* Seem., *Parmentiera edulis* DC., *Pithecoctenium cynanchoides* DC., *Pyrostegia venusta* (Ker. Gawl.) Miers, *Saritaea magnifica* (Sprague ex Steenis) Dugand, *Spathodea campanulata* P.Beauv., *Tecoma stans* (L.) Kunth and *Tecomaria capensis* (Thunb.) Spach have naturalised in Australia. *T. stans* is considered a serious weed in some areas of Queensland. Other introduced ornamentals that have not been recorded as naturalised include *Tabebuia* spp. and the African sausage tree, *Kigelia africana* (Lam.) Benth. (Batianoff and Butler, 2002; Harden, 1992; Henderson 1997; http://plantnet.rbgsyd.nsw.gov.au/avh.html). Other families in the order Lamiales represented in the Australian flora are: Acanthaceae, Avicenniaceae, Buddlejaceae, Gesneriaceae, Lamiaceae, Lentibulariaceae Myoporaceae, Oleaceae, Orobanchaceae, Pedeliaceae, Plantaginaceae, Scrophulariaceae, and Verbenaceae. Buddlejaceae is represented by introduced species only.

2. Distribution

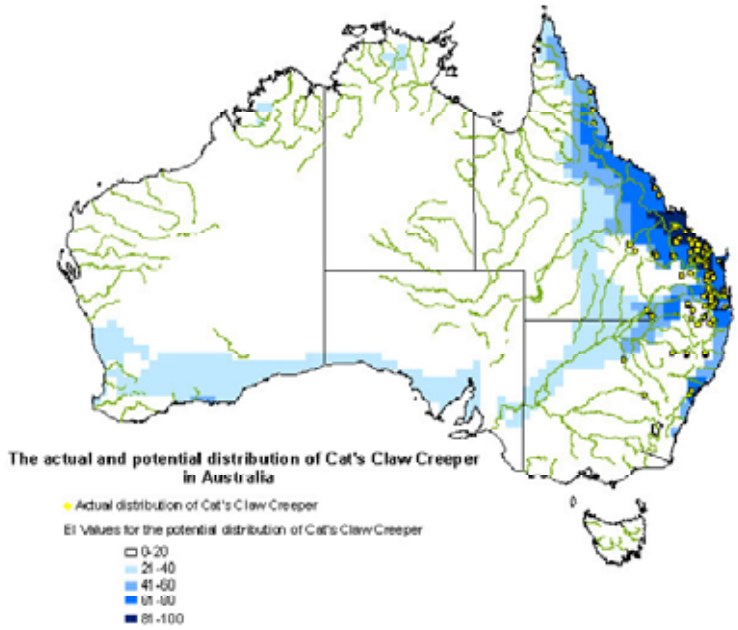
2.1. Native geographic range

Macfadyena unguis-cati is endemic from Mexico through Central America (Belize, Costa Rica, El Salvador, Guatemala, Honduras, Nicaragua & Panama) to tropical South America (Argentina, Bolivia, Brazil, Chile, Colombia, Ecuador, French Guiana, Guyana, Paraguay, Peru, Suriname, Uruguay, & Venezuela), and the Caribbean Islands (Bahamas, Barbados, Bermuda, Cuba, Dominical Republic, Haiti, Jamaica, Leeward Island, Puerto Rico, Tobago, Trinidad and Virgin Islands (Howard 1989; Sparks 1999).

2.2. Distribution in Australia

In Australia *M. unguis-cati* occurs in coastal and subcoastal Queensland and New South Wales (Fig. 1; Csurhes and Edwards, 1998; Swarbrick and Skarratt, 1994). In Queensland *M. unguis-cati* occurs in 61 shires and in 32 shires infestation levels of the weed are on the increase. In NSW *M. unguis-cati* occurs in five of the sub-divisions in the northern coast. Cat's claw creeper has the potential to spread along the entire eastern coast (Fig. 1).

Fig. 1. Current and potential distribution of *M. unguis-cati* in Australia. Areas with EI (Eco-climatic Index) more than 20 are suitable for *M. unguis-cati*.



2.3. Distribution in other countries

Cat's claw creeper is also an invasive weed in the northeastern provinces of South Africa (Sparks, 1999), Florida and Hawaii in USA, India, Mauritius, St Helena Island (Holm *et al.*, 1991) and New Caledonia (Meyer, 2000). Cat's claw creeper is also occur in Europe (France, Greece, Switzerland & Sicilia), Africa (Kenya, Uganda & Zimbabwe), Asia (China, India, Malaysia, Indonesia, Nepal & Sri Lanka) and Oceania (New Caledonia, Micronesia & New Zealand) (http://www.mobot.org/).

3. Control methods

3.1. Chemical and physical methods

Chemical control options for cat's claw creeper are available (Armstrong *et al.*, 2003), but are not often used due to the sensitive riparian ecosystems it occurs in. In other areas, mechanical and chemical controls are difficult and expensive over large areas. Whilst aboveground growth can be effectively treated (Armstrong *et al.*, 2003), regeneration will continue over the long term from subterranean tubers (Vivian-Smith & Panetta, 2004), which are extremely difficult to access. Whether the control methods are mechanical, physical or chemical, there is a need to treat infested areas repeatedly over a number of years. This severely limits the size of areas that can be treated. Biological control is considered as the most viable option for the long-term management of this weed.

3.2. Biological control

Surveys in Brazil, Argentina, Paraguay, Venezuela and Trinidad have identified several potential biological control agents for host specificity tests (Sparks, 1999). Simulated herbivory studies have suggested that specialist leaf-feeding herbivores are desirable as biocontrol agents for cat's claw creeper (Raghu and Dhileepan, 2005). A leaf-feeding beetle, *Charidotis auroguttata* (Chrysomelidae: Coleoptera) was the first agent to be introduced in to South Africa (Sparks, 1999), but this agent was not approved for release in Australia due to its perceived non-target risk to a native plant (Dhileepan *et al*., 2005). Subsequently, the leaf-sucking tingid from South Africa has been imported for host specificity tests in Australia.

4. Importance

4.1. Detrimental aspects

Cat's claw creeper is a serious threat to biodiversity in riparian and rainforest communities in eastern Australia. It is a high climbing woody vine, with stems to 6 cm in diameter and roots becoming elongated-tuberous with age. The plant flowers in spring and the seeds are dispersed by wind and water (Vivian-Smith & Panetta, 2004). It thrives in full sun or partial shade and in a wide variety of soils and also produces stolons and root tubers, which grow vigorously and produce dense mats on the forest floor. It climbs over standing trees in vine scrubs, gallery forests, rainforests, closed forests and open forests. Trees can be crushed by the weight of vines, allowing further light to enter the forest and promoting invasion by more light-demanding species. Furthermore, *M. unguis-cati* infestations may cause an inward collapse of the forest margin, as individual trees are colonised and killed.

4.2. Beneficial aspects

Cat's claw creeper, with its dense evergreen foliage and attractive yellow flowers was once a popular ornamental plant used commonly in gardens to cover trellises and fences. However, due to its invasive nature, it is not recommended for garden use.

4.3. Legislation

In Queensland, cat's claw creeper is a declared class 3 weed under the *Land Protection (Pest and Stock Route Management) Act 2002*.. In NSW also cat's claw creeper is a category W4C (A weed that is a threat to agriculture, the environment or the community, and shall not be sold, propagated or knowingly distributed) under *Noxious Weeds Act 1993*.

4.3.1. Approval As A Target Species For Biological Control

Natural Resource Management Standing Committee approved cat's claw creeper as a target species for biological control in 2005.

THE APPROVED TEST LIST

1. The approved list

The list (Table 1) is built using the centrifugal phylogenetic method (Wapshere 1974; Briese 2003) and commences with the nearest relatives of the target within the family Bignoniaceae in Australia and proceeds to less closely related plants in other families in the order Lamiales (Angiosperm Phylogeny Group, 2003; Schwarzbach and McDade, 2002; Spangler and Olmstead, 1999; Fig. 2). In the list, the Bignoniaceae are represented by a minimum of one native species of each Australian genus as well as by introduced species that are available in commercial nurseries (Table 1). More plant species representing the family Myoporaceae are included in view of the adult and larval feeding of *C. auroguttata* on one of the native *Myoporum* species in quarantine (Dhileepan *et al*., 2005). The families Acanthaceae, Gesneriaceae, Avicenniaceae, Myoporaceae, Oleaceae, Pedeliaceae, Scrophulariaceae, Plantaginaceae and Verbenaceae are also represented in the list. Lentibulariaceae (bladderwort family) are mainly submerged plants and are unlikely to be exposed to terrestrial insects. Hence these were not included in the list. Members of Orobanchaceae (Broomrape family) are parasitic succulent plants with much reduced leaves and were not included in the test list. Test plants representing various out-groups, including crops that were tested in South Africa (Annexure I), were also not included in the approved test list (Table 1). However, one representative species from order Solanales (*Lycopersicon esculentum* Mill.; Solanaceae) outside this order (Lamiales) but within the Euasterids I was included as an outgroup (Fig. 2).

Fig. 2. Phylogenetic relationship of families included in the host-specificity tests

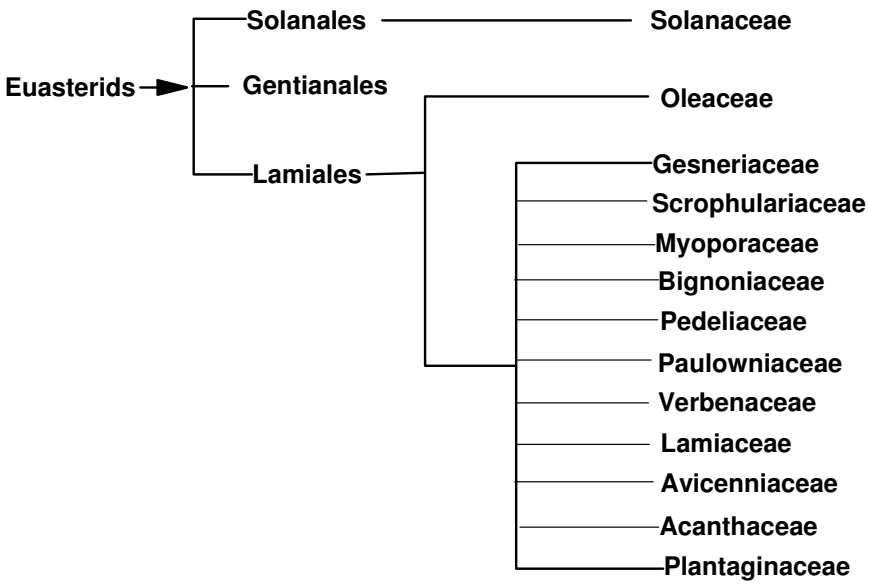


Table. 1. Approved test plants list for host-specificity tests.

ORDER/Family	Species	Native/ornamental	Habit
LAMIALES			
Bignoniaceae	*Macfadyena unguis-cati* (L.) Gentry	target weed	vine
	Deplanchea tetraphylla (R.Br.) F.Muell.	native	tree
	Dolichandrone heterophylla (R.Br.)F.Muell.	native	tree/shrub
	Neosepicaea jucunda (F.Muell.) Steenis	native	vine
	Pandorea jasminoides (L.) K.Schum.	native - ornamental	vine
	Pandorea pandorana (Andrews) Steenis	native	vine
	Tecomanthe hillii (F.Muell.) Steenis	native	vine
	Jacaranda mimosifolia D.Don	ornamental	tree
	Pyrostegia venusta (Ker-Gawl.) Miers	ornamental	vine
	Tabebuia palmeri Rose	ornamental	tree
	Tecoma stans (L.)	ornamental/invasive	shrub
	Spathodea campanulata P.Beauv.	ornamental/invasive	tree
Acanthaceae	*Graptophyllum excelsum* (F.Muell.) Druce	native	shrub/small tree
	Hypoestes floribunda R.Br.	native	shrub
	Thunbergia grandiflora (Roxb.) ex. (Rottler) Roxb.	invasive	vine
Avicenniaceae	*Avicennia marina* (Forssk.) Vierh.	native mangrove	tree
Gesneriaceae	*Boea hygroscopica* F.Muell.	native	ground cover
	Saintpaulia ionantha Wendl.	ornamental	ground cover
Myporaceae	*Myoporum acuminatum* R.Br.	native -ornamental	shrub/small tree
	Myoporum boninense ssp. *australe* Chinnock.	native -ornamental	shrub/small tree
	Myoporum montanum R.Br.	native -ornamental	shrub/small tree
	Eremophila maculata (Ker Gawl.) F.Muell.	native -ornamental	shrub
	Eremophila bignoniiflora x polyclada	native -ornamental	shrub
Oleaceae	*Olea europaea* L.	crop	tree
	Olea paniculate R. Br.	native	tree
	Jasminum suavissimum Lindl.	native	vine
Pedaliaceae	*Uncarina grandidieri* (Baill.) Stapf	ornamental	tree
	Sesamum indicum L.	crop	shrub
Scrophulariaceae	*Antirrhinum* sp.	ornamental	herb
	Artanema fimbriatum (Graham) D.Don	native	herb
	P*aulownia tomentose* (Thunb.) Sieb .& Zucc. ex Steud.	Invasive/crop	tree
Verbenaceae	*Citharexylem spinosum* L.	ornamental	tree
Lamiaceae	*Vitex lignum-vitae* Schauer.	native	tree
	Ocimum basilium L.	crop	herb
Plantaginaceae	*Plantago lanceolata* L.	invasive/medicinal	herb
SOLANALES			
Solanaceae	*Lycopersicon esculentum* Mill	crop	herb

2. Variation from the approved list

Nil.

INFORMATION ON THE PROPOSED BIOLOGICAL CONTROL AGENT, *Carvalhotingis visenda*

1. Taxonomy

Order: Hemiptera
Family: Tingidae
Genus: *Carvalhotingis*
Species: *visenda* (Drake & Hambleton)
Specimens identified by: Dr Thomas J. Henry, Systematic Entomology Laboratory, USDA-ARS, Beltsville, Maryland 20705-2350, USA.

Voucher specimens have been lodged with the Australian Quarantine and Inspection Service (AQIS) in the following form: Genus name, species name & collection locations.

2. Description

Adults are creamy-white on emergence, turning grey as their elytra hardened. Elytra with two dark marks. The sizes of nymphs (head to the tips of the abdomen) and adults (head to the tips of the wings) are as follow: 1st instar nymph = 0.22 ± 0.04 mm; 2nd instar nymph = 0.73 ± 0.03 mm; 3rd instar nymph = 0.96 ± 0.10 mm; 4th instar nymph = 1.36 ± 0.12 mm; 5th instar nymph = 1.70 ± 0.09 mm; adult female = 2.78 ± 0.14 mm and adult male adult = 2.64 ± 0.13 mm.

3. Biology of Agent

Eggs: Females lay their eggs closely together in groups of about 19 along the main vein on the undersides of leaves. After about 15 days the nymphs emerge.

Nymphs: On emergence, the nymphs fed as a group, usually on the underside of leaves, by sucking out the cell contents of leaves, causing white speckling and eventual abscission of the leaves. The nymphal period occupied about 15 days, during which the nymphs developed through 5 nymphal instars.

Adults: The adults fed in the same manner as the immatures, but were found singly on the leaves, readily dropping when disturbed. After a period of about 8 days, females started laying eggs, up to 187 in their lifetime. Adults were relatively long-lived; some adults were found alive after 2 months.

4. Native range and probable centre of origin

The genus *Carvalhotingis* is known only from Central to South America (Mexico, Guatemala, Argentina, Brazil, Bolivia and Peru) (Froeschner 1995). *C. visenda* is known to occur in Argentina, Brazil and Peru (Drake and Ruhoff, 1965). *C. visenda* was originally collected from Argentina and Brazil (Stefan Neser, Plant Protection Research Institute of South Africa, Personal communication).

5. Related species

The genus *Acanthocheila* Stal was revised and redefined in 1995 with the erection of the new genus *Carvalhotingis* (Froeschner 1995). Of the 16 species formerly known under the genus *Acanthocheila*, 8 were retained as *Acanthocheila*, but reduced to 7 with 1 new synonymy. The other 8 species were transferred to *Carvalhotingis* and

reduced to 5 with the following new combinations and new synonymies: *C. comitis* (Drake), *C. hollandi* (Drake) (= *comentis* Drake, = *denieri* Monte, = *rustica plana* Drake, = *rustica rustica* Monte), *C. nexa* (Drake), *C. tumida* (Drake), and *C. visenda* (Drake and Hambleton) (Froeschner 1995).

The genus *Carvalhotingis* is known only from Central to South America (Mexico, Guatemala, Argentina, Brazil, Bolivia and Peru) (Froeschner 1995). *Carvalhotingis hollandi (=Acanthocheila holandi)* was recorded on Bignoniaceae and *Carvalhotingis visenda* (=*Acanthocheila visenda)* was recorded on *M. unguis-cati* (=*Bignonia exoleta*), but the host plants of other species are not known. (Drake and Ruhoff, 1965).

Surveys by Stefan Neser (PPRI, South Africa) over a 4-year period have only recorded *C. visenda* and *C. hollandi* only on *M. unguis-cati* in Argentina and Brazil (S. Neser pers. comm.). All individuals imported into S. Africa and subsequently into Australia were collected only on cat's claw creeper. We therefore considered the South African host specificity test results as the basis for selecting test plants. The South African results (Annexure I) suggest that this insect is host specific and does not appear to reproduce on any plants other than cat's claw creeper.

6. Source of agent

C. visenda was sourced from a laboratory colony maintained at the Plant Protection Research Institute, Pretoria. The colony was established from material collected on *M. unguis-cati* by Dr S. Neser in Argentina and Brazil in April 2002. Dr Thomas J. Henry, from the Systematic Entomology Laboratory, USDA, Beltsville, Maryland, USA, has confirmed the identification of the tingid species.

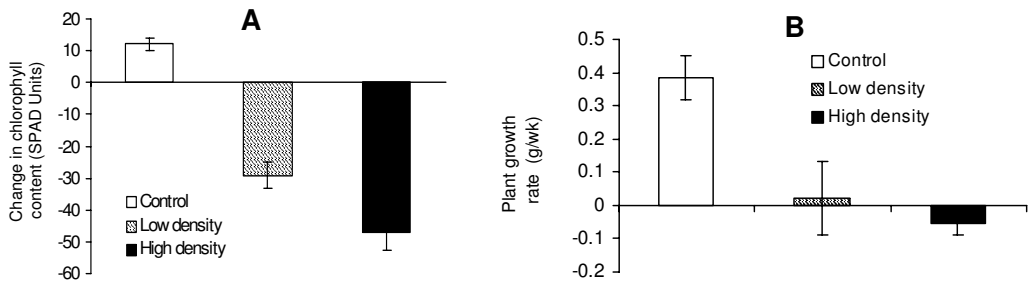
7. Mode of action

Both adults and nymphs pierce the epidermis and feed on the intracellular contents, resulting in feeding injury and plasmolysis. Damage to the foliar tissue increases in severity with increasing insect population. Severe tingid damage in general causes shoot and leaf discolouration and premature leaf drop off, which results in stunted growth of plants and reduced plant vigour (Drake and Ruhoff, 1965).

8. Potential for control

In South Africa, under laboratory conditions, *C. visenda* displayed several promising biological characteristics that may contribute to its success as a biocontrol agent. These included longevity, short generation times, high rates of reproductive output, and impressive visible damage to the host plant caused by nymphal and adult feeding.

Fig. 3. Impact of *Carvalhotingis visenda* on leaf chlorosis (A) and the growth rate (dry weight) (B) in cat's claw seedlings under low and high insect densities.



Under quarantine conditions, feeding by *C. visenda* caused severe chlorosis (Fig. 3A), resulting in a significant negative impact on growth rates of cat's claw creeper seedlings due to feeding (Fig. 3B) (Conrad 2006).

9. Non-target organisms at risk

The tingid feed on the intracellular contents, resulting in chlorosis. Hence feeding by the tingid on target and non-target plants was quantified by measuring the level of leaf chlorophyll using a chlorophyll meter before and after feeding. Risk assessment by measuring feeding induced changes in leaf chlorophyll levels suggests that *C. visenda* does not pose any threat to non-target plants (refer section 11.2.2. below). Research done in South Africa also suggests that no plants other than the target weed are at risk. (South African host specificity report enclosed; Annexure I). There are no indications that non-target organisms are at risk.

10. Possible interactions

There have been no previous agents released against *M. unguis-cati* in Australia with which *C. visenda* could interact. A leaf-tying moth *Hypocosmia pyrochroma* Jones (Lepidoptera: Pyralidae) from Brazil and Argentina is currently being investigated in quarantine for its host specificity and suitability as a biocontrol agent for cat's claw creeper in Australia.

11. Host specificity testing

11.1. Host specificity testing in South Africa

Host testing for South Africa was carried out in Pretoria against 24 plant species in 11 families (Annexure I), focussing on species of Bignoniaceae and of the related families Pedeliaceae, Acanthaceae and Scrophulariaceae. Results indicated that the tingid is highly specific to *M. unguis-cati*. An application to release the tingid in South Africa has been lodged (test results enclosed; Annexure I), and has been approved for field release in Mpumalanga Province in South Africa (Stefan Neser, pers. comm.).

11.2. Host specificity testing in Australia

Detailed biological and host specificity testing was conducted using potted plants in a temperature-controlled (22-27°C) quarantine insectary at the Alan Fletcher Research Station (AFRS), Sherwood, Queensland. The potential host range of *C. visenda* was evaluated on the basis of nymphal development, adult feeding and survival, and oviposition preference using choice and no-choice tests involving 38 plant species in 10 families (Table 1).

11.2.1. No choice adult survival and oviposition tests

Method: Batches of test plants were tested as they became available. In each batch a control *M. unguis-cati* plant was included and each test was repeated for a minimum of five times. On each small test plant, 10 newly emerged adults were enclosed in a cylindrical transparent perspex tube (34 cm high and 12 cm diameter) with a gauze cap.

Table 2. Adult survival (days) and oviposition (eggs/plant) (Means ± SE) in no-choice trials.

ORDER / Family	Test plants	Rep	longevity (days)	Oviposition (eggs/plant)
LAMEALES				
Bignoniaceae	***Macfadyena unguis-cati***	**27**	**53.3 ± 3.81**	**1478 ± 156**
	Tecomanthe hillii	5	12.2 ± 1.52	0
	Pandorea jasminoides	5	34.2 ± 4.26	0
	Pandorea pandorana	5	4.6 ± 0.65	0
	Jacaranda mimosifolia	5	6.5 ± 2.57	0
	Deplanchea tetraphylla	5	9.5 ± 3.53	0
	Pyrostegia venusta	6	22.1 ± 5.52	0
	Tabebuia palmeri	5	6.9 ± 2.43	0
	Tecoma stans	5	47.6 ± 8.98	0
	Dolichandrone heterophylla	5	11.6 ± 3.31	0
	Neosepicaea viticoides	5	2.9 ± 1.2	0
	Spathodea campanulata	5	9.2 ± 3.11	0
Gesneriaceae	*Boea hygroscopica*	5	4.0 ± 0.58	0
	Saintpaulia ionantha	5	2.3 ± 0.26	0
Acanthaceae	*Hypoestes floribunda*	6	19.1 ± 8.93	0
	Hypoestes phyllostachia	5	4.6 ± 0.73	0
	Graptophyllum excelsum	16	6.8 ± 2.46	0
	Thunbergia grandiflora	5	27.2 ± 5.02	0
Scrophulariaceae	*Antirrihinum sp.*	5	5.6 ± 0.9	0
	Artanema fimbriatum	5	1.8 ± 0.22	0
	Paulownia tomentosa	5	1.9 ± 0.19	0
Myoporaceae	*Myoporum accuminatum*	5	17.8 ± 4.07	0
	Myoporum montanum	5	5.9 ± 1.02	0
	Myoporum boninense ssp. australe	5	14.1 ± 5.3	0
	Eremophila maculata	5	7.9 ± 1.4	0
	Eremophila bignoniiflora x polyclada	6	9.5 ± 3.1	0
Plantaginaceae	*Plantago lanceolata*	5	6.9 ± 1.34	0
Pedeliaceae	*Sesamum indicum*	5	2.9 ± 0.54	0
	Uncarina grandidieri	5	1.1 ± 0.04	0
Verbenaceae	*Citharexylem spinosum*	8	15.1 ± 3.4	0
Lamiaceae	*Vitex lignum-vitae*	5	10.7 ± 1.3	0
	Ocimum basilium	5	1.9 ± 0.23	0
Avicenniaceae	*Avicennia marina*	8	14.1 ± 3.12	0
Oleaceae	*Olea europaea*	6	12.1 ± 2.6	0
	Olea paniculata	5	7.5 ± 1.52	0
	Jasminum suavissimum	6	7.4 ± 3.37	0
SOLANALES				
Solanaceae	*Lycopersicon esculentum*	5	3.0 ± 0.38	0
NEGATIVE CONTROL	Water alone	3	3.9 ± 0.17	0

On larger test plants, 10 newly emerged adults were enclosed in fine nylon gauze bags secured around potted test plant branches with healthy leaves. The plants were sampled three times each week and the surviving adults in each cage were counted, along with signs of feeding and oviposition. In test plants where oviposition occurred, bags were replaced and egg hatching and nymphal development, if any, were monitored until the emergence of adults or death of all nymphs.

Results: The duration of adult survival different significantly on different test plant species (Table 2; One-way ANOVA, $F_{37,191} = 15.1$, $P < 0.001$). The duration of adult survival on *M. unguis-cati* was significantly higher than other plants (Tukey test, $P > 0.05$). Adults not exposed to any test plants (water alone, negative control) survived up to a week. The duration of adult survival remained higher (but less than on *M. unguis-cati*) on a few native (*P. jasminoides* and *H. floribunda*) and non-native plants (*T. stans*, *T. grandiflora* and *P. venusta*)(Table 2), but no visible feeding damage was

evident on any of the plants other than *M. unguis-cati*. Oviposition was evident only on *M. unguis-cati*.

11.2.2. No choice adult feeding damage: chlorophyll loss

Method: Effects of *C. visenda* feeding damage on target and non-target plants on which adult survival remained higher (Table 2) were evaluated by measuring chlorophyll (SPAD values) loss using a Chlorophyll meter (SPAD-502, Konica-Minolta®). On each test plant (*M. unguis-cat, A. marina, C. spinosum, P. jasminoides, T. stans, M. acuminatum, V. lignum-vitae, E. excelsum* and *P. venusta*) with two opposite leaves (all other leaves removed), 20 *C. visenda* adults were enclosed in a cylindrical transparent Perspex tube (34 cm high and 12 cm diameter) with a gauze cap. Simultaneously equal numbers of control plants with the same number of leaves were maintained free of *C. visenda* in cylindrical transparent Perspex tubes.

The chlorophyll content (SPAD values) of individual leaves on each test plant (control and treatment plants) was recorded before introducing the tingids (week-0). The chlorophyll content of each leaf in all plants was recorded after the first and second weeks, along with the number of adults on each plant. The experiment was repeated at least three times.

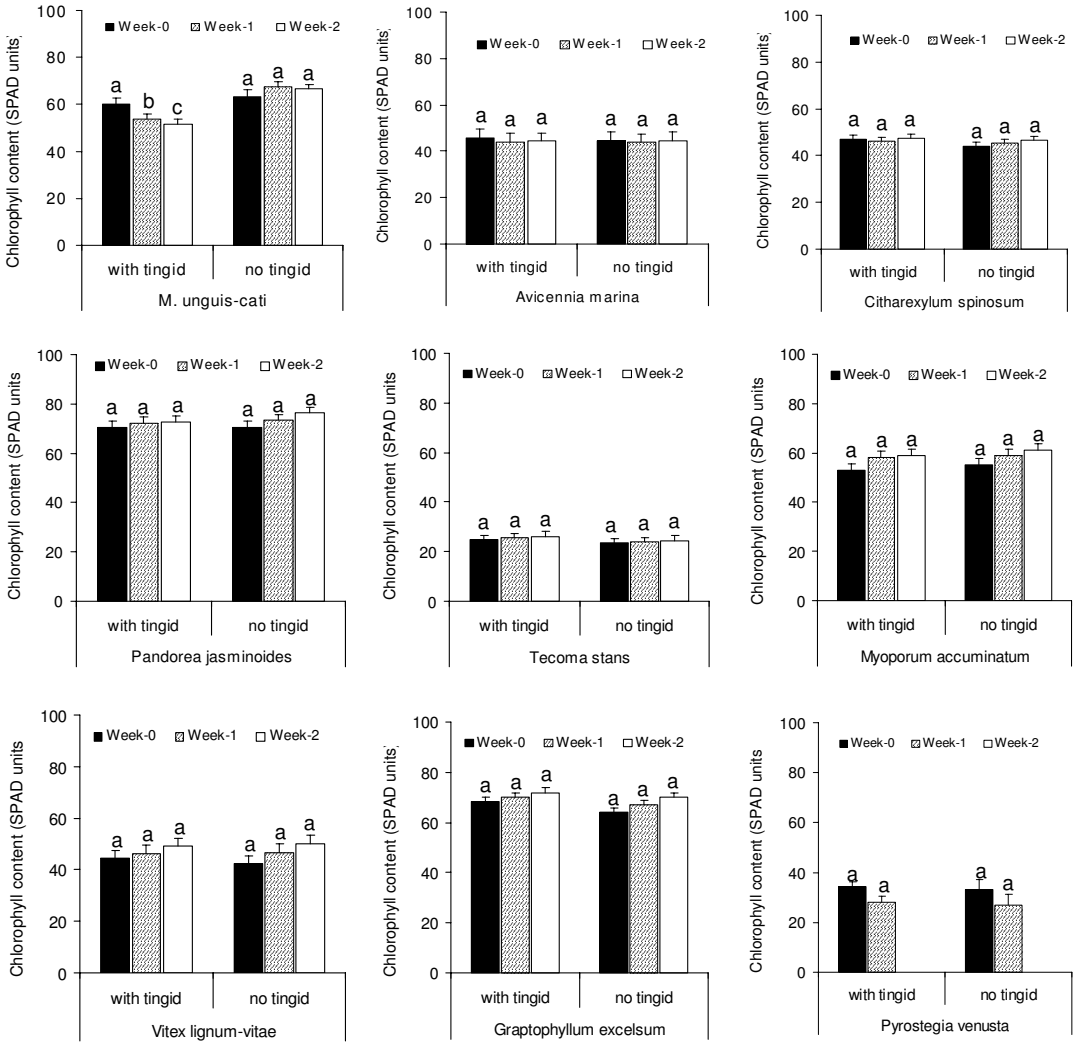
Results: On *M. unguis-cati* tingids caused significant chlorophyll loss, and the chlorophyll loss increased with time ($F_{1,2,38} = 5.15$, $P = 0.01$) (Fig. 4). On the non-target test plant, *A. marina* ($F_{1,2,30} = 0.03$, $P = 0.97$), *C. spinosum* ($F_{1,2,30} = 0.19$, $P = 0.83$), *P. jasminoides* ($F_{1,2,30} = 0.28$, $P = 0.76$), *T. stans* ($F_{1,2,30} = 0.33$, $P = 0.72$), *M. acuminatum* ($F_{1,2,38} = 0.04$, $P = 0.96$), *V. lignum-vitae* ($F_{1,2,30} = 0.17$, $P = 0.85$), *E. excelsum* ($F_{1,2,30} = 0.21$, $P = 0.81$) and *P. venusta* ($F_{3,20} = 1.18$, $P = 0.34$) there was no chlorophyll loss due to tingid feeding (Fig. 4).

11.2.3. No choice nymphal survival and development tests

Method: Batches of test plants were tested as they became available. In each batch a control *M. unguis-cati* plant was included and each test was repeated at least five times. Ten newly emerged nymphs were enclosed in fine nylon gauze bags secured around potted test plant branches with healthy leaves or enclosed in a cylindrical transparent perspex tube containing a potted test plant with a gauze cap. The nymphs were checked thrice weekly and dead nymphs and their instar were recorded. Emerging adults were counted.

Results: On *M. unguis-cati* more than 81% (80.6 ± 1.87%; N = 36) of the newly emerged nymphs completed development and became adults in 24.9 ± 0.38 days (N = 50). No feeding or any nymphal development occurred on any of the non-target plants.

Fig. 4. Leaf chlorophyll levels in target and non-target plants with and without *C. visenda* adults. Vertical bars represent SEM. Tukey test: bars with same letter are not significantly different.



11.2.4. Multiple choice feeding and oviposition preference tests

Methods: Those test plant species on which adults survived more than 15 days in no choice tests (Table 2) were subjected to multiple-choice oviposition and feeding tests. In Trial 1, two introduced but invasive plants on which the adult survival remained high (*Tecoma stans* & *Thunbergia grandiflora*; Table 2) were screened; and in Trial 2, 10 plant species which include both native plants and introduced ornamentals were screened (Fig. 5).

In Trial 1, two plants each of *M. unguis-cati, Thunbergia grandiflora and Tecoma stans* were placed randomly in a cage with 50 adults released in the middle of the cage. All plants were sampled on day 2, 3, 4, 5 and 7 and the numbers of adults in each plant were recorded along with any feeding and oviposition marks. The test was repeated three times.

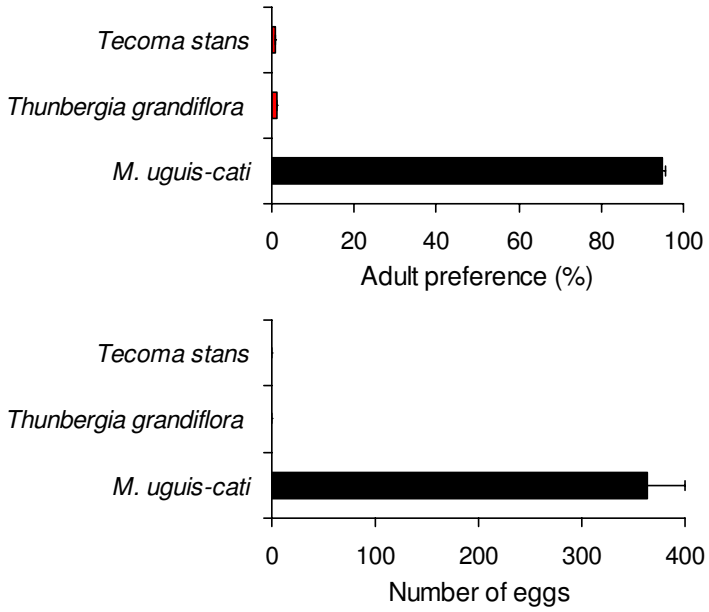
In Trial 2, a plant each of *M. unguis-cati, Olea europea, Pandorea jasminoides, Citharexylem spinosum, Pyrostegia venusta, Eremophilla bignoniflora x polyclada, Graptophyllum excelsum, Jasminum suavissimum, Hypoestes floribunda, Myoporum acuminatum, Avicennia marina* were placed randomly in a cage with 50

adults. All plants were sampled on day 3,4,5,6,7 and 10 and the number of adults in each plant was recorded along with any feeding and oviposition marks. The test was repeated four times.

Results:

Trial 1: The proportion of adults on *M. unguis-cati* (95 ± 0.8%) remained significantly higher than on *T. grandiflora* (1.1 ± 0.2%) and *T. stans* (0.6 ± 0.2%) ($F_{3, 8} = 8137$, $P < 0.001$) (Fig. 5). The average number of adults on the cage walls (3.7 ± 0.6%) remained significantly higher than on both *T. grandiflora* and *T. stans.* Oviposition was also evident only on cat's claw (364 ± 36 eggs) with no eggs laid on other non-target plants (Fig. 5).

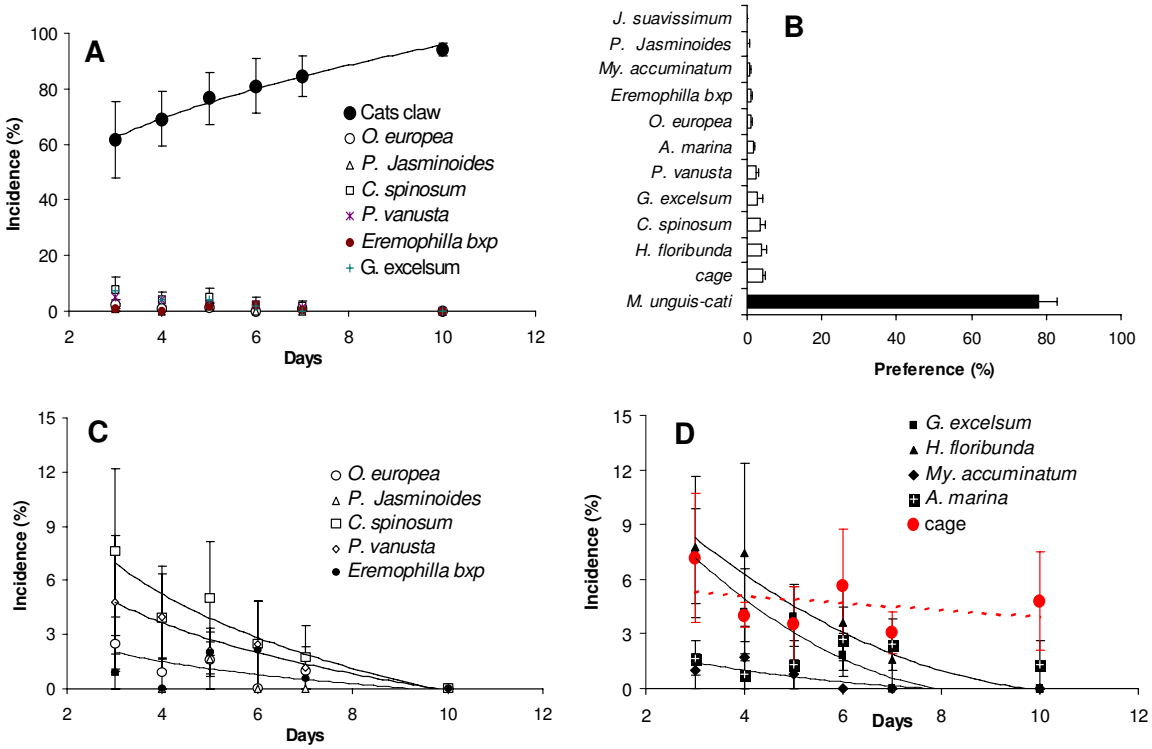
Fig. 5. Preference for feeding and oviposition by *M. unguis-cati* over introduced non-target plants in choice trials.



Trial 2: The proportion of adults on *M. unguis-cati* increased with time ($y = 42.51x^{0.353}$; $R^2 = 0.988$) (Fig. 6A), and correspondingly the proportion of adults on non-target plants declined (Fig. 6C & 6D). On the 10th day no adults were evident on any of the non-target plants. In contrast, the proportion of adults present on the walls of the cages did not decline with time ($R^2 = 0.106$; $F = 0.473$, $P = 0.53$; Fig. 6D), and always remained higher than on any of the non-target plants (Fig. 6B). This suggests that the reduction in the proportion of adults in the non-target plants was not random and confirms the tingid's preference to *M. unguis-cati*. More over, there was also no visible feeding damage on any of the non-target plants supporting the above argument.

Oviposition was evident on *M. unguis-cati* (398 ± 138 eggs/plant) in all four trials, and on *G. excelsum* (4.8 ± 3.82 eggs/plant) only in two of the four trials. No oviposition was recorded on any other non-target plants in the trials. On *M. unguis-cati* oviposition was evident even on the first day after introducing the tingids, while on *G. excelsum* oviposition was noticed only after the 6th day. However, none of the nymphs emerged in *G. excelsum* survived and developed.

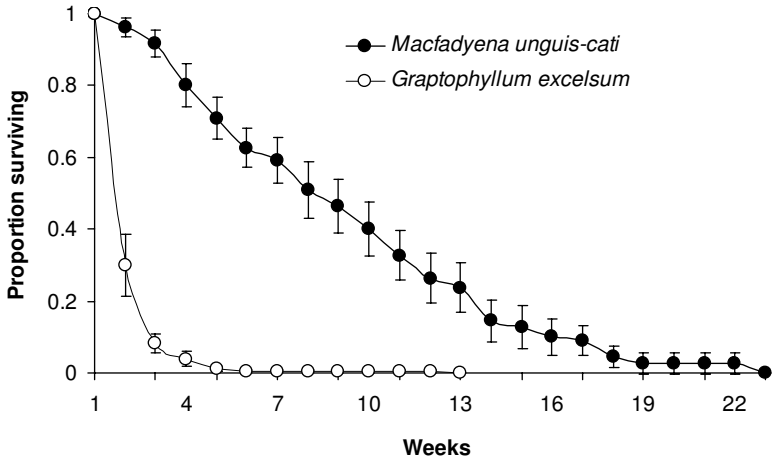
Fig. 6. Preference (Mean + SEM) for *M. unguis-cati* over non-target plants in choice trials.



11.2.5. No-choice demography studies

Methods: Since oviposition was evident on *G. excelsum* in the choice trial, a no-choice demography trial was carried out to ascertain the ability of *G. excelsum* to sustain the tingid population. Newly emerged adults (N = 30) were introduced to individual *G. excelsum* plants enclosed in transparent cylindrical perspex tubes and the proportion of surviving adults and oviposition, if any, were recorded at weekly intervals. The trial was repeated 10 times.

Fig. 7. Proportion of adults surviving on *M. unguis-cati* and *G. excelsum* in no-choice demography studies.



Results: More than 90% of the tingids did not survive on *G. excelsum* beyond three weeks (Fig. 7). Oviposition was observed in only one of the 10 trials, with two eggs

on one leaf. However, none of the eggs hatched, confirming that *G. excelsum* is not suitable host to sustain the tingid population.

On *M. unguis-cati*, in contrast, females laid 6.8 ± 0.8 eggs/day with an average of 73.9 ± 11.2 eggs each in their lifetime. Females (48.0 ± 7.3 days) lived longer than males (24.4 ± 7.9 days).

11.3. Conclusions

- In no-choice tests, although adults survived on a few of the non-target plants, no eggs were laid on any of the non-target plants. There was also no visible feeding damage on any of the non-target plants.
- Further evidence from chlorophyll estimation studies indicates that there is no feeding induced chlorosis in any of the non-target plants.
- In no-choice tests, nymphs developed and became adults only on the target weed and no nymphal development occurred on any of the non-target plants.
- In choice tests, adults showed distinct preference for the target weed, and the number of adults on the cage wall was consistently more than on any non-target plants. At the end of the trial no adults were evident on any non-target plants.
- In the choice trials, though very few eggs were laid on non-target *G. excelsum* plants, all nymphs died soon without further development.
- No choice demography studies on *G. excelsum* confirmed that a viable population could not be sustained on this non-target plant.
- The results overall suggest that *C. visenda* is specific to *M. unguis-cati* and it does not pose risk to any of the non-target plants tested. Host specificity tests conducted in South Africa further support this.

12. Use of agent in other countries

An application to release the tingid in South Africa has been lodged (Annexure I & II; Hester Williams, pers. comm.). The tingid has been approved for field release in Mpumalanga Province in South Africa (Stefan Neser, pers. comm.).

13. Acknowledgments

We thank Dr Stefan Neser and Ms Hester Williams of Plant Protection Research Institute, Pretoria, Republic of South Africa, for providing tingid colonies and for information on its biology; Wilmot Senaratne, Kathryn Conrad, Michele Rogers and Tanya Grimshaw for technical assistance.

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Annexure I

Assessment of the environmental Impact of releasing *Carvalhotingis visenda* (Drake & Hambleton) and *C. hollandi* (Drake) (Hemiptera: Tingidae) as biological control agents for *Macfadyena unguis-cati* (L.) Gentry (Bignoniaceae) in South Africa.

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July 2003

EXECUTIVE SUMMARY

Macfadyena unguis-cati is a frost-tolerant, perennial vine whose native range extends from Mexico and the tropical Americas, including Trinidad and Tobago. The plant was introduced into South Africa as an ornamental and was planted widely, and is now found to be almost impossible to remove from overrun gardens. It has also invaded natural vegetation, particularly woodlands and forests, as well as cultivated orchards, forestry plantations, roadsides and open urban spaces. Cat's claw creeper has been reported causing problems in several provinces of South Africa, including Limpopo, Mpumalanga, Gauteng, North West and KwaZulu-Natal. In natural forests, the weed forms a thick carpet on the forest floors and clambers up tree trunks draping itself over the tree canopy, where a combination of weight and shading can kill even the largest canopy trees. In addition, it excludes light from the undergrowth, out competes shallow-rooted understorey plants and suppresses seed germination.

Cat's claw creeper produces copious amounts of seeds that are widely spread by wind and water. The plant also reproduces vegetatively very efficiently through an extensive system of roots and tubers. These tubers hamper mechanical and chemical control methods, as new plants are regenerated if any attempts are made to control the aboveground parts. Currently no herbicide is registered against cat's claw creeper. Biological control, using host-specific natural enemies is thus the only promising approach towards solving the problem on the long term.

Although this weed is considered to be in the early stages of invasion, if left unrepressed it will become widespread causing irreversible damage to sensitive and economically important eco-systems in many areas in South Africa. It is recognized as a most important invasive plant in NSW and Queensland in Australia, and is already targeted for biological control in Queensland. This weed represents an opportunity to apply biological control at a relatively early stage of its spread in southern Africa, and thus to prevent costly and time consuming eradication practices in future, as is found with many of the more widespread weeds in South Africa.

Two leaf-sucking tingid species, *Carvalhotingis visenda* and *C. hollandi*, were collected at sites in Brazil and Argentina in April 2002 and imported to the ARC-PPRI quarantine facility in Pretoria, South Africa. The insect species were subjected to meticulous host specificity testing, including nymphal no-choice and adult multi-choice trials, during which they were exposed to several related and other indigenous and commercially used plant species (Table 1 on page 11). Results indicated that these two tingid species are highly specific to *M. unguis-cati*.

Under laboratory conditions, both tingid species displayed several promising biological characteristics that will contribute to their success as biocontrol agents. These included long-lived adults, short generation times, high rates of reproductive increase; and impressive visible damage to the host plant caused by nymphal and adult feeding.

These studies indicate that *C. visenda* and *C. hollandi* are completely specific to *Macfadyena unguis-cati*, and will pose no threat to indigenous or commercial plant species in South Africa. Once released and established, they will contribute towards exerting pressure on *M. unguis-cati* and curbing the aggressiveness of this weed.

4. INTRODUCTION

4.1 The target weed: *Macfadyena unguis-cati*

Macfadyena unguis-cati (L.) Gentry is a frost-tolerant, perennial vine whose native range extends from Mexico through to the tropical Americas, including Trinidad and Tobago (Everett 1980). Each leaf consists of two small leaflets, between which lies a tendril ending in three, tiny, hooked claws. The name "cat's claw creeper" is derived from these clawed tendrils that enable the plant to climb up against walls, rocks, tree trunks and other vegetation. Also typical are the large, yellow, trumpet-shaped flowers that are displayed during spring and summer. Winged seeds are produced in long, slender, pod-like capsules and are released when the capsules split (Auld and Medd 1987).

The species was introduced into South Africa as an ornamental plant (Henderson 1995), and is used as a fast-growing creeper for hedges and walls. However, it has since escaped cultivation and, in 2001, was declared a category 1 weed in South Africa (Act No. 43 of 1983, amended in 2001), which means that this species is prohibited in South Africa and must be controlled or eradicated where possible, except in biological control reserves (Henderson 2001). Cat's claw creeper invades natural vegetation, particularly woodlands and forests, as well as cultivated orchards, forestry plantations, roadsides and open urban spaces. In natural forests, the weed forms a thick carpet on the forest floors and clambers up tree trunks draping itself over the tree canopy, where a combination of weight and shading can kill even the largest canopy trees (Neser 1996). In addition, it excludes light from the undergrowth, out competes shallow-rooted understorey plants and suppresses seed germination. The largest remaining indigenous forest in northern South Africa, the Grootvadersbosch of Magoebaskloof, is threatened by a large infestation of *M. unguis-cati* on its eastern boundary. This infestation has already severely degraded some of the bordering indigenous forest on the Westfalia Estate of the Hans Merensky Foundation, despite several costly attempts at herbicide control (H. G. Zimmermann, pers. comm.).

Macfadyena unguis-cati has already been reported as problematic in several provinces, in particular Mpumalanga and Limpopo, but also Gauteng, North West and KwaZulu-Natal (Henderson 1995). Langel and Craddock-Burks (2000) report *M. unguis-cati* to be a weed in south and northern Florida, where it is persistent around former habitation and has spread to become the dominant ground cover in undisturbed hammocks at Lake George and hammock preserves in Dade Country. *Macfadyena unguis-cati* has also been reported as a weed in Australia, causing considerable damage (R. Macfadyen, pers. comm.), India, Mauritius and St. Helena Island (Holm et al. 1991, Swarbrick and Sharratt 1994), where it is particularly invasive in coastal and subcoastal regions (e.g. Australia) and in floodplain and forest habitats (e.g. St. Helena Island). The climatic attributes of these areas, mostly temperate to subtropical regions with medium to high rainfall, suggest that large parts of South Africa are suitable for invasion.

The potential for further spread is also high because the plant is present in gardens throughout South Africa. These plants act as sources for the wind and water dispersed seeds. However, the plant also reproduces vegetatively through its extensive root system. Large tubers, formed along the lateral roots, each produce climbing runners that are also capable of forming tuber-like roots wherever the nodes touch the soil. All tubers produce new plants if separated from the parent plant.

4.2 Control methods

Herbicide and Mechanical control

Mechanical and chemical control methods have proved largely unsuccessful in South Africa and no herbicides are registered for use on *M. unguis-cati*. Control operations are hampered by the weed's tuber-like roots which break off during mechanical weeding and which allow the plants to regenerate, even when the above-ground parts are sprayed (Neser 1996). This is often aggravated by the inaccessibility of plants and the difficulty in locating all of the points where stems are rooted. The use of broad-leaf herbicides is also undesirable in most of the situations invaded, particularly orchards, forests and plantations and biological control is thus the only viable option.

Biological Control

The biological control programme against *M. unguis-cati* was initiated in 1996, and the first agent, the golden spotted tortoise beetle, *Charidotis auroguttata* released in 1999 (Williams 2002). Attempts at mass rearing this insect have been problematic and establishment disappointing, suggesting that the

beetle may not be ideally suited to South African conditions. Additional agents are needed to augment it.

4.3 The candidate biocontrol agents: *Carvalhotingis visenda* (Drake & Hambleton) and *C. hollandi* (Drake)

The two cat's claw leaf-sucking tingids, *C. visenda* and *C. hollandi* were collected in Argentina and Brazil in April 2002 and imported into South Africa under Import Permit No. P0001308. Voucher specimens were deposited in the National Collection of Insects in Pretoria. The final identification was done by Thomas J. Henry, from the Systematic Entomology Laboratory, USDA, Beltsville, Maryland, USA.

Studies on the biology and host specificity of *C. visenda* and *C. hollandi* are represented below in support of this application for permission to release the two tingids in South Africa.

5. MATERIALS AND METHODS

Similar materials and methods were used for the biology and host specificity studies on the two tingid species, unless other otherwise stated.

5.1 Biological studies

Biological studies were done in a quarantine laboratory with overhead daylight fluorescent light-banks with conditions maintained at a 12/12 hour light/dark cycle, with temperatures ranging from 22ºC to 29ºC, and relative humidity from 55% to 65%. All studies were conducted on potted plants of *M. unguis-cati* unless otherwise stated.

Adult longevity and female fertility were studied by enclosing newly-emerged adults in ventilated containers into which live stems of cat's claw creeper were inserted through a small hole on the side of the container. The date on which oviposition started was noted for all females. The number of eggs laid by each female was noted twice weekly, until the females died. To study the number and duration of the nymphal instars, newly emerged nymphs were separated into small containers with freshly-cut leaves of cat's claw creeper, replaced regularly. The containers were checked every day and the instars of the nymphs recorded.

5.2 Host specificity studies

The selection of test plant species was done according to Wapshere's (1974) proposed centrifugal phylogenetic testing method. Test plant species (Table 1 on page 11)
consisted of 24 representative species in total; 9 species in the family Bignoniaceae; 7 species in related families that fall under the same order (Lamiales) as Bignoniaceae; and 7 economically important species representing several families. Host specificity studies were conducted in a quarantine glasshouse with natural photoperiod, with temperatures ranging from 22ºC at night to 29ºC during the day. Relative humidity ranged between 40% and 80%.

Nymphal no-choice trials

Ten newly-emerged nymphs were transferred to potted plants of the test species including *M. unguis-cati* as control. The plant pots were caged and the nymphs allowed to feed and develop. The number of nymphs surviving to adulthood was recorded. Each test plant species was exposed to nymphs on at least 3 separate occasions.

Adult multi- choice trials

Test plant species were exposed to adult feeding and oviposition during adult multi-choice trials. These trials were conducted in cages measuring 1.5m x 2m x 1m with the plants arranged according to a 3x4 rectangular lattice design. This design consisted of 3 replications, with the test plant species each time arranged according to a pre-defined layout. Test plant species included 10 species in the family Bignoniaceae, while one species each in the closest related families Pedaliaceae and Scrophulariaceae were included (Table 1 on page 11) to complete the numbers needed for a 3x4 lattice design layout. Twenty pairs of adults for *C. visenda* and 10 pairs for *C. hollandi* were released into the cage and removed after one week. At the conclusion of each test, any feeding marks and the number of adults on the test plant species were noted and recorded. *Carvalhotingis visenda* females laid their eggs tightly grouped along the main vein on the underside of leaves, and thus the number of such groups was

recorded. *Carvalhotingis hollandi* females laid their eggs in more scattered groups on the upperside of leaves and in this case the actual number of eggs was recorded.

6. RESULTS

6.1 Biological studies

Carvalhotingis visenda

Eggs: Females laid their eggs closely together in groups of about 19 along the main vein on the undersides of leaves. After about 15 days the nymphs emerge.
Nymphs: On emergence, the nymphs fed as a group, usually on the underside of leaves, by sucking out the cell-matter of leaves, causing white speckling and eventual abscission of the leaves. The nymphal period occupied about 15 days, during which the nymphs developed through 5 nymphal instars.

Adults: New adults were creamy-white on emergence, but turned grey in a few hours as their elytra hardened. A conspicuous feature of this species is the two black protruding knobs (bullae) on their elytra. The adults fed in the same manner as the immatures, but were found singly on the leaves, readily dropping when disturbed. After a period of about 8 days, females started laying eggs, up to 187 during a lifetime. Adults were relatively long-lived; some adults were found alive after 2 months.

Carvalhotingis hollandi

Eggs: Females laid their eggs in scattered groups on the upper (axial) aspect of leaves. The duration of the egg stage was about 15 days.

Nymphs: Like *C. visenda*, the nymphs fed as a group throughout the nymphal stage, also causing white speckling and premature leaf-drop. The nymphal period of five instars occupied about 19 days.

Adults: Adults were creamy-white on emergence, turning grey as their elytra hardened. Adults are more flat in appearance than *C. visenda*, also with two dark marks on their elytra, but these do not bulge out as those on *C. visenda*. Adults fed singly on the leaves, and displayed the same defense and escape mechanism as *C. visenda*, dropping rapidly when disturbed. About 10 days after emergence, females started laying eggs, about 4 to 5 eggs per day. Adults were relatively long-lived, with some adults found alive after 2 months.

6.2 Host specificity studies

Carvalhotingis visenda

Nymphal no-choice trials: Only *M. unguis-cati* proved to be suitable for nymphal feeding and development, with an average of 90% nymphs (45 out of 50) developing through to adulthood on it (Table 2 on page 12). Even under the deprived conditions of no-choice trials no other test species were suitable for nymphal development, or accepted for feeding (Table 2 on page 12).

6.2.2 Adult multi-choice trials

Feeding and oviposition took place only on *M. unguis-cati* with an average of 16.7 (16 to 17) groups of eggs laid on it per replicate (Table 3 on page 13). No other test plant species provided suitable sites for oviposition and/or feeding (Table 3 on page 13).

Carvalhotingis hollandi

Nymphal no-choice trials: Nymphal feeding and development took place on *M. unguis-cati* only, with an average of 80% of nymphs (24 out of 30) developing through to adulthood (Table 2 on page 12).

Adult multi-choice trials: *Macfadyena unguis-cati* qualified as the only plant species acceptable for feeding and oviposition by *C. hollandi* adults. No feeding or oviposition took place on any of the other test plant species (Table 3 on page 13).

7. DISCUSSION

Laboratory studies showed the two tingid species to be **highly** specific to *M. unguis-cati*, even within the family Bignoniaceae. No adult feeding and oviposition took place on any of the test plant species during any of the tests to which the insect species were submitted. A combination of non-selection by

females and non-development of nymphs even in no-choice situations is seen as a clear indication of host-specificity. The host specificity testing thus clearly indicates *M. unguis-cati* to be the only suitable host amongst closely-related and other plants for *C. visenda* and *C. hollandi* and that no threat is posed to other flora should these insect species be released as biocontrol agent for *M. unguis-cati* in South Africa.

Carvalhotingis visenda and *C. hollandi* displayed several promising biological factors under laboratory conditions that will contribute to their success as biocontrol agents. These included short generation times of about 30 days and 34 days respectively (under rearing conditions used), that will insure rapid population build-up in the field. Another positive aspect is the typical predator- and parasitoid-avoidance tactic by the adults that drop when disturbed or threatened.

Different species of Tingidae have been used as biocontrol agents in South Africa, some with great success, e.g. *Teleomemia scrupulosa* (Stål), which is considered to be one of the most valuable agents against the notorious invader, *Lantana camara* L. (Baars and Neser 1999).

8. RECOMMENDATION

Given that the tingid species, *Carvalhotingis visenda* and *C. hollandi*, are potentially highly damaging to the weed, *Macfadyena unguis-cati*, and that they pose no threat to economic or indigenous plants, it is strongly and unreservedly recommended that permission be granted to release the two species from quarantine for the biological control of *M. unguis-cati* in South Africa.

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Table 1: Plant species used during host specificity testing of *Carvalhotingis visenda* and *C. hollandi*, two potential biocontrol agents for *M. unguis-cati*

Family	Plant species	Common name
Bignoniaceae (Order: Lamiales Bromhead)	*Macfadyena unguis-cati*	Cat's claw creeper
	Podranea ricasoliana (Tanf.) Sprague*	Port St. John's creeper
	Rhigozum zambesiacum Bak.	Mopane pomegranate
	Jacaranda mimosifolia D. Don*	Jacaranda
	Tecoma capensis (Thunb.) Lindl.	Cape honey suckle
	Tecoma stans (L.)*	Yellow Bells
	Markhamia obtusifolia (Bak.) Sprague	Bell bean tree
	Markhamia zanzibarica (Bojer ex DC.) K. Schum.	
	Kigelia africana (Lam.) Benth.	Sausage tree
	Pyrostegia venusta (Ker Gawl.) Miers*	Golden shower
Related families: (Order: Lamiales Bromhead)		
Pedaliaceae	*Ceratotheca triloba* (Bernh.) Hook.	Wild foxglove
Acanthaceae	*Thunbergia grandiflora* (Roxb.) ex (Rottler) Roxb.*	Sky vine
	Thunbergia alata Sims	Black-eyed Susan
Scrophulariaceae	*Freylinia tropica* S. Moore	
	Halleria lucida L.	Tree fuchsia
Oleaceae	*Jasminum officinale* L.*	Yellow jasmine
	Jasminum multipartitum Hochst.	White jasmine
Other plant families:		
Solanaceae	*Lycopersicon esculentum* Mill.*	Tomato
Alliaceae	*Allium cepa* L.*	Onion
Brassicaceae	*Brassica oleracea* L.*	Cabbage
Chenopodiaceae	*Beta vulgaris* sub.sp. *vulgaris* L.*	Beetroot
	Beta vulgaris var. *cida* L.*	Spinach beet
Asteraceae	*Lactuca sativa* L.*	Lettuce
Umbelliferae	*Daucus carota* L.*	Carrot

* Non-indigenous crop or ornamental plants

Table 2: Results of no-choice trials with nymphs of *Carvalhotingis visenda* and *C. hollandi*.

Plant species	*n*	*C. visenda* **Mean no of nymphs surviving (out of 10)**	*n*	*C. hollandi* **Mean no of nymphs surviving (out of 10)**
Bignoniaceae				
Macfadyena unguis-cati	5	9	3	8
Podranea ricasoliana	3	0	3	0
Rhigozum zambesiacum	3	0	3	0
Jacaranda mimosifolia	3	0	3	0
Tecoma capensis	3	0	3	0
Tecoma stans	3	0	3	0
Markhamia obtusifolia	3	0	3	0
Markhamia zanzibarica	3	0	3	0
Kigelia africana	3	0	3	0
Pyrostegia venusta	3	0	3	0
Pedaliaceae				
Ceratotheca triloba	3	0	3	0
Acanthaceae				
Thunbergia grandiflora	3	0	3	0
Thunbergia alata	3	0	3	0
Schrophulariaceae				
Freylinia tropica	-	-	2	0
Halleria lucida	3	0	3	0
Oleaceae				
Jasminum officinale	3	0	3	0
Jasminum multipartitum	4	0	3	0
Solanaceae				
Lycopersicon esculentum	3	0	3	0
Alliaceae				
Allium cepa	-	-	3	0
Brassicaceae				
Brassica oleracea	3	0	3	0
Chenopodiaceae				
Beta vulgaris sub. sp. *vulgaris*	3	0	3	0
Beta vulgaris *var.* cida	-	-	3	0
Umbelliferae				
Daucus carota	3	0	-	-
Asteraceae				
Lactuca sativa	-	-	3	0

- Not included in test

Table 3: Results of multi-choice feeding and oviposition trials with adults of *Carvalhotingis visenda* and *C. hollandi*.

Plant species	*n*	*C. visenda*		*C. hollandi*	
		Feeding intensity	**No of groups of eggs deposited (Mean ± SE)**	**Feeding intensity**	**No of eggs deposited (Mean ± SE)**
Bignoniaceae					
Macfadyena unguis-cati	3	+++	16.7 ± 0.3	+++	114.7 ± 21.7
Podranea ricasoliana	3	-	0	-	0
Rhigozum zambesiacum	3	-	0	-	0
Jacaranda mimosifolia	3	-	0	-	0
Tecoma capensis	3	-	0	-	0
Tecoma stans	3	-	0	-	0
Markhamia obtusifolia	3	-	0	-	0
Markhamia zanzibarica	3	-	0	-	0
Kigelia africana	3	-	0	-	0
Pyrostegia venusta	3	-	0	-	0
Pedaliaceae					
Ceratotheca triloba	3	-	0	-	0
Schrophulariaceae					
Halleria lucida	3	-	0	-	0

+++ Normal feeding
- No feeding