Biological Control 55 (2010) 58–62

Contents lists available at ScienceDirect

Biological Control

journal homepage: www.elsevier.com/locate/ybcon

Introduction and establishment of *Carvalhotingis visenda* (Hemiptera: Tingidae) as a biological control agent for cat's claw creeper *Macfadyena unguis-cati* (Bignoniaceae) in Australia

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ARTICLE INFO

Article history:
Received 26 October 2009
Accepted 30 June 2010
Available online 6 July 2010

Keywords:
Tingidae
Mass-rearing
Field release
Community groups
Establishment
Weed
Biological control
Cat's claw creeper
Australia

ABSTRACT

Carvalhotingis visenda (Hemiptera: Tingidae) is the first biological control agent approved for release against cat's claw creeper *Macfadyena unguis-cati* (Bignoniaceae) in Australia. The mass-rearing and field releases of *C. visenda* commenced in May 2007 and since then more than half a million individuals have been released at 72 sites in Queensland and New South Wales. In addition, community groups have released over 11,000 tingid-infested potted cat's claw creeper plants at 63 sites in Queensland. Establishment of *C. visenda* was evident at 80% of the release sites after three years. The tingid established on the two morphologically distinct 'long-pod' and 'short-pod' cat's claw creeper varieties present in Australia. Establishment was more at sites that received three or more field releases (83%) than at sites that received two or less releases (73%); and also at sites that received more than 5000 individuals (82%) than at sites that received less than 5000 individuals (68%). In the field, the tingid spread slowly (5.4 m per year), and the maximum distance of *C. visenda* incidence away from the initial release points ranged from 6 m to approximately 1 km.

1. Introduction

Cat's claw creeper *Macfadyena unguis-cati* (L.) Gentry (Bignoniaceae) is a perennial woody climbing vine native to tropical America (Rafter et al., 2008). It is regarded as an invasive in several countries, including Australia, South Africa, India, Mauritius, China, Hawaii and Florida in the USA, and New Caledonia (King and Dhileepan, 2009). In Australia, cat's claw creeper is a major environmental weed in Queensland and New South Wales (NSW), where it poses a significant threat to biodiversity in riparian and rainforest communities (Vivian-Smith and Panetta, 2004).

Cat's claw creeper is a structural parasite that grows vigorously, sprawling over other standing vegetation including large trees and shrubs, eventually causing canopy collapse. In areas without standing vegetation or man-made structures (e.g. fences) the vines grow along the ground and form a dense mat which precludes the growth and seed germination of understory vegetation. Cat's claw creeper produces stolons and subterranean root tubers. It does not have a persistent seed bank, so while it spreads through seeds, it persists through the tuber bank (Vivian-Smith and Panetta, 2004; Osunkoya et al., 2009).

Chemical control options for managing cat's claw creeper are available, but are not often used due to the sensitive ecosystems in which it occurs. Mechanical control of above-ground growth provides only temporary relief, as regeneration from subterranean tubers continue over many years. As a result, there is a need to treat infested areas with mechanical or chemical control options repeatedly. This severely limits the size of areas that can be treated. Susceptibility of cat's claw creeper to herbivory (Raghu and Dhileepan, 2005; Raghu et al., 2006) suggests that biological control is the most desirable option to manage this weed.

Biological control of cat's claw creeper in Australia was initiated in 2001 in collaboration with the Agricultural Research Council-Plant Protection Research Institute (ARC-PPRI) in South Africa. A leaf-feeding beetle, *Charidotis auroguttata* (Boheman) (Chrysomelidae: Coleoptera) from Venezuela (Sparks, 1999; Williams, 2002) was the first agent to be imported into Australia for host specificity tests. But the application for its release was not approved because it was judged to be a risk to *Myoporum boninense australe* Chinnock (Myoporaceae), a native non-target plant (Dhileepan et al., 2005). Subsequently, the leaf-sucking tingid *Carvalhotingis visenda* (Drake

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doi:10.1016/j.biocontrol.2010.06.016

& Hambleton) (Hemiptera: Tingidae) from Brazil and Argentina (Williams et al., 2008), was imported to Australia for host specificity tests (Treviño et al., 2006). The tingid was approved for field release (Dhileepan et al., 2007), and mass-rearing and field release of this agent in Queensland and NSW commenced in June 2007. This paper describes various rearing and field release methods adopted, along with its field establishment status in Australia.

2. Materials and methods

2.1. Insect source

Carvalhotingis visenda, originally collected on cat's claw creeper from Curitiba (Brazil) and Posadas (Argentina) in April 2002 by Dr. Stefan Neser, was imported from the ARC-PPRI, Pretoria, South Africa into a quarantine facility at the Alan Fletcher Research Station in Australia in 2004. After obtaining approval for field release, *C. visenda* was moved to glasshouses in March 2007 for mass-rearing and field release.

2.2. Life cycle

Life cycle studies were conducted using potted plants in a quarantine glasshouse at 20–27 °C. Ten pairs of newly emerged male and female adults were each transferred onto separate potted cat's claw creeper plants, each enclosed in a cylindrical transparent perspex tube (34 cm high and 12 cm diameter) with a gauze cap. The adults were transferred onto a fresh cat's claw creeper plant each week, and the longevity, pre-oviposition period, and the number of eggs per female per day were recorded. The eggs laid in the first week in each replication were retained and durations of egg and nymphal development recorded.

2.3. Mass-rearing and field release

Potted cat's claw creeper plants raised from field-collected tubers were used for rearing *C. visenda* in the glasshouse. A colony of *C. visenda* was maintained on these plants in insect-proof cages ($90 \times 80 \times 75$ cm) at 20–27 °C, and natural photoperiod. Each insect-proof cage with 12 potted plants (10–12 cm shoot length) was inoculated with 40 *C. visenda* adults. The plants were irrigated manually twice a week. After eight weeks, plants with adults and nymphs (~1500 per cage) were taken to cat's claw creeper infested sites and placed on the surface of the ground or with the pot buried in the soil.

Mass-rearing and field release of *C. visenda* commenced in June 2007 and continued till December 2009, in partnership with four landcare groups, four shire councils, six catchment groups, and four schools. The schools were involved as part of the Weeds Warriors program. Field releases of *C. visenda* were made at sites from northern NSW in the south to Mackay in northern Queensland, and from Nerang and other coastal sites in the east to Taroom in the west (Fig. 1), covering both riparian and non-riparian sites (Supplementary material, Table 1).

Two morphologically distinct cat's claw creeper varieties occur in Australia (Osunkoya et al., 2009). An abundant 'short-pod' variety, with narrow, shiny leaves with smooth margins, is widespread throughout Queensland and NSW. A less-abundant 'long-pod' variety, with broad, hairy leaves with serrated margins and pods that are twice as long as those in the 'short-pod' variety, occurs along with 'short-pod' variety at two sites (Oxley and Carindale) in south-eastern Queensland. Releases were made on both varieties of cat's claw creeper (Supplementary material, Table 1).

2.4. Field monitoring

Post release surveys were undertaken during April–May 2008, May–June 2009, and March–April 2010 to determine the establishment status of *C. visenda* in Queensland and NSW. Among the 23 sites where field releases commenced in 2007, post release surveys were conducted at 22 sites in April–May 2008, at 17 sites in May–June 2009 and at 19 sites in March–April 2010. Among the 40 sites where field releases commenced in 2008, post release surveys were conducted at 26 sites in May–June 2009 and at 19 sites in March–April 2010. Preliminary observations showed that the tingids were commonly found on prostrate plants at ground level and not on the plants climbing on trees, so surveys were only conducted at ground level. At each site in each year, a total of 20 min was spent by one person visually examining cat's claw creeper leaves and recording the total number of *C. visenda* eggs, nymphs and adults encountered.

2.5. Field dispersal and damage levels

Studies on dispersal and damage levels were carried out at 15 release sites in south-eastern Queensland during Aug–Sep 2008. This included 10 riparian (Canungra, Coulson Creek, Croftby, Fassifern, Latimer's Crossing, Maroon, Moggill, Moogerah, Mooloolah and Nerang) and five non-riparian (Bardon, Carindale, Maleny, Oxley and Pine Mountain) sites (Supplementary material, Table 1). At each site, a transect was run horizontally at ground level from the release point in each cardinal direction (north, south, east and west). For each transect, a maximum of 10 quadrats (each 20×20 cm) were sampled at 1 m intervals along each transect. Within each quadrat, total number of leaves and proportion of leaves with feeding damage were counted, and proportion of leaf area with feeding damage was estimated by visual inspection of all the leaves in the sample to determine the proportion of surface area damaged. All leaves within each quadrat were visually inspected to count the number of leaves with tingid eggs and the number tingid adults. The average rate of spread of tingid damage at these sites was calculated using the equation: rate of spread = d/m, where d = furthest distance (metres) from release point where 50% of leaves were damaged and m = months since release of agent.

2.6. Data analysis

One-way ANOVA was used to compare the lifespan of males and females under glasshouse conditions. Chi-square test was used to test if the proportions of field established sites for the tingid were equal across riparian and non-riparian zones, and between years. Polynomial regression analysis was used to study the changes in the average number of adults m^{-2}, the proportion of leaves damaged and the proportion of leaves with eggs, in relation to distance from release sites. Regression analysis was used to study the relationship between the average number of adults m^{-2} and the average% of leaf area with feeding damage. Values are presented as means ± standard error.

3. Results

3.1. Life cycle

In the glasshouse, the lifespan of females (48.0 ± 7.3 days) was significantly longer (one-way ANOVA: $F_{1,15} = 5.34$, $P = 0.04$) than that of males (24.4 ± 7.9 days). Females laid 82.2 ± 13.2 eggs in their lifetime (6.8 ± 0.8 eggs day^{-1}) after a pre-oviposition period of 3.4 ± 0.7 days. The eggs hatched in 13.2 ± 1.4 days, and 88% of

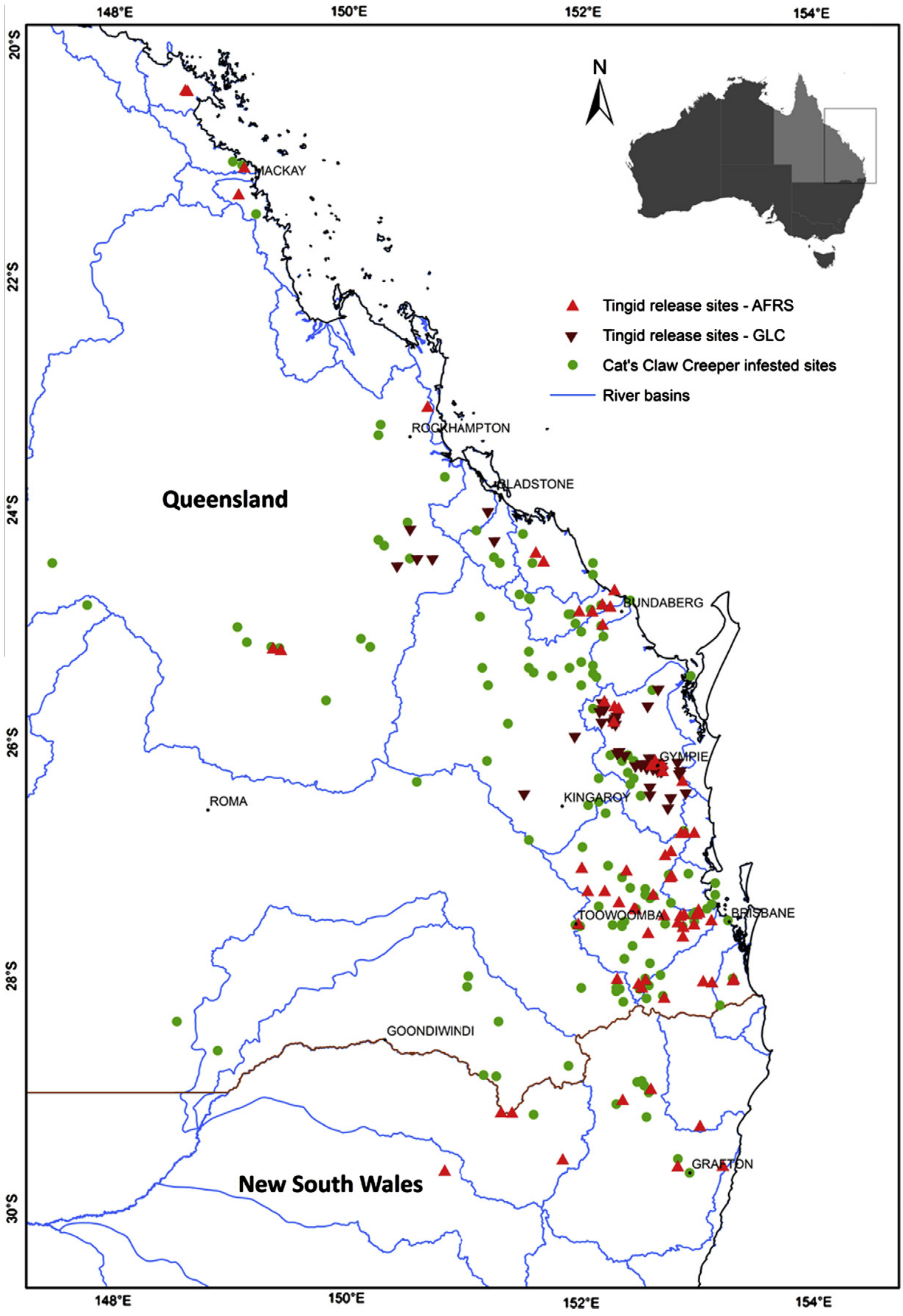

Fig. 1. Cat's claw creeper infestations and release sites for *Carvalhotingis visenda* in Queensland and New South Wales, Australia. AFRS = Releases made by the Alan Fletcher Research Station; GLC = Releases made by the Gympie Landcare Group.

neonates matured to adulthood in 16.9 ± 1.4 days. The generation time (egg to egg) was 34 days.

3.2. Mass-rearing and field release

More than half a million *C. visenda* adults and nymphs have been released from June 2007 to December 2009 at 72 sites in Queensland and northern NSW (Fig. 1; Supplementary material, Table 1). The majority of the release sites (80%) were in riparian zones (Fig. 1). Field releases commenced at 23 sites in 2007, 40 sites in 2008 and nine sites in 2009. Field releases continued throughout the year, including the winter months, and the numbers of *C. visenda* released at each site ranged from 1000 to 26,670 (Supplementary material, Table 1). At more than half of the sites (57%), *C. visenda* was released only once. The remaining sites received either two (25%) or three (11%) or four (6%) or five (1%) releases over one to three year period (Supplementary material, Table 1). The Gympie landcare group released around 11,000 tingid-infested potted cat's claw creeper plants (∼1 million individuals) at 63 sites in south-eastern Queensland (Fig. 1).

3.3. Field establishment

At sites where field releases commenced in 2007, establishment was evident at 95% ($n = 22$), 82% ($n = 17$) and 76% ($n = 190$) of the sites in 2008, 2009 and 2010, respectively. At sites where field releases commenced in 2008, establishment was evident at 85% ($n = 26$) and 90% ($n = 19$) of the sites in 2009 and 2010, respectively. There were no significant differences in the proportion of established sites between riparian (83–100%) and non-riparian (73–91%) zones, and between years (2007 release sites: $\chi^2 = 1.64$, $P = 0.439$; 2008 release sites: $\chi^2 = 0.70$, $P = 0.705$). Establishment was higher at sites that received three or more releases (83%, $n = 12$) than at sites that received either single (74%, $n = 31$) or two releases (73%, $n = 15$). Likewise, sites that received from 5000 to 10,000 individuals (90% establishment, $n = 20$; 6483 ± 292 individuals per site) and more than 10,000 individuals (83% establishment, $n = 18$; 14721 ± 1112 individuals per site) exhibited higher establishment rates than the sites that received less than 5000 individuals (68% establishment, $n = 19$; 3291 ± 201 individuals per site). Field establishment of *C. visenda* was evident on both 'long-pod' and 'short-pod' cat's claw creeper varieties at Oxley and Carindale sites (Supplementary material, Table 1).

3.4. Field dispersal and damage levels

The tingid caused severe and visible feeding damage, mostly at ground level. There was a significant positive relationship between the number of *C. visenda* adults m^{-2} and the proportion of leaf area damaged, and populations in excess of five adults m^{-2} resulted in feeding damage to more than 50% of the leaf area (Fig. 2). The average number of adults m^{-2}, proportion of leaves with eggs, and proportion of leaves with feeding damage all declined with increase in distance from the initial release point (Fig. 3). The average rate of spread of *C. visenda*, as evident from its feeding damage was 5.4 m per year. The maximum distance that *C. visenda* was located away from the initial release points ranged from a minimum of 6 m at Neurum Road to a maximum of almost 1 km at Moogerah.

4. Discussion

In many weed biological control programs, either the research organisation or an associated extension agency has been responsible for the rearing and field release of biological control agents. This arrangement has restricted the areas over which releases could be made because of limitations in human resource availability and travelling costs. For the cat's claw creeper program, involvement of 18 community groups enabled the release of around 1.5 million individuals across 135 sites within three years. Other weed biological control programs have also successfully engaged community groups, but more often to develop agent release networks for a more rapid delivery of biological control to the end users (e.g. Briese and McLaren, 1997; Swirepik et al., 2004).

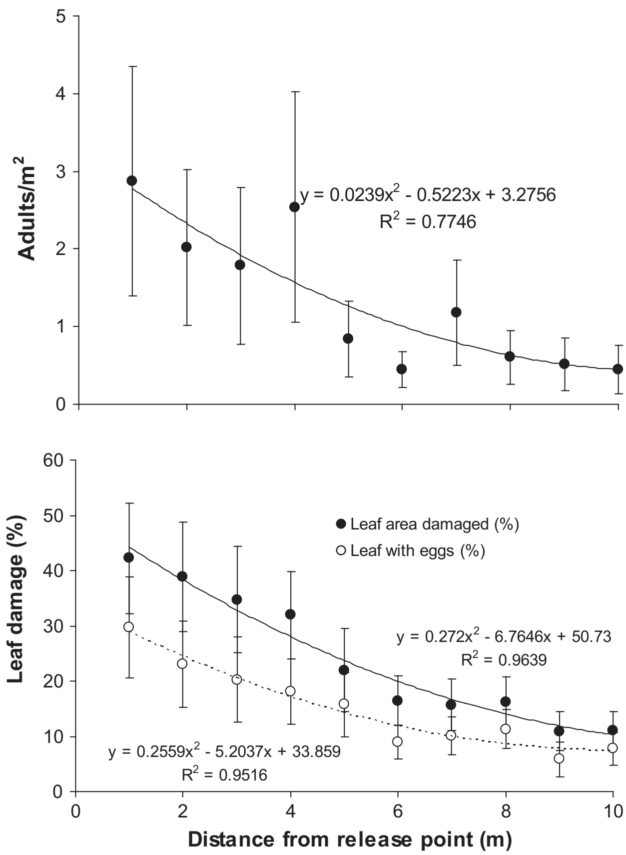

Fig. 2. Changes in the number of adults m^{-2} (Mean ± SE), the proportion of leaves with eggs (Mean ± SE), and the proportion of leaf area damaged (Mean ± SE) in relation to distance from initial point of release of *Carvalhotingis visenda*.

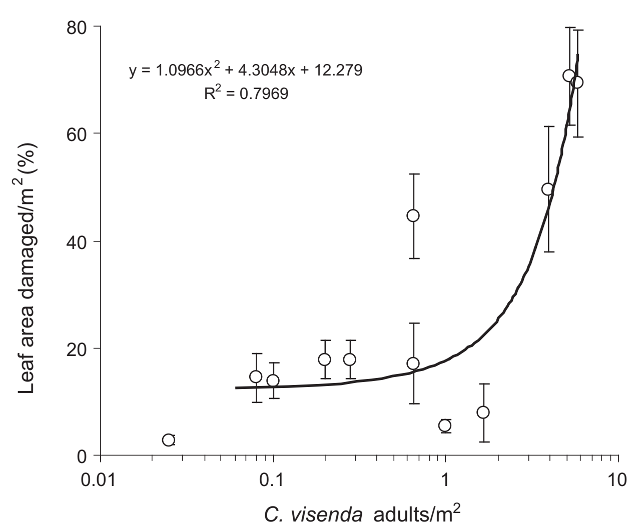

Fig. 3. The relationship between the number of *Carvalhotingis visenda* adults m^{-2} and the percentage of leaf area damaged m^{-2} (Mean ± SE).

As predicted by the climate matching model (Rafter et al., 2008), *C. visenda* became established widely, soon after field release. Establishment was evident across a range of climatic conditions, from sub-tropical northern NSW to tropical north Queensland and arid western Queensland. Establishment of the tingid on the two cat's claw creeper varieties in Australia suggest that the tingid is a suitable agent for both varieties, and hence could be exploited in other countries in the introduced range, with either cat's claw creeper variety. Establishment was dependent on the number of individuals released, as well as the number of releases. Hence, future efforts should focus on redistributing the tingid regularly from the established sites to release sites where field establishment is yet to be confirmed.

The tingid survived and reproduced throughout the year, including winter, and there was no evidence of winter diapause. As a result, the tingid has the potential to complete from three generations per year in northern NSW to eight generations per year in north Queensland (Dhileepan et al., 2010). Field establishment of *C. visenda* has been reported from South Africa also (King and Dhileepan, 2009). Similar widespread establishments soon after field releases have been reported for other weed biological control agents also (e.g. Wilson and Flanagan, 1990; Dhileepan and Strathie, 2009; Hourgh-Goldstein et al., 2009).

The time-based sampling, a method not commonly used in monitoring weed biological control agents, provided information on the incidence and abundance of the tingid to confirm its field establishment status. Sampling using transects, a method often used in monitoring weed biological control agents (e.g. Hourgh-Goldstein et al., 2009), on the other hand, provided baseline information on rates of expansion of the tingid populations and damage levels from the initial release points. In the current study, only plants that were accessible at ground level were included in the transects. Future sampling at these sites will include vertical sampling, to check for tingid establishment and abundance on cat's claw creeper climbing on trees as well as horizontal sampling at ground level to show changes in rate of spread over time.

In the field, the tingid spread slowly, mostly at ground level. The rate of spread of about 0.5 m per month is a conservative estimate as it represents the dispersal in the first year of release, and is likely to increase over time. It is only three years since *C. visenda* was first released in Australia, and it may take several years before population levels peak.

Acknowledgments

We thank Dr Stefan Neser, Ms Hester Williams and Mr Anthony King of ARC-PPRI, Republic of South Africa for providing tingid colonies and for information on its biology, Laura Simmons for technical support; Fernando Chandima for the ArcGIS map; Dr S. Raghu for statistical advise; and the various community groups (Atkinson & Buaraba Catchment Landcare Groups, Brisbane City Council, Burnett Catchment Care Association, Fraser Coast Regional Council, Gympie & District Landcare, Ipswich City Council, Queensland Parks & Wildlife Service, Moggill Creek Catchment Group, Noosa & District Landcare Group Inc., Pine Rivers Catchment Association, South East Queensland Water, Sunshine Coast Regional Council, Whitsunday Landcare Catchment Association Inc., Boonah, Rathdowney, Homebush and Moggill State Schools) in Queensland and New South Wales (Environmental Training and Employment Inc) for participating in the tingid rearing and release program. We also thank Bill Palmer, Dane Panetta, John Hoffmann and the two anonymous reviewers for comments on the earlier versions of the manuscript.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.biocontrol.2010.06.016.

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