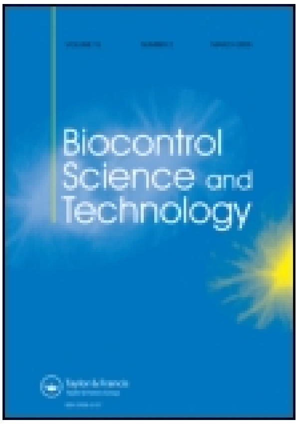

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Field and laboratory studies to determine the suitability of Cissoanthonomus tuberculipennis (Coleoptera: Curculionidae) for release against Cardiospermum grandiflorum (Sapindaceae) in South Africa

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RESEARCH ARTICLE

Field and laboratory studies to determine the suitability of *Cissoanthonomus tuberculipennis* (Coleoptera: Curculionidae) for release against *Cardiospermum grandiflorum* (Sapindaceae) in South Africa

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Cardiospermum grandiflorum is an invasive creeper that was targeted for biological control in South Africa in 2003. To determine ecological host range of its natural enemies, surveys were conducted on *C. grandiflorum* and 11 other Sapindaceae at 40 sites in the weed's native range (Argentina). These surveys indicated that the seed-feeding weevil *Cissoanthonomus tuberculipennis* was restricted to *C. grandiflorum*, and that it was among the common natural enemies, occurring at most sites where *C. grandiflorum* was recorded. Open-field tests were conducted under natural conditions in Argentina to determine the host preference of *C. tuberculipennis* and other natural enemies of *C. grandiflorum* among three *Cardiospermum* species. These tests revealed that *C. tuberculipennis* and the bug *Gargaphia* sp. were restricted to *C. grandiflorum* though the latter subsequently developed on non-target *Cardiospermum* species in the laboratory. *C. tuberculipennis* was found to be highly damaging, destroying up to 44% of the seeds per plant in Argentina. In all the host-specificity tests, including no-choice, paired-choice and multi-choice tests, *C. tuberculipennis* only fed and developed on *C. grandiflorum.* Failure of *C. tuberculipennis* to feed and develop on all congeners of *C. grandiflorum* shows that the weevil is highly host-specific to the target weed. Results of host-specificity tests, open field tests and long-term monitoring of *C. tuberculipennis* populations demonstrate that the weevil poses no threat to non-target plant species, and therefore safe for release against *C. grandiflorum* in South Africa. Permission to release *C. tuberculipennis* in South Africa has been granted by the relevant regulatory authorities.

Keywords: *Cardiospermum grandiflorum*; *Cissoanthonomus tuberculipennis*; open-field tests; ecological host range; host-specificity tests

Introduction

Balloon vine *Cardiospermum grandiflorum* Sw (Sapindaceae) has become a prolific invader in South Africa following its introduction in the early 1900s as an ornamental creeper (Cowan, 1983; Moraes, 1990). The increasing infestations of *C. grandiflorum* in the riparian and suburban areas during the past 20 years has been a concern in South Africa (Henderson, 2001), hence the initiation of a biological

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control programme against this weed in 2003 (Mc Kay, Olerio, Fourie, & Simelane, 2010; Simelane, Fourie, & Mawela, 2011).

In the Americas, the Neotropical native range distribution of *C. grandiflorum* extends from southern Mexico to Brazil (Cowan, 1983; Ferrucci, 1991). Other than South Africa, the plant has also become invasive in Australia, the Cook Islands, Hawaii and New Zealand (Carroll, Mathieson, & Loye, 2005; Meyer, 2000; United States Department of Agriculture-National Resource Conservation Service [USDA-NRCS], 2009). *C. grandiflorum* is still grown for ornamental purposes in many warm regions of the world (Carroll et al., 2005), suggesting that it may have escaped horticultural plantations in countries where it has become invasive. Although *C. grandiflorum* was introduced into South Africa in 1912 (Henderson, 2006), massive infestations of this climber have only been reported in the eastern low altitude regions of the Mpumalanga and KwaZulu-Natal provinces in 2000 (Olckers, 2004), suggesting that it may have been a sleeper weed (i.e. non-invasive phase) for almost 90 years. Although considered to be in the early stages of invasion in South Africa, the Department of Agriculture, Forestry and Fisheries (DAFF) has acknowledged the potential threat of *C. grandiflorum* to the ecosystems, thus declaring it as a Category 1 weed (Henderson, 2001), meaning that the plant is prohibited and it must be controlled. The magnitude of invasion by *C. grandiflorum* in recent years has been quite severe, threatening local biodiversity, especially along the coastal regions of KwaZulu-Natal. Mechanical control of *C. grandiflorum* is extremely difficult and costly as dead plant material has to be removed to restore exposure of the understory to sunlight. Chemical control is similarly problematic because of non-target damage to underlying vegetation. Biological control is thus considered the only feasible and sustainable option to curb the invasion of this vine in South Africa.

Biological control against *C. grandiflorum* was initiated by the Plant Protection Research Institute of the Agricultural Research Council (ARC-PPRI) in 2003, and search for potential agents commenced in 2005 at the native range of the weed in the northern part of Argentina (Mc Kay et al., 2010; Simelane et al., 2011). Considering that *C. grandiflorum* reproduces and spreads through seeds, seed-feeding agents were prioritised as candidate biological control agents. Seeds play an essential role in the maintenance and spread of certain weeds, yet they are vulnerable to seed predators which often affect weed population dynamics (Crawley, 1992; Hoffmann & Moran, 1998; van Klinken, Kriticos, Wilson, & Hoffmann, 2004). Seed-feeders are often used as weed biological control agents, and their use often prevent the development of conflict of interest where the vegetative part of the weed is of economic or social value. The South African agroforestry industry has successfully used seed-attacking agents in resolving conflicts of interest (Moran, Hoffmann, & Olckers, 2004; Zimmermann & Neser, 1999). Despite being relatively easy to rear with a relatively high host-specificity, seed predators can reduce invasion rates of certain weeds even at low seed predation rates (van Klinken, Colasanti, & Buckley, 2008).

It is generally accepted that field host range studies in the native range are of critical importance in host-specificity determination of insects used in the biological control of weeds (Balciunas, Burrows, & Purcell, 1996; Clement & Cristofaro, 1995). Apart from plant suitability for adult-feeding and larval development, open-field testing of potential biological control agents in the native range also encompasses features of the host selection process (e.g., host habitat finding and oviposition site selection), thereby generating host-specificity data under more natural conditions than can be obtained via cage tests (Clement & Cristofaro, 1995). The ecological information obtained during field surveys in the native range also aid in reducing the number of non-target plant species that need to be tested in quarantine laboratory in the exotic country, thus avoiding the need to maintain cultures of a large number of non-target plant species which could make quarantine screening cumbersome and time-consuming. Field studies are also useful for agents that are difficult to rear under laboratory conditions (Sheppard, van Klinken, & Heard, 2005). Although field host range studies in native countries have proved their worth in several cases involving host-specificity determination (Clement & Cristofaro, 1995; Sheppard et al., 2005), high costs coupled with logistical constraints remain serious challenges that often limit the execution of these studies in foreign countries.

The host-specificity procedure currently employed in biological control systems attempts to predict which host plants are likely to be attacked in the release environment. This procedure, however, neglects to screen control agents efficiently for effectiveness in the exotic range. Due to an increasingly contentious concern of non-target effects of weed biological control (Louda, Kendall, Connor, & Simberloff, 1997; McEvoy & Coombs, 2000), it is generally accepted that the total number of biological control agents utilised be minimised, with the aim of reducing the risk to non-target plant species while maximising the likelihood of effective weed control (McEvoy & Coombs, 2000). Therefore, the current pre-release studies conducted in the native range ascertained the safety of *C. tuberculipennis* as a biological control agent and its ability to inflict significant damage on *C. grandiflorum.*

To determine the host range of *C. tuberculipennis*, host preference and relative abundance of *C. tuberculipennis* in open-field sites at the native range and laboratory host-specificity tests were conducted on selected Sapindaceae in South Africa. The contribution of *C. tuberculipennis* to seed destruction in relation to other seed feeders in the native range was also determined, and this was aimed at predicting the potential impact of this weevil on *C. grandiflorum*.

Materials and methods

Laboratory conditions

Laboratory studies were conducted in quarantine at the ARC-PPRI (Weeds Unit) in Pretoria. Glasshouse temperatures, ranging from 22 to 30°C, and a relative humidity of 23–88%, were maintained during rearing and laboratory studies. Flood lights (300W) were installed in the glasshouse, and a 12/12 hour light/dark cycle was maintained during the studies.

Study species

C. grandiflorum is a perennial, slightly woody climber with widely spaced biternate leaves and extensive tendrils. It flowers throughout the year, and each flower produces a membranous and inflated fruit containing three angled capsules or chambers. Although the plant regrows from root fragments, fruit capsules with seeds are carried by wind and float freely on water, dispersing the plant along waterways (Henderson, 2001). *C. grandiflorum* plants used for the experiments were propagated from seeds and transplanted in 10-litre pots in a nursery shade house before they were transferred to cages in a quarantine glasshouse.

Adult *C. tuberculipennis* is light brown to grey in colour and are 3–5 mm in length. Adults feed on the flower buds and open flowers, and occasionally on the leaves and shoot tips. Females insert their eggs into the young green fruits (±1 cm) and the emerging larvae feed on the seeds inside the developing fruit. Larvae are highly damaging, with one or two larvae capable of destroying all the three seeds within a single fruit capsule. Pupation occurs inside the fruit capsule. Development between oviposition and adult emergence is completed in 40–45 days. Adult *C. tuberculipennis* are long-lived, surviving for two to five months (Mc Kay et al., 2010; Simelane et al., 2011).

Rearing of **C. tuberculipennis**

A culture of *C. tuberculipennis* was established from adults collected from various sites in Misiones Province, and export of the weevil from Argentina was authorised by the Dirección de Fauna Silvestre and Dirección Nacional de Ordenamiento Ambiental y Conservación de la Biodiversidad (Permit No: 2917/2007; 5175/09). Newly emerged *C. tuberculipennis* adults were confined in a gauze-covered cage (0.5 m × 0.5 m × 0.95 m) with a potted *C. grandiflorum* plant. After seven days, a clump of young excised balloon vine fruits (approximately 1 cm long) whose pedicels were inserted into 200-ml flask full of water was placed inside the cage to allow oviposition by females. After egg hatch, which occurred after 12-day exposure of fruits to adults, fruits were dissected under a microscope and early instars were removed and transferred to larger fruits where they developed till pupation.

Ecological host range and relative abundance of **C. tuberculipennis** *in the native range, Argentina*

As part of the pre-release evaluation of potential agents of *C. grandiflorum*, extensive field surveys were conducted in the native range (Argentina) (Mc Kay et al., 2010), and these were primarily aimed at predicting the ecological host ranges of the weed's natural enemies in South Africa. Field studies were made possible through the scientific collaboration that has been in existence between ARC-PPRI (South Africa) and the Fundación para el Estudio de Especies Invasivas (FUEDEI), formerly, USDA-ARS-SABCL, Argentina.

To determine the presence of *C. tuberculipennis* and thus its ecological host range, extensive exploratory surveys were conducted on *C. grandiflorum* and 11 other selected Sapindaceae at 40 sites in Argentina from 2005 to 2013. Among the 11 selected plant species were the congeners of *C. grandiflorum* (i.e., *Cardiospermum corindum* L., *Cardiospermum halicacabum* var. *halicacabum,* L. and *C. halicacabum var. microcarpum* (Kunth) Blume) and other closely related species within the Sapindaceae such as *Paullinia elegans* Cambessèdes, *Serjania fuscifolia* Radlkofer, *Serjania glabrata* Kunth, *Serjania laruotteana* Cambessèdes, *Serjania meridionalis* Cambessèdes, *Urvillea chacoensis* Hunziker, *Urvillea ulmacea* Kunth and *Urvillea uniloba* Radlkofer. The sites were concentrated in northern Argentina, and inspection was mainly conducted along the natural forest edges and main roads in the provinces of Chaco, Corrientes, Formosa, Jujuy, Misiones and Salta. The relative abundance of *C. tuberculipennis* on selected Sapindaceae plant species was determined in 2007, 2009 and 2013, and the majority of sites were inspected twice per year. In each site, inspection was carried-out on all the plants within a 10 m radius of a marked plant.

Abundance of *C. tuberculipennis* per site was ranked as follows; none (N) when there were no beetles or feeding damage, rare (R) when the beetle or feeding was present at less than 10%, occasional (O) at 10–30% and common (C) at more than 30% of the fruits per site. Voucher specimens of plants and insects collected were deposited at the FUEDEI, Museo Argentino de Ciencias Naturales (MACN), Buenos Aires and at the Instituto de Botánica del Nordeste (IBONE), Corrientes, Argentina.

Host preference of C. tuberculipennis *in an open-field in the native range*

Open-field trials were carried out to determine the host preference of *C. tuberculipennis* among three *Cardiospermum* species (i.e., *C. grandiflorum, C. corindum and C. halicacabum*). Although the native ranges of the two *Cardiospermum* spp. (*C. corindum* and *C. halicacabum*) are still unknown, they also occur in both South Africa and South America (Simelane et al., 2011). The three *Cardiospermum* species were planted on three plots (8 × 10 m each) at two separate sites located at about 40 km apart in Misiones Province, Argentina. At both study sites, *C. tuberculipennis* and its natural host (*C. grandiflorum*) were located at 10–300 m away from the plots. Plants were grown in August 2008, and each plant species was grown in its own row at an inter- and intra-row spacing of 2 m. At the beginning of summer season (October), seedlings were transplanted individually into pits (0.5 m × 0.5 m × 0.5 m) and were allowed to grow till the flowering stage, and inspection commenced at the end of summer season in February when fruit pods of all the *Cardiospermum* spp. had fully developed. The feeding damage and the abundance of *C. tuberculipennis* and other natural enemies were assessed on all the plants eight times between September 2008 and September 2010 on each *Cardiospermum* species at both sites. Based on the number of plant species on which *C. tuberculipennis* was found feeding or developing, its host-specificity index was determined using a method developed by Palmer and Pullen (1995). The host-specificity index was ranked as follows: **** = when *C. tuberculipennis* was found on *C. grandiflorum* only, *** = when found on *C. grandiflorum* and *C. corindum* or *C. halicacabum,* ** = when found on *C. grandiflorum, C. corindum* and *C. halicacabum*, * = when found on either *C. corindum* or *C. halicacabum* or both but not on *C. grandiflorum*, and — = not found on any species.

Seed predation by C. tuberculipennis *in an open-field in the native range*

Percentage of *C. grandiflorum* seeds destroyed by *C. tuberculipennis* and other seed-feeders at site 1 was determined four times between September 2008 and September 2010. However, plants in site 2 were destroyed by frost in 2008, so seed predation data were only collected from site 1. Sampling of sites was undertaken in April–May and September–October as these had been shown to be the peak periods for *C. tuberculipennis* populations at these particular sites during previous surveys. Non-parametric Kruskal–Wallis tests (Statistica, 2012) were used to compare seed predation by *C. tuberculipennis* and other seed-feeders during the sampling periods.

Laboratory no-choice feeding and reproductive performance tests

The test plants were selected on the basis of their taxonomic relatedness to the target weed, and this is often referred to as the centrifugal phylogenetic method which was initially developed by Wapshere (1989) and later refined by Briese (2003). No-choice feeding and reproductive performance tests were carried out under quarantine (laboratory) conditions described previously and were mainly designed to determine whether the plant species was suitable for oviposition and development of *C. tuberculipennis*. These tests were conducted on 21 plant species in nine families. Test plants, each grown in a 10-litre pot, were washed with a jet of water to remove any unwanted insects and then transferred to the quarantine glasshouse where they were placed in separate gauze-covered cages (0.55 m × 0.55 m × 0.95 m). At flowering or early fruiting stage, each test plant was infested with 10 unsexed adult *C. tuberculipennis* obtained from the laboratory-reared colony. After 25 days, all the adults were removed and counted to determine their survival on each test plant. At this stage, the palatability of each test plant to adults was evaluated by rating the feeding damage on the flowers, shoots or fruits as follows: 0 = no feeding, * = small feeding punctures (exploratory feeding) and *** = large feeding holes (normal feeding). Pods were also checked for the seed-feeding damage by larvae and presence of immature or adult progeny. Each plant species was tested four times (four replicates).

Laboratory paired-choice feeding and reproductive performance tests

Paired-choice tests were conducted to determine the ability of the adult *C. tuberculipennis* to make oviposition choice between its natural host (*C. grandiflorum*) and one of the three *Cardiospermum* species. These tests were carried out under quarantine (laboratory) conditions. The plants used in the study were at flowering and early fruiting stages. Two potted plants, *C. grandiflorum* and one of its three congeners (i.e., *C. corindum, C. halicacabum* and *Cardiospermum pechuelii*), were confined with 12 newly emerged unsexed *C. tuberculipennis* adults in a gauze-covered cage (0.55 m × 0.55 m × 0.95 m). After a 25-day exposure to plants, adult insects were removed, and flowers of each plant species were inspected to establish the degree of feeding damage by *C. tuberculipennis* adults. Feeding damage was evaluated and rated as described previously. Pods of both plant species were dissected to determine seed-feeding damage, presence of immature or adult progeny of *C. tuberculipennis*.

Laboratory multi-choice feeding and reproductive performance tests

The multi-choice tests were carried out to determine whether *C. tuberculipennis* displays feeding and oviposition preference among four *Cardiospermum* plant species. The four *Cardiospermum* species included *C. grandiflorum, C. corindum, C. halicacabum* and *C. pechuelii*. Although *C. corindum* and *C. halicacabum* are cosmopolitan species, their native ranges are unknown and are now under investigation (Simelane et al., 2011). The native range of *C. pechuelii* is in the semi-desert of western Namibia. At flowering or early fruiting, which occurs from January to March for all the four *Cardiospermum* species, all the potted four species were placed in a nylon-screened walk-in cage (4 m × 4 m × 2 m) in quarantine. Four plants of each species were arranged randomly in a cage, and 24 unsexed newly emerged *C. tuberculipennis* adults were released into the cage. To allow oviposition on young fruits, the beetles were confined with plants for 25 days. After 25 days, the beetles were removed and flowers of each plant species were examined to establish the degree of feeding damage by *C. tuberculipennis* adults. After 37 days, pods of all plant species were dissected to determine the seed-feeding damage by larvae and presence of immature or adult progeny of *C. tuberculipennis.*

Results

Ecological host range and relative abundance of C. tuberculipennis *in the native range*

Out of nine insect and two fungal species recorded on selected Sapindaceae during the survey, only two insect species (i.e., the seed-feeding weevil *C. tuberculipennis* and the fruit-galling midge *Contarinia* sp. (Diptera: Cecidomyiidae)) were found feeding and developing exclusively on the target weed *C. grandiflorum* (Mc Kay et al., 2010). However, several attempts to rear the midge *Contarinia* sp. in the laboratory have not been successful, thus impeding further pre-release studies on this potential biological control agent. The seed-feeding weevil *C. tuberculipennis* was the most widespread and abundant natural enemy, occurring in all the provinces where *C. grandiflorum* was recorded in the native range (Table 1). Despite the high species richness of non-target Sapindaceae species at Misiones sites, the populations of *C. tuberculipennis* were confined to their natural host (*C. grandiflorum*). Because of its significant feeding damage, wide geographic distribution and narrow ecological host range in its native range, the weevil *C. tuberculipennis* was prioritised for further pre-release studies, including open-field and host-specificity tests in Argentina and South Africa, respectively.

Host preference of C. tuberculipennis *in an open-field at the native range*

Eighteen months of monitoring two open-field sites in Misiones, Argentina, revealed that the seed weevil *C. tuberculipennis* and the leaf-sucking bug *Gargaphia* sp. (Heteroptera: Tingitidae) were the only natural enemies that were restricted to *C. grandiflorum* (Tables 2 and 3). However, *Gargaphia* sp. subsequently developed on non-target *Cardiospermum* spp. in caged experiments. Other seed-damaging insects such as the butterfly *Chlorostrymon simaethis sarita* Skinner (Lepidoptera: Lycaenidae), *Moodnopsis* sp. Dyar (Lepidoptera: Pyralidae) and *Lisseurytomella flava* (Ashmead) (Hymenoptera: Eulophidae) larvae were found feeding on *C. grandiflorum* and two other *Cardiospermum* spp. Fungal pathogens such as *Phyllacora rimulosa* (Phyllachoraceae) and *Puccinia arechavaletae* Speg. (Pucciniales: Pucciniaceae) were also recorded on *C. grandiflorum* and two other *Cardiospermum* species at both experimental sites. Although the flower-galling midge *Contarinia* sp. was not recorded at either of open-field sites, it had been recorded only on *C. grandiflorum* at various sites in the Northern part of Argentina (Mc Kay et al., 2010).

Seed predation by C. tuberculipennis *in an open-field in the native range*

Seed predation by *C. tuberculipennis* was significantly higher (44%) in September 2008 than during the other three sampling dates (Kruskal–Wallis test: $\chi^2 = 8.18$; $P = 0.017$) (Figure 1). Predation by *C. tuberculipennis* was almost the same in April 2009, April 2010 and September 2010, ranging from 19% to 29%. Seed predation by *C. tuberculipennis* remained steady from April 2010 to September 2010 despite competitive interaction with other seed feeders whose incidence of predation

Table 1. Relative abundance of *C. tuberculipennis* on selected (Sapindaceae) in eight provinces of Argentina from 2007 to 2013.

		Relative abundance of *C. tuberculipennis*		
Province	Plant species (Sapindandaceae)	2007	2009	2013
Chaco	*C. halicacabum* var. *microcarpum*	N	N	N
	C. corucorindumndum	N	N	N
	P. elegans	N	N	N
	U. chacoensis	N	N	N
Corrientes	*C. grandiflorum*	O	C	C
	C. halicacabum var. *microcarpum*	N	N	N
	P. elegans	N	N	N
	S. fuscifolia	N	N	–
	S. meridionalis	N	N	–
	U. uniloba	N	N	–
Formosa	*C. grandiflorum*	C	C	C
	C. corindum	N	N	N
Jujuy	*C. corindum*	N	N	N
	S. glabrata	N	N	–
Misiones	*C. grandiflorum*	C	C	C
	C. halicacabum var. *microcarpum*	N	N	N
	C. corindum	N	N	N
	P. elegans	N	N	–
	S. fuscifolia	N	N	N
	S. laruotteana	N	N	N
	S. meridionalis	N	N	–
	U. ulmacea	N	N	N
Salta	*C. corindum*	N	N	N
	C. halicacabum var. *halicacabum*	N	N	N
	S. glabrata	N	N	–
	S. meridionalis	N	N	–
	U. chacoensis	N	N	N
Santiago del Estero	*U. chacoensis*	N	N	N
Tucuman	*U. chacoensis*	N	N	N

Note: Relative abundance of *C. tuberculipennis* from 2007 to 2013.
N = None; R = Rare; O = Occasional; C = Common.

increased during the same period. Other predators of *C. grandiflorum* seeds included *C. simaethis sarita*, *Moodnopsis* sp. and *L. flava* which also fed on two non-target *Cardiospermum* species.

Laboratory no-choice feeding and reproductive performance tests

Of the 21 plant species tested in isolation cages, *C. tuberculipennis* only fed, oviposited and developed on the target weed *C. grandiflorum* (Table 4). Both visual and microscopic examination of flower buds and fruits showed no sign of damage by *C. tuberculipennis* to *C. corindum*, *C. halicacabum* and *C. pechuelii.*

Table 2. Host-specificity index of natural enemies associated with three *Cardiospermum* species at open-field site 1 (S 25°58′ 42.68″; W 53°48′ 14″) in the native range, Argentina.

	Host specificity index during sampling dates							
Natural enemy species	September 2008[a]	December 2008[ab]	March 2009[bd]	May 2009[d]	July 2009[ac]	December 2009[abc]	May[d] 2010	September 2010[ad]
C. tuberculipennis	****	–	–	****	****	–	****	****
Contarinia sp.	–	–	–		–	–		–
C. simaethis sarita	**	**	*	****	****	–	***	****
Moodnopsis near *perangusta*	–	–	*	****	****	–		****
L. flava	***	–	*	****	****	–	*	****
Gargaphia sp.	–	****	****	****	–	–		****
P. arechavaletae	****	*	****	****	–	*	****	****
P. rimulosa	**	–	****	–	–	–		****

Host-specificity index: ****Found on *C. grandiflorum* only, ***Found on *C. grandiflorum* and *C. corindum* or *C. halicacabum,*s**Found on *C. grandiflorum*, *C. corindum* and *C. halicacabum,* *Found on either *C. corindum* or *C. halicacabum* or both but not on *C. grandiflorum,* –= Not found on any species.

[a] No *C. corindum* fruits available, [b] no *C. grandiflorum* fruits available, [c] no *C. halicacabum* fruits available, [d] no *C. halicacabum* plants available, [e] no *C. corindum* plants available.

Table 3. Host-specificity index of natural enemies associated with three *Cardiospermum* species at open-field site 2 (S 25°51′ 53.9″; W 53°58′ 59.8″) in the native range, Argentina.

	Host specificity index during sampling dates							
Natural enemy species	September 2008[abc]	December 2008[abc]	March 2009[b]	May 2009	July 2009[ac]	December[abc] 2009	May[a] 2010	September 2010[ad]
C. tuberculipennis	–	–	–	–	****	–	****	****
Contarinia sp.	–	–	–	–	–	–	–	–
C. simaethis sarita	–	–	*	**	****	–	***	–
Moodnopsis near *perangusta*	–	–	–	–	–	–	–	–
L. flava	–	–	*	**	–	–	*	–
Gargaphia sp.	–	–	–	–	–	–	–	–
P. arechavaletae	–	–	****	–	–	–	–	****
P. rimulosa	–	–	–	–	–	–	–	_

Host-specificity index: **** = Found on *C. grandiflorum* only, *** = Found on *C. grandiflorum* and *C. corindum* or *C. halicacabum*, ** = Found on *C. grandiflorum*, *C. corindum* and *C. halicacabum,* *Found on either *C. corindum* or *C. halicacabum* or both but not on *C. grandiflorum*, — = not found on any species.

[a] No *C. corindum* fruits available, [b] no *C. grandiflorum* fruits available, [c] no *C. halicacabum* fruits available, [d] no *C. halicacabum* plants available.

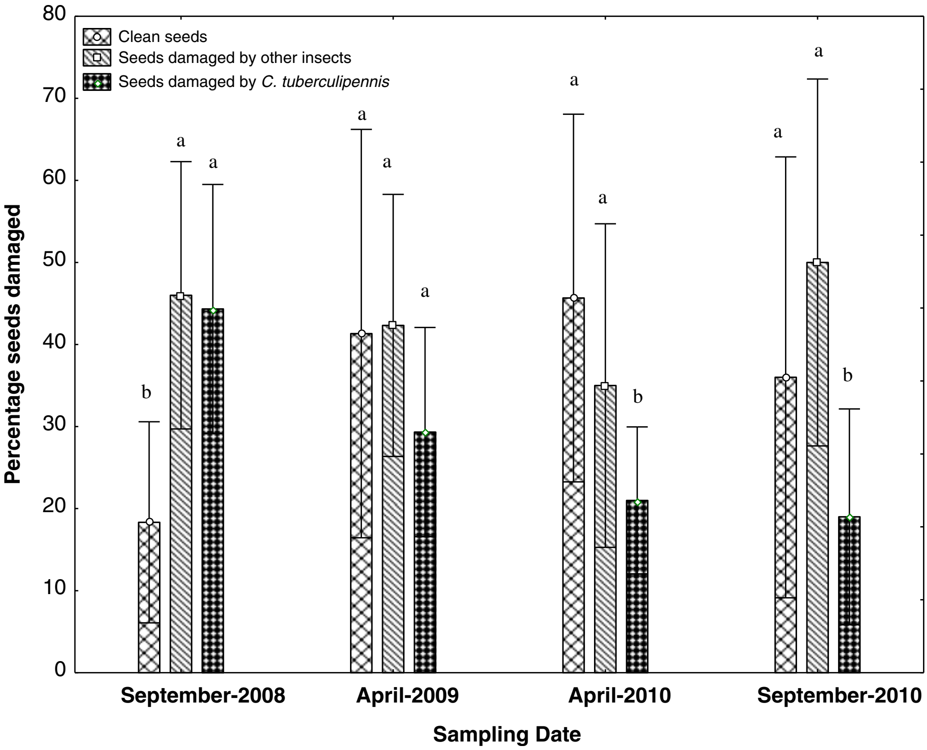

Figure 1. Percentage of *C. grandiflorum* seeds destroyed by *C. tuberculipennis* and other seed-feeders in open-field site 1 (S25°58′ 42.68; W 53°48′14″) at Misiones, Argentina, between September 2008 and September 2010. Means (SE) with the same letter within the sampling date are not significantly different at $P = 0.05$ (Kruskal–Wallis test).

Paired- and multi-choice feeding and reproductive performance tests

Other than the target weed *C. grandiflorum*, none of the three other *Cardiospermum* species were selected for either feeding or oviposition by *C. tuberculipennis* during paired-choice tests (Table 5). When all four *Cardiospermum* species were placed together in the same cage, *C. tuberculipennis* only fed and reproduced on *C. grandiflorum* (Table 6). Failure of *C. tuberculipennis* to feed and reproduce on three *Cardiospermum* species during these tests is a clear indication that the weevil is highly host-specific and suitable for release against *C. grandiflorum* in South Africa.

Discussion

It is generally accepted that simplistic laboratory-based host-specificity tests estimate physiological host range well but tend to overestimate the ecological host range because host acceptance or rejection mechanisms are compromised due to constraints associated with the use of cages (Balciunas et al., 1996; Cullen, 1990; Ding, Wang, & Jin, 2007). Prior to development of its progeny within a host, a female must overcome various ecological and behavioural barriers to ensure host-

Table 4. Feeding damage and total number of adult progeny of *C. tuberculipennis* emerged from *Cardiospermum* species during no-choice feeding and reproductive performance tests (n = 4).

Plant family/ species	Flower-feeding damage	Seed-feeding damage	No. of adult progeny (mean ±SE)
Sapindaceae			
C. grandiflorum Swartz	***	***	15 ± 4.5
C. corindum L.	0	0	0
C. halicacabum L.	0	0	0
C. pechuelii Kuntze	0	0	0
Allophylus dicipiens (Sond.) Radlk	0	0	0
Allophylus dregeanus (Sond.) De Winter	0	0	0
Atalaya natalensis R.A. Dyer	0	0	0
Dodonea viscose Jacz.	0	0	0
Litchi chinensis Sonn.	0	0	0
Paullinia pinnata L.	0	0	0
Rosaceae			
Prunus persica (L.) Batsch (Peach)	0	0	0
Rutaceae			
Citrus sinensis (L.) Osbeck (Sweet orange)	0	0	0
Meliaceae			
Melia azedarach L.	0	0	0
Asteraceae			
Helianthus annuus L. (Sunflower)	0	0	0
Malvaceae			
Gossypium hirsitum L. (Cotton)	0	0	0
Solanaceae			
Solanum melongena L. (Egg plant)	0	0	0
Capsicum frutescens L. (Green pepper)	0	0	0
Lycopersicum esculentum Mill. (Tomato)	0	0	0
Fabaceae			
Phaseolus vulgaris L. (Bean)	0	0	0
Pisum sativum L. (Pea)	0	0	0
Poaceae			
Zea mays L. (Maize)	0	0	0

0 = no feeding; *** = large feeding holes (normal feeding).

finding, niche-finding, habitat preference and life cycle synchrony. Therefore, the ecological information obtained through field surveys in the native range took into consideration all the factors that affect host selection by *C. tuberculipennis*, and this was crucial when assessing the risk of non-target attack by this weevil.

The weevil *C. tuberculipennis* was not only confined to *C. grandiflorum* throughout its native range but was also widespread and abundant, particularly in the Misiones province. Although Misiones sites had the highest species richness of

Table 5. Feeding damage and total number of adult progeny of *C. tuberculipennis* emerged from *Cardiospermum* species during paired-choice feeding and reproductive performance tests.

Tested plants	Flower-feeding damage by adults	Seed-feeding damage by larvae	No. of progeny (Mean ± SE)
C. grandiflorum	***	***	6.5 ± 0.5
C. grandiflorum	***	***	7.5 ± 0.4
C. grandiflorum	***	***	13.2 ± 0.7
C. corindum	0	0	0
C. grandiflorum	***	***	10.2 ± 0.2
C. halicacabum	0	0	0
C. grandiflorum	***	***	9.7 ± 1.0
C. pechuelii	0	0	0

0 = no feeding; *** = large feeding holes (normal feeding).

Sapindaceae, the non-target Sapindaceae species were not exploited by *C. tuberculipennis* and yet these species were often found growing sympatrically with weevil-infested *C. grandiflorum* plants at survey sites. The fact that the populations of weevil *C. tuberculipennis* and its natural host *C. grandiflorum* were widespread in four provinces in the north of Argentina is a clear indication that the non-target host species in those provinces were not providing appropriate cues for host selection mechanism and exploitation by the weevil.

The results of laboratory no-choice, paired-choice and multi-choice tests were very similar, showing no attack of non-target plant species by *C. tuberculipennis.* Although there are a number of cases in the literature where insects tend to feed on non-suitable host plants when held in captivity (Hill & Hulley, 1995; Kaufman & Landis, 2000; Olckers, 2000), this was not the case with *C. tuberculipennis. C. tuberculipennis* adults were instead starved to death without feeding on any of the 20 test plants under caged conditions. Apart from the laboratory host-specificity tests, long-term monitoring of *C. tuberculipennis* populations on non-target species in the open-field sites and 40 other survey sites demonstrate beyond reasonable doubt that the weevil is monophagous, and therefore poses no threat to non-target plant species in South Africa.

Seeds are the primary unit by which *C. grandiflorum* spreads over time and space. Because *C. grandiflorum* reproduces and spreads through seeds, it was logical to prioritise seed-attacking insects such as *C. tuberculipennis* as candidate biological

Table 6. Feeding damage and total number of adult progeny of *C. tuberculipennis* emerged from *Cardiospermum* species during multi-choice feeding and reproductive performance tests.

Plant species	Flower-feeding damage	Seed-feeding damage	No. of progeny (mean ± SE)
C. grandiflorum	***	***	8 ± 2.3
C. halicacabum	0	0	0
C. corindum	0	0	0
C. pechuelii	0	0	0

0 = no feeding; *** = large feeding holes (normal feeding).

control agents for *C. grandiflorum*. In fact, the current study and field surveys conducted by Mc Kay et al. (2010) showed that *C. tuberculipennis* and other seed-feeding insects were the most common and destructive agents, and probably the key factors that regulate the populations of *C. grandiflorum* in its native range. In contrast to previous studies which suggested that seed predation rates need to be very high (excess of 90%) to regulate plant populations (Buckley et al., 2005; Edwards et al., 2009; Myers & Risling, 2000), predictive modelling by van Klinken et al. (2008) showed that seed predators can reduce invasion rates of certain weeds even at low seed predation rates. *C. tuberculipennis* alone destroyed up to 44% of seeds per site despite competitive interaction with other seed-attacking insects in the native range. Because September–October 2008 was a relatively wet season, *C. grandiflorum* yielded more fruits per plant than in 2009 and 2010, and this may explain the higher seed predation by *C. tuberculipennis* in September 2008. The positive relationship between seed predation by *C. tuberculipennis* and fruit density could also suggest that the impact by the weevil is density-dependent. Lack of seed-feeding competitors on *C. grandiflorum* in South Africa is likely to increase the reproductive output of *C. tuberculipennis* and thereby increase seed destruction by the weevil. In fact, seed predation by polyphagous insects in South Africa has been found to be negligible (unpubl. data). Apart from the destructive ability of *C. tuberculipennis* larvae, the short generation time (approximately 40 days) of the immature stages and the long-lived (two to four months) adult stage suggest that this weevil has a great potential as a biological control agent for *C. grandiflorum*.

During the nine-year survey in the native range of *C. tuberculipennis*, we have not detected any natural enemy associated with this weevil. It is therefore conceivable that *C. tuberculipennis* would reach high densities and enhance seed predation of *C. grandiflorum* in South Africa as parasitism is significantly associated with the failure of agents to suppress weed populations (Paynter et al., 2010). In fact, a study by Cornell and Hawkins (1993) showed that the numbers of primary parasitoid species acquired by insect herbivores in their introduced range are correlated with the numbers of primary parasitoid species attacking them in their area of origin.

Although native range studies continue to pose logistical challenges, they enable sensible prioritisation of potential agents, increase screening efficiency in quarantine and improve the chances of releasing safe and effective agents. Given that *C. tuberculipennis* is highly destructive on *C. grandiflorum* seeds, and poses no threat to economic and indigenous plant species, the beetle is the best candidate for biological control of this weedy creeper in South Africa and elsewhere. Permission to release *C. tuberculipennis* from quarantine for the biological control of *C. grandiflorum* in South Africa has been granted by the relevant regulatory authorities. Field releases of *C. tuberculipennis* commenced at the beginning of spring season in 2013.

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