Suitability of the defoliating beetle *Physonota maculiventris* (Coleoptera: Chrysomelidae) for release against *Tithonia diversifolia* (Hemsl.) A. Gray (Asteraceae) in South Africa

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ABSTRACT

This study was conducted to assess the suitability of the defoliating beetle *Physonota maculiventris* Boheman (Coleoptera: Chrysomelidae: Cassidinae) for release as a biological control agent against Mexican sunflower, *Tithonia diversifolia* (Hemsl.) A. Gray (Asteraceae), in South Africa. The biology and host range as well as the potential impact and distribution of *P. maculiventris* were studied under quarantine conditions to determine its safety and effectiveness. Under favourable conditions, females laid 5.3 ± 0.3 (mean ± SE) egg batches during their lifetime, with each batch consisting of approximately 33 eggs. Larvae are highly gregarious as early instars and both larvae and adults feed voraciously, often defoliating the plants completely. The life cycle of the beetle was completed in 67.5 ± 7.5 days under quarantine conditions. Among the 58 test plant species subjected to no-choice tests, *P. maculiventris* developed successfully on *T. diversifolia* but on very few non-target species. However, only minor damage was recorded on non-target species, notably the exotic weed *Xanthium strumarium* L. and some sunflower (*Helianthus annuus* L.) cultivars. Also, survival to adulthood was considerably lower on sunflower cultivars than on the target weed during these tests. During choice tests, *P. maculiventris* oviposited and developed successfully on *T. diversifolia* only, with minor feeding damage on some *H. annuus* cultivars, suggesting that the beetle's field host range will be confined to the target weed. Risk analysis also showed that *P. maculiventris* presents an extremely low risk to non-target plant species, notably those within the tribe Heliantheae and other close relatives. The effectiveness of *P. maculiventris* was assessed on the basis of its impact on the growth and biomass production of the weed. Significant foliar damage by the adult and larval stages of *P. maculiventris* was recorded at low and high insect densities, causing a 50.2 % and 55.0 % reduction in plant biomass, respectively. Climatic modelling (CLIMEX) suggested that the beetle is likely to establish over the entire range of *T. diversifolia* in South Africa and neighbouring countries. The study concludes that *P. maculiventris* is safe for release and is likely to become widely established and cause significant damage to populations of *T. diversifolia* in South Africa. An application to release *P. maculiventris* into the field is thus being prepared for submission to the relevant South African regulatory authorities.

Key words: Agent impact, Host-specificity, *Physonota maculiventris*, *Tithonia diversifolia*, Weed biocontrol

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CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1
GENERAL INTRODUCTION

1.1. Origin and distribution of *T. diversifolia*

Several species of invasive alien plants have been introduced into South Africa, both accidentally and deliberately (Van Wilgen *et al*. 2008), and these include two species of *Tithonia* (Mexican and red sunflowers) that are currently causing negative impacts on various biomes in KwaZulu-Natal, Mpumalanga and Limpopo provinces. *Tithonia diversifolia* (Helms.) A. Gray (Asteraceae: Heliantheae), which has become naturalised in the country's tropical and subtropical regions (Fig. 1.1), is the focus of this study. This plant is a noxious weed of farmlands, disturbed lands and roadsides in several countries, including South Africa (Agboola *et al*. 2006).

Tithonia diversifolia, which is native to Central America (including Mexico) (Nash 1976), is reported to be an aggressive weed in South East Asia, South America and tropical Africa (Lazarides *et al*. 1997; Meyer 2000; Henderson 2001; Varnham 2006). It has become an important weed of arable crops in Oyo, Gbongan and Ogun States of Nigeria, forcing some farmers to abandon their lands (Chukwuka *et al.* 2007). The rapid and extensive invasion of *T. diversifolia* in South Africa followed its initial introduction into the country in the early 1930s as an ornamental plant (Henderson 2001), which later escaped into natural systems during the same decade (Henderson 2006). According to the National Environmental Management and Biodiversity Act (NEMBA) and the Conservation of Agricultural Resources Act (CARA) of South Africa, *T. diversifolia* has been declared a category 1b weed and category 1 weed, respectively, implying that control in invaded areas is compulsory (Henderson 2001).

The second species of invasive sunflower, *Tithonia rotundifolia* (Mill.) S.F. Blake (red sunflower), is predominant in the inland areas of the south-eastern regions of Africa, including the middleveld and lowveld regions of South Africa (Henderson 2001). Although not yet confirmed in South Africa, a dense population of a third invasive sunflower, *Tithonia tubiformis* (Jacq.) Cass., was recently spotted in the North East of Swaziland (D.O. Simelane. pers. comm.), which is some 5km away from the border separating Swaziland and the Mpumalanga Province of South Africa. In total, there are 11 species of *Tithonia* and their

native ranges include Mexico, the south-western USA and other Central American countries (Muoghalus & Chuba 2005).

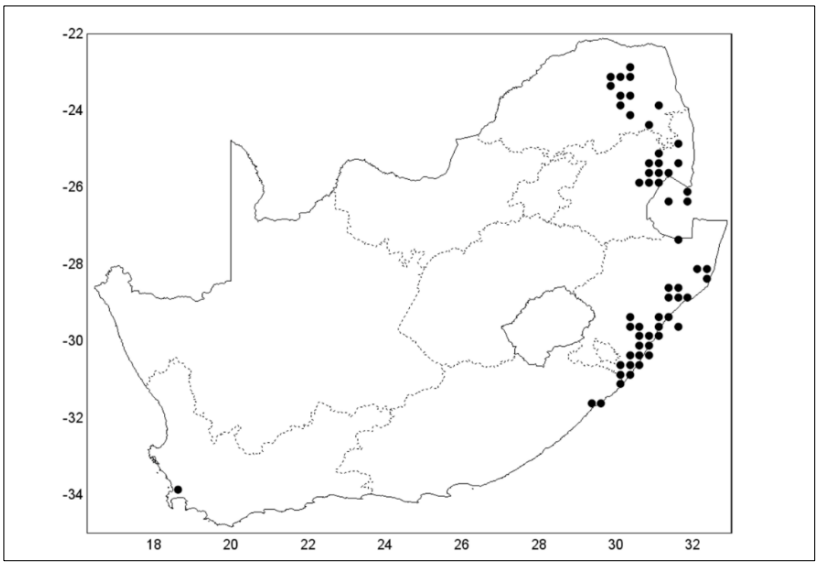

Fig. 1.1 *Tithonia diversifolia* distribution in South Africa (from Simelane *et al*. 2011).

1.2 Botanical information on *T. diversifolia*

Tithonia diversifolia, commonly known as Mexican sunflower, is an annual herbaceous shrub that grows up to 5m tall, particularly along the humid east coast of South Africa (Fasuyi *et al*. 2010).

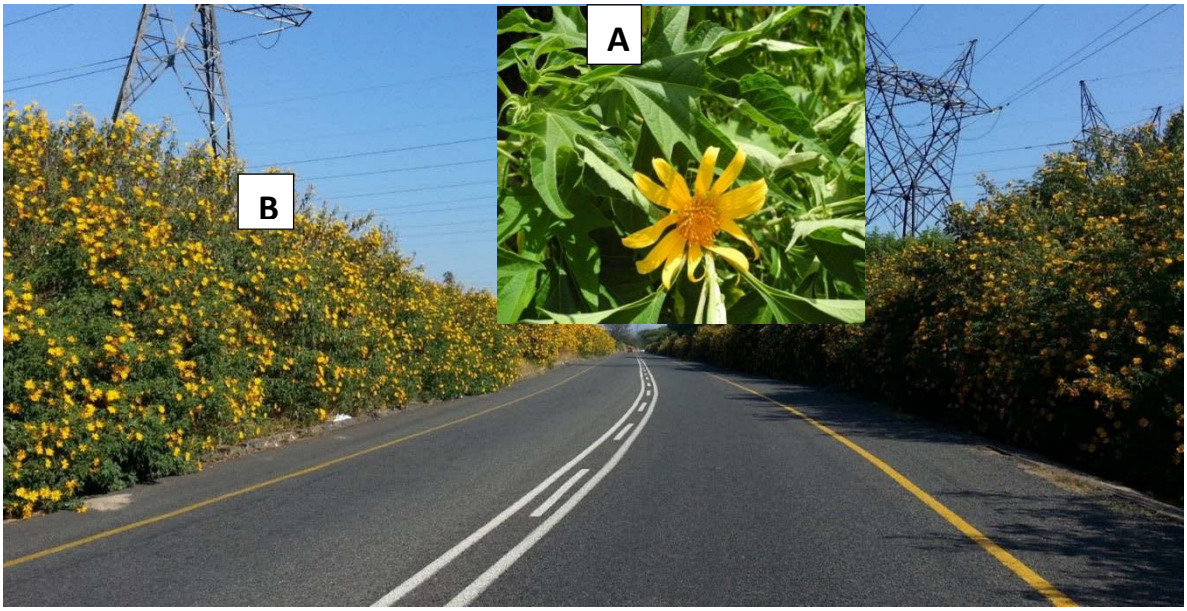

Fig. 1.2 *Tithonia diversifolia* close-up (A) and infestation in Mpumalanga (B).

The plant has 13-15 cm long leaves that are alternatively arranged; each with 3-5 lobed blades. In South Africa, flowering occurs in May and June when bright yellow flowers (Fig. 1.2A,B) that are up to 100 mm in width become apparent (lpou *et al*. 2011; Simelane *et al*. 2011; Mphephu *et al*. 2014a). The fruits constitute brown achenes that form a rounded spiky mass (Henderson 2001). The plant is rich in nutrients due to its ability to extract high levels of nutrients from the soil (Jama *et al*. 2000). It is also well adapted to heat and drought and can easily be propagated from both cuttings and seeds (lpou *et al*. 2011).

1.3 Attributes and ecological impact of the weed

Tithonia diversifolia is adaptable to various habitats, including road sides, brown fields (i.e., abandoned areas that were previously utilized for urbanization), disturbed areas, river banks and ecosystems that are exposed to high levels of sunlight (Yang *et al*. 2012). The plant produces large numbers of light-weight seeds which are often spread by wind over a large area (Muoghalu & Chuba 2005). It also coppices from stem cuttings, forming dense stands along roadsides; often soon after weed clearing operations (Simelane *et al*. 2011). In Nigeria, invasion by *T. diversifolia* was reported to be caused mainly by effective seed dispersal strategies (Ayeni *et al*. 1997). The seeds can also be dispersed by humans, livestock and water currents (Yang *et al*. 2012). High rates of clonal proliferation have also been observed after heavy rains (Ayeni *et al*.1997).

The seeds of *T. diversifolia* can tolerate dry seasons, remaining dormant prior to the induction of germination by rain (Agboola *et al*. 2006). The plant has an extensive root system, enabling it to tolerate low levels of soil nutrients and recover after fires (Wanjau *et al*. 1998). *Tithonia diversifolia* is well known to have allelopathic properties which inhibit the growth of other plants in close proximity (Taiwo & Makinde 2005; Oyerinde *et al*. 2009). Stunted growth of shoots and roots of certain plants growing in habitats that were previously occupied by *T. diversifolia* have also been reported as a result of allelopathy (Tongman *et al*. 1998). The plant's secondary metabolites include phenols, tannins, sesquiterpene lactones (tagitinin A and tagitinin C) and flavonoids (hispidulin) which are known to deter feeding by various insect species (Taiwo & Makinde 2005; Oyerinde *et al*. 2009) and ensure that the plants escape insect damage in invaded areas.

1.4 Utilization of *T. diversifolia*

Tithonia diversfolia is widely cultivated for ornamental purposes, particularly in West Africa (Nash 1976; Akobundu & Agyakwa 1987). The plant also has several medicinal, agricultural and other uses. Leaf infusion of *T. diversifolia* has been used to treat malaria, intestinal parasites and domestic animal skin diseases (Rios 1999). The plant is also utilized as animal fodder in some East African countries (Rios 1999; Agboola *et al*. 2006). It is often used as a component of manure or compost, resulting in high yields of crops in some Central African countries (Ojeniyi & Adetoro 1993).While the ability of *T. diversifolia* to control termite infestations was first reported by Spore (1998), its insecticidal properties were later reported by Orwa *et al*. (2009). Farmers have used the plant to repel various insect pests by introducing it into their agricultural crops (Orwa *et al*. 2009). It has also been used as a hedge around farms and homesteads (Orwa *et al*. 2009) in some countries in East Africa. Although the plant's medicinal and agricultural benefits are acknowledged in several countries, the problems that it causes as an invasive weed far outweigh these benefits. In South Africa, neither medicinal nor agricultural value has been acknowledged for *T. diversifolia* and there are therefore no conflicts of interest in this country.

1.5 Control of *T. diversifolia*

1.5.1 Chemical and mechanical control

Currently, no conventional control measures are effective against infestations of *T. diversifolia* in South Africa. No herbicides have been registered for use against *T. diversifolia* in the country (Simelane *et al*. 2011), although some herbicides such as Glyphosate™ have been used with limited success (Bio-hazard 2001). Mechanical control is largely ineffective due to the plant's ability to coppice from stems and because of the rapid recruitment of seedlings in cleared areas. Although mechanical and chemical control measures are often applied by land owners in South Africa, these are expensive, generally ineffective and unsustainable (Simelane *et al*. 2011).

1.5.2 Biological control

Classical biological control, which involves the introduction and release of natural enemies from the pest's country of origin, has been deployed against invasive weeds in South Africa for more than 100 years and has a good track record of success (Moran *et al*. 2013). These natural enemies (i.e., biological control agents) include mostly host specific insects,

but also pathogens, and have the potential to contribute to the permanent suppression of populations of target weeds (Olckers *et al*. 1998). Biocontrol is the most viable option for prolific weeds like *T. diversifolia* as it constitutes an environmentally friendly and self-sustaining regulatory system with minimal costs (Simelane *et al*. 2011). *Tithonia diversifolia* has been targeted for biological control by the ARC-Plant Protection Research Institute since 2007 and South Africa is currently the only country that is involved with biological control research on this weed (Simelane *et al*. 2011).

A total of eight natural enemy species (Table 1.1) have been recorded during surveys of *T. diversifolia* that were conducted in the native range of the weed from 2007 to 2012 (Mawela & Simelane 2014). The natural enemy complex consists of leaf feeders, stem borers and flower feeders. Whilst some of these potential agents were difficult to rear under laboratory conditions (e.g. the stem-boring weevil *Rhodobaenus auctus* Chevrolet (Curculionidae)), others (e.g. the leaf-feeding butterfly *Chlosyne* sp. (Nymphalidae)) were unsuitable for release due to wide host ranges recorded in their native range (Mawela & Simelane 2014). The current study was conducted to assess the suitability of the defoliating beetle *Physonota maculiventris* Boheman (Chrysomelidae) for release as a biological control agent of *T. diversifolia* in South Africa.

1.5.3. *Physonota maculiventris* Boheman (Coleoptera: Chrysomelidae)

Physonota maculiventris was imported from Mexico in 2009 and 2012 for host range evaluation tests at the ARC-PPRI quarantine facility in Pretoria, South Africa. The selection of this tortoise beetle was based on its potential to inflict high levels of leaf damage on *T. diversifolia* (Fig. 1.3) which could result in significant loss of photosynthetic area and high levels of stress on the plants.

Fig. 1.3 Adult and larva of *P. maculiventris* on a severely damaged host plant leaf.

1.6 Taxonomic position of *P. maculiventris*

Physonota maculiventris belongs to the coleopteran family Chrysomelidae which is regarded as the largest group of insects, comprising mainly leaf-feeding species (Blatchley 1924) (Fig. 1.4). There are 19 subfamilies (Lawrence 1982) and some 35 000 identified species (Jolivet & Petitpierre 1981) within the Chrysomelidae. *Physonota maculiventris* falls under the Cassidinae, which is the second largest subfamily in terms of species richness and contains some 35 tribes (Chaboo 2007). There are 6 genera within the tribe *Physonotini* and 39 species within this tribe belong to the genus *Physonota*. The tribe *Physonotini* is widely distributed throughout the New World (Chaboo 2007) and their host plants include species in the families Asteraceae, Ehretiaceae and Lamiaceae. Three of the 39 species of *Physonota* (i.e., *P. unipunctata*, *P. helianthi* and *P. alutacea*) are closely related to *P. maculiventris* (Jolivet & Petitpierre 1981). Several species within the Chrysomelidae are specific to their host plants (Jolivet & Petitpierre 1981) suggesting that they are suitable as candidate biocontrol agents.

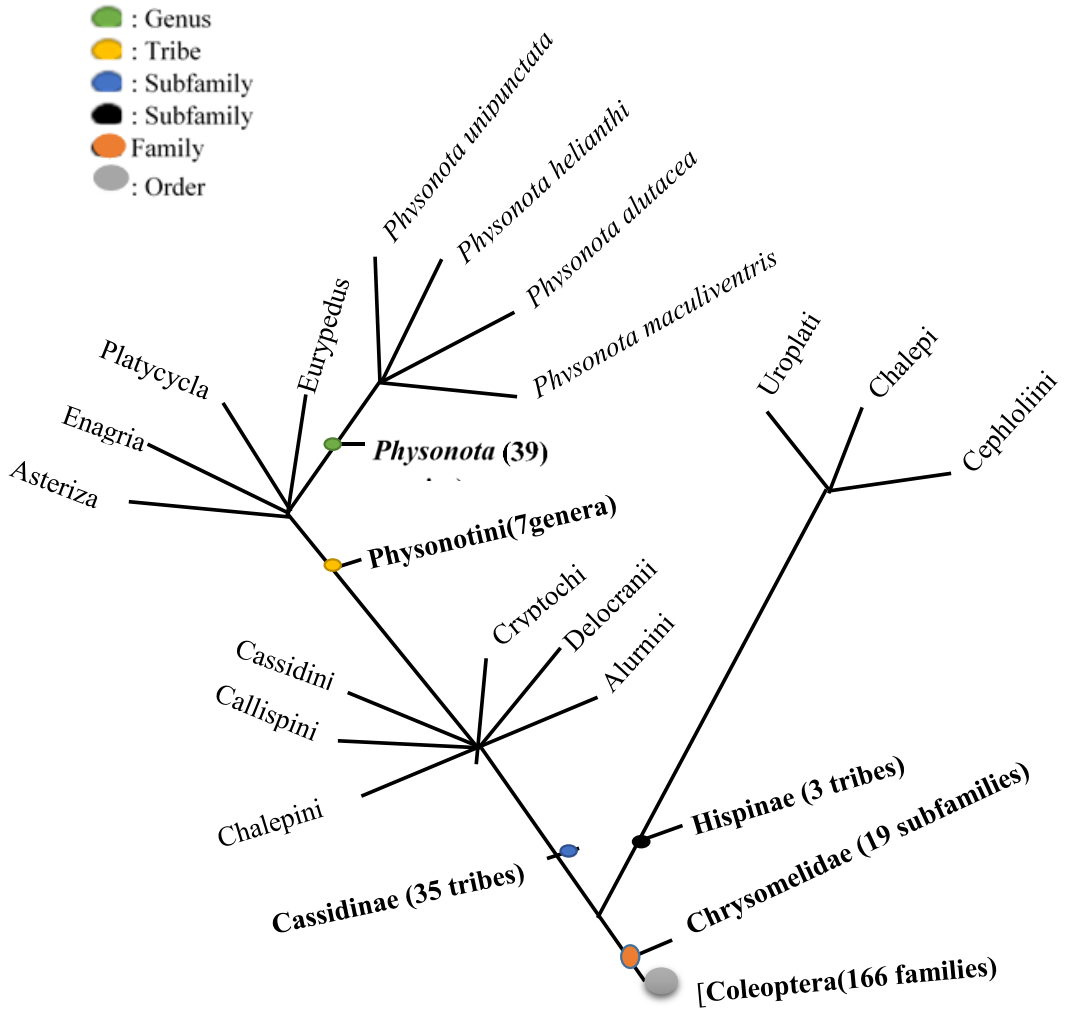

Fig. 1.4 Taxonomic position of *Physonota maculiventris* in relation to other tribes in the Cassidinae. Information from ITIS Report (Taxonomic Serial No. 839554) and Chaboo (2007).

Table 1.1 Natural enemies considered for the biological control of *T. diversifolia* in South Africa.

Order: Family	Potential agent species	Location	Current status
Lepidoptera: Nymphalidae	*Chlosyne* sp.	Cuarnavaca, Mexico	Host-specificity tests conducted; wide host range; culture culled
Lepidoptera: Tortricidae	Unidentified shoot-borer	Puerto Angel City, Mexico	Not tested; low priority; culture culled
Uredinales: Pucciniaceae	*Puccinia tithoniae**	Various locations in Mexico	Incompatible with South African *Tithonia* biotypes
Coleoptera: Chrysomelidae	*Physonota maculiventris*	Various locations in Mexico	Host-specificity testing completed (this study)
Coleoptera: Cerambycidae	*Canidia mexicana*	Mexico	Shelved due to culturing difficulties
Coleoptera: Curculionidae	*Rhodobaenus auctus*	Mexico	Shelved due to culturing difficulties
Coleoptera: Curculionidae	*Lixus fimbriolatus*	Mexico	Host-specificity testing in progress
Lepidoptera: Gracillariidae	Unidentified leaf-miner	Mexico	Shelved
Lepidoptera: Unknown family	Unidentified flower-feeder	Mexico	Imported but failed to culture

*Pathogen.

1.7 Aim and objectives of this study

The aim of this study was to assess the suitability of *P. maculiventris* for release as a biological control agent against *T. diversifolia* in South Africa. In order to achieve this, my objectives were to: (i) study certain biological aspects of *P. maculiventris* that could influence its success as a biological control agent; (ii) determine the host-specificity of *P. maculiventris* under laboratory conditions and hence its safety for release and; (iii) gain some insight into the potential impact and distribution of *P. maculiventris* on *T. diversifolia* in the field.

CHAPTER 2

Biological studies on the defoliating beetle *Physonota maculiventris* Boheman (Coleoptera: Chrysomelidae: Cassidinae)

ABSTRACT

The tortoise beetle *Physonota maculiventris* Boheman 1854 was collected from its native range in Mexico and introduced into South Africa as a potential biological control agent for Mexican sunflower *Tithonia diversifolia* (Hemls.) A. Gray (Asteraceae). Studies on the fecundity, duration of development of various life stages, sex ratio and adult longevity of *P. maculiventris* were conducted under quarantine conditions to determine whether the beetle has the necessary attributes to be an effective biological control agent. Egg batches were deposited strictly on the under-surfaces of the leaves, often towards the apex of the leaf. Each female laid 5 to 6 egg batches [(mean ± SE= 5.3 ± 0.3 batches (n = 4)] during its life time, with each batch consisting of around 33 eggs. From each egg batch, all eggs hatched successfully, and larval survival to adulthood was 85.6 % [28.3 ± 0.8 eggs (n = 4) of the 33 eggs per batch)] on the host plant. *Physonota maculiventris* larvae were highly gregarious during their early stages, and both adult and larval stages fed voraciously, often defoliating the plants completely. Four larval instars were recorded and larval development was completed in 18 days. The generation time of the beetle was completed in 67.5 ± 7.5 days. The adults were relatively long-lived, with males and females surviving for 50.0 ± 1.3 and 45.0 ± 2.2 days, respectively, after emergence. Based on these studies and the track record of other species of Cassidinae that had been used previously as biocontrol agents, *P. maculiventris* has the necessary biological attributes to be a successful biocontrol agent for *T. diversifolia*.

Key words: Agent fecundity, Biological weed control, Host plant-insect interactions, Natural enemies, *Tithonia diversifolia*.

1. INTRODUCTION

The Cassidinae are generally referred to as tortoise beetles and represent one of the largest subfamilies of the Chrysomelidae, comprising more than 2,850 known species worldwide (Borowiec & Moragues 2005). Cassidinae species are all phytophagous and known to be highly specific to their host plants (Jolivet *et al.* 1988). Most species of Cassidinae feed gregariously during their larval stages, while the adults disperse rapidly and also feed extensively on the plant, particularly the females during their pre-oviposition period (Jolivet *et al*. 1988). Tortoise beetles also display prolonged reproductive periods, enabling them to sustain high population densities in the field (Nakamura *et al.* 1989; Cappuccino 2000). Because of their high degree of host specificity and voracious feeding capabilities, together with extensive defensive strategies during their life stages, tortoise beetles have often been selected as biological control agents for invasive alien weeds (Chaboo 2007). Their defensive strategies include egg batches that are covered with a coat of mucous, short egg incubation periods, larval furculae on the last abdominal segment which contain an accumulation of larval exuviae and faeces and are used to deter predators, short pupation periods and hard adult exoskeletons (Rabaud 1921; Rothschild 1972; Jolivet *et al.* 1988; Bacher & Luder 2005).

Cassidinae species have been utilized in many countries as biocontrol agents of weed species, and their impact on their targets has varied from negligible to substantial (e.g. Hill & Hulley 1995a; Olckers *et al.* 1999; Broughton 2000; Medal & Cuda 2010). The biological control of tropical soda apple in Florida (USA), recorded two years after the release of *Gratiana boliviana* Spaeth (Chrysomelidae: Cassidinae), is one of the most recent successes (Medal & Cuda 2010). Although predation is known to retard the build-up of populations of tortoise beetles after release into new environments, this is often mitigated by their high fecundity (Manrique *et al*. 2011).

Physonota maculiventris Boheman, a tortoise beetle native to the Chiapas Province of Mexico, was first introduced into South Africa as a candidate biocontrol agent for the Mexican sunflower, *T. diversifolia*, in 2010 (Mphephu *et al*. 2014a,b). The beetle has only been recorded on the target weed *T. diversifolia* in Mexico (Fig. 2.1) and was thus considered very likely to be host specific (D.O. Simelane, pers. comm.; Mphephu *et al*. 2014a,b). Although *P. maculiventris* was first described in 1854 (Boheman 1854), no biological studies had ever been undertaken on this beetle. The current study thus considered various biological

aspects of *P. maculiventris* in order to determine whether it has the necessary attributes to be a suitable biological control agent for *T. diversifolia*.

2. MATERIALS AND METHODS

2.1 Insect cultures

Insect cultures were maintained on potted *T. diversifolia* plants under quarantine laboratory conditions, in which temperatures of around 28ºC and 22ºC were maintained during the day and night, respectively. Relative humidity ranged from 45 % to 90 %. The beetles were confined on their host plants in gauze-covered cages (55 x 55 x 75 cm) in the quarantine facility of the ARC-PPRI at Rietondale, Pretoria. The laboratory was fitted with overhead lights that included OSRAM L 36W/77 FLUORA (grow lux) and OSRAM L 36W/740 cool white to provide a 16:8 (L: D) hour photoperiod.

Fig. 2.1 The site near Comitan City ( ) where *P. maculiventris* was collected in Chiapas Province, in Mexico.

2.2 Plants

Cuttings of *T. diversifolia* were sourced from sites in KwaZulu-Natal, Limpopo and Mpumalanga and were collected from infestations along roadsides and invaded lands. Cuttings were initially planted in pure sand to facilitate quick stimulation of root formation. Rooted cuttings were then transferred into a standard soil mixture of sand, Styrofoam™ and

compost at a ratio of 1:1:1, in 2-litre pots. Potted cuttings were kept under 50 % shade conditions in the shade house and were irrigated twice a day with electronic overhead sprinklers.

2.3 Life history of *P. maculiventris*

All experiments were conducted in small gauze-covered cages (26 x 15 x 21 cm) under the laboratory conditions described previously.

To determine the egg incubation period (i.e., period between oviposition and hatching), 10 newly-deposited egg batches were tagged and monitored daily until egg hatch. The number of eggs contained in each batch was determined by counting individual eggs within a dissected batch using a compound microscope.

Larval developmental period was determined using 20 newly-emerged larvae collected from the culture, with each larva placed separately on a host plant grown in a 2-litre pot. The plants were placed some15cm apart in the cages to prevent overlapping of leaves and were monitored daily. Based on the number of larval skins shed during moulting, the number of larval instars and their respective developmental periods were determined. The study was replicated five times, ensuring that 100 larvae were monitored.

Larval survival was measured using 33 newly-emerged larvae collected from the culture and placed on a host plant that contained sufficient leaves for larval development. Survival was monitored on a daily basis and the number of individuals that reached adulthood was recorded. This experiment was replicated four times, with each plant representing a replicate.

To determine the duration of pupation, eight pre-pupal final instar larvae were monitored on plants from pupation until adult emergence. The period between the formation of the pupa and adult emergence was recorded as the pupal period.

Newly-emerged adults were then sexed (see below), weighed and paired (one male and one female) into five groups. To determine the female's pre-oviposition period, each pair of adults was monitored daily until the female had deposited her first egg batch. Fecundity was determined by the number of egg batches that each female in each of the five pairs (see above) deposited during its life time. Egg viability was determined by the number of larvae produced in relation to the total number of eggs contained in each egg batch. The longevity of

each of the five male and female beetles was determined by recording the number of days from adult emergence until death.

Sex ratios were determined using three randomly selected egg batches that were deposited in the cultures. Larvae arising from these egg batches were reared until the adult stage. Based on the shape of the abdomen, which was somewhat oblong for females and more circular for males (Fig. 2.2), the sexes of the newly-emerged adults were determined. The proportion of females to males was referred to as the sex ratio.

Fig. 2.2 Differences in the shape of the abdomen between adult males (A) and females (B) of *P. maculiventris*.

The duration of the immature stages (generation period) was determined by summing the developmental periods of all the different life stages for all the replicates (i.e., egg incubation, larval and pupation periods).

2.4 Data analysis

The developmental periods were analysed with descriptive statistics. Differences in body size between the genders were compared using *t*-tests.

3. RESULTS

3.1 Life history of *P. maculiventris*

Newly-emerged adults have conspicuous black and white streaks on their elytra. The colour often turns to golden brown (Fig. 2.3) as the beetles age and become sexually mature. The shape of the female abdomen is somewhat oblong while that of the male is circular. The females are significantly longer (13.5 ± 0.1 mm; n = 10) than the males (10.6 ± 0.1 mm; n = 10) (t = 14.8; df = 18; $P < 0.05$).

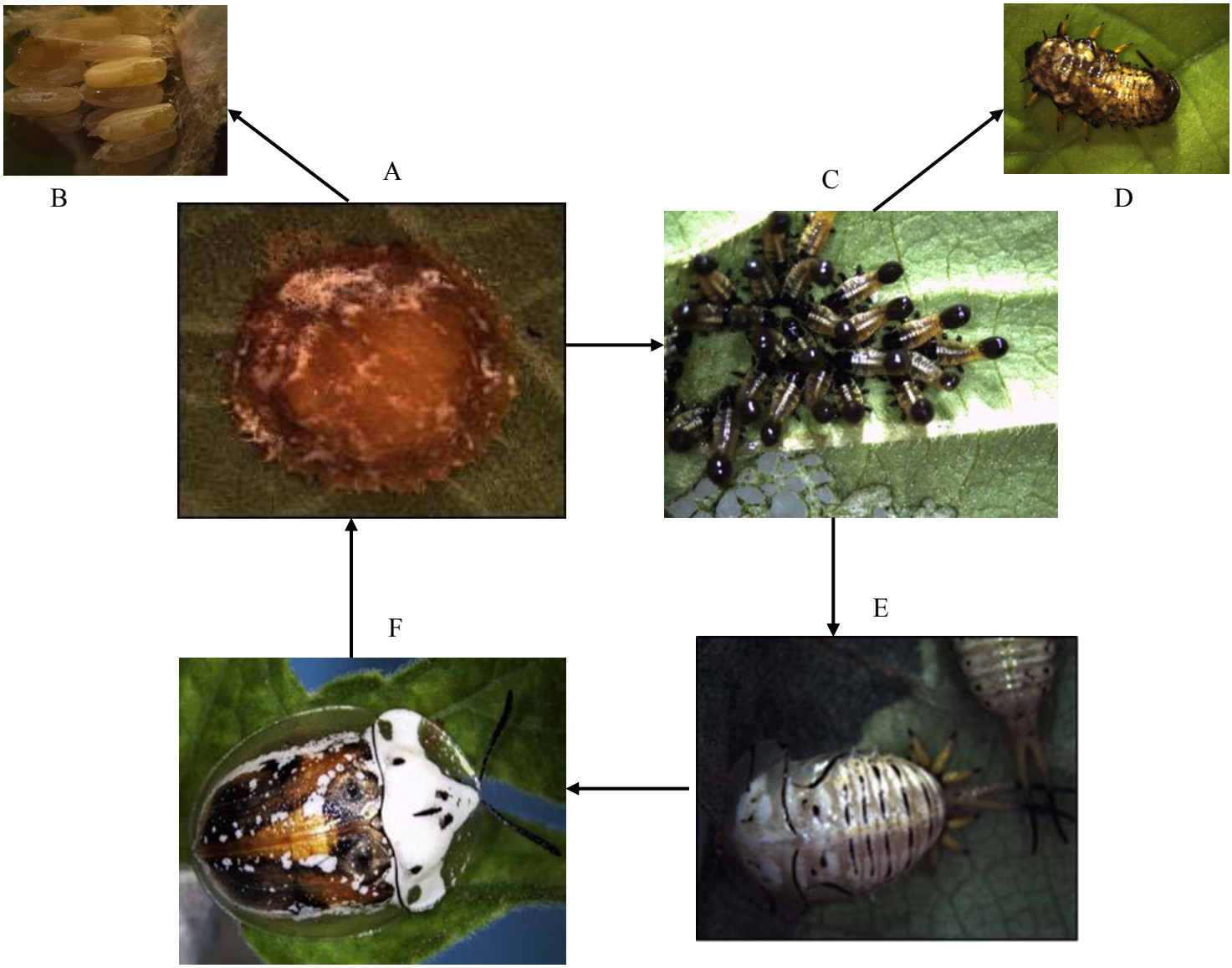

Fig. 2.3 Life cycle of *P. maculiventris*, indicating an egg batch (A), exposed eggs (B), early instar larvae (C), late instar larva (D), pupa (E) and mature adult (F).

On average, females deposited five to six egg batches (Fig. 2.3A) (n = 4), and each batch contained an average of 33 eggs. Eggs were tubular in shape and were around 2mm in length (Fig. 2.3B). The incubation period ranged between 11 and13 days (mean ± SE = 11.8 ± 0.5; n = 10), and 100% of the eggs hatched. Each larva shed three cast skins (exuviae) during its development, indicating four larval instars (Fig. 2.3C, D).

The larval developmental period (all four instars combined) ranged from 15 to 20 days (mean ± SE = 18.1 ± 0.5; n = 8). Survival to adulthood of a cohort of 33 larvae produced by an egg batch was around 85.6 % on average (mean ± SE = 28.3 ± 0.8 larvae; n = 3). Pupation (Fig. 2.3E) often occurred on dead leaves and the duration of pupation ranged from four to eight days (mean ± SE = 6.3 ± 0.5; n = 8).

Adult females were on average around 45 % larger than males, with newly-emerged females weighing from 76 to 87mg (mean ± SE = 81 ± 3; n = 5) and males from 51 to 62 mg (mean ± SE = 56.0 ± 2.0; n = 5). The female pre-oviposition period was relatively long and ranged from 22 to 24 days (mean ± SE = 22.8 ± 0.5; n = 4).

The number of egg batches deposited by each female ranged from 5 to 6 (mean ± SE = 5.3 ± 0.3; n = 4) during its life time. Males lived slightly longer (around 10 %) than females, with males surviving from 47 to 53 days (mean ± SE = 50.3 ± 1.3; n = 5) and females from 39 to 49 days (mean ± SE = 45.5 ± 2.2; n = 5) after adult emergence.

The number of adults that resulted from randomly selected egg batches ranged from 27 to 30 (mean ± SE = 28.7 ± 0.9; n = 3). From these adults, there were slightly more females (mean ± SE = 15.3 ± 2.9; n = 3) than males (mean ± SE = 13.3 ± 3.5; n = 3), giving a female to male ratio of around 1:1.2 and indicating that the sex ratio was largely equivalent.

The duration of the immature stages (egg to adult) ranged from 36-45 days (mean ± SE = 40.8± 1.9; n = 10) and the generation time (adult to adult) ranged from 60-81days (mean ± SE = 67.5 ± 7.5; n =10).

4. DISCUSSION

The combined feeding damage by both the larval and adult stages of *P. maculiventris* is high, which is consistent with other species of Cassidinae (Appendix 1) (Jolivet *et al*. 1988; Nakamura *et al*. 1989; Cappuccino 2000). Despite the possibility of attack by native parasitoids and predators (Hill & Hulley 1995a,b; Olckers & Hulley 1995), the beetle's short life cycle coupled with a high reproductive output are some of the important biological attributes possessed by *P. maculiventris* which could influence its success as a biological control agent. Also, the relativeness shortness of the developmental stages in relation to the longevity of the adults, may be an additional advantage that may help the beetle to avoid parasitism (see below).

An egg batch of *P. maculiventris* can contain up to 33 eggs, and a single adult female can lay up to 6 egg batches (i.e. around 200 eggs), which (assuming high rates of survival) should be sufficient to maintan high population densities of the beetle in the field. Also, egg hatch rates were maximized (100%), and an average of 85.6% of hatching larvae survived to adulthood under quarantine conditions. Assuming good climatic compatability and a low recruitment of native natural enemies in South Africa, the high reproductive output and survival of the immature stages of *P. maculiventris* are likely to sustain high population densities within the range of the target weed. High population densities of insect agents are generally essential for success in weed biological control (Nakamura *et al.* 1989; Cappuccino 2000). From each egg batch, a large number of larvae are produced, which feed gregariously and remove large amounts of leaf material from the host plant. Gassmann (1996) also argued that geographical populations with the best fitness traits should be selected to favour proliferation of the insect in the area of introduction.

Eggs of *P. maculiventris* are deposited in protective cases (oothecae) on the undersides of the leaves, which facilitates protection from abiotic mortality factors such as sun and rain (Rabaud 1921). Although Jolivet *et al*. (1988) argued that concealed oviposition sites could also facilitate the protection of eggs from potential predators, Olckers & Hulley (1995) and Hill & Hulley (1995b) later found that tortoise beetle eggs and pupae were vulnerable to parasitism. Both the larval and adult stages of tortoise beetles such as *P. maculiventris* also possess defensive mechanisms to protect them against their natural enemies (Rothschild 1972). Larvae feed voraciously and retain their faecal masses on the spikes (furculae) extending from the ends of their abdomens and from these a yellow fluid

which covers their entire bodies is produced. These faecal masses are believed to deter potential predators like ants and other generalist species (Jolivet *et al.* 1988; Bacher & Luder 2005). Rothschild (1972) also reported a similar mechanism in which secondary compounds secreted by other tortoise beetles facilitated chemical defensive mechanisms against potential predators. Adults are also well protected by the hardened elytra which provide cover for their body parts. Pupation of the 4th instar larvae of *P. maculiventris* often occurs on dead leaves of the host plant, ensuring that the pupae are not disturbed by larvae and adults that are still feeding on fresh leaves.

The pupation period takes up to 8 days which is relatively short compared to the other life stages (around 8.8% of the average life cycle). Jolivet *et al*. (1988) argued that the susceptibility of tortoise beetle pupae, which seem to lack sufficient defence mechanisms to natural enemies, is somewhat mitigated by their relatively short pupation period which enables them to escape parasitism and predation to some degree. During the onset of pupation, the late instar larva glues itself onto the leaf cuticle or dead leaf material of the plant that remains after larval feeding damage. The colour of the pupae becomes whitish to mimic the leaf cuticle and the dead leaf material, and this could also mitigate attack by predators and parasitoids. Although larval and adult stages are likely to escape parasistim, eggs and pupal stages may be attacked by native parasitoids (see Hill & Hulley 1995a,b; Olckers & Hulley 1995). For example, a high percentage of the pupae of *Gratiana spadicea* (Klug) (Chrysomelidae: Cassidinae), an agent of *Solanum sisymbriifolium* Lam. (Solanaceae), were attacked in the field in South Africa, particularly during overwintering, ensuring that relatively low numbers of adults emerged at the start of each growing season (King *et al*. 2011). There is also the possibility that *P. maculiventris* eggs and pupae may be attacked by ants, as ocurred with the congeneric *Physonota alutacea* Boheman (Chrysomelidae: Cassidinae), which failed to establish on black sage, *Cordia curassavica* (Jacq.) Roem. & Schult. (Boraginaceae), in Mauritius due to interference by ants (Winston *et al.* 2014).

Apart from the possibility of attack by native parasitoids and predators in the introduced range, the biological studies reported here (and additional studies discussed in Chapters 3 and 4) demonstrate that *P. maculiventris* has the necessary biological attributes to be successful as a biocontrol agent of *T. diversifolia*. These baseline data are also important for conducting host-specificty tests and impact studies and predicting field outcomes.

CHAPTER 3

Host range of *Physonota maculiventris* Boheman 1854 (Coleoptera: Chrysomelidae: Cassidinae), a potential biological control agent for *Tithonia diversifolia* (Hemsl.) A. Gray (Asteraceae) in South Africa

ABSTRACT

The defoliating tortoise beetle *Physonota maculiventris* Boheman 1854 (Coleoptera: Chrysomelidae), a promising candidate biocontrol agent for the weedy Mexican sunflower *Tithonia diversifolia* (Hemsl.) A. Gray (Asteraceae), was collected from Mexico and introduced into quarantine in South Africa. Host-specificity tests were conducted to determine whether the beetle is suitable for release in South Africa. No-choice, paired-choice and multi-choice tests were conducted under quarantine conditions on cultivated and native South African plant species that are closely related to the target weed. Among the 58 test plant species subjected to no-choice tests, *P. maculiventris* developed to adulthood on only *T. diversifolia* and three cultivars of sunflower, *Helianthus annuus* L. (Asteraceae). Although, the beetle caused similar damage on the three *H. annuus* cultivars during the no-choice tests, significantly fewer egg batches were deposited on them than on the controls, with significantly fewer larvae surviving to adulthood. Similarly, the exotic weed *Xanthium strumarium* L. (Asteraceae) supported significantly less feeding and oviposition, but no survival to adulthood, during the no-choice tests. Similarly, survival to adulthood was considerably lower on sunflower cultivars than on the target weed during the larval survival tests. During multi-choice tests involving plant species that were attacked during no-choice tests, *P. maculiventris* oviposited on the target weed only, causing only minor feeding damage on some *H. annuus* cultivars. In paired-choice tests involving sunflower cultivars and the target weed, significantly lower levels of feeding and oviposition were recorded on sunflowers. These results suggest that the field host range of this beetle will be confined to the target weed. Risk analysis also showed that *P. maculiventris* presents an extremely low risk to non-target plant species within the tribe Heliantheae and other closely related species. These findings strongly suggest that *P. maculiventris* is suitable for release against *T. diversifolia* in South Africa.

Key words: Biological weed control, Host-specificity testing, Mexican sunflower, Risk assessment.

1. INTRODUCTION

Host-specificity testing is an important process that is undertaken during the selection of suitable weed biological control agents. This procedure is aimed at reducing the risk of releasing insects that are likely to have non-target impacts on cultivated and native plant species in the country of introduction (Wan & Harris 1997; Olckers 2000; Louda *et al*. 2003; Sheppard *et al*. 2005). The major focus of the host range determination of insect agents is to verify the plant species that fall within their basic (fundamental) host range (Van Klinken & Heard 2000) and elucidate their realized host range by interpreting their ecology.

During host-specificity testing, a wide range of test plant species are selected, largely on the basis of their taxonomic and phylogenetic relatedness to the target weed. Each test plant species is then confined with the potential insect agent to determine its ability to feed, oviposit and develop successfully on the plant (Wapshere 1974; Marohasy 1998; Briese 2003). There are generally three major types of host-specificity tests for screening potential insect agents, namely no-choice, choice and open-field tests. No-choice tests ascertain the fundamental (potential) host range of an insect which includes a set of plant species that are capable of being utilized when its natural host is not present. Choice tests, which include the presence of the natural host and one or many alternative host(s) under less confined laboratory conditions, is a better predictor of the insect's field (ecological) host range (Sheppard *et al*. 2005). Open-field tests, which are conducted under outdoor conditions in the native range, also encompass more natural features of the host selection process (e.g. host habitat finding and oviposition site selection), thereby generating more realistic host-specificity data under natural conditions that would otherwise not be obtained via cage tests in the laboratory (Clement & Cristofaro, 1995; Goolsby *et al*. 2006).

It is crucial to test the different life stages of the insect as part of this process, as these provide a detailed response of the insect to each test plant species with regard to oviposition site selection and the subsequent feeding, development and survival of its immature stages (Sheppard *et al*. 2005). In addition to its host range, biological aspects of the candidate insect agent should also be studied as part of the pre-release assessment as these can indicate whether it has the necessary attributes to be effective (Hanson 1976; Briese 2005; Chapter 2). Based on the results of the different host-range tests, risk assessments of potential biocontrol agents have been undertaken to quantify any risks posed by the agent to non-target plant

species in the introduced range (Wan & Harris 1997; Olckers 2000; Louda *et al*. 2003; Sheppard *et al*. 2005).

In this study, a promising leaf-feeding tortoise beetle, *Physonota maculiventris* Boheman (Coleoptera: Chrysomelidae: Cassidinae), was subjected to host-specificity tests in quarantine to determine its suitability for release against the weedy Mexican sunflower *Tithonia diversifolia* (Hemsl.) A. Gray (Asteraceae) in South Africa. Host-specificity tests included no-choice and choice tests conducted on the adult and larval stages. The results of these tests were interpreted in relation to the beetle's response to the target (control) plants. The host-range studies also included a risk assessment of *P. maculiventris* which ascertained the extent of any risks posed by the release of this beetle to non-target plant species in South Africa.

2. MATERIALS AND METHODS

2.1 Insect culture

Adult and larval stages of *P. maculiventris* that were used in the experiments were initially collected from *T. diversifolia* in Mexico in 2010 and 2012. The beetle was reared on *T. diversifolia* in cages (55 x 55 x 75 cm) under quarantine conditions where daily temperatures ranged from 22ºC to 32ºC and relative humidity from 35 % to 41 %. The quarantine laboratory was constructed of transparent glass to facilitate the penetration of natural light. One potted plant was confined with adult beetles in each cage to allow feeding and oviposition. Defoliated plants were replaced with fresh ones whenever necessary. Some of the newly-emerged larvae and adults from these cultures were then utilized to initiate the various host-range trials on *P. maculiventris*.

2.2 Test plants

Test plants were grown from field-collected seedlings obtained from localities in Gauteng, KwaZulu-Natal, Mpumalanga and Limpopo provinces. Commercial sunflower seeds were provided by Agricol Seeds and some of the ornamental flowering plant species were purchased from nurseries around Pretoria. The test plant species were selected on the basis of their taxonomic and centrifugal phylogenetic relatedness to *T. diversifolia* (see Wasphere 1974; Briese 2003). Some 58 plant species from seven families were selected as test plants (Table 3.1), and these included indigenous and other species of commercial value. Test plants were planted in 2-litre pots with a standard soil mixture of sand, vermiculite and compost at a ratio of 1:1:1, respectively. Plants were maintained in a nursery under 50 % shade and were irrigated twice daily via an automatic irrigation system.

2.3 Adult feeding and oviposition during no-choice tests

Four pairs of *P. maculiventris* adults (i.e. four males and four females) were confined with each potted test plant species in a cage (55 x 55 x 75 cm) for 40 days. *Tithonia diversifolia* was also confined with the same number of beetles to serve as a control. Daily inspections were conducted to ascertain feeding damage, oviposition and survival to adulthood of the larvae for the duration of the trial. The trials were terminated after 40 days, by which time sufficient egg batches had been laid on the control plants (*T. diversifolia*). At the end of the experiment, the numbers of egg batches were recorded on all test plants, and these were then monitored throughout their incubation, larval and pupation stages. Each test

plant species, including the control, was tested on at least three separate occasions. The damage level was assessed using the following rating scale where; 0 = no feeding; 1 = exploratory feeding; 2 = minor feeding and; 3 = normal feeding.

2.4 Adult feeding and oviposition during multi-choice tests

These tests were conducted to predict the ecological (realized) host range of *P. maculiventris*. The tests were conducted in large cages (95 x 141 x 123 cm) under laboratory conditions where the average room temperatures were approximately 25°C during the day and 18°C at night. Two separate choice trials were conducted under the same laboratory conditions. The first trial involved four different plant species that supported feeding and oviposition of *P. maculiventris* during no-choice tests. These included *Helianthus annuus* L., *Xanthium strumarium* L., *Tithonia rotundifolia* (Mill.) S.F. Blake and *T. diversifolia* (control) (Fig. 3.1A). The second trial involved five sunflower (*H. annuus*) cultivars and *T. diversifolia* (control) (Fig. 3.1B). In each trial, 20 pairs (40 adults) of newly-emerged *P. maculiventris* adults were confined with the plants for 40 days, and both trials were replicated three times. The adults were released in the centre of the cage, and feeding and oviposition was evaluated on each plant during the trial period. To eliminate interference by the pots and facilitate easier access of the adult beetles to the plants, the cages were filled with vermiculite up to the base of the stems of the test plants (Fig, 3.1A, B).

Fig. 3.1 (A) Multi-choice trial involving test plant species that were attacked during no-choice tests.

Fig. 3.1 (B) Multi-choice trial involving five sunflower (*H. annuus*) cultivars and *T. diversifolia* (control).

2.5 Adult feeding and oviposition during paired-choice tests

As a support for the multi-choice tests, these tests were conducted to determine the feeding and oviposition preferences of *P. maculiventris* when presented with the host plant (*T. diversifolia*) and each of five sunflower (*H. annuus*) cultivars. One potted plant of a *H. annuus* cultivar was paired with one *T. diversifolia* plant in the same cage (55 x 55 x 75 cm). Cages were filled with vermiculite up to the base of the plants' stems to facilitate easier access of the beetles to the plants. Two pairs (four individuals) of adults were released at the centre of the cages, and the plants were inspected daily to record feeding and oviposition for the duration of the experiment. Each experiment was replicated three times and was terminated after 40 days.

2.6 Comparison of larval survival of *P. maculiventris* between *T. diversifolia* and sunflower (*H. annuus*) cultivars

These tests were conducted to compare the survival and duration of development of *P. maculiventris* larvae on the natural host (*Tithonia diversifolia*) with that on five sunflower (*H. annuus*) cultivars. Ten newly emerged larvae were placed on each test plant and these were housed in separate cages (55 x 55 x 75 cm) under the laboratory conditions described in section 2.1. Daily inspections focused on ascertaining the feeding damage, mortality and duration of development of the larval stages on the different test and control plants. Each

experiment was terminated after 20 days, by which time all larvae had developed to adulthood on the control plants.

2.7 Risk assessment of *P. maculiventris* on non-target plant species

The potential risks posed by *P. maculiventris* to non-target *H. annuus* cultivars and *X. strumarium* were determined by means of its feeding and oviposition performance on each non-target species, as a proportion of that on the target weed, *T. diversifolia* (Wan & Harris 1997). The performance criteria used were plant preference (R^1), food acceptability (R^2), oviposition preference (R^3), larval survival (R^4) and oviposition potential (R^5), and these were based on the beetle's feeding and reproductive performance during choice (R^1, R^3) and no-choice (R^2, R^4, R^5) tests. Plant preference was measured by where the beetles were located while oviposition preference was measured by where they laid eggs. Food acceptability was measured by the amount of feeding while oviposition potential was measured by the numbers of eggs deposited. Larval survival was measured by the numbers of larvae that survived to adulthood. The feeding risk was determined as a product of the scores for plant (location) preference and food acceptability ($R^1 \times R^2$) while the reproductive risk was determined as a product of the scores for oviposition preference, larval survival and oviposition potential ($R^3 \times R^4 \times R^5$). To facilitate calculations, a score of 0.001 was used to replace zero values.

Table 3.1 List of test plants (58 species) used in the different host-specificity tests on *Physonota maculiventris*.

Family	Tribe	Genera and species
Amaranthaceae		*Amaranthus cruentus* L. #**
		Amaranthus spinosus L. #**
		Amaranthus thunbergii Moq. #**
		Spinacia oleracea L. #**
Apiaceae		*Daucus carota* L. #**
Asteraceae	Anthemideae	*Argyranthemum* sp.**
		Artemisia afra Jacq. Ex Willd.*
		Chrysanthemum segetum L. #
		Chrysanthemum 'mermaid' #**
		Ursinia nana DC.*
	Eupatorieae	*Ageratum conyzoides* L.**
		Campuloclinium macrocephalum (Less.) DC. #
	Coreopsideae	*Bidens bipinnata* L.#
		Bidens formosa (Bonato) Sch. Bip. #
		Bidens pilosa L. #
		Coreopsis 'garnet' #**
		Dahlia 'maryevelin' sp. #**
	Cynareae	*Centaurea cyanus* L. #
		Cirsium arvense (L.) Scop. #
	Astereae	*Conyza bonariensis* (L.) Cronq. *
		Conyza canadensis (L.) Cronq. #
		Conyza sumatrensis (Retz.) E.H. Walker #
		Felicia sp. 1 #**
		Felicia sp. 2 #**
		Nolletia rarifolia (Turcz.) Streetz.*
	Helenieae	*Flaveria bidentis* (L.) Kuntze #
Asteraceae	Heliantheae	*Galinsoga parviflora* Cav. #
		Helianthus annuus L. #*
		(Agsun 5278 k2)
		(Agsun 8251 k3)
		(Agsun 8251 k2)
		(Agsun 8251 kia 53)
		(Agsun 5174 cl k3)
		Tithonia diversifolia (Hemsl.) A. Gray #
		Tithonia rotundifolia (Mill.) S.F. Blake #
		Xanthium strumarium L. #
		Aspilia spp. DC *
		Sphagneticola calendulacea (L.) Pruski #
		Zinnia elegans Jacq. #
		Zinnia peruviana (L.) L. #

Indigenous plants*, Ornamental/ economic plants**, incidental introduced /weedy plants# in South Africa. [Continued on next page]

Table 3.1 continued

Family	Tribe	Genera and species
Asteraceae	Arctotideae	*Berkheya montana* J.M. Wood & M.S. Evans *
		Gazania 'sundance' *
		Gazania rigens (L.) Gaertn.*
	Mutisieae s.s	*Gerbera jamesonii* Bolus ex Hooker. F.*
	Gnaphalieae	*Athrixia elata* Sond. *
		Helichrysum pilosellum (L.f) Beentje*
		Hypochoeris radicata (L.) Hill #
	Senecioneae	*Cineraria deltoidea* Sond.*
		Euryops pectinatus (L.) Cass. #**
		Senecio affinis DC. #**
		Senecio angulatus L. f.*
		Senecio serratuloides DC.*
		Senecio sp.*
		Senecio venosus Harv.*
	Cichorieae	*Lactuca serriola* L. #
		Sonchus asper (L.) Hill #
		Taraxacum officinale F.H. Wigg. #
	Tageteae	*Tagetes minuta* L. #
		Tagetes patula L. #
Brassicaceae		*Brassica oleracea* L. #**
Fabaceae		*Phaseolus vulgaris* L. #**
Poaceae		*Zea mays* L. #**
Solanaceae		*Solanum tuberosum* L. #**

Indigenous plants*, Ornamental/ economic plants**, incidental introduced /weedy plants# in South Africa.

2.8 Data analysis

Where necessary, raw data were analysed using descriptive statistics, and all results were presented as means and standard errors (mean ± SE). Data on plant species that supported feeding during the no-choice and multi-choice tests were subjected to Kruskal-Wallis tests to determine if there were significant differences in feeding scores between the plants. Data on plant species that supported oviposition and survival of the beetle during the no-choice tests were subjected to ANOVA, and Fisher's LSD tests were used to separate the means where significant differences ($P < 0.05$) were recorded. Data from the paired-choice tests were subjected to Mann-Whitney U tests to determine if the differences in feeding and oviposition between the pairs of plants were significant.

3. RESULTS

3.1 Adult feeding and oviposition during no-choice tests

Of the 58 plant species that were exposed to pairs of adult *P. maculiventris* during the no-choice tests (Table 3.1 and 3.2), only three (*T. diversifolia*, *X. strumarium* and *H. annuus*) supported feeding, oviposition and larval development to adulthood. The remaining plant species were not accepted by the beetle for feeding or oviposition. Further tests showed that all five tested cultivars of *H. annuus* were susceptible to *P. maculiventris.* Although there were significant differences in feeding between the susceptible test plants (χ^2 =12.88; $P <$ 0.045), feeding levels on some *H. annuus* cultivars were not significantly different from that on *T. diversifolia* (Table 3.3). However, the highest survival to adulthood on any test species did not exceed 10% of that on the host plant. There were also significant differences in oviposition ($F_{(6, 14)} = 45.444$, $P < 0.05$) and survival to adulthood ($F_{(6, 14)} = 129.77$, $P < 0.05$) between the susceptible test plants (Table 3.3). The number of egg batches laid and number of emerging adults was significantly lower on all sunflower cultivars than on the target weed (Table 3.3). Numbers of egg batches deposited and survival to adulthood on the most susceptible sunflower cultivar were 63% and 10%, respectively, of that on the target weed *T. diversifolia* (Table 3.3). The levels of feeding on some *H. annuus* cultivars (i.e., Agsun 5278k2, Agsun 8251k3 and Agsun 8251k2) did not differ significantly from that on *T. diversifolia*. However, significantly lower feeding levels were recorded on *X. strumarium* and two varieties of *H. annuus* (Agsun 8251kia53 and Agsun 5174clk3) than on *T. diversifolia*.

Table 3.2 Plant families and the numbers of species within each family that displayed susceptibility to feeding and oviposition by *P. maculiventris* during no-choice tests.

Family	Number of plant species tested	No. of plant species susceptible to feeding and oviposition
Amaranthaceae	4	0
Apiaceae	1	0
Asteraceae	49	3
Brassicaceae	1	0
Fabaceae	1	0
Poaceae	1	0
Solanaceae	1	0

Table 3.3 Plant species which supported feeding, oviposition and development of *P. maculiventris* to adulthood during no-choice tests.

Plant species	Leaf feeding damage*	No. of egg batches/plant	No. of adults emerged	
			Range	Mean (± SE)
T. diversifolia**	**3**[a]	**9.0 ± 0.58**[a]	**234-270**	**234.67 ± 20.21**[a]
X. strumarium	1.33 ± 0.33[c]	5.67 ± 0.33[b]	0	0
H. annuus (Agsun 5278 k2)	2.33 ± 0.33[ab]	5.0 ± 0.58[bc]	22-26	23.33 ± 1.33[b]
H. annuus (Agsun 8251 k3)	2.67 ± 0.33[ab]	4.0 ± 0.58[c]	5-16	7.0 ± 4.73[b]
H. annuus (Agsun 8251 k2)	2.33 ± 0.33[ab]	5.0 ± 0.58[bc]	21-23	20.0 ± 1.45[b]
H. annuus (Agsun 8251 kia 53)	2[bc]	3.67 ± 0.33[c]	0	0
H. annuus (Agsun 5174 cl k3)	2[bc]	1.33 ± 0.33[d]	0	0

*Feeding damage ranged from 0 to 3 where 0 = no feeding; 1 = exploratory feeding; 2 = minor feeding and; 3 = normal feeding. Means with the same letter did not differ significantly ($P > 0.05$) and all zero scores (0) were not analysed statistically. **Control or target plant species.

3.2 Adult feeding and oviposition during multi-choice tests

During multi-choice tests, oviposition and survival of *P. maculiventris* was only recorded on the target weed *T. diversifolia* (Tables 3.4 and 3.5). Although *X. strumarium* and some cultivars of *H. annuus* displayed minor damage, no oviposition was recorded on these plants (Table 3.4). Furthermore, the levels of feeding damage were significantly higher ($\chi^2 = 9.82$; $P = 0.02$) on the target weed *T. diversifolia* than on any of the test plants (Table 3.4). Among the five cultivars of *H. annuus* that were tested, only three (Agsun_8251 kia 53, Agsun 5174 cl k3 and Agsun 5278 k2) were fed on during adult-choice tests (Table 3.5), with damage always significantly higher on *T. diversifolia* ($\chi^2 = 14.44$; $P = 0.013$).

Table 3.4 Feeding and reproductive performance of *P. maculiventris* during multi-choice tests involving plant species that were attacked during no-choice tests.

Plant species	Leaf feeding damage*	No. of egg batches/plant	No. of adults emerged	
			Range	Mean (± SE)
T. diversifolia**	**2.7 ± 0.33[a]**	**3.67 ± 0.88**	**54-135**	**99 ± 23.81**
T. rotundifolia	0	0	0	0
X. strumarium	0.33 ± 0.24[b]	0	0	0
H. annuus (Agsun 8251 k3)	1.0 ± 0.0[b]	0	0	0

*Feeding damage ranged from 0-3 where, 0= no feeding; 1= exploratory feeding; 2= minor feeding and; 3= normal feeding. Means with the same letter did not differ significantly ($P > 0.05$; Kruskal-Wallis test). Zero scores (0) were not analyzed statistically. **Control or target plant species.

Table 3.5 Feeding and reproductive performance of *P. maculiventris* during multi-choice tests involving sunflower cultivars and the target weed.

Plant species	Leaf feeding damage*	No. of egg batches/plant	No. of adults emerged	
			Range	Mean (± SE)
T. diversifolia**	**3.0 ± 0.0[a]**	**2.33 ± 0.33**	**54-81**	**63.0 ± 9.0**
H. annuus (Agsun 8251 k3)	0	0	0	0
H. annuus (Agsun 8251 k2)	0	0	0	0
H. annuus (Agsun 8251 kia 53)	1.33 ± 0.33[b]	0	0	0
H. annuus (Agsun 5174 cl k3)	1.0 ± 0.58[b]	0	0	0
H. annuus (Agsun 5278 k2)	1.67 ± 0.33[b]	0	0	0

*Feeding damage ranged from 0-3 where, 0= no feeding; 1= exploratory feeding; 2= minor feeding and; 3= normal feeding. Means with the same letter did not differ significantly ($P > 0.05$; Kruskal-Wallis test). Zero scores (0) were not analyzed statistically. **Control or target plant species.

3.3 Adult feeding and oviposition of *P. maculiventris* during paired-choice tests

During paired-choice tests involving *T. diversifolia* and *H. annuus* cultivars, the beetle generally preferred the target weed *T. diversifolia* for feeding and oviposition (Table 3.6). Although feeding on one *H. annuus* cultivar (Agsun 8251 k3) did not differ significantly from that on the target weed ($Z = 2.236$, $P > 0.05$), the remaining cultivars were significantly less preferred for both feeding and oviposition (Table 3.6).

Table 3.6 Feeding and reproductive performance of *P. maculiventris* on susceptible *H. annuus* cultivars, relative to *T. diversifolia*, during paired-choice tests.

Plant species	Leaf feeding damage (mean ± SE)*	No. of egg batches/plant (mean ± SE)
H. annuus (Agsun 5278 k2)	0.67 ± 0.33^{b}	1.33 ± 0.33^{b}
T. diversifolia*	**2.67 ± 0.33^{a}**	**3.67 ± 0.33^{a}**
H. annuus (Agsun 8251 k3)	2.0 ± 0.0^{a}	1.67 ± 0.33^{b}
T. diversifolia*	**2.33 ± 0.33^{a}**	**3.33 ± 0.33^{a}**
H. annuus (Agsun 8251 k2)	0	0
T. diversifolia*	**2.67 ± 0.33**	**5.33 ± 0.33**
H. annuus (Agsun 8251 kia 53)	0	0
T. diversifolia*	**3.0 ± 0.0**	**5.0 ± 0.0**
H. annuus(Agsun 5174 cl k3)	1.0 ± 0.0^{b}	0.33 ± 0.33^{b}
T. diversifolia*	**2.67 ± 0.33^{a}**	**4.67 ± 0.33^{a}**

*Feeding damage ranged from 0-3 where, 0= no feeding; 1= exploratory feeding; 2= minor feeding and; 3= normal feeding. Means with the same letter did not differ significantly ($P > 0.05$; Mann-Whitney tests). Zero scores (0) were not analyzed statistically. **Control or target plant species.

3.4 Comparison of larval survival of *P. maculiventris* between *T. diversifolia* and *H. annuus* cultivars

Survival of *P. maculiventris* to adulthood was generally significantly higher on the target weed *T. diversifolia* than on the sunflower cultivars ($F_{(5, 12)} = 2.71$, $P < 0.05$). Differences in survival were significant in three of the five cultivars relative to *T. diversifolia* (Table 3.7). Survival of *P. maculiventris* on the natural host plant was 93% versus 53 to 67% on the most susceptible *H. annuus* cultivars (Table. 3.7).

Table 3.7 Larval survival of *P. maculiventris* during no-choice tests on susceptible cultivars of *H. annuus* and *T. diversifolia.*

Plant species	Number of adults emerged*	
	Range	Mean (± SE)
T. diversifolia*	**9-10**	**9.33 ± 0.33[a]**
H. annuus (Agsun 5278 k2)	5-7	6.67 ± 0.58[ab]
H. annuus (Agsun 8251k3)	3-7	5.33 ± 1.20[b]
H. annuus (Agsun 8251 k2)	5-6	5.33 ± 0.33[b]
H.annuus (Agsun 8251 kia 53)	4-9	6.00 ± 1.53[ab]
H. annuus (Agsun 5174 cl k3)	0-7	3.33 ± 2.03[b]

*Adult emergence resulting from 10 first-instar larvae. Means with the same letter did not differ significantly ($P > 0.05$; Fisher's LSD test). *Control or target plant species.

3.5 Risk analysis

The risk analysis on *P. maculiventris* was determined by the feeding and reproductive performance of the beetle on the different hosts during the various no-choice and choice tests. The risk of 'spillover' feeding damage (i.e. feeding risk) was low in most non-target species, but was relatively higher (22 to 44 %) in three cultivars of *H. annuus* (Table 3.8). However, the risk of these plants supporting viable populations of the beetle in the field (reproductive risk) was extremely low (<1%) in all cases (Table 3.8).

Table 3.8 Risk analysis on the feeding and reproductive performance of *P. maculiventris* on non-target plant species in the tribe Heliantheae (Asteraceae).

Test plants	Plant preference (R^1)	Food acceptability (R^2)	Feeding risk (R^1 x R^2)	Oviposition preference (R^3)	Larval survival (R^4)	Oviposition potential (R^5)	Reproductive risk (R^3 x R^4 x R^5)
T. diversifolia	1.0	1.0	1.0	1.0	1.0	1.0	1.0
X. strumarium	0.001	0.44	4.4×10^{-3}	0.001	0.001	0.63	6×10^{-6}
H. annuus (Agsun 5278 k2)	0.56	0.78	0.44	0.001	0.10	0.61	6.1×10^{-4}
H. annuus (Agsun 8251 k3)	0.001	0.89	8.9×10^{-3}	0.001	0.03	0.44	1.32×10^{-4}
H. annuus (Agsun 8251 k2)	0.001	0.78	7.8×10^{-3}	0.001	0.11	0.61	6.71×10^{-4}
H. annuus (Agsun 8251 kia 53)	0.44	0.66	0.29	0.001	0.001	0.41	4×10^{-6}
H. annuus (Agsun 5174 cl k3)	0.33	0.66	0.22	0.001	0.001	0.15	2×10^{-6}

4. DISCUSSION

During the various host range tests conducted on *P. maculiventris* under quarantine conditions, the beetle fed, oviposited and developed to adulthood on only two of the 58 plant species tested. *Physonota maculiventris* clearly displayed a high degree of host-specificity considering that 49 of these test plant species were in the family Asteraceae. Even the closest related species, *T. rotundifolia*, was not attacked by the beetle. However, it is common for insects to avoid the closest related species while attacking others in different genera. For example, Simelane (2002) found that *Lantana rugosa* Thunb. (congeneric with *Lantana camara*) was not attacked by *Ophiomyia camara* Spencer (Diptera: Agromyzidae) while the fly attacked a number of *Lippia* species that are related at the family level (Verbenaceae).

The two species (i.e., the natural host *T. diversifolia* and cultivated sunflower *H. annuus*) that were attacked by *P. maculiventris* belong to the same tribe (i.e., Heliantheae) within the family Asteraceae. It is not uncommon for an insect to utilize unnatural hosts during laboratory host-range trials (e.g. Olckers 2000), as cages place restrictions on its natural host searching ability. It is generally accepted that simplistic laboratory-based host-specificity tests effectively estimate the physiological (potential) host range of insects, but tend to overestimate their field (realized) host range. This is because host acceptance or rejection mechanisms are often compromised by the experimental design, enabling the agent to utilize and develop on a wider range of plants than it would under field conditions (e.g. Balciunas *et al*. 1996). Indeed, the narrower host range displayed by *P. maculiventris* during the paired-choice and multi-choice tests suggests that it's ecological or field host range will be narrowed even further. Interestingly, *T. rotundifolia*, the congeneric test plant species, was totally avoided for feeding and oviposition by *P. maculiventris*, strongly suggesting that the beetle is specific to *T. diversifolia* and that feeding and development on sunflower cultivars are likely to be laboratory artefacts.

The probability of the beetle expanding its host range to native asteraceous plant species is also extremely low because multiple aspects of its biology, including host location, adult feeding and larval survival, would need to change simultaneously to facilitate this (e.g. Cullen 1990; Balciunas *et al.* 1996). Indeed, post-release evaluations of specialist weed biocontrol agents have revealed very little evidence of host shifts outside the agents' physiological host range (i.e., the plant species that are utilized by a potential agent during no-choice tests) (Pemberton 2000; van Klinken & Edwards 2002; Louda *et al*. 2003).

Although sunflower (*H. annuus*) is widely grown in Mexico, *P. maculiventris* has never been recorded as a pest of this crop (Knodel *et al*. 2010). However, a congener of *P. maculiventris*, the sunflower tortoise beetle *Physonota helianthi* Boheman 1854 (Coleoptera: Chrysomelidae: Cassidinae), is a well-known pest of sunflower in the United States and parts of Canada (Campbell *et al*. 1989). The distribution of *P. helianthi* is confined to North America and does not extend to Mexico. Other *Physonota* species that have been used successfully as weed biocontrol agents include *Physonota alutacea* Boheman 1854 and *Physonota arizonae* Boheman 1854, which were released against wild olive *Cordia macrostachya* (Jacq.) Roem.Schult (Boraginaceae) in Canada and Mauritius (Simmonds 1949) and against ragweed *Ambrosia ambrosioides* (Cav.) Payne (Asterales: Asteraceae) in the United States (Manuel & Eloy 2003), respectively. Since their introduction as weed biocontrol agents, neither *P. alutacea* nor *P. arizonae* have extended their host ranges beyond their target weed species in their introduced ranges (Manuel & Eloy 2003).

In the unlikely event that some sunflower cultivars are colonized by *P. maculiventris* in South Africa, following 'spillover' from nearby *T. diversifolia* populations, it is possible that temporary feeding could occur. However, the beetle is unlikely to sustain itself on sunflower in the absence of its natural host because sunflower is largely grown in the inland highveld region of South Africa (Department of Agriculture, Fisheries & Forestry 2010) while *T. diversifolia* is prevalent in the lowveld and humid eastern coastal regions of South Africa (Henderson 2001; see Fig. 1.1). Since there is virtually no overlap between sunflower cultivations and *T. diversifolia* infestations, the risk possed by *P. maculiventris* to this crop is minimal. Risk assessments have also shown that the probability of the beetle establishing viable populations on sunflower, or any other non-target plant species, is extremely low (<1%).

The results presented in this and the previous chapter suggest that *P. maculiventris* will not only be safe for release against *T. diversifolia* but will also be highly prolific in building up populations and inflicting damage on the weed. Consequently, it is concluded that this beetle is suitable for release as a biological control agent of *T. diversifolia* in South Africa and neighbouring countries.

CHAPTER 4

Potential impact of *Physonota maculiventris* Boheman (Coleoptera: Chrysomelidae: Cassidinae) on *Tithonia diversifolia* (Hemsl.) A. Gray (Asteraceae) and the prediction of its distribution range in South Africa

ABSTRACT

Weed biological control programmes are focused on locating and selecting the most suitable specialist candidate agents, as well predicting their impact and distribution in the introduced range. As part of this programme, a leaf-feeding tortoise beetle, *Physonota maculiventris* (Coleoptera: Chrysomelidae: Cassidinae), was selected as a promising candidate agent for the aggressive Mexican sunflower *Tithonia diversifolia* (Hemsl.) A. Gray (Asteraceae) in South Africa. The potential effectiveness of the tortoise beetle was assessed on the ability of low and high population densities to negatively affect the growth and biomass production of the weed. Its potential distribution in areas invaded by *T. diversifolia* in South Africa was estimated using the climate-matching programme CLIMEX. Severe foliar damage by the adults and immature stages at low population densities caused significant reductions of 57.8% and 42.6% in the above-ground (i.e., shoots, leaves and stems) and below-ground (roots) biomass of the plant, respectively. At high population densities, above- and below-ground plant biomass was reduced by 57% and 51%, respectively. CLIMEX predicted that *P. maculiventris* is likely to establish widely in the areas invaded by *T. diversifolia* in South Africa as well as in neighbouring countries. These findings suggest that *P. maculiventris* could be very effective in suppressing the growth of *T. diversifolia* over a wide range in South Africa.

Key words: Agent impact, CLIMEX, Distribution range, Mexican sunflower, Weed biocontrol.

1. INTRODUCTION

Studies on plant demography, plant-insect interactions, and the potential impact and distribution of prioritized agents are all useful during the process of selecting effective candidate biocontrol agents (Dhileepan *et al*. 2005). Evaluations of the potential impact and distribution of candidate agents on their target weeds in the introduced range are crucial in reducing the risk of releasing ineffective agents (Conrad & Dhileepan 2007). In weed biocontrol programmes, however, more effort is often placed on locating specialist herbivores in their native ranges and determining their safety through host-specificity studies in quarantine, under the assumption that host-specific agents will control their target weeds when released (Myers 1985; McFadyen 2003; Conrad & Dhileepan 2007). However, a successful biological control programme depends largely on the ability of specialist herbivores to spread widely and cause significant negative impacts on the population densities of their target weeds.

To predict the efficacy of candidate biocontrol agents, it is important that pre-release studies are undertaken to ascertain their ability to cause significant damage to certain parts of the host plant such as leaves, stems, roots and flowers (McClay & Balcianus 2005; Conrad & Dhileepan 2007). Success also depends on their ability to spread widely over the distribution range of the target weed, and these predictions should also be made prior to the release of the agents. Predictions of agent distribution are generally determined by the climate-matching programme CLIMEX, which compares the climatic conditions in the insect's native range with that in its introduced range, and generates maps that highlight areas where the insect agent is likely to establish and proliferate (Spafford & Briese 2003). However, despite their importance, these types of pre-release studies are not routinely undertaken by biocontrol practitioners because of aspects like limited quarantine space, time constraints and pressure from funding agencies to release agents and thereby demonstrate progress (e.g. McFadyen 2003; Conrad & Dhileepan 2007).

The invasiveness of the Mexican sunflower *Tithonia diversifolia* (Hemsl.) A. Gray (Asteraceae) in South Africa has necessitated the release of suitable biological control agents. Based on surveys conducted in Mexico from 2010 to 2012, the leaf-feeding tortoise beetle *Physonota maculiventris* (Coleoptera: Chrysomelidae: Cassidinae) was studied in quarantine to determine its suitability for release against *T. diversifolia* in South Africa (see Chapters 2 and 3). It was anticipated that the beetle could greatly impact on the weed's productivity by

directly reducing growth and indirectly reducing reproduction through a reduction of the plant's photosynthetic capacity. Biological control studies have shown that defoliating insects can be effective in reducing weed infestations (e.g. Raghu *et al*. 2006) and should therefore be considered as candidate agents.

To predict the effectiveness of *P. maculiventris* in controlling *T. diversifolia*, this study was conducted to assess the effect of low and high population densities of the beetle on the biomass production and growth of leaves, stems and shoots of *T. diversifolia.* CLIMEX was also used to provide an estimate of the potential distribution of *P. maculiventris* within South African biomes that are invaded by *T. diversifolia*.

2. MATERIALS AND METHODS

2.1. Host plants

Tithonia diversifolia seedlings were established from seeds collected from field sites in KwaZulu-Natal and Mpumalanga provinces. Seeds were sown in a mixture of red soil, sand, vermiculite and compost at a ratio of 1:2:1:1 in 10-litre pots. Individual seedlings were then propagated in 10-litre pots and used in the different experiments (see below) that were conducted under quarantine conditions with daily fluctuating temperatures of 22 to 32^{o}C. The plants were watered three times a week.

2.2. Impact of *P. maculiventris* on plant height, stem diameter and shoot growth

The effect of feeding damage by *P. maculiventris* was assessed in order to predict its effectiveness in controlling *T. diversifolia*. Twelve established seedlings with the same plant size parameters were selected. To ensure that the plants were of uniform sizes, four plants were randomly selected among the test plants at the beginning of the experiment, and their stem height, numbers of shoots and leaves, and root lengths were measured and compared. Based on these results, the measurements of the various parameters were almost the same [Leaves: $F_{(2, 9)} = 0.0006$, $P = 0.999$; Root length: $F_{(2, 9)} = 0.0027$, $P = 0.997$; Number of shoots: $F_{(2, 9)} = 0.2143$, $P = 0.811$; stem height: $F_{(2, 9)} = 0.687$, $P = 0.9341$]. Seedlings were used instead of fully grown plants to ensure similar-sized plants and because fully grown plants become too large for the cages.

The selected plants were divided into three groups (treatments), each of which consisted of four plants (replicates) that were confined in separate cages (55 x 55 x 75 cm). The first treatment was confined with a low population density of beetles (i.e., two pairs of adults). The second treatment was confined with a high population density (four pairs of adults), and the third treatment was confined without beetles (control). In each experimental treatment, the beetles were exposed to the plants for two weeks, during which oviposition and larval hatching occurred. From the newly-emerged larvae (F_1), 110 and 50 were confined on the same plants for another six weeks as part of the high and low population density treatments, respectively. Plants in all treatments were watered three times a week. The numbers of emerging adults (F_1) that arose from each experimental treatment were recorded. The experiments were terminated after eight weeks, after which plant parameters (i.e., leaf and shoot numbers, stem diameters and stem heights) were measured and compared between

treatments. The percentage survival of the F_1 larvae at the low and high population density treatments was also determined. The effect of beetle population density on the different plant parameters were tested with one-way ANOVA and where significant differences were present, the means were compared with Fisher's LSD tests.

2.3 Effect of *P. maculiventris* on biomass production of above- and below-ground plant components

The impact of *P. maculiventris* on the biomass of *T. diversifolia* following the different treatments was determined at the termination of the above experiments (section 2.2). Each of the four plants in each treatment was separated into roots, shoots, stems and leaves, and then oven-dried at 60^{o}C for 72 hours. The dry masses of plant shoots, stems and leaves in each treatment were measured separately, and later combined to determine the above-ground biomass. The root system in each treatment comprised the below-ground biomass. The means of the above- and below-ground biomass of the two experimental treatments and the controls were compared with one-way ANOVA and Fisher's LSD tests, to determine the effect of *P. maculiventris* population densities on plant biomass.

2.4 Survival of *P. maculiventris* at low and high population density treatments

Populations of 50 and 110 F_1 first-instar larvae that comprised the low and high density treatments were confined on the plants until pupation (see section 2.2). Percentage survival of these larvae to adulthood was determined and compared between the low and high population density treatments using *t*-tests.

2.5 Potential distribution of *P. maculiventris* in South Africa

The distribution of *P. maculiventris* in South Africa was predicted by the CLIMEX programme, based on the average temperatures found in the beetle's native range (i.e., around Comitan City, Mexico). Using comparisons with mean temperatures in Africa, a map, showing the regions in Africa that are unsuitable, suitable or highly suitable for the establishment of *P. maculiventris* was generated by the CLIMEX programme.

3. RESULTS

3.1. Impact of *P. maculiventris* on plant growth

3.1.1 Plant height

Feeding damage by *P. maculiventris* at both low and high population densities had a significant negative effect on plant height ($F_{2, 9}$ = 45.051, P = 0.001). By the end of the experiment, the mean plant height was reduced by 33% and 45.2% in the low and high population density treatments, respectively (Fig. 4.1). Plant height was also significantly lower in the high density treatment than in the low density treatment.

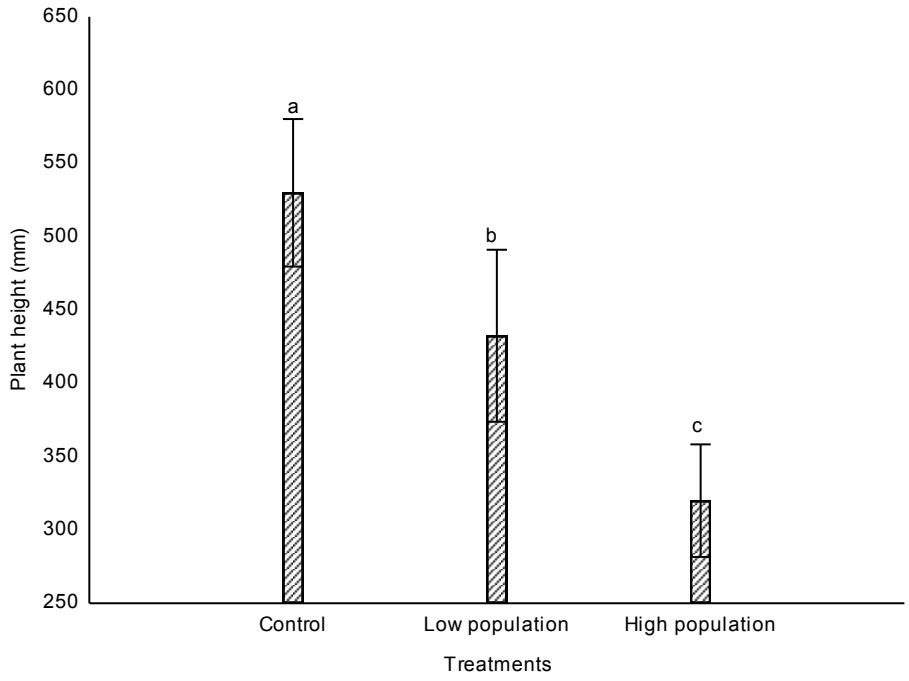

Fig. 4.1 Impact of low and high population densities of *P. maculiventris* on plant height (mean ± SE) of *T. diversifolia*. Means with different letters are significantly different (P < 0.05).

3.1.2 Stem diameter

The radial growth of plants exposed to low and high densities of *P. maculiventris* were reduced, but not significantly, compared with those of the control plants ($F_{2, 9}$ = 8.0224, P = 0.08). At the end of the experiment, stem diameter was reduced by 17.1% and 14.6% in the low and high population density treatments, respectively (Fig. 4.2).

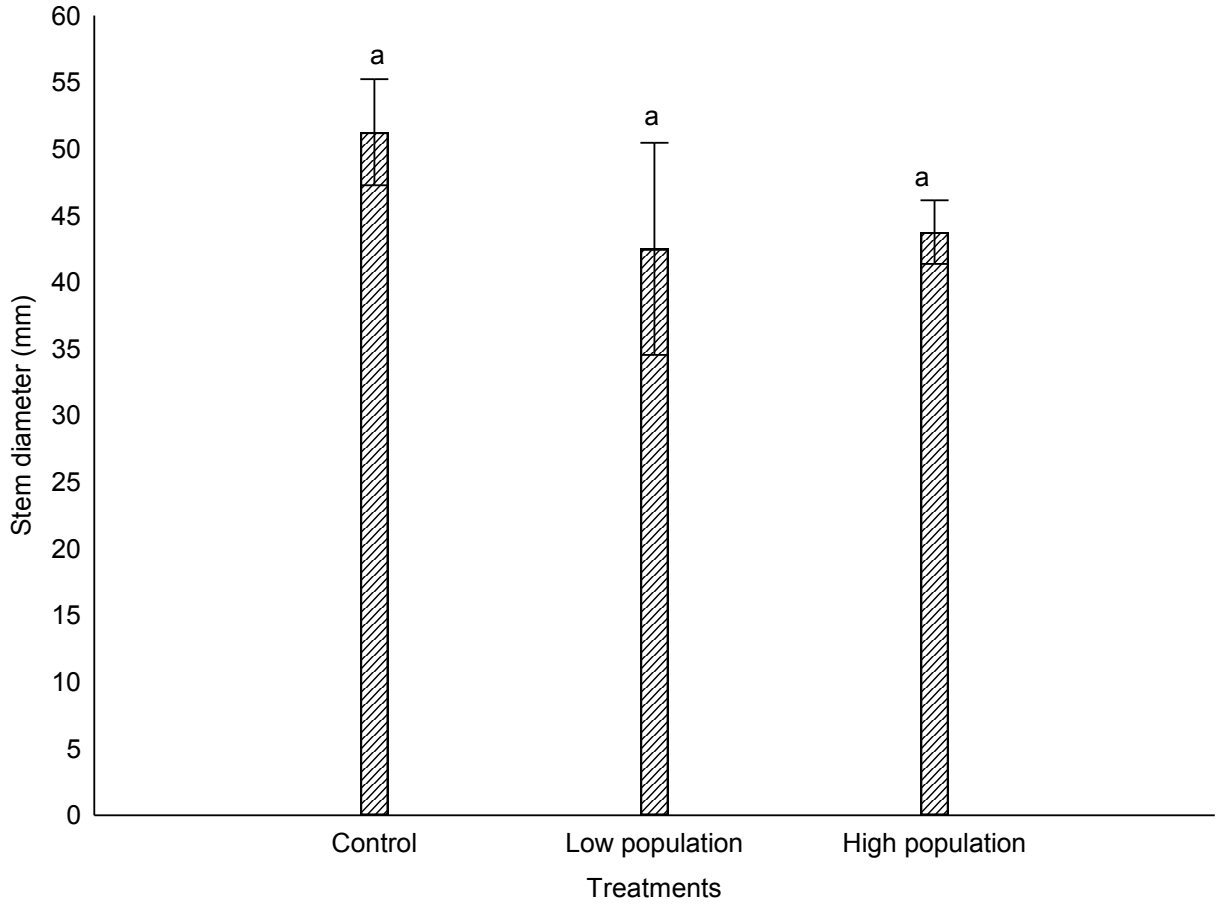

Fig. 4.2 Impact of low and high population densities of *P. maculiventris* on stem diameter (mean ± SE) of *T. diversifolia*. Means with the same letter are not significantly different ($P > 0.05$).

3.1.3 Number of shoots

Herbivory by *P. maculiventris*, at both low and high population densities, had a negative effect on shoot production (Fig. 4.3). After an eight-week period, shoot numbers were significantly reduced at both densities of *P. maculiventris* ($F_{2, 9} = 9.7500$, $P = 0.006$), although the number of shoots produced did not differ significantly between the low and high treatments (Fig. 4.3). After the eight-week exposure period, shoot numbers were reduced by 23.8% and 38% at the low and high population densities, respectively.

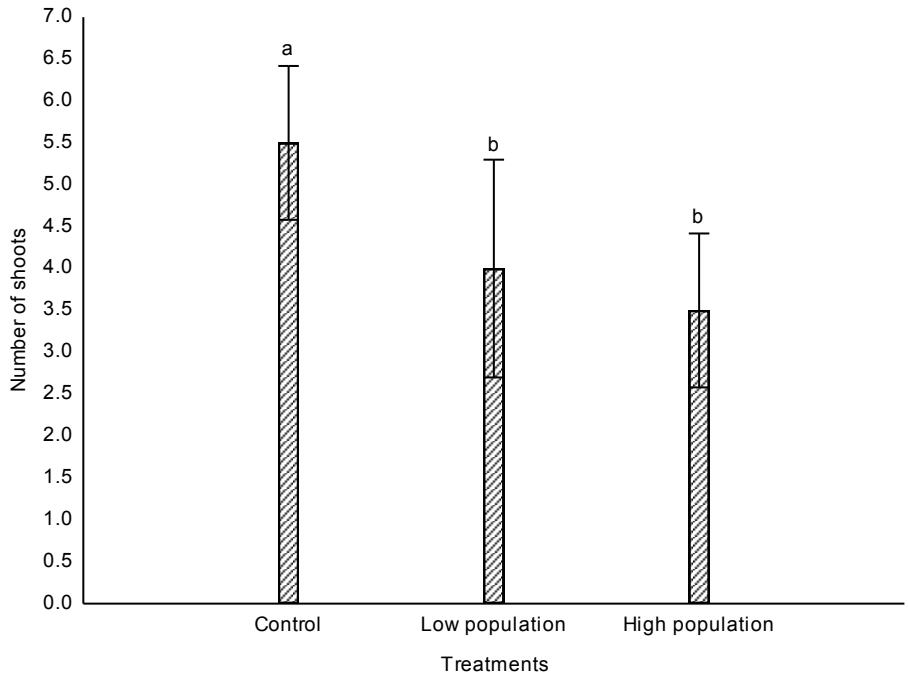

Fig. 4.3 Impact of low and high population densities of *P. maculiventris* on the number of shoots (mean ± SE) produced by *T. diversifolia* plants. Means with different letters are significantly different ($P < 0.05$).

3.1.4 Numbers of leaves

In the control plants that were free of herbivory, the number of leaves per plant increased over the eight-week period (Fig. 4.4). In contrast, herbivory by *P. maculiventris* significantly reduced the mean numbers of leaves ($F_{2,9}$ = 31.603, P =0.00009), with significantly fewer leaves on plants exposed to high beetle densities than on those exposed to low beetle densities (Fig. 4.4). At the end of the eight-week exposure period, leaf numbers were reduced by 86% in the high beetle density treatments versus 44% in the low beetle density treatments.

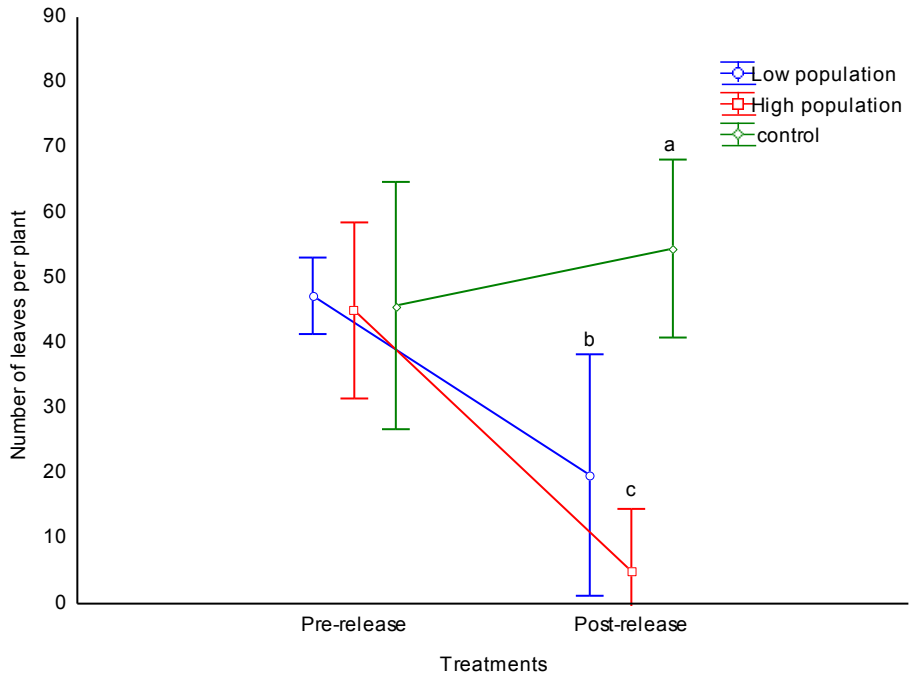

Fig. 4.4 Impact of low and high population densities of *P. maculiventris* on the number of leaves (mean ± SE) on *T. diversifolia* plants. Post-release means with different letters are significantly different ($P < 0.05$).

3.2. Effect of *P. maculiventris* on biomass production of above- and below-ground plant components

With the exception of the shoots, the biomass of all plant components (Fig. 4.5) was significantly reduced by the beetle at both low and high population density levels (leaf biomass: $F_{2, 9} = 27.54$, $P = 0.002$; stem biomass: $F_{2, 9} = 9.65$, $P = 0.006$; shoot biomass: $F_{2, 9} = 0.89$, $P = 0.505$; root biomass: $F_{2, 9} = 5.43$, $P = 0.028$). At the end of the experiment, the above-ground biomass of plants (i.e., stems, shoots and leaves) exposed to low and high population densities of the beetle were substantially reduced by 57.8% in both cases. The low and high population density treatments also substantially reduced the plants' below-ground biomass by 44.6% and 51.6%, respectively, during the same period (Fig. 4.5). With the exception of leaf biomass, the impact of *P. maculiventris* herbivory on plant biomass was independent of beetle density, as the differences between the low and high density treatments were not significant for the different plant components (Fig. 4.5).

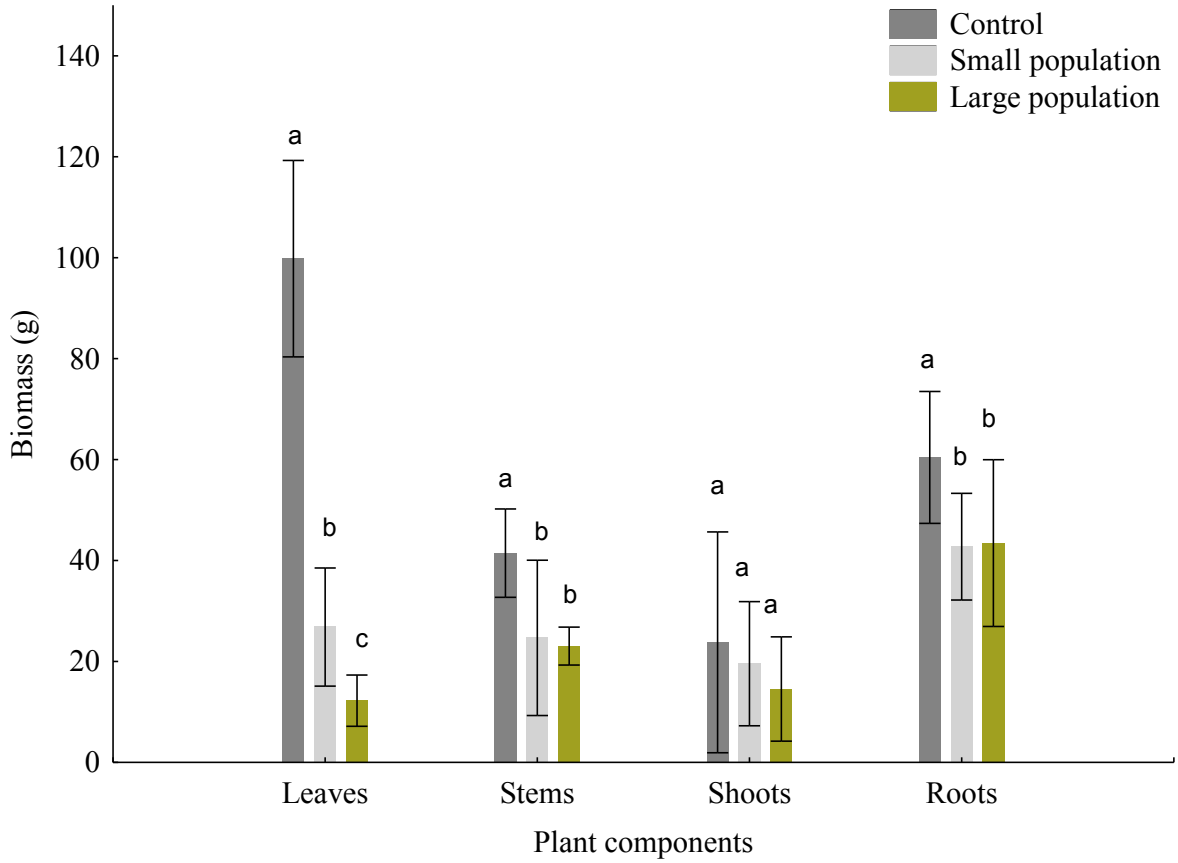

Fig. 4.5 Impact of small and large populations of *P. maculiventris* on the biomass of the different plant components (mean ± SE). Means with different letters are significantly different ($P < 0.05$).

3.3. Survival of *P. maculiventris* larvae at low and high density treatments

The population density of *P. maculiventris* larvae had a significant effect on their ability to survive to adulthood, indicating the effect of intra-specific competion ($t = -2.7124$; $df = 6$; $P = 0.034$). The number of F_1 *P. maculiventris* larvae that survived to adulthood during the experiment period was 28.0 ± 5.8 (mean ± SE) out of 50 in the low density treatment versus 40.3 ± 7.43 out of 110 in the high density treatment, which amounted to 56% and 36.6% larval survival in low and high density treatments, respectively.

3.4. Potential distribution of *P. maculiventris*

CLIMEX predictions that were based on broad temperature comparisons between the native and introduced ranges of *P. maculiventris* suggested that the beetle should be able to establish and spread throughout the current distribution range of *T. diversifolia* in southern

Africa (Fig. 4.6). Much of the range invaded by *T. diversifolia* in South Africa (see Fig. 1.1) included areas that ranged from suitable to highly suitable for the survival of *P. maculiventris*.

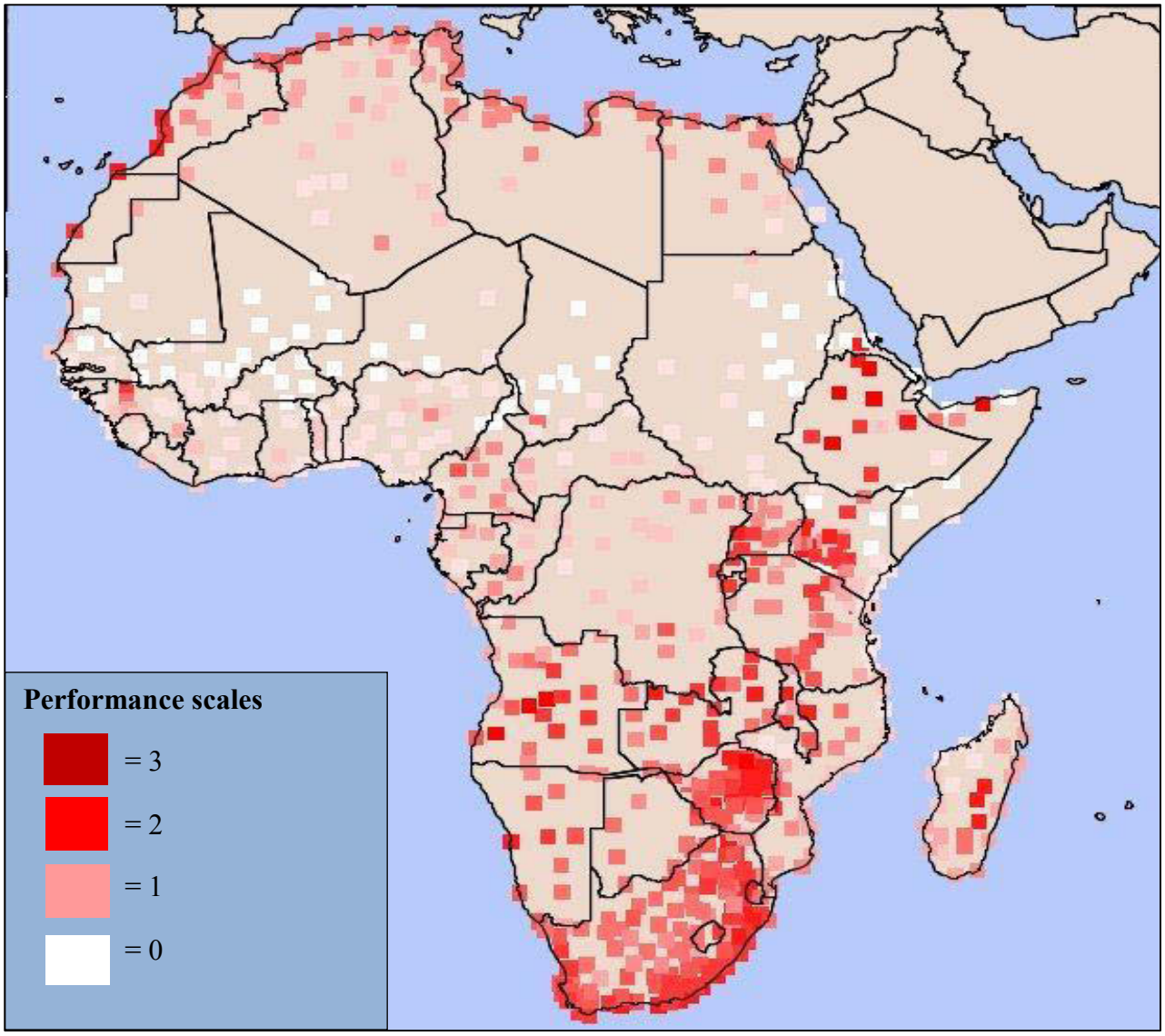

Fig. 4.6 Predicted distribution of *P. maculiventris* in Africa using CLIMEX. Predictions were based on average temperatures and rainfall in its native range. Performance scales: 0 = unsuitable, 1 = marginally suitable, 2 = suitable and 3 = highly suitable areas.

4.4. DISCUSSION

The results of this study have suggested that *P. maculiventris* has considerable potential as a biological control agent for *T. diversifolia*. Both larval and adult feeding damage significantly reduced the growth of *T. diversifolia* at both low and high beetle densities under quarantine conditions.

The larval stages of *P. maculiventris* feed gregariously during their development from first instars to pupation, causing extensive defoliation of *T. diversifolia* plants. The beetle is highly prolific, with each female laying 5-6 egg batches during its lifetime, and each batch containing around 33 eggs (Chapter 2). Multiple generations (i.e. four to five generations per year under laboratory conditions) coupled with high egg hatch rates and larval survival (Chapter 2) should result in rapid population increases, and this is likely to increase herbivore pressure on the weed over time. Extensive feeding damage by *P. maculiventris* appears to be effective in suppressing the growth and biomass of *T. diversifolia*, and this may greatly limit the densification of weed infestations in the introduced range. These findings are consistent with the results of herbivory trials carried out on *Macfadyena unguis-cati* (Bignoniaceae) in Australia (Raghu *et al*. 2006; Conrad & Dhileepan 2007). The latter studies suggested that insect herbivory studies, either through actual or simulated herbivory, should form part of the agent selection process, as has also been demonstrated in other weed biological control programmes (e.g. Lehtila & Boalt 2004; Schooler *et al*. 2006). McClay & Baciunas (2005) suggested that the use of agents that are insufficiently damaging to their targets, even at high densities, is one of the causes of failure that can be avoided by pre-release efficacy assessments.

Furthermore, since *P. maculiventris* significantly reduces leaf and shoot production, it may indirectly suppress flowering and seed production, thus reducing the weed's reproductive output and its invasive potential (Simelane & Phenye 2005). A similar tortoise beetle, *Gratiana boliviana* Spaeth (Coleoptera: Chrysomelidae), released on tropical soda apple, *Solanum viarum* Dunal (Solanaceae), in Florida (USA), established and dispersed widely, causing extensive defoliation that reduced flower production and resulted in the replacement of *S. viarum* infestations with native plant species within two years of its release (Medal & Cuda 2010). Cumulative herbivory by a leaf-feeding weevil, *Oxyops vitiosa* (Pascoe) (Curculionidae), resulted in a 94.5% defoliation of its host, *Melaleuca quinquenervia* (Cav.) S.T. Blake (Myrtaceae), in the USA, with damaged trees sustaining a

significant decline in reproductive output (Pratt *et al*. 2009). It is therefore likely that intense foliar feeding by adults and larvae of *P. maculiventris* will also suppress the reproductive capacity and invasion potential of *T. diversifolia*, which could result in a long-term suppression of recruitment, spread and possibly abundance of the weed. However, it should be noted that since these trials were carried out on smaller plants (initiated at seedling stage), the results may not necessarily apply to large fully grown planst which may be more resilient to damage.

Although the results demonstrated that both low and high population densities of *P. maculiventris* caused similar levels of plant damage, the beetles were unable to disperse from overexploited plants as they were confined in cages, thus resulting in subsequent larval mortality caused by overcrowding. However, such intra-specific competition will mostly be prevented under field conditions as the beetles will be able to disperse to unexploited plants nearby and to adjacent areas that are invaded by the weed.

The climate-matching programme CLIMEX has predicted a wide distribution for *P. maculiventris* that covers most of the present range of *T. diversifolia* in South Africa, extending to other neighbouring southern African countries. Based on this prediction, the beetle seems likely to proliferate in all areas invaded by *T. diversifolia* in South Africa, particularly along the humid eastern coastal region where *T. diversifolia* is abundant. With abundant food resources, and a temperature range of 15–32°C, the lowveld and eastern coastal regions of South Africa appear to be a good match with the native region of *P. maculiventris* in Mexico. However, surveys conducted so far in Mexico (D.O. Simelane pers. comm. 2012; 2013; 2014; Mphephu *et al*. 2014a) have revealed that *P. maculiventris* is abundant and somewhat localized around the city of Comitan in the south-eastern part of Mexico. These observations may suggest that *P. maculiventris* could struggle to adapt to varying environments and that, despite the CLIMEX predications, its spread and distribution may actually be limited in South Africa. However, extensive surveys to ascertain the geographic distribution of the beetle in Mexico are planned for the future in order to resolve this uncertainty.

In summary, the results of this study suggest that *P. maculiventris* should be highly prolific and damaging on *T. diversifolia* while exhibiting a narrow host range (Chapter 3) and a wide potential distribution in southern Africa. These results thus support the contention that

the beetle should be considered for release as a biocontrol agent of *T. diversifolia* in South Africa and other neighbouring countries.

CHAPTER 5

GENERAL DISCUSSION AND CONCLUSIONS

5.1 Biological attributes of *P. maculiventris* and its prospects as a biocontrol agent

The combination of an insect's survivorship, developmental rate and fecundity is referred to its intrinsic rate of increase (*r*), and is an expression of fitness (Odum 1959). Intrinsic rate of increase is a key component in determining the potential effectiveness of a weed biological control agent (Gassmann 1996). The short life cycle coupled with a high reproductive output and good defence mechanism against potential natural enemies are some of the important biological attributes of *P. maculiventris* which could influence its fitness and success as a biocontrol agent. Indeed, several species of tortoise beetle have been released against various weed species elsewhere in the world with varying degrees of success (see Appendix 1). With some exceptions, tortoise beetles appear to establish easily and some have inflicted substantial levels of damage on their target plants (Appendix 1).

Although it is uncertain that high levels of herbivory by *P. maculiventris* will bring about control of *T. diversifolia*, the conventional wisdom is that the probability of success will be higher if the herbivore reaches high population densities. Studies by Nakamura *et al.* (1989) and Cappuccino (2000) demonstrated that high population densities of insect agents are generally essential for success in weed biocontrol. In a camparative study on the intrinsic rates of increase of two congeneric agents [i.e., *Cyrtobagous singularis* Calder & Sands and *C. salviniae* Calder & Sands (Coleoptera: Curculionidae)] on *Salvinia molesta* D. Mitch. (Salviniaceae), *C. salviniae* laid seven times more eggs than *C. singularis* under similar laboratory conditions and attained higher population levels and feeding impact in the field (Sands *et al.*1986). Provided that egg and pupal mortality are not exacerbated by parasitism and predation, as occured in other biocontrol programmes (e.g. Hill & Hulley 1995b; Olckers & Hulley 1995; Lockett & Palmer 2003; King *et al*. 2011), the high fecundity (200 eggs per female) of *P. maculiventris* should enable it to maintain high population densities in the field.

Clumped distributions of insect herbivore attack, resulting from gregarious feeding behaviour, are known to occur in some successful cases of weed biocontrol (Lawton 1985) and a wave-like process of defoliation was described for *Zygogramma suturalis* Stål (Coleoptera: Chrysomelidae) on *Ambrosia artemisiifolia* L. (Asteraceae) (Kovalev 1989). A similar form of gregarious feeding behaviour is displayed by the first three larval instars of *P.*

maculiventris and this combined feeding effort results in complete defoliation of the plant. In contrast, the fourth instars disperse to feed in a more solitary manner and later pupate on dead leaves. Other successful cases of weed biocontrol involving insect species with gregarious feeding behaviour include *Pareuchaetes pseudoinsulata* Rego Barros (Lepidoptera: Arctiidae) on *Chromoleana odorata* (L.) King and Robinson (Asteraceae) in Asian countries (Muniappan *et al*. 1988), *Leptinotarsa texana* Schaeffer (Coleoptera: Chrysomelidae) on *Solanum elaeagnifolium* Cav. (Solanaceae) in South Africa (Hoffmann *et al.* 1998) and *Zygogramma bicolorata* Pallister (Coleoptera: Chrysomelidae) on *Parthenium hysterophorus* L. (Asteraceae) in Australia (Dhileepan *et al*. 2000).

5.2 Safety of *P. maculiventris* as a biocontrol agent

The primary goal of host-specificity tests is to ensure that any proposed insect agent does not have an unacceptable impact, either ecological or economic, on the environment into which it is being introduced (Briese 2005). Despite some concerns regarding possible negative effects on non-target plants (Louda *et al*. 1997), classical weed biocontrol remains the most sustainable, cost-effective, environmentally friendly, and internationally accepted method of managing invasive alien plants (Sheppard *et al*. 2005). Through host-specificity testing, the risk of releasing agents that may become pests on plants of economic or environmental importance is, by and large, eliminated (Wapshere 1974; Sands & Van Driesche 2000).

Host-specificity tests conducted in the current study have demonstrated that *P. maculiventris* is safe for release against *T. diversifolia* in South Africa. When subjected to 58 plant species from seven families, the beetle displayed a very restricted host range, developing successfully on only the target weed *T. diversifolia* and on three of the five cultivars of sunflower (*H. annuus*) that were tested. However, the feeding and reproductive performance of *P. maculiventris* on these 'susceptible' *H. annuus* cultivars was very poor, with the highest adult emergence (i.e., only 9.8% of that recorded on *T. diversifolia*) recorded on one cultivar (Agsun 5278k2) during no-choice tests. Bearing in mind that laboratory studies are well known to overestimate the host range of potential weed biocontrol agents (e.g. Balciunas *et al.* 1996; Briese 2005), the host range of *P. maculiventris* is highly likely to be restricted to the target weed in the field. This was also true of the tortoise beetle *Gratiana boliviana* Spaeth (Chrysomelidae: Cassidinae) which did not attack unsprayed cultivations of eggplant (*Solanum melongena* L. (Solanaceae)) that were growing within or near patches of

its natural host *Solanum viarum* L. (Solanaceae), despite having been reared sucessfully on eggplant under laboratory conditions (Gandolfo *et al.* 2000). Similar arguments were put forward by Hill & Hulley (1995a) and Olckers (2000) for attacks by biocontrol agents of *Solanum* weeds on cultivated eggplant, which was able to support limited development of these agents during host-specificity tests. Hasan & Delfosse (1995) justified the release of a rust fungus on *Heliotropium europaeum* L. (Boraginaceae) in Australia, despite some infection of native *Heliotropium* species. Similarly, Simelane (2002) defended the release of *Ophiomya camarae* Spencer (Diptera: Agromyzidae) on *Lantana camara* L. (Verbenaceae) in South Africa, despite feeding on some native *Lippia* species. The above examples all culminated in the release of the agents with no reports of significant adverse effects to date. The results and arguments put forward in Chapter 3 strongly suggest that non-target plants are unlikely to be attacked by *P. maculiventris* under field conditions.

The results of the current study are consistent with those of several other studies in which tortoise beetles have been reported to be highly specific to their host plants (Maw 1984; Bain & Kay 1989; Kay 1990; Hill & Hulley 1995a; Gandolfo *et al.* 2000; Kok 2001; Manuel & Elroy 2003; Ghorbanali *et al*. 2013). Although the congeneric wild olive tortoise beetle, *Physonota alutacea* Boheman (Chrysomelidae: Cassidinae), failed to establish in Mauritius, apparently due to ant predation (Winston *et al.* 2014), it established and remained confined to the target weed *Cordia macrostachya* (Jacq.) Roem. & Schult. (Boraginaceae) in the West Indies (Simmonds 1949). *Physonota maculiventris*, with biological attributes very similar to those of *P. alutacea*, is thus expected to be confined to *T. diversifolia* in South Africa.

5.3 Prediction of impact and distribution range of *P. maculiventris* in South Africa

Pre-release impact evaluations (e.g. Raghu *et al*. 2006; Conrad & Dhileepan 2007) are often carried out in laboratories and glasshouses, or in the field in the weed's native range, to predict the impact of candidate agents on individual plants or populations and to assist in selection of the most promising agents. The results of this impact study, conducted under laboratory conditions, demonstrate that sustained attack by *P. maculiventris* should reduce the vegetative growth of *T. diversifolia* in the field. During these studies, *P. maculiventris* significantly reduced leaf density, shoot formation, stem thickening and biomass accumulation of both subterranean and aerial parts, at both low and high insect density treatments. Often, there were no significant differences in plant growth features between the

low and high insect density treatments, suggesting that even smaller numbers of beetles might be able to exert appreciable herbivore pressure on *P. maculiventris* populations in the field. However, plants growing in the wild are expected to have a greater ability to compensate for defoliation (Kleinjan *et al*. 2004) than the experimental plants which were much smaller and presumably more vulnerable to insect attack. Nonetheless, the reduction of growth in most of the measured plant parameters indicate that *P. maculiventris* is capable of stunting plants which could in turn reduce their competitive ability and reproductive output, thus maintaining weed infestations at lower levels. The leaf-feeding beetle *Zygogramma bicolorata* Pallister (Coleoptera: Chrysomelidae), which is capable of causing 100% defoliation of *Parthenium hysterophorus* L. (Asteraceae) plants in Australia, significantly reduced plant density, biomass, flowering and soil seed banks by 65 to 100% (Dhileepan *et al*. 2000).

Tithonia diversifolia is a fast-growing perennial plant that possesses a number of biological attributes which are the main drivers behind its invasion in South Africa. The plant produces a large number of light-weight seeds which are easily spread by wind over a large area (Muoghalus & Chuba 2005). In addition, *T. diversifolia* seeds can tolerate dry seasons, remaining dormant prior to the induction of germination by rain (Agboola *et al*. 2006). Both seedlings and mature *T. diversifolia* plants are also tolerant of low levels of soil nutrients and fires (Wanjau *et al*. 1998). To curb the invasiveness of *T. diversifolia* in the field, a biological control agent must be capable of reducing not only the growth but also the reproductive capacity of the plant. Although herbivory by *P. maculiventris* could result in stunted plant growth and indirectly reduce the reproductive capacity of *T. diversifolia*, additional agents that directly attack the reproductive parts will probably be required to complement the beetle. The introduction of a flower- or seed-feeding agent, rather than an additional defoliator, is desirable as biocontrol agents with extensive niche overlap could result in competitive interactions (April *et al.* 2011).

The use of multiple agents has proved successful in facilitating the control of asteraceous weeds. For example, successful biocontrol of tansy ragwort *Senecio jacobaea* L. (Asteraceae) in Oregon (USA) was achieved by three agents [i.e., the leaf-feeding cinnabar moth *Tyria jacobaeae* L. (Lepidoptera: Arctiidae), the root-feeding flea beetle *Longitarsus jacobaeae* L. (Coleoptera: Chrysomelidae) and the seed fly *Botanophila seneciella* Meade (Diptera: Anthomyiidae)] that attack three different niches on the plant (Isaacson *et al*. 1996). Field exploration in the native range revealed the existence of an unidentified flower head-feeding moth on *T. diversifolia* (see Table 1.1), and this was introduced into South Africa in

2012 as a candidate agent (D.O. Simelane, pers. comm. 2014). However, the rearing of flower head-attacking insects is difficult as *T. diversifolia* flowers only once per year, and can only do so outside of quarantine. Hence, attempts to rear the unidentified flower head-feeding moth were unsuccessful.

The distribution and occurence of *P. maculiventris*, like any other phytophagous insect, will depend not only on the availability of host plants but also on the prevailing biotic and abiotic conditions in the introduced range. Although predictions by CLIMEX suggest that *P. maculiventris* should establish throughout the regions invaded by *T. diversifolia* in South Africa, the limited distribution of the beetle observed so far in its native Mexico remains a concern (D.O. Simelane, pers. comm. 2014). Surveys in Mexico have revealed that *P. maculiventris* is abundant, but somewhat localized around the city of Comitan in the south-eastern part of Mexico, and very rare in other areas where *T. diversifolia* is prevalent (D.O. Simelane, pers. comm. 2012; 2013; 2014; Mphephu *et al*. 2014a,b). However, this could be an indication that *P. maculiventris* is being kept in check by its natural enemies in the native range. If this is true, then "enemy free space" (see Lawton & Jeffries 1984) in the introduced range should enable populations of *P. maculiventris* to flourish in South Africa and other regions, as predicted by CLIMEX. In the event that *P. maculiventris* displays poor dispersal abilities in South Africa, it will take longer to achieve region-wide impacts on the target weed (e.g. Sullivan & Hosking 1995) and will require the development of specific mass-rearing techniques and release strategies to re-distribute the beetle throughout *T. diversifolia*-invaded regions. It would be advantageous to involve the Natural Resource Programme teams of the Department of Environmental Affairs as well as landowners in the mass-rearing and re-distribution of *P. maculiventris.* Indeed, the involvement of landowners and local community groups in Australia increased the number of release sites for *Lixus cardui* Olivier (Coleoptera: Curculionidae) on *Onopordum acanthium* L. (Asteraceae) by 20-fold over three years (Briese *et al* 1996).

Another concern is the possibility that native parasitoids and predators could influence the efficacy of *P. maculiventris*, particularly because its sedentary life stages (i.e., eggs and pupae) are highly vulnerable. Post-release studies have shown that introduced tortoise beetles have encountered parasitism and predation by native natural enemy species (Olckers and Hulley 1995; King *et al.* 2011). Hill & Hulley (1995b) also reported that about 40% of established biocontrol agents in South Africa were attacked by parasitoids. Also, high levels of predation by ants was reported as the reason for the failure of the congeneric *P.*

alutacea against black sage *Cordia curassavica* (jacq.) Roem. & Schult in Mauritius (Quinn 2009; Winston *et al*. 2014). Parasitism of both egg cases and pupae of *Gratiana spadicea* (Klug) (Chrysomelidae: Cassidinae), an agent of *Solanum sisymbriifolium* Lam. (Solanaceae) in South Africa, has been reported (King *et al*. 2011), although the extent of this interference has not been fully quantified. Although parasitism and predation of *P. maculiventris* are likely to occur, it is uncertain as to whether this will hamper its efficacy in controlling *T. diversifolia* in South Africa. Another tortoise beetle, *Cassida rubiginosa* O.F. Müller (Coleoptera: Chrysomelidae), that was accidentally introduced onto Canada thistle *Cirsium arvense* (L.) Scop. in the USA, is reported to be having a significant impact on the weed, despite being attacked by native predators and parasitoids (Winston *et al*. 2014).

5.4 Conclusions

Based on the biological studies reported here (Chapter 2), it is concluded that *P. maculiventris* has the necessary biological attributes (e.g., short life cycle, high reproductive output and good defence mechanisms against native natural enemies) to be a successful biocontrol agent for *T. diversifolia* in South Africa.The results of the host-specificity tests (Chapter 3) and impact studies (Chapter 4) have also indicated that *P. maculiventris* is adequately host specific and sufficiently damaging to *T. diversifolia*, and poses no risk to non-target plant species that are either native or of commercial value in South Africa. Preliminary climatic matching (Chapter 4) also suggests that *P. maculiventris* should be able to establish throughout the regions invaded by *T. diversifolia* in South Africa. Given these conclusions, it is strongly recommended that permission be granted for the release of this beetle from quarantine, to facilitate the biological control of *T. diversifolia* in South Africa. An application to release *P. maculiventris* into the field is thus being prepared for submission to the relevant South African regulatory authorities. Following releases and establishment of the beetle, it will be important to conduct post-release evaluations to determine whether the predictions made by this study (i.e. host range, impact and distribution) were accurate.

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APPENDIX 1

List of tortoise beetles (Chrysomelidae: Cassidinae) that were released as biological control agents of invasive weeds around the world, with an assessment of the outcomes of the releases.

Weed species	**Tortoise beetle species**	**Countries**	**Outcomes of releases***	**References**
Calystegia sepium (L.) R. Br. (Convolvulaceae)	*Charidotella sexpunctata bicolor* (F.)	Sub-regions of Canada	Established; trivial damage; negligible control.	Julien (1992)
Carduus nutans (L.) (Asteraceae)	*Psylliodes chalcomera* (Illiger)	USA	Not established.	Winston *et al*. (2014)
Cirsium arizonicum (A. Gray) Petr. (Asteraceae)	*Cassida rubiginosa* O.F. Müller	USA, Canada	Established; trivial damage; negligible control.	Winston *et al*. (2014)
Cirsium arvense (L.) Scop. (Asteraceae)	*Cassida rubiginosa* O.F. Müller	USA, Canada	Established; extensive damage.	Winston *et al*. (2014)
Convolvulus arvensis (L.) (Convolvulaceae)	*Chelymorpha cassidea* (F.) *Chirida guttata* (Olivier)	Alberta, Canada Alberta, Canada	Not established. Not established.	Julien (1992) Julien (1992)
Cordia macrostachya (Jacq.) Roem. & Schult. (Boraginaceae)	*Physonota alutacea* Boh.	West Indies	Established; unknown damage and degree of control.	Simmonds (1949)
Ipomoea carnea Jacq. (Convolvulaceae)	*Aspidomorpha miliaris* F.	India	Established; extensive damage (about 84%).	Bhuyan *et al*. (2008)
Macfadyena unguis-cati	*Charidotis auroguttata* (Boh.)	South Africa	Established; trivial damage;	Klein (2011)

(Bignoniaceae)			negligible control.	
Solanum sisymbriifolium Lam. (Solanaceae)	*Gratiana spadicea* (Klug)	South Africa	Established; extensive damage; substantial control	Klein (2011)
Solanum viarum Dun. (Solanaceae)	*Gratiana boliviana* Spaeth	Florida, USA	Established; extensive damage; substantial control.	Medal & Cuda (2010)

*Definitions of key terms: Extensive damage - most leaves attacked, few survive; Trivial damage - few leaves attacked; Unknown damage - no information on the effectiveness of the agent in the literature; Negligible control - unsatisfactory impact of the agent; Substantial control - major impact of the agent.