Pre-release evaluation of absolute spillover impact risk of Physonota maculiventris (Chrysomelidae: Cassidinae) on non-target plant species Helianthus annuus (Asteraceae) and Zea m…

Some of the authors of this publication are also working on these related projects:

Biological control of Invasive Alien Weeds View project

Effect of Helicosporidium sp. (Chlorophyta; Trebouxiophyceae) infection in Cyrtobagous Salviniae Calder and Sands (Coleoptera; Curculionidae) populations introduced onto the invasive Salvinia molesta D.S. Mitchell (Salviniaceae) in South Africa. View project
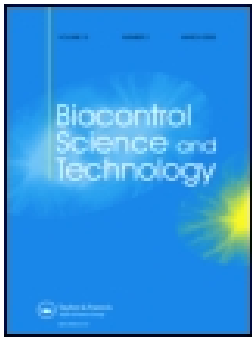
Pre-release evaluation of absolute spillover impact risk of *Physonota maculiventris* (Chrysomelidae: Cassidinae) on non-target plant species *Helianthus annuus* (Asteraceae) and *Zea mays* (Poaceae) in South Africa

Tshililo E. Mphephu & Ludzula Mukwevho

Published online: 18 Apr 2019.

Submit your article to this journal ↗

View Crossmark data ↗

Check for updates

Pre-release evaluation of absolute spillover impact risk of *Physonota maculiventris* (Chrysomelidae: Cassidinae) on non-target plant species *Helianthus annuus* (Asteraceae) and *Zea mays* (Poaceae) in South Africa

Tshililo E. Mphephu[a]* and Ludzula Mukwevho[b]

[a]ARC-Plant Health and Protection, Queenswood, South Africa; [b]School of Biology and Environmental Sciences, University of Mpumalanga, Mbombela, South Africa

ABSTRACT

The tortoise beetle, *Physonota maculiventris* (Coleoptera: Chrysomelidae), a candidate biological control agent of *Tithonia diversifolia* (Hemsl.) A. Gray (Asteraceae) was screened for spillover risks on non-target plant species in South Africa. Studies were conducted to measure the absolute feeding damage and reproductive performance of *P. maculiventris* on non-target plant species, *Helianthus annuus* L. (Asteraceae) and *Zea mays* L. (Poaceae). The influence of spillover on generational build-up performance on the non-target plant species was also investigated. Adult female beetles were switched from *T. diversifolia* at 14 (actively feeding colony) or 24 (gravid colony) days to the non-target species. Likewise, as a backup or control, female beetles were exposed to *H. annuus* in a no-choice situation and switched to *T. diversifolia* and *Z. mays*. Feeding damage, adult longevity and egg production of *P. maculiventris* were significantly lower on *H. annuus*, compared to those metrics on *T. diversifolia*. Gravid *P. maculiventris* adults switched from *T. diversifolia* on the 14th day after emergence laid a few egg batches on the leaf surfaces of *Z. mays*, but no signs of feeding were observed. Furthermore, the population of *P. maculiventris* significantly increased by 11.7 fold (26.8–312.5 adults) between the first (*F1*) and second (*F2*) generations on *T. diversifolia*, while on the non-target, *H. annuus,* it decreased from 6.3 to zero (0). The study concludes that *P. maculiventris* will sustain its populations entirely on the target, *T. diversifolia* population stands associated with or without *H. annuus* and *Z. mays* cultivations at different scales in South Africa.

Introduction

Host range studies in classical biological control are aimed at selecting biological control agents that show minimal risk of feeding damage on non-target plant species (Fowler, Paynter, Dodd, & Groenteman, 2012; Mphephu, Olckers, & Simelane, 2017; Tingle,

CONTACT Tshililo E. Mphephu ✉ mphephu.te@gmail.com 🖂 ARC-Plant Health and Protection, Private Bag X134, Queenswood 0121, South Africa
*Current address: Centre for Biological Control, Department of Zoology and Entomology, Rhodes University, P. O. Box 94, Grahamstown 6140, South Africa.

Cook-Patton, & Agrawal, 2016). Because of these studies, dozens of effective and prolific candidate biological control agents have been released against their prospective target plant species across the world and have not attacked the native non-target plant species (Wheeler, Duncan, & Wright, 2017). Nevertheless, unintended feeding damages on non-target still occur through spillover. For example, a leaf feeding beetle, *Zygogramma bicolorata* Chevrolat *in* Dejean (Coleoptera: Chrysomelidae), a biocontrol agent of *Parthenium hysterophorus* (Asteraceae), had spillover feeding onto commercial sunflower, *H. annuus* cultivations in India (Visalakshy, Jayanth, Ghosh, & Chaudhary, 2008). Based on this example, spillover feeding and reproductive performance tests of candidate biological control agents were recommended by the USA regulatory authorities to form part of the pre-release evaluation of potential biocontrol agents (Tingle et al., 2016). It has been argued that no-choice, paired choice and multi-choice test studies alone cannot predict absolute feeding damage risks that occur as unintended impacts by candidate biological control agents on non-target plants (McClay & Balciunas, 2005; Van Klinken, 2000).

Biological control researchers often conduct post-release assessments on spillover feeding damage by insect agents on non-target plants (Dhileepan, Trevino, & Raghu, 2006; Lake, Smith, Dray, & Pratt, 2015; Tingle et al., 2016), an assessment that should be a fundamental part of pre-release evaluation (Coetzee, Byrne, Hill, & Center, 2009; Louda, Pemberton, Johnson, & Follett, 2003; Simberloff & Stiling, 1996; Wheeler et al., 2017). A spillover test is an instrumental measure used to predict the absolute feeding risk by the candidate biological control agents (Tingle et al., 2016). The spillover test uses the candidate biocontrol agents that previously fed on their primary host plants and confine them with the selected test plant species, either commercial or indigenous. Integration of the choice, no-choice, paired-choice and spillover tests, can accurately predict direct and indirect risks of candidate biological control agents (Hopper, 2001; Center & Hill, 2002; Wheeler et al., 2017). However, the spillover test is rarely incorporated during pre-release evaluation studies across the world (Wheeler et al., 2017), particularly in South Africa where a number of biocontrol programmes are actively involved with the pre-release screening of potential biocontrol agents.

In South Africa, the weedy Mexican sunflower *Tithonia diversifolia* (Hemsl.) A. Gray (Asteraceae) is the more aggressive of the two *Tithonia* species which have been subjected to classical biological control programmes since 2007 (Mphephu et al., 2017; Simelane, Mawela, & Fourie, 2011). A defoliating beetle *Physonota maculiventris* Boheman (Chrysomelidae: Cassidinae) was screened as a candidate agent for *T. diversifolia* using no-choice, choice and paired-choice tests. Based on these tests, *P. maculiventris* was found to be adequately host specific to be released against *T. diversifolia* in South Africa, and an application to release the beetle from quarantine was sought from the relevant regulatory authorities (Mphephu et al., 2017). However, the beetle had caused minor feeding damage and oviposited on non-target species such as sunflower, *Heliathus annuus* L. (Asteraceae) and *Xanthium strumarium* L. (Asteraceae), although only a few adults developed on sunflower and these were not sustained beyond the first generation on any of the non-target plants (Mphephu et al., 2017).

Whilst it is not uncommon for host-specific biocontrol agents to feed and oviposit on non-target plant species when kept in captivity under cage conditions (i.e. no-choice tests), the regulatory authority tasked with the review of the release application for

P. maculiventris was critical of the findings, particularly with regards to the feeding and development on sunflower during no-choice tests. The regulatory authority resolved to call for further public participation as part of the review process of the release application. The regulatory authority specifically suggested the Sunflower Growers Association as the major stakeholder to be informed of the imminent release of *P. maculiventris* by the applicant (ARC-PHP). Although the Sunflower Growers Association has since supported the release of *P. maculiventris* in South Africa, feeding and development of potential agents on non-target crop species under cage conditions will remain a contentious issue. For example, a release application with similar host-specificity results involving *Ceratapion basicorne* (Illiger) (Coleoptera: Apionidae) which attacked a non-target commercial safflower *Carthamus tinctorius* L. (Asteraceae) in the western USA (Cristofaro, De Biase, & Smith, 2013) was denied due to perceived risk to safflower. To resolve this challenge, a field study was conducted in the native range to evaluate the potential risk of feeding and reproductive performances, that is likely spillover, by *C. basicorne* on *C. tinctorius*. The study showed that *C. basicorne* posed no risk to safflower cultivations in the USA (Cristofaro et al., 2013).

While confined in a cage under quarantine conditions, during culture maintenance, *P. maculiventris* exploited all the leaves and stem epidermal layers of its host plant *T. diversifolia*, resulting in some spillover feeding on an unidentified grass species (Poaceae). The grass species had emerged with many unknown herbaceous plant species in the same pot which contained the host plant, *T. diversifolia*. Although the beetle nibbled on grass leaves, its nibbling activities were ephemeral (lasting approximately 7 days) and no egg batch was recorded on the grass species. However, this raised concerns regarding the prospects of *P. maculiventris* spilling over onto non-target species in the Poaceae family in the field. As part of the process to predict the unintended spillover of *P. maculiventris* onto non-target species, a study was conducted to predict the absolute spillover feeding damage on non-target *H. annuus* and *Zea mays* L. (Poaceae) crop species. Because the spillover damage had been observed on the unidentified grass species, its congener *Z. mays* was selected to represent plant species within the Poaceae. The selection of *Z. mays* was also motivated by its importance as a staple crop in South Africa. Although the previous study (Mphephu et al., 2017) had concluded that *P. maculiventris* posed an insignificant risk to non-target *H. annuus*, the absolute impact risk of the beetle on the non-target species after it has fed on *T. diversifolia* leaves was unknown.

Material and methods

Insect colony of *P. maculiventris*

Experimental colonies of *P. maculiventris* were initially collected within the beetle's native range in Comitan City, Mexico (Mphephu et al., 2017; Simelane et al., 2011). The colonies were maintained from 2012 to 2015 on *T. diversifolia* in large cages (55 cm × 55 cm × 75 cm) at the Agricultural Research Council-Plant Health and Protection's (ARC-PHP) quarantine facility, Roodeplaat, South Africa. The quarantine conditions were maintained at a temperature range of 22°C to 32°C, relative humidity at 35% to 70% and a photoperiod of 14 L; 10 D (Mphephu et al., 2017).

Experimental plants source

Tithonia diversifolia plants were collected from roadsides, riparian and mountainous areas to the west of KwaMhlushwa village, Mpumalanga Province. The seeds of *H. annuus* (Agsun 5278K2) were supplied by AGRICOL. The *Z. mays* seedlings were sourced from the ARC-PHP, insect ecology nurseries. *Helianthus annuus* cultivar Agsun 5278K2 was selected for the spillover assessments due to the relatively high level of vulnerability to *P. maculiventris* observed during previous no-choice and choice tests (Mphephu et al., 2017). The seeds were sown in large numbers to produce sufficient numbers of experimental plants at the ARC-PHP, Roodeplaat nursery. Seedlings were transplanted into 2-litre pots with a standard soil mixture of sand, red soil, vermiculite and compost at a ratio of 1:1:1:2, respectively. Plants were maintained in the nursery under 50% shade, and were irrigated twice daily via an automatic irrigation system. Experimental tests were conducted on leaves of all the experimental plant species. Leaves were excised from these plants when required for the tests. These leaves were subsequently inserted with their petioles in the containers that were quarter-filled with a moistened vermiculite. This vermiculite allowed some of the leaf petioles to develop thin roots, and all the leaves remained alive and fresh for a long time, and this further limited interference with the adults, for replacement of leaves replacing. Only the severely damaged leaf materials were replaced with a new and clean leaf material of the respective plant species.

Leaf feeding damage and reproductive performance

To determine unintended spillover feeding damage by *P. maculiventris* on non-target plants, two colonies comprising of 16 newly emerged adult females each, were selected from the colony maintenance cages and released onto *T. diversifolia* (Figure 1). Male beetles were also introduced for mating purposes, and the selection of male beetles followed the ratio of male to female of 1:1.2 that was often produced from a cohort of larvae (Mphephu et al., 2017). The first colony was initially exposed to their natural host *T. diversifolia* for 14 or 24 days. We chose 14- and 24-day exposure periods because an earlier study (Mphephu et al., 2017) indicated that active feeding, mating and oviposition by *P. maculiventris* adults occur during these periods. As a backup, in order to further determine the role of sunflower, *H. annuus* on *P. maculiventris*, the last colony was exposed to the sunflower for obligatory feeding until 14 and 24 days were reached. *Zea mays* was excluded from the initial feeding set up as the beetle had failed to feed during the previous study (Mphephu et al., 2017).

To determine the feeding and reproductive performance of *P. maculiventris* on the non-target, eight females were switched at each of 14 and 24 days from *T. diversifolia* to *H. annuus* and *Z. mays*, where each plant was confined with four beetles. Similarly, the obligatorily reared female beetles were switched from *H. annuus* after each of 14 and 24 days of feeding to *T. diversifolia* and *Z. mays* (Figure 1). Then, feeding, egg production and longevity by *P. maculiventris* were determined on both the target and non-target species. As adapted from Wheeler et al. (2017), a wet paper towel (WPT) was used to detect egg batches that were laid on non-target plant species by coincidence. The WPT was placed horizontally against the walls of the containers and confined with both the colonies. The experimental setup emulates the standard protocols of the
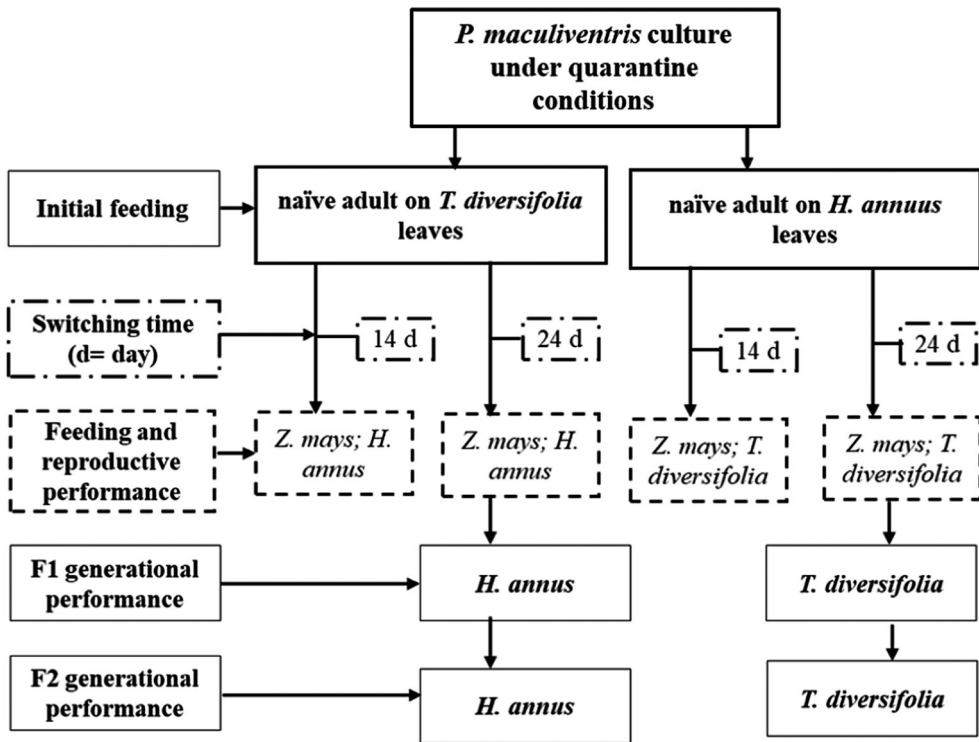
Figure 1. Schematic presentation of the experimental approach followed to test absolute spillover impact risk of P. *maculiventris*.

no-choice tests used during the beetle's host range studies (Mphephu et al., 2017). The tests were conducted in a ventilated container (10 L) with ample space and were replicated four times.

The feeding and reproductive performance of *P. maculiventris* on its host plant, *T. diversifolia* recorded from the host range studies (Mphephu et al., 2017), was used as a control or guideline of normal performance of the beetle. Egg production ranged between 5 and 6 batches (mean ± SE = 5.25 ± 0.25), while larval survival to adulthood was 85.6% (27.33 ± 0.95) from a cohort of larvae and female adult longevity ranged from 39 to 49 days (45.5 ± 2.22), and feeding was 3 (normal) (3 ± 0.00) from one generation to another (Mphephu et al., 2017).

Feeding damage scores ranged from 0 to 3 where 0 = no feeding; 1 = exploratory feeding; 2 = minor feeding and 3 = normal feeding, and reproductive performance was determined as the number of egg batches laid per plant species (Mphephu et al., 2017).

Insect generational build-up

The switched colonies used for the first spillover feeding damage and reproduction tests were further exposed to the experimental plants (target and non-target species) to determine the influence of a spillover feeding damage on generational build-up performance of the beetle (Figure 1). Thus, a colony of the beetles that showed high feeding and

reproductive performances in the leaf feeding damage and reproductive performance test were continuously monitored as they were, to measure their feeding damage, survival rates and reproductive output on the susceptible non-target plant species. This was important to determine if the actual first generation of *P. maculiventris* population influenced by spillover on *H. annuus* could continue to grow or not, without further access to *T. diversifolia*. The experiment was monitored daily and recorded parameters included the feeding damage, egg oviposition, adult longevity and adult emergence. The experiments were replicated four times.

Statistical analyses

Adult feeding damage, longevity and egg batch production data were subjected to a two-way analysis of variance (ANOVA) to compare the performances within and between switching date and combination (feeding plant source and experimental plant), followed by post-hoc tests (Feeding damage and egg batch production were subjected to Tukey's HSD, whereas adult longevity was subjected to Fisher's LSD) to measure statistical differences between treatment combinations. Student's t-test was used to determine the significant differences ($P < 0.05$) in adult emergence, feeding damage and oviposition rates between *T. diversifolia* or *H. annuus* infested with *P. maculiventris* over two generational periods (F1 and F2).

Results

Leaf feeding damage

The leaf damage by adults was significantly greater for adult colonies switched from *H. annuus* to *T. diversifolia*, compared with those switched from *T. diversifolia* to *H. annuus*, and absolute zero (0) feeding damage was observed on *Z. mays* ($F_{3,24} = 5.1538$; $P = 0.007$) (Figure 2). Although feeding damage was observed on *H. annuus*, the damage was exploratory to minor (between 1.25 and 1.75 feeding score), but different for beetles switched at 14 days than 24 days old from *T. diversifolia*. The leaf feeding damage score on *T. diversifolia* by the adult females switched from *H. annuus* at 14 days differed (minor to normal feeding = 2.5) to that of *H. annuus* switched from *T. diversifolia* at the same age. The damage score significantly decreased from 2.5 to 1.5 (exploratory to minor) for the adults switched at 24 days, while the feeding damage differed significantly between the plant species for beetles switched at 14 ($F_{3,12} = 16.667$; $P < 0.001$) and 24 ($F_{3,12} = 43.857$; $P < 0.001$) days. Therefore, the leaf damage by adults was significantly affected by both the switching time and plant species (Table 1).

Adult longevity

Mean longevity was significantly greater for the colonies that were switched to *T. diversifolia* after previously fed *H. annuus* leaves for either 14 or 24 days, compared to those that were switched to *H. annuus* from *T. diversifolia* after feeding for the same periods ($F_{3,24} = 118.93$; $P < 0.001$) (Figure 3). Adult colonies switched from *H. annuus* to *T. diversifolia* at 14 and 24 days lived significantly longer (36.25 and 17.75 days) compared to those switched from
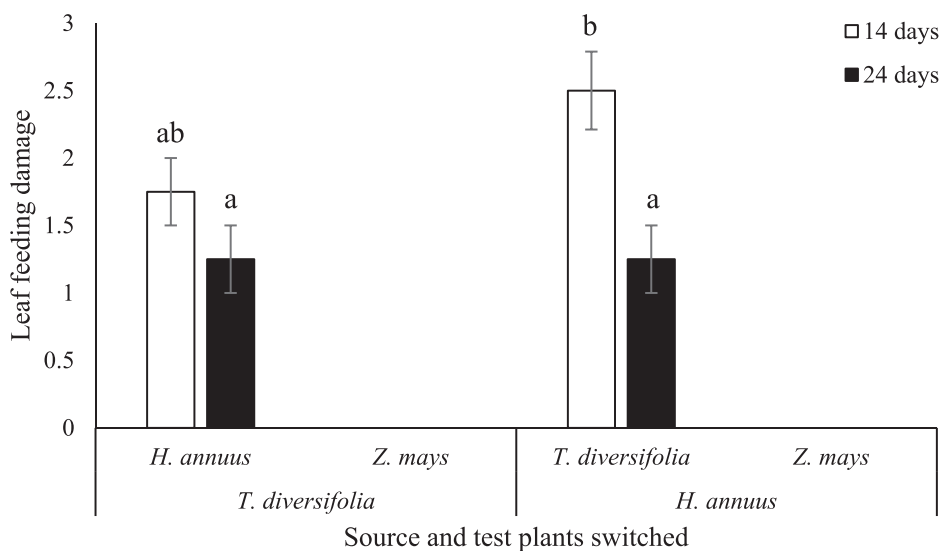
Figure 2. Mean (+SE) leaf feeding damage of 14- and 24-days old, P. *maculiventris* females fed on either *T. diversifolia* or *H. annuus* and then switched to either of two commercial crops (*H. annuus* or *Z. mays*) or the host plant, T. *diversifolia*. Means with the same letter are not significantly different at <0.5 (Tukey's HSD test for unequal sample sizes).

Table 1. Statistical details (ANOVA) on the effect of host-plant switching on leaf feeding damage, adult longevity and egg production when *P. maculiventris* was switched from its natural host *T. diversifolia*, onto the non-target commercial crops, *Z. mays* and *H. annuus*.

Effect	DF	MS	F-value	P-value
Leaf feeding damage				
Switching date (SD)	1	1,53	11,31	0,003
Switching combination (SC)	3	7,78	57,46	<0,001
SD*SC	3	0,7	5,15	0,007
Total	31			
Adult longevity (days)				
Switching date (SD)	1	162	72	<0,001
Switching combination (SC)	3	517,71	230,09	<0,001
SD*SC	3	267,58	118,93	<0,001
Total	31			
Egg batch production/plant				
Switching date (SD)	1	0,02	0,06	0,81
Switching combination (SC)	5	6,37	18,72	<0,001
SD*SC	5	4,07	11,96	<0,001
Total	47			

Some colonies were obligatorily reared on *H. annuus* and then shifted onto the host, *T. diversifolia* and non-target *Z. mays*. Significant *P*-values are in bold.

T. diversifolia to *H. annuus* at 14 and 24 days that lived for 21.25 and 12.00 days, respectively (Figure 3). We found a significant date effect for longevity indicating that the colonies that previously fed on *T. diversifolia* leaves lived longer after being switched on the non-target, *Z. mays* and *H. annuus*, compared to colonies that have obligatory fed on *H. annuus* leaves and switched to non-target, *Z. mays* (Table 1). Longevity increased significantly only for the colony that was switched from *H. annuus* leaves after 14 days of
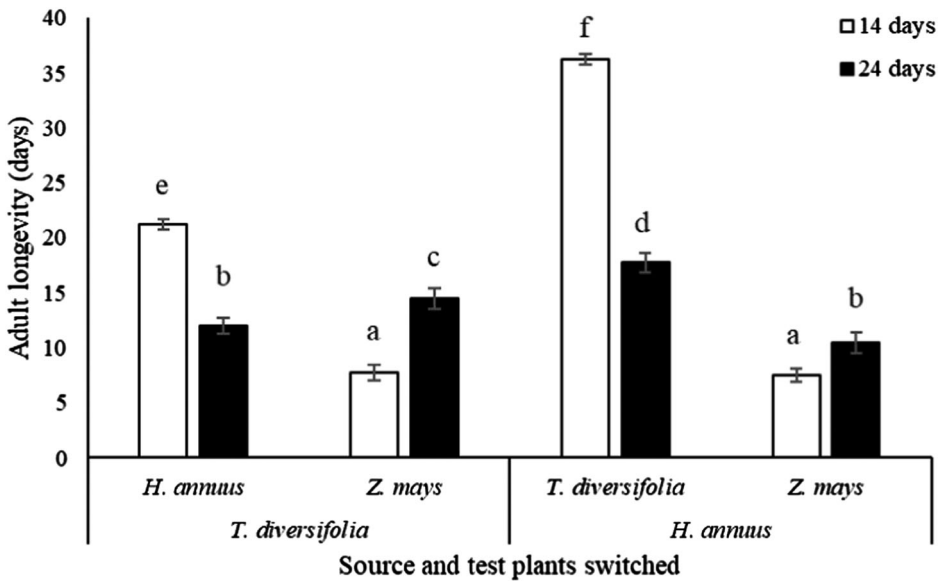
Figure 3. Mean (+SE) longevity of adult female *P. maculiventris* switched to either of two commercial crops (*H. annuus* or *Z. mays*) or the host-plant, T. *diversifolia* after 14- and 24- days of feeding on either of the two susceptible plants (*T. diversifolia* or *H. annuus*). Different letters indicate significant difference among all means at $P < 0.05$ (Fisher LSD).

feeding to *T. diversifolia*, compared to the colony that was switched from *T. diversifolia* leaves after feeding for the same period to *H. annuus* (Figure 3). The interaction between date and species for longevity was significant (Table 1).

Egg production

The overall oviposition was significantly different for the colonies that were switched from either *H. annuus* or *T. diversifolia*, to either of *H. annuus*, *T. diversifolia*, *Z. mays* or WPT at 14 and 24 days ($F_{5,36} = 11.963$; $P < 0.001$). The oviposition rate was significantly different between beetles switched from either *H. annuus* or *T. diversifolia*, to either of *H. annuus*, *T. diversifolia*, *Z. mays* or WPT at 14 days ($F_{5,18} = 34.263$; $P < 0.001$), than those that were switched at 24 days ($F_{5,18} = 3.3600$; $P = 0.026$) (Figure 4; Table 1). The colonies that were switched to *Z. mays* after either 14 or 24 days of feeding on either *T. diversifolia* or *H. annuus* produced 0.00 and 1.3 egg batches, respectively. The colonies switched to *T. diversifolia* produced 3.8 and 1 egg batches after previously being fed *H. annuus* leaves for 14 and 24 days, respectively. The colonies that were switched to *H. annuus* produced 1 and 1.3 egg batches after previously being fed *T. diversifolia* leaves for 14 and 24 days, respectively. Egg batch productions was greatly affected by the switching date for *P. maculiventris* colonies that were switched from *H. annuus* and exposed to *T. diversifolia* (Figure 4).

Insect generational build-up performance

The first generation (*F1*) colony feeding damage was exploratory to minor (1.5) (±0.29) and minor to normal 2.75 (±0.25) on *H. annuus* and *T. diversifolia*, respectively. Although
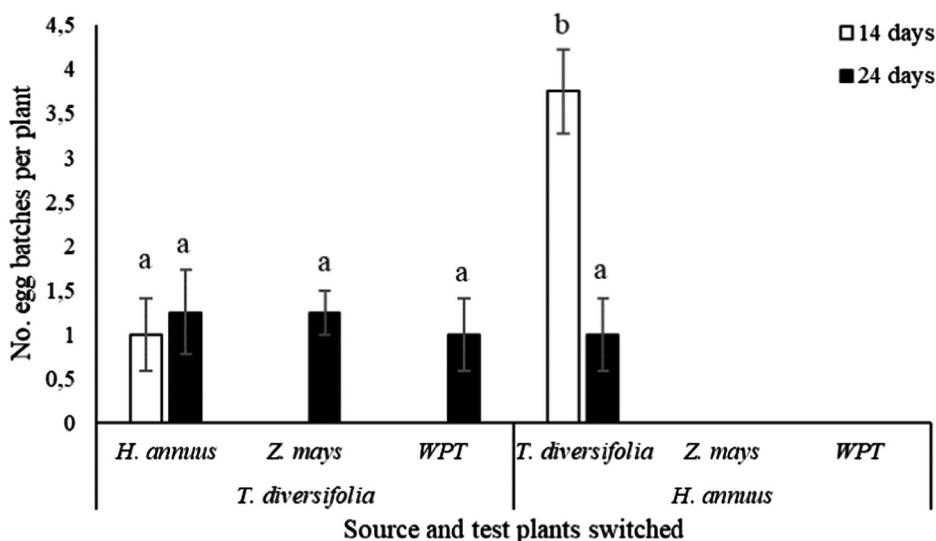
Figure 4. Mean (+SE) number of egg batches laid by female *P. maculiventris* that were switched to either of two commercial crops (*H. annuus* or *Z. mays*) or the host plant, T. *diversifolia* after 14 and 24 days of feeding on either of *T. diversifolia* and *H. annuus*. Means with the same letter are not significantly different at *P* < 0.5 (Tukey's HSD test).

the first colony produced on *H. annuus* constituted lower adult number of 6.25 (±2.43), compared to 26.75 (±1.49) adults on *T. diversifolia* during *F1* generation, the performance of these *F1* adults on *H. annuus* was poor and resulted in 0 adults in the *F2* generation. In

Table 2. Nested analysis of variance (ANOVA) on the feeding damage, adult emergence and egg production differences between two generations (F1 and F2) of *P. maculiventris* switched at 14 days from *T. diversifolia* (*Tithonia*) to *H. annuus* (*Helianthus*) and vice versa.

Variable	Switching combination (A)	Switching combination (B)	Mean Difference (A–B)	SE	Sig.	95% Confidence interval	
						Lower bound	Upper bound
Feeding Damage	[1]*Tithonia*Helianthus*	[2]*Tithonia*Helianthus*	1,500	0,368	0,002	0,70	2,30
		[1]*Helianthus*Tithonia*	−0,750	0,368	0,064	−1,55	0,05
		[2]*Helianthus*Tithonia*	−1,000	0,368	0,019	−1,80	−0,20
	[2]*Tithonia*Helianthus*	[1]*Helianthus*Tithonia*	−2,250	0,368	0,000	−3,05	−1,45
		[2]*Helianthus*Tithonia*	−2,500	0,368	0,000	−3,30	−1,70
	[1]*Helianthus*Tithonia*	[2]*Helianthus*Tithonia*	−0,250	0,368	0,510	−1,05	0,55
Adults emerged	[1]*Tithonia*Helianthus*	[2]*Tithonia*Helianthus*	6,000	19,646	0,765	−36,81	48,81
		[1]*Helianthus*Tithonia*	−20,500	19,646	0,317	−63,31	22,31
		[2]*Helianthus*Tithonia*	−306,250	19,646	0,000	−349,06	−263,44
	[2]*Tithonia*Helianthus*	[1]*Helianthus*Tithonia*	−26,500	19,646	0,202	−69,31	16,31
		[2]*Helianthus*Tithonia*	−312,250	19,646	0,000	−355,06	−269,44
	[1]*Helianthus*Tithonia*	[2]*Helianthus*Tithonia*	−285,750	19,646	0,000	−328,56	−242,94
Egg production	[1]*Tithonia*Helianthus*	[2]*Tithonia*Helianthus*	−0,750	0,854	0,397	−2,61	1,11
		[1]*Helianthus*Tithonia*	−2,750	0,854	0,007	−4,61	−0,89
		[2]*Helianthus*Tithonia*	−10,000	0,854	0,000	−11,86	−8,14
	[2]*Tithonia*Helianthus*	[1]*Helianthus*Tithonia*	−2,000	0,854	0,037	−3,86	−0,14
		[2]*Helianthus*Tithonia*	−9,250	0,854	0,000	−11,11	−7,39
	[1]*Helianthus*Tithonia*	[2]*Helianthus*Tithonia*	−7,250	0,854	0,000	−9,11	−5,39

Superscript numbers represent the generations: [1]First, and [2]Second generation; represent two different switching combinations being compared; statistically significant *P*-values (<0.05) are highlighted in bold.

contrast, the number of adults on *T diversifolia* increased substantially and significantly in the second generation (*F2*) by 11.8 fold, producing 316.3 (±28.39) adults (Table 2).

Discussion

The results presented in this study confirm the findings of the biology and host range study, which indicated that *P. maculiventris* can only sustain populations on the target weed (Mphephu, 2015; Mphephu et al., 2017). Although the beetle displayed minor feeding damage and poor development on *H. annuus* cultivars (Mphephu et al., 2017), the present study indicated that *H. annuus* populations cannot sustain multiple generations of *P. maculiventris*. Neither could *Z. mays* support feeding and reproduction by *P. maculiventris*. Egg production by the gravid female colonies previously fed on *T. diversifolia* leaves, decreased when they were switched to *H. annuus* and *Z. mays*. Given the failure of *P. maculiventris* to survive for two generations on *H. annuus*, any larval production that could occur on *H. annuus* would not be sustained to ensure a large colony, without the beetles having access to *T. diversifolia*. However, during the choice test studies, at 14 or 24, *P. maculiventris* did not oviposit on any of *H. annuus* cultivars, but minor feeding damage occurred (Mphephu et al., 2017). Between these two non-target plant species, *H. annuus* is the one that is most vulnerable to a non-significant rate of spillover feeding damage.

Under field conditions, spillover feeding damage is expected to occur mostly on non-target species that suffered minor feeding damage by the candidate biocontrol agents during host range studies. However, this is most likely to occur when the geographic distribution range of the non-target species overlaps with that of target weed (Sheppard, Van Klinken, & Heard, 2005; Thomas, Casula, & Wilby, 2004). Furthermore, this usually happens following a large population outbreak of the biocontrol agent (Wheeler et al., 2017). In South Africa, large-scale and small-scale (recently detected) cultivations of *H. annuus* cultivars are largely distributed in the Highveld and Lowveld regions of the country, respectively (Department of Agriculture, Fisheries and Forestry, 2010), whilst *T. diversifolia* is most prevalent in the Lowveld and humid eastern regions of the country (Simelane et al., 2011). In these overlapping ranges of *T. diversifolia* and *H. annuus* in the Lowveld regions of South Africa, some spillover feeding damage may occur on *H. annuus* (Rand & Louda, 2004). This occurred in India, where *Z. bicolorata* introduced against *P. hysterophorus* subsequently fed and reproduced (however poorly) on *H. annuus* cultivations (Visalakshy et al., 2008). In such situations, pre-release spillover studies can play a valuable role in predicting the absolute likelihood of candidate biocontrol agents exhibiting spillover in the field (Wheeler et al., 2017).

In a spillover situation with *P. maculiventris* adults, with or without previous feeding on *T. diversifolia*, the beetles are likely to cause only exploratory to minor feeding damage on non-target species. Feeding damage by both adult females switched at both 14 and 24 days from *T. diversifolia* to *H. annuus,* was significantly lower than those beetles switched from *H. annuus* to *T. diversifolia*. Although we predicted some minor feeding on *Z. mays* by the adult females previously fed on *T. diversifolia* leaves, as occurred on an unidentified grass species during culture maintenance, no feeding damage occurred on *Z. mays*. Although the adults switched from *T. diversifolia* to *H. annuus* exhibited some non-target feeding damage, this did not significantly increase their longevity on *H. annuus*. However, when females were switched from

H. annuus to *T. diversifolia*, their longevity was significantly improved. Interestingly, both the 14 and 24-day-old beetles switched from *T. diversifolia* to *H. annuus* produced fewer egg batches compared to those switched from *H. annuus* to *T. diversifolia*. We suspect that the poor production of eggs by *P. maculiventris* on *H. annuus* was because the leaves of *H. annuus* have lower nutritional quality compared to those of *T. diversifolia*. Seemingly, this was possible because the 14-day-old females produced significantly more egg batches when switched from *H. annuus* to the primary host plant, *T. diversifolia*. This confirms that, *P. maculivetris* will choose to oviposit on *T. diversifolia* in the presence of *H. annuus*.

Likewise, with the previous results of the host range studies (Mphephu et al., 2017), our results are further supported by a global study by Rogers (1992) who found no records of *P. maculiventris* (or any other Cassidinae species) being reported as pests of commercial sunflower (*H. annuus*) in North and South America. The lack of a single record of *P. maculiventris* utilising any cultivars of commercial sunflower in its native range further suggests that the risk of spillover damage on *H. annuus* cultivations in South Africa and elsewhere are very low (Janet et al., 2010; Mphephu et al., 2017).

Predictions of spillover impact can help to avoid releasing insects that could damage non-target native plants, as occurred in the USA where the seed-feeding weevil *Larinus planus* (Coleoptera: Curculionidae), which was introduced against noxious thistle species of rangelands, was subsequently recorded on a native plant, *Cirsium pitcheri* (Asteraceae) (Havens et al., 2012). *Larinus planus* was later prohibited for further field collection and re-distribution in the country (Havens et al., 2012). Therefore, we conclude that the risk of spillover damage by *P. maculiventris* onto non-target, *H. annuus* and *Z. mays* is extremely low, and it would be highly unlikely for *P. maculiventris* to sustain a viable population on *H. annuus*. Furthermore, we advocated that an application to release *P. maculiventris* in South Africa should be re-submitted to regulatory authorities, since we have no doubts that *P. maculiventris* will not become a pest of either *H. annuus* or *Z. mays* cultivations in South Africa.

Disclosure statement

No potential conflict of interest was reported by the authors.

References

Center, T. D., & Hill, M. P. (2002). Field efficacy and predicted host range of the pickerelweed borer, *Bellura densa*, a potential biological control agent of water hyacinth. *Biocontrol*, 47, 231–243.

Coetzee, J. A., Byrne, M. J., Hill, M. P., & Center, T. D. (2009). Should the mirid, *Eccritotarsus catarinensis* (Heteroptera: Miridae), be considered for release against water hyacinth in the United States of America? *Biocontrol Science and Technology*, 19, 103–111.

Cristofaro, M., De Biase, A., & Smith, L. (2013). Field release of a prospective biological control agent of weeds, *Ceratapion basicorne*, to evaluate potential risk to a nontarget crop. *Biological Control*, 64, 305–314.

Department of Agriculture, Forestry and Fisheries. (2010). Sunflower production guidelines. Retrieved from http://www.nda.agric.za/docs/Brochures/prodGuideSunflower.pdf

Dhileepan, K., Trevino, M., & Raghu, S. (2006). Temporal patterns in incidence and abundance of *Aconophora compresa* (Hemiptera: Membracidae), a biological control agent for *Lantana camara*, on target and nontarget plants. *Environmental Entomology*, 35, 1001–1012.

Fowler, S. V., Paynter, Q., Dodd, S., & Groenteman, R. (2012). How can ecologists help practitioners minimize non-target effects in weed biocontrol? *Journal of Applied Ecology*, 49, 307–310.

Havens, K., Jolls, C. L., Marik, J. E., Vitt, P., McEachern, A. K., & Kind, D. (2012). Effects of a non-native biocontrol weevil, *Larinus planus*, and other emerging threats on populations of the federally threatened Pitcher's thistle, *Cirsium pitcheri*. *Biological Conservation*, 155, 202–211.

Hopper, K. R. (2001). Research needs concerning non-target impacts of biological control introductions. In E. Wajnberg, J. K. Scott, & P. C. Quimby (Eds.), *Evaluating indirect ecological effects of biological control* (pp. 39–56). Wallingford, New Zealand: CABI Publishing.

Janet, J. K., Laurence, D. C., & John, G. (2010). Integrated pest management of sunflower insect's pests in the Northern Great Plains. Extension Service, Fargo, North Dakota 58108.

Lake, E. C., Smith, M. C., Dray, F. A., & Pratt, P. D. (2015). Ecological host-range of *Lilioceris cheni* (Coleoptera: Chrysomelidae), a biological control agent of *Dioscorea bulbifera*. *Biological Control*, 85, 18–24.

Louda, S. M., Pemberton, R. W., Johnson, M. T., & Follett, P. A. (2003). Nontarget effects-the Achilles' heel of biological control? Retrospective analyses to reduce risk associated with biocontrol introductions. *Annual Review of Entomology*, 48, 365–396.

McClay, A. S., & Balciunas, J. K. (2005). The role of pre-release efficacy assessment in selecting classical biological control agents for weeds—Applying the Anna Karenina principle. *Biological Control*, 35, 197–207.

Mphephu, T. E. (2015). Suitability of the defoliating beetle *Physonota maculiventris* (Coleoptera: Chrysomelidae) for release against *Tithonia diversifolia* (Hemsl.) A. Gray (Asteraceae) in South Africa (M.Sc. thesis). University of KwaZulu-Natal, Pietermaritzburg.

Mphephu, T. E., Olckers, T., & Simelane, D. O. (2017). The tortoise beetle *Physonota maculiventris* (Chrysomelidae: Cassidinae) is suitable for release against the weedy Mexican sunflower *Tithonia diversifolia* (Asteraceae) in South Africa. *Biocontrol Science and Technology*, 27(4), 510–524.

Rand, T. A., & Louda, S. M. (2004). Exotic weed invasion increases the susceptibility of native plants to attack by a biocontrol herbivore. *Ecology*, 85, 1548–1554.

Rogers, C. E. (1992). Insect pests and strategies for their management in cultivated sunflower. *Field Crops Research*, 30, 301–332.

Sheppard, A. W., Van Klinken, R. D., & Heard, T. A. (2005). Scientific advances in the analysis of direct risks of weed biological control agents to nontarget plants. *Biological Control*, 35, 215–226.

Simberloff, D., & Stiling, P. (1996). How risky is biological control? *Ecology*, 77, 1965–1974.

Simelane, D. O., Mawela, K. V., & Fourie, A. (2011). Prospective agents for the biological control of *Tithonia rotundifolia* (Mill.) S.F. Blake and *Tithonia diversifolia* (Hemsl.) A. Gray (Asteraceae) in South Africa. *African Entomology*, 19, 443–450.

Thomas, M. B., Casula, P., & Wilby, A. (2004). Biological control and indirect effects. *Trends in Ecology and Evolution*, 19, 61.

Tingle, J. L., Cook-Patton, S. C., & Agrawal, A. A. (2016). Spillover of a biological control agent (*Chrysolina quadrigemina*) onto native St. Johnswort (*Hypericum punctatum*). *Peereviewed Journal*, *4*, e1886.

Van Klinken, R. D. (2000). Host specificity testing: Why do we do it and how we can do it better. In R. G. Van Driesche, T. A. Heard, A. McClay, & R. Reardon (Eds.), *Proceedings: Host specificity testing of exotic arthropod biological control agents: The biological basis for improvement in safety* (pp. 54–68). Morgantown, WV: USDA Forest Service.

Visalakshy, G., Jayanth, K. P., Ghosh, S. K., & Chaudhary, M. (2008). Post introductory risk assessment on *Zygogramma bicolorata* (Coleoptera: Chrysomelidae), a classical biological control agent of *Parthenium hyterophorus* (Asteraceae) in India. *Biocontrol Science and Technology*, *18*(10), 1083–1086.

Wheeler, G. S., Duncan, J. G., & Wright, S. (2017). Predicting spillover risk to non-target plants pre-release: *Bikasha collaris* a potential biological control agent of Chinese tallowtree (*Triadica sebifera*). *Biological Control*, *108*, 16–21.