



Using pre-release efficacy as a candidate agent prioritisation tool for the weed *Trichocereus spachianus* (Caryophyllales: Cactaceae)

T. C. Griffith · M. B. Aguirre · G. F. Sutton · L. Chari ·
I. D. Paterson

Received: 28 January 2025 / Accepted: 17 July 2025 / Published online: 30 July 2025
© The Author(s) 2025

Abstract Pre-release efficacy assessments (PREA) can be used to prioritise the most damaging candidate agents for further studies in biological control programmes against invasive alien plants. This study assessed the efficacy of various potential enemies of *Trichocereus spachianus* (Riccob.) (Caryophyllales: Cactaceae), an invasive cactus in South Africa, in order to prioritise the most damaging candidates for further studies, including host specificity testing. *T. spachianus* is considered indigenous in Argentina and Bolivia, but the original description was based on a cultivated plant, and it is either very rare or absent in its indigenous range, with no verified records of natural populations. This necessitates the use of new associations collected off close relatives of the target plant as potential biocontrol agents. We evaluated eight potential biocontrol agents by deriving host suitability

indices (HSI) and performing efficacy trials. The HSI analysis demonstrated that only one out of the eight candidate agents, a cochineal insect *Dactylopius confertus* De Lotto (Hemiptera: Dactylopiidae), had high fitness (high survival, fecundity and complete development) on *T. spachianus*. This helped us eliminate ineffective agents early on in the programme. The cochineal insect was prioritised for efficacy trials, which demonstrated its high potential efficacy, killing approximately 89% of *T. spachianus* plants over 60 weeks. Taken together, our results indicated that *D. confertus* is a highly promising potential agent for the biocontrol of *T. spachianus* in South Africa. By prioritising candidate biocontrol agents based on efficacy prior to host specificity testing, we ensured that the highest priority candidate agent was identified for detailed and resource-intensive host specificity testing.

Handling Editor: Michelle Rafter.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s10526-025-10337-x>.

T. C. Griffith (✉) · G. F. Sutton · L. Chari · I. D. Paterson
Department of Zoology and Entomology, Centre
for Biological Control, Rhodes University, Grahamstown,
South Africa
e-mail: t.camillagr@gmail.com

M. B. Aguirre
Fundación Para el Estudio de Especies Invasivas
(FuEDEI), Hurlingham, Buenos Aires, Argentina

Keywords Cactaceae · Dactylopiidae · *Dactylopius confertus* · Pre-release efficacy · *Trichocereus spachianus*

Introduction

Selecting and prioritising biological control agents is essential for the success of biological control programmes. Historically, the selection of biological control agents has primarily focused on host specificity (McClay and Balcianus 2005), which remains

the most important factor in ensuring that introduced agents do not adversely affect non-target species. Several other metrics have also been used alongside host specificity testing to further prioritise candidate agents, such as the availability of the insect, potential transfer programmes, phenology of attack, ease of mass rearing, and most notably, potential efficacy (Harris 1973; Goeden 1983; Sheppard 2003; van Klinken and Raghu 2006; Paynter 2024). More recently, regulatory authorities often require detailed information on the expected efficacy of a candidate agent to assess the risks and benefits, ensuring justification for the release of a biological control agent (van Klinken and Raghu 2006; TAG-BCAW Manual 2021). Efficacy refers to the agent's ability to significantly reduce the target species' population density and reproductive success (Balciunas 2004; McClay and Balciunas 2005).

Invasive plants often have many insect species associated with them in their native range that could be considered as potential biocontrol agents. However, the economic costs, logistics and time required for conducting host specificity testing on all the candidate biological control agents can quickly become infeasible and impractical (McFadyen 1998). Integrating pre-release efficacy assessments (PREAs) early in the prioritisation process can help identify agents likely to achieve desired control levels (Balciunas 2004; McClay and Balciunas 2005), as well as reducing the time and costs of developing effective biocontrol programmes by prioritising only the most suitable candidate agents for detailed host specificity testing (Sutton et al. 2021). By prioritising agents based on their demonstrated or potential efficacy, PREAs along with host specificity testing helps to ensure that only those agents with a high likelihood of efficacy and safety are considered for release (McClay and Balciunas 2005; van Klinken and Raghu 2006; Paynter 2024). In addition to being cost-effective, incorporating the PREA alongside host specificity testing may reduce the total number of agents that need to be released, thereby lowering the risk of unintended negative consequences (McClay and Balciunas 2005). Combining host specificity testing with PREAs offers a more streamlined and targeted approach to biological control by enhancing the selection and release of safe and effective agents, increasing the likelihood of successful control of invasive weed populations.

Although many programmes conduct host specificity testing before PREAs, this paper used predicted efficacy as a prioritisation tool to identify the most promising candidate biological control agents for *Trichocereus spachianus* (Riccob.) (Caryophyllales: Cactaceae) to ensure that only the most effective potential agent was considered for host specificity testing. Recently, *T. spachianus* has undergone a taxonomic change to *Soehrensia spachianus* (Lem.) Schlumpb., a classification accepted by certain authorities (Korotkova et al. 2021). Nonetheless, cactus experts argue, based on morphology, that it should remain under the *Trichocereus* genus (R. Kiesling, pers. comm.) and will therefore be referred to as *T. spachianus*, hereafter. *T. spachianus*, an invasive cactus native to South America, has become a problematic weed in South Africa, where it disrupts ecosystems and agriculture with no effective management strategies (Paterson et al. 2021).

While *T. spachianus* is believed to originate from north-western Argentina and southern Bolivia, it is either rare or absent in its native range, with no verified records of indigenous populations (L. Varone, pers. comm.), and the species' original description was based on cultivated plants and herbarium specimens (Kiesling 1978; Albesiano Hoyos 2015). Despite the lack of confirmed native populations, closely-related cactus species have been successfully controlled through biological control, indicating a high likelihood that biocontrol will be a successful control method against *T. spachianus* (Paterson et al. 2021). While taxonomic uncertainties can complicate agent collection and host specificity testing, biological control of cacti has been achieved using agents from new associations, where the agent has not co-evolved with the target plant (Hokkanen and Pimentel 1984; Hokkanen and Sailer 1985; Klein et al. 2020). Thus, collecting insect agents from related cactus species within the suspected native range remains a promising strategy for controlling this invasive cactus. A biological control programme was initiated in 2018 and several potential agents, collected from species closely related to *T. spachianus*, were identified through surveys conducted in both the invaded and native ranges (Griffith 2024).

In this study, we conduct a PREA for all the potential agents for *T. spachianus* in order to select the most promising potential agent to be subjected to host specificity testing. These potential agents included

one species of the galling mealybug *Hypogeococcus* that is already used in South Africa for the control of closely related target weeds, namely *Hypogeococcus* sp., as well as several other lineages of *Hypogeococcus* and a cochineal insect that are indigenous to South America and have not been used as biocontrol agents before. The absence of any indigenous populations of the target weed necessitated the collection of these potential agents from closely related cactus species (Paterson et al 2021).

Materials and methods

This study used insect fitness experiments and efficacy trials to prioritise candidate biological control agents for *Trichocereus spachianus* in South Africa. Specifically, we sought to identify agents exhibiting high host suitability and effectiveness in reducing the target weed population.

Biological material collection

Several insect species, including various *Hypogeococcus* (mealybugs) species/lineages and *Dactylopius confertus* De Lotto (Hemiptera: Dactylopiidae), were collected as candidate biological control agents for *T. spachianus* during field surveys in both its invaded range (South Africa and Namibia) and its native range in Argentina (Griffith 2024). The first candidate agent considered was a lineage of *Hypogeococcus* sp. (Hemiptera: Pseudococcidae) that has already been released in South Africa and proved to be a successful biological control agent against several cactus species closely related to *T. spachianus*, including: *Harrisia pomanensis* (F.A.C. Weber ex K. Schum.) (Caryophyllales: Cactaceae), *Harrisia martinii* (Labour.) Britton (Caryophyllales: Cactaceae), *Harrisia balansae* (K. Schum.) N.P. Taylor and Zappi (Caryophyllales: Cactaceae) and *Cereus jamacaru* De Candolle (Caryophyllales: Cactaceae) (Muskett et al. 2024). If found to be a promising candidate, this agent could significantly reduce the cost and time needed to develop a biological control programme for this cactus, as no further host specificity testing would be required for release in South Africa, nor would there be costs associated with importing the agent. The *Hypogeococcus* spp. used in the experiments were sourced

from the Centre for Biological Control's Mass Rearing facility in Kariega, South Africa, where the insect is cultured on *H. martinii* (Hill et al. 2021). The lineage that has already been released in South Africa will be referred to as *Hypogeococcus* sp., as this is how it has been referred to in other recent studies (Poveda-Martínez et al. 2020; Paterson et al. 2021; Zachariades 2021).

Since no wild populations of *T. spachianus* were found in its suspected native range in Argentina (Griffith 2024), candidate agents could not be collected directly from this cactus. Instead, all candidate agents identified during native-range surveys were collected from cactus species closely related to *T. spachianus*. Surveys for other candidate *Hypogeococcus* species and lineages of *Hypogeococcus* sp. were conducted in northern Argentina in 2019 at locations previously surveyed by researchers at Fundación para el Estudio de Especies Invasivas (FuEDEI) (Griffith 2024). Six other *Hypogeococcus* lineages were collected from various locations and host plants in Argentina (Table S2).

Hypogeococcus sp. is part of the *Hypogeococcus pungens* species complex, and the members of this complex show a high degree of specificity at the host plant level (Poveda-Martínez et al. 2020). Species delimitation analysis identified two well-supported potential species within *H. pungens*: one comprising mealybugs that feed on Amaranthaceae (*H. pungens sensu stricto*), and a distinct, undescribed species that feeds on Cactaceae (*Hypogeococcus* sp.) (Poveda-Martínez et al. 2020). The authors also identified genetic heterogeneity among the *Hypogeococcus* sp. lineages feeding on cacti in Argentina, as well as the one introduced in South Africa for the biological control of cactus weeds (Poveda-Martínez et al. 2020; Ezech et al. 2023).

D. confertus was collected just outside Windhoek, Namibia on *H. pomanensis* (Table S2). Although collected in Namibia, *D. confertus* is native to South America and was likely introduced into Namibia accidentally, possibly through the cactus horticulture trade. While most cochineal species primarily target *Opuntia* species, *D. confertus* has been found to include *Trichocereus* species in its host range in its native range of Argentina (Jujuy and Catamarca) (van Dam 2013). Therefore, *D. confertus* may also target *T. spachianus* established in South Africa. All plants used to test the suitability and efficacy of candidate

agents were collected from field sites in South Africa (Table S1).

Host suitability index (HSI)

As none of the candidate agents could be directly collected from *T. spachianus*, it was necessary to determine whether this cactus was included in their host range before conducting efficacy trials. A Host Suitability Index (HSI) was calculated for each of the eight potential insect biocontrol agents on *H. martinii*, *H. pomanensis*, *H. balansae*, *C. jamaru*, and the target plant, *T. spachianus*. HSI was calculated for the other cactus species to allow a relative comparison with insect performance on *T. spachianus*.

The HSI calculation requires several developmental and reproductive parameters to be estimated, including nymphal survival (proportion of crawlers that survive to F₁ generation), days to egg production and fecundity (number of crawlers produced per gravid female). These parameters were calculated from laboratory-based rearing trials. Each trial consisted of placing a single piece of cut cactus in a plastic container (19.5 cm × 27.5 cm × 9 cm) with holes (5 cm) covered with a fine mesh for ventilation to prevent insects from escaping. For *C. jamaru*, plastic containers measuring 21.5 cm × 33.4 cm × 16.8 cm were used owing to their larger diameter. Gravid females from each insect culture were collected, dewaxed using fine forceps and placed into Petri dishes (9 cm), which were then sealed with parafilm to ensure no crawlers could escape. Once crawlers hatched, 30 first instars (less than 24 h old) were carefully collected using a fine paintbrush and placed onto the cactus piece in each plastic container. The cacti were left for three days to allow the crawlers to settle before being checked every two days to record the number of settled crawlers (indicated by white waxy filaments). After 14 days, all crawlers were considered either settled or dead, and the F₁ survival rate (proportion of the 30 crawlers that were added to the cactus plant) of the crawlers was determined. Cactus pieces were checked weekly for gravid females (identified by red egg-shaped masses in their bodies), and they were collected and placed into separate sealed Petri dishes. The number of crawlers produced by each female was recorded after 30 days to estimate relative fecundity. Five replicates were conducted for each plant species. All insect performance trials were

conducted under controlled conditions at 26 °C and with a L:D 12:12 photoperiod.

A host suitability index, adapted from Maw (1976), was used to identify and compare test plant species suitable for *Hypogeococcus* species/lineages and *D. confertus* hosts. A single measure or parameter cannot determine host suitability of a plant, so a combination of factors was considered to obtain a reliable indication of host suitability through the use of the Maw's host suitability index (MHSI) equation:

$$MHSI = \frac{\text{pupal mass} \times \text{percentage pupation}}{\text{developmental time}} \quad (1)$$

However, the index for this research was modified to cater to the biology of the candidate agents since they do not undergo pupation. The proportion of the initial 30 crawlers that settled on each cactus piece was recorded as F₁ survival, while the time crawlers took to reach maturity (when females started producing eggs) was recorded as the developmental time (in days). Fecundity referred to the number of crawlers produced per female. The HSI scores were interpreted as the higher the units, the greater the host suitability. The modified host suitability index (HSI) was employed:

$$HSI = \frac{\text{fecundity} \times F1 \text{ survival}}{\text{developmental time}} \quad (2)$$

Efficacy trials

Efficacy trials were conducted for *D. confertus*, as it was identified as having the highest potential for damage during the HSI trials. We also conducted trials for *Hypogeococcus* sp. as a comparison, because *T. spachianus* was a poor host in HSI trials, but it is a successful agent of other cactus species in South Africa (Muskett et al. 2024). Efficacy trials were performed as whole plant potted trials under greenhouse conditions for *Hypogeococcus* sp., and quarantine conditions for the imported *D. confertus*. The efficacy of *Hypogeococcus* sp. was tested on the target weed and the four species that the agent is used to control (*H. martinii*, *H. balansae*, *H. pomanensis*, *C. jamaru*), while *D. confertus* was tested on its host plant, *H. pomanensis*, and the target weed, *T. spachianus*. Individual cactus plants were placed inside a fine-meshed white breeding cage and infested with

either a *Hypogeococcus* sp. gall (approximately 2 cm in diameter, that contained a mixture of adults and crawlers), or 100 crawlers of *D. confertus*.

All treatments were run simultaneously, and plants were monitored weekly to determine the insects' acceptance of each cactus as a host and the success of initial establishment. Establishment was successful if the crawlers settled and produced white waxy filaments and was unsuccessful if the crawlers did not settle (100% mortality of the crawlers). Time to mortality for each cactus was recorded in months. Mortality was recorded as the point where no living tissue (green tissue) was observed on the cactus plant. Plant mortality was scored as a binary variable, where alive = 1 and dead = 0 for each month over 17 months. Five replicates and a negative control (for each replicate) (cactus plants not infested with either *Hypogeococcus* sp. or *D. confertus*) were conducted for each cactus species.

Statistical analysis

Data analyses were performed using Julia version 1.10.3 (Bezanson et al. 2017). All modelling was conducted using the 'GLM.jl' and 'MixedModels.jl' packages in R (R Core Team 2024) (Bates et al. 2023, 2024). All hypothesis testing was performed assuming statistical significance at $P < 0.05$. Post-hoc comparisons were made using the 'Effects.jl' package (Alday et al. 2024).

Host suitability indices

To investigate potential differences in insect HSI when reared on different cactus plants, a generalized linear model (GLM) was used. HSI score was modelled as a function of two fixed effect variables, namely: the insect lineage (eight lineages tested), and the cactus plant on which they were reared (five plant species). The model was specified assuming Gaussian error distributions and an identity link function. A multiplicative interaction term was included in the model formula to test the hypothesis that any differences in insect HSI scores may be driven by the cactus plant on which they were reared. This hypotheses were assessed using likelihood ratio tests comparing the log-likelihood of the multiplicative model versus a simplified model, whereby only the two fixed

effect variables were included in the model as additive terms.

Two sets of post-hoc comparisons were made to allow us to identify high-priority candidate biological control agents for *T. spachianus* in South Africa based on HSI. Firstly, we performed within-groups contrasts of HSI scores of the eight insect species/lineages when reared on *T. spachianus*. If any of the eight species/lineages were found to have higher HSI scores than the others, this would indicate higher potential suitability as a biological control agent. Thereafter, we performed a series of custom between-groups contrasts, whereby the HSI scores of the most suitable insect species/lineage identified on *T. spachianus* above was compared to the HSI scores of *Hypogeococcus* sp. If the HSI score of the most suitable insect species/lineage identified on *T. spachianus* was not significantly different, or potentially higher, than the HSI scores of the existing biological control agent (*Hypogeococcus* sp.) in South Africa, this would further indicate the potential suitability of the novel insect species/lineage. A Wald's test was computed to compare the estimated marginal means of pairwise comparisons, where necessary.

Efficacy trials

Given that the mortality experiments for *Hypogeococcus* sp. and *D. confertus* were performed under greenhouse and quarantine conditions, respectively, we analysed these data separately. The potential of the top-performing insect species/lineage identified during the HSI assessments to kill *T. spachianus* plants over 60 weeks, and to compare its performance to the existing *Hypogeococcus* sp. in South Africa on its host plants, was assessed using a generalised linear mixed model (GLMM) (Bolker et al. 2009). Here, the survival of each plant was specified as a Bernoulli response variable, each week for 60 weeks (1 = dead, 0 = alive), which was modelled as a function of time since inoculation of insects (weeks). Insect species/lineage (*D. confertus*/*Hypogeococcus* sp.) and plant species (*C. jamacaru*, *H. martinii*, *H. balansae*, *H. pomanesis*, *T. spachianus*) were specified as categorical fixed effects. A multiplicative interaction term was included in the model formula to test the hypothesis that the mortality rate was variable between plant species. The model was specified with a logit link function. A random intercept term was

included to account for repeated measurements taken from the same plant in different weeks (Bolker et al. 2009). Fixed effect hypothesis testing was performed with a series of likelihood ratio tests, as above. Post-hoc comparisons of mean mortality were performed using pairwise contrasts amongst insect species/lineages and plant species at week 60 (end-point of the experiment).

Results

Host suitability indices

There was evidence for a statistically significant multiplicative term between the insect species/lineage treatment and the cactus species upon which they

were reared, explaining differences in HSI scores ($\chi^2=264.29$, $df=28$, $P<0.001$). This indicates that HSI scores were significantly different between insect species/lineages. However, the sign and magnitude of these differences were dependent on the cactus species upon which each species/lineage was reared (Fig. 1). When averaged across all plant species tested, the *Hypogeococcus* sp. Alemania lineage consistently showed the highest HSI scores (92.2 ± 12.6), while *Hypogeococcus* sp. had the lowest (19.3 ± 3.7) (Fig. 1).

However, there was noticeable variation in HSI scores across plant species for some insect lineages. For example, *Hypogeococcus* sp. Catamarca lineage performed well on *C. jamararu* (70.4 ± 20.1) and *H. balansa* (202.2 ± 28.8), but performed poorly on *H. pomanensis* (35.5 ± 10.5), and could not be reared

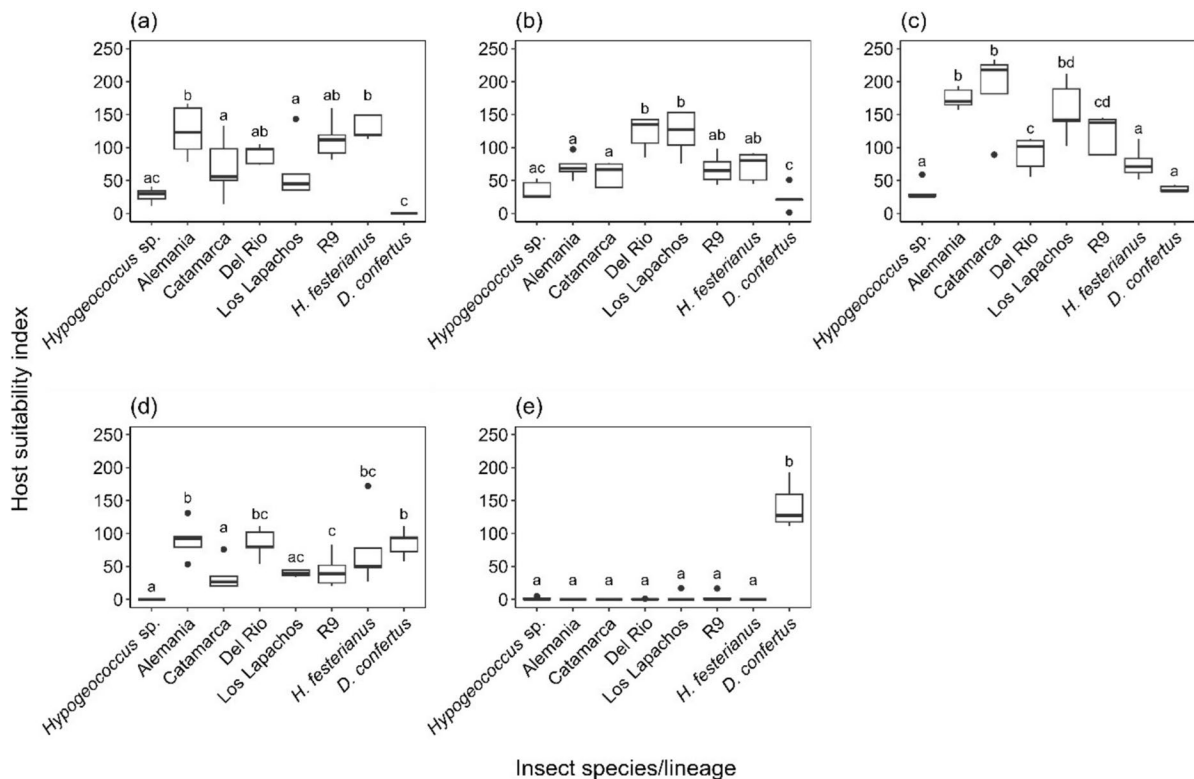


Fig. 1 Box and whisker plot showing the Host suitability indices (HSI) for eight potential biological control agents when reared on five invasive cactus species from South Africa, including the target weed *Trichocereus spachianus*. Each panel represents one of the five invasive cacti tested, namely: **a** *Cereus jamararu*, **b** *Harrisia martinii*, **c** *Harrisia balansa*, **d** *Harrisia pomanensis*, and **e** *Trichocereus spachianus*. Whisk-

ers represented the minimum and maximum values, the middle line represents the median, and the box the interquartile range. Outliers were represented by a closed circle. Post-hoc within-plant comparisons of HSI means, following adjustment for multiple comparisons, were indicated with different significance letters ($P<0.05$)

on *T. spachianus* (0.0). Similar patterns of host-plant dependent variability in HSI scores were evident for the *Hypogeococcus* sp. Alemania and *Hypogeococcus* sp. Los Lapachos lineages, in particular (Fig. 1). On the other hand, there was little evidence for host plant dependent variability in HSI scores for *Hypogeococcus* sp., which produced consistently low HSI scores across all plant species tested (19.3 ± 3.7) (Fig. 1).

The performance of the existing *Hypogeococcus* sp. in South Africa on *T. spachianus* was poor (1.2 ± 0.9), with only two out of the five replicates producing any F_1 adults. The only insect lineage that was able to be consistently reared and produce F_1 adults and viable offspring on *T. spachianus* was *D. confertus* (Fig. 1e). The HSI of *D. confertus* on *T. spachianus* (142.0 ± 15.3) was significantly higher than the HSI for all other insect lineages tested, which all produced HSI scores on *T. spachianus* < 5.0 (Fig. 1, Table S3). The HSI score of *D. confertus* on *T. spachianus* was statistically significantly higher than all four plant hosts of *Hypogeococcus* sp. (Table S4).

Efficacy trials

All control plants survived until the end of the experiment, so any plant mortality observed was attributed to the presence of the insects. There was statistical support for a significant difference in mortality rate varying between cactus species over time when exposed to *Hypogeococcus* sp. ($\chi^2 = 26.21$, $df = 4$, $P < 0.001$). This result was driven by a higher mortality rate earlier in the experiment of *C. jamacaru* relative to *H. balansae* and *H. martinii*, and more noticeably, *H. pomanensis* and *T. spachianus* (Fig. 2). By the end of the experiment, *Hypogeococcus* sp. caused significantly higher mortality of *C. jamacaru* (99%), *H. martinii* (80%), and *H. balansae* (64%), than either *H. pomanensis* (3%) or *T. spachianus* ($< 1\%$) (Fig. 2). The mortality caused by *Hypogeococcus* sp. was not statistically significantly different between *H. pomanensis* and *T. spachianus* ($z = 0.001$, $P = 1.000$) (Fig. 2).

On the other hand, there was no statistical support for a significant difference in mortality rate varying between cactus species over time when exposed to *D. confertus* ($\chi^2 = 0.65$, $df = 1$, $P = 0.419$) (Fig. 3). By the end of the experiment, the mean expected mortality of *H. pomanensis* was marginally higher, with

99.7% (95% CI: 82.9–99.9%), than the mortality of *T. spachianus*, which was 95.4% (95% CI: 50.6–99.9%) (Fig. 3). Although this experiment was conducted in a different environment to the *Hypogeococcus* sp. efficacy trial and is therefore not directly comparable, the mortality rates of both *T. spachianus* and *H. pomanensis* when exposed to *D. confertus* are either similar or greater than the most damaging agent-plant combinations of the biocontrol agent *Hypogeococcus* sp..

Discussion

Biological control programmes rely heavily on prioritising the most suitable agents to efficiently manage invasive weeds. Host specificity testing has traditionally been the sole criterion for determining the suitability of novel biological control agents. However, this method can be impractical, resource-intensive, and time-consuming, especially when evaluating multiple potential agents, and it does not consider the importance of selecting biological control agents with the potential to provide effective control over the target weed. Integrating PREAs into the agent selection process provides an opportunity to prioritise agents that are not only host-specific to the target weed but also have the greatest chance of suppressing target weed populations (McClay and Balciunas 2005).

Moreover, using PREAs (field or laboratory based) at an early stage in the biological control programme may increase resource efficiency by only subjecting the highest-priority candidate agents to detailed and resource-intensive quarantine-based host specificity testing (Sutton et al. 2021). Our study demonstrated using PREA to prioritise potential biological control agents for the invasive cactus *T. spachianus* in South Africa. To do this, we initially screened candidate agents using Host Suitability Indices (HSI), followed by efficacy assessments of plant mortality for the most promising agent. The modified HSI successfully aided in the early rejection of candidate agents that did not feed on *T. spachianus*. HSI values revealed that the highly effective *Hypogeococcus* sp., already released in South Africa to control closely related invasive cacti, as well as six *Hypogeococcus* species/lineages collected from the putative native range of *T. spachianus* in Argentina, showed low suitability for *T. spachianus* as a host.

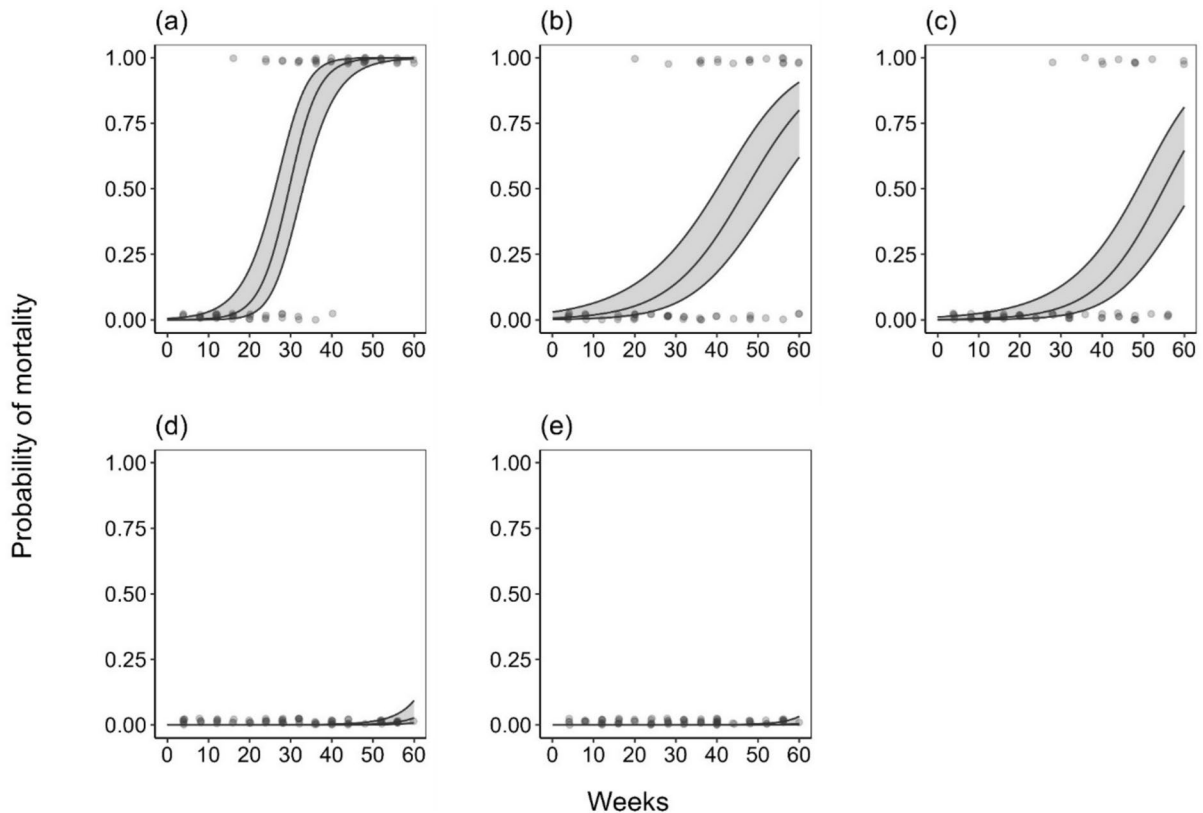


Fig. 2 Marginal effects plots, derived from the fitted GLM, indicating the probability of plant mortality over time associated with *Hypogeococcus* sp. when reared on five invasive cactus species from South Africa, including the target weed *Trichocereus spachianus*. Each panel represents one of the five

invasive cacti tested, namely: **a** *Cereus jamacaru*, **b** *Harrisia martinii*, **c** *Harrisia balansae*, **d** *Harrisia pomanensis*, and **e** *Trichocereus spachianus*. Shaded areas indicate the 95% confidence interval of the mean expected probability of mortality

These species exhibited low nymphal survival, prolonged time to maturity, and reduced fecundity on *T. spachianus*, resulting in a low HSI value compared to *Hypogeococcus* sp. on its targeted cactus hosts. The low HSI value indicates that population growth of these *Hypogeococcus* lineages/species on *T. spachianus* would likely be slow, as high survival and fecundity are critical for achieving significant population densities (Augustinus et al. 2020). Based on the low HSI score, these *Hypogeococcus* lineages/species were deemed poor candidates and were rejected from further efficacy testing. Rejecting these unsuitable agents early in the selection process prevented wasting resources on efficacy and host specificity testing on these agents.

On the other hand, the HSI identified a potential candidate based on its compatibility with the target

host plant, *T. spachianus*. *D. confertus* demonstrated a higher HSI score on *T. spachianus* than on its known host plant, *H. pomanensis*. Although several performance parameters were similar between the two cactus species, higher survival rates and more established colonies on *T. spachianus* suggest that *D. confertus* is better suited to this cactus. The HSI value for *T. spachianus* was higher than that for *Hypogeococcus* sp. on its targeted host plants. Since *Hypogeococcus* sp. is an effective agent against its hosts (Muskett et al. 2024), an equal or higher HSI value for *D. confertus* on *T. spachianus* strongly indicates its potential efficacy. Moreover, the high survival, fast reproduction times and high fecundity indicated by the high HSI scores for *D. confertus* on *T. spachianus* are critical for achieving significant population densities, which has been strongly correlated with high

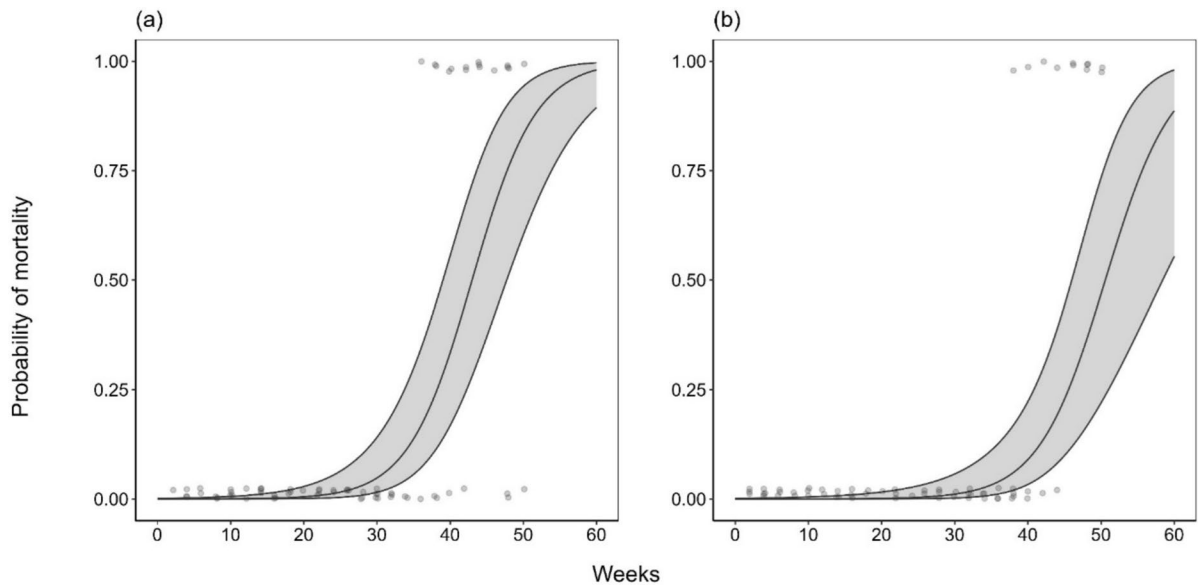


Fig. 3 Marginal effects plots, derived from the fitted GLM, indicating the probability of plant mortality over time associated with *Dactylopius confertus* when reared on two invasive cactus species from South Africa, including the target weed

Trichocereus spachianus. Each panel represents one of the invasive cacti tested, namely: **a** *Harrisia pomanensis*, and **b** *Trichocereus spachianus*. Shaded areas indicate the 95% confidence interval of the mean expected probability of mortality

plant mortality/biological control efficacy for *Hypogeococcus* sp. and other cochineal insects (e.g., Sutton et al. 2018; Jones et al. 2023).

The HSI is an effective rapid screening tool but does not provide a complete picture of an agent's potential efficacy. Therefore, integrating HSI with efficacy trials is essential for a comprehensive assessment. *D. confertus* was identified as the only promising agent due to its high HSI value and was therefore selected for efficacy trials. Mortality caused by *D. confertus* on *T. spachianus* was approximately 88% higher than that caused by *Hypogeococcus* sp., which had less than a 1% probability of causing mortality by the end of the experiment. The mortality caused by *D. confertus* on *T. spachianus* at the end of the experiment was comparable to the highly effective *Hypogeococcus* sp. on its hosts in South Africa. Given the high HSI value for *D. confertus* on *T. spachianus* and the promising efficacy results, we expect that the release of *D. confertus* could achieve the same level of control over *T. spachianus* as *Hypogeococcus* sp. has over its host plants. Other studies that have performed PREAs under greenhouse conditions have found these agents to be very effective in the field. For example, multiple lineages of *Dactylopius*

tomentosus (Lamarck) (Hemiptera: Dactylopiidae) were assessed as potential agents for *Cylindropuntia pallida* (Rose) F.M. Knuth (Caryophyllales: Cactaceae). PREAs identified the 'californica var. Parkerii' lineage as having the greatest potential for success. After its release on large infestations of *C. pallida* in Australia, long-term monitoring data suggest that the cactus will be under complete control within 24 to 36 months (Paterson et al. 2021). This has also been observed in non-cactus biological control efforts. For example, greenhouse studies demonstrated that *Tetramesa romana* Walker (Hymenoptera: Eurytomidae) could significantly impede the growth of *Arundo donax* L. (Poaceae: Arundinoideae) (Goolsby et al. 2009). The release of *T. romana* in the field, following promising PREA results, led to a significant decrease in *A. donax* biomass after five years of monitoring (Goolsby et al. 2016).

Focusing on efficacy for initial screening could prove advantageous for agents that, like cactophagous insects, are likely to be host-specific. PREAs can be an effective early prioritisation tool in biological control programmes, when the target weed has few closely related species in the invaded range or, as with *T. spachianus*, lacks a native-range distribution.

This approach is also particularly useful for invasive hybrids, which often have limited or no native range where potential agents can be sourced. In such cases, combining HSI and PREAs can help identify promising candidate agents early in the process. Examples include *Lantana camara* L. (Lamiales: Verbenaceae) and *Nymphaea mexicana* Zuccarini (Nymphaeaceae), where hybridisation complicates native-range-based agent selection (Williams and Madire 2008; Reid et al. 2023). This approach prioritises assessing an agent's efficacy before conducting detailed host specificity testing and may be particularly valuable for other invasive cactus species in South Africa, as well as for weeds without native congeners in their introduced range.

Our findings suggest that integrating HSI with PREAs can enhance the selection process for biological control agents by ensuring that candidate agents are both host-specific and effective. The higher HSI score and observed plant mortality indicate that *D. confertus* has significant potential as a biological control agent for *T. spachianus*. Therefore, it stands out as a strong candidate for further testing and prioritisation in the control of this invasive species. By using PREAs as a first step in this biocontrol programme, resources were optimised by concentrating host specificity tests on the potentially effective biocontrol agent only. The efficacy of an agent is a useful tool for prioritisation. However, it does not eliminate the need to ensure released agents are host-specific. The safety of biological control relies solely on preventing non-target effects on native or commercially important species once an agent is released (Paynter et al. 2020). Although *D. confertus* is a promising agent, its safety must be confirmed through host specificity testing before it is considered for release.

Acknowledgements The authors wish to thank the following Argentine authorities for the permits obtained during the course of this project: government of Salta (No. 0090227-267680-2017-0), government of Tucumán (No.1888-330-2015), government of Corrientes (No. 00021155), Ministry of Environment (No. CE-2020-66116233-APN-DNBI#MAD) and National Food Safety and Quality Service—SENASA (No. NO-2020-72988681-APN-DNPV#SENASA). The importation of the biological control agent candidates into South Africa was made under a permit from the Department of Agriculture, Forestry and Fisheries (Permit No. P0102395).

Author contributions TCG Investigation, methodology, formal analysis, writing—original draft, writing—review and editing; MBA Supervision, methodology; GFS Formal

analysis, visualization, writing—review and editing; LC Investigation, writing—review and editing; IDP: Conceptualization, funding acquisition, resources, project administration, supervision, writing—review and editing.

Funding Open access funding provided by Rhodes University. This research was funded by the South African South African National Research Foundation Research Chairs Initiative of the Department of Science and Technology (DST) (SARChI #84643) and the National Research Foundation (NRF) of South Africa. Any opinion, finding, conclusion or recommendation expressed in this material is that of the authors, and the funding agencies do not accept any liability in this regard.

Declarations

Conflict of interest All authors certify that they have no affiliations with or involvement in any organisation or entity with any financial or non-financial interest in the subject matter or materials discussed in this manuscript.

Ethical approval The Centre for Biological Control has a general ethical clearance number for all 2024 projects if required: 2024-8120-9068.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

- Albesiano Hoyos AS (2015) Taxonomía y filogenia de *Trichocereus* (Berg.) Riccob. (Trichocereaceae—Cactaceae). PhD thesis, Universidad Nacional de La Plata, Argentina
- Alday PM, Kleinschmidt DF, Arslan AR (2024) Effects.jl, version 1.3. <https://zenodo.org/doi/10.5281/zenodo.7054546>
- Augustinus B, Sun Y, Beuchat C, Schaffner U, Müller-Schärer H (2020) Predicting impact of a biocontrol agent: integrating distribution modeling with climate-dependent vital rates. *Ecol Appl* 30:e02003
- Balcunas JK (2004) Are mono-specific agents necessarily safe? The need for prerelease assessment of probable impact of candidate biocontrol agents, with some examples. XI International symposium on biological control of weeds: 252

- Bates D, Alday P, Kleinschmidt D, Santiago Calderón JB, Zhan L, Noack A, Bouchet-Valat M, Arslan A, Kelman T, Baldassari A, Ehinger B, Karrasch D, Saba E, Quinn J, Hatherly M, Piibeleht M, Mogensen PK, Babayan S, Holy T, Gagnon YL, Nazarathy Y (2024) MixedModels.jl, version 4.26. <https://doi.org/10.5281/zenodo.13754074>
- Bates D, Noack A, Kornblith S, Bouchet-Valat M, Borregaard MK, Arslan A, White JM, Kleinschmidt D, Alday P, Lynch G, Dunning I, Mogensen PK, Lendle S, Aluthge D, Dutta M, pdeffebach, Santiago Calderón JB, Patnaik A, Born B, Setzler B, DuBois C, Quinn J, Slámečka O, Bastide P, Shah VB, Blaom A, König B (2023) GLM.jl, version 1.9. <https://zenodo.org/doi/10.5281/zenodo.3376013>
- Bezanson J, Edelman J, Karpinski S, Shah VB (2017) Julia: a fresh approach to numeric computing. *SIAM Rev* 59:65–98
- Bolker BM, Brooks ME, Clark CJ, Geange SW, Poulsen JR, Stevens MHH, White JSS (2009) Generalized linear mixed models: a practical guide for ecology and evolution. *Trends Ecol Evol* 24:127–135
- Ezeh AE, Hereward JP, Day MD, Taylor T, Furlong MJ (2023) Confirming the identity of the *Hypogeococcus* species (Hemiptera: Pseudococcidae) associated with *Harrisia martinii* (Labour.) Britton (Cactaceae) in Australia: implications for biological control. *Aust Entomol* 62:235–245
- Goeden RD (1983) Critique and revision of Harris' scoring system for selection of insect agents in biological control of weeds. *Protect Ecol* 5:287–301
- Goolsby JA, Moran PJ, Racelis AE, Summy KR, Jimenez MM, Lacewell RD, Perez de Leon A, Kirk AA (2016) Impact of the biological control agent *Tetramesa romana* (Hymenoptera: Eurytomidae) on *Arundo donax* (Poaceae: Arundinoideae) along the Rio Grande River in Texas. *Biocontrol Sci Technol* 26:47–60
- Goolsby JA, Spencer D, Whitehand L (2009) Pre-release assessment of impact on *Arundo donax* by the candidate biological control agents *Tetramesa romana* (Hymenoptera: Eurytomidae) and *Rhizaspidiotus donacis* (Hemiptera: Diaspididae) under quarantine conditions. *Southwest Entomol* 34:359–376
- Griffith TC (2024) Biological control of torch cactus in South Africa: finding a suitable agent for a non-native weed with an unknown indigenous distribution. PhD Thesis, Rhodes University
- Harris P (1973) The selection of effective agents for the biological control of weeds. *Can Entomol* 105:1495–1503
- Hill MP, Conlong D, Zachariades C, Coetzee JA, Paterson ID, Miller BE, Foxcroft L, van der Westhuizen L (2021) The role of mass-rearing in weed biological control projects in South Africa. *Afr Entomol* 29:1030–1044
- Hokkanen HM, Sailer RI (1985) Success in classical biological control. *Crit Rev Plant Sci* 3:35–72
- Hokkanen HM, Pimentel D (1984) New approach for selecting biological control agents. *Can Entomol* 116:1109–1121
- Jones PK, Day MD, Mcconnachie AJ, Holtkamp RH, Conroy J, Pidgeon G, Clark E, Clark P, Fletcher A, Savage M (2023) The successful biological control of *Cylindropuntia fulgida* var. *mamillata* (A. Schott ex Engelm.) Backeb. (Cactaceae) (coral or boxing glove cactus) at two field sites in Queensland, Australia. *Biol Control* 182:105235
- Kiesling R (1978) El género *Trichocereus* (Cactaceae): I: Las especies de la Rep. Argentina. *Darwiniana* 21:263–330
- Klein H, Zimmermann HG, Xivuri T (2020) Exceptional biological control of two varieties of *Cylindropuntia fulgida* (Cactaceae) in South Africa using a recently identified different biotype of the cochineal insect, *Dactylopius tomentosus* (Dactylopiidae). *Biol Control* 149:104314
- Korotkova N, Aquino D, Arias S, Eggli U, Franck A, Gómez-Hinostrosa C, Guerrero PC, Hernández HM, Kohlbecker A, Köhler M, Luther K, Majure LC, Müller A, Metzger D, Nyffeler R, Sánchez D, Schlumberger B, Berendsohn WG (2021) Cactaceae at Caryophyllales.org – a dynamic online species-level taxonomic backbone for the family. *Willdenowia* 51:251–271
- Maw MG (1976) Biology of the tortoise beetle, *Cassida hemisphaerica* (Coleoptera: Chrysomelidae), a possible biological control agent for bladder campion, *Silene cucubalus* (Caryophyllaceae), in Canada. *Can Entomol* 108:945–954
- Mcclay AS, Balciunas JK (2005) The role of pre-release efficacy assessment in selecting classical biological control agents for weeds—applying the Anna Karenina principle. *BioControl* 35:197–207
- Mcfadyen REC (1998) Biological control of weeds. *Annu Rev Entomol* 43:369–393
- Muskett P, Sutton GF, Paterson ID (2024) Long-term post-release evaluation shows *Hypogeococcus* sp. is an effective biological control agent for queen of the night cactus in South Africa. *Biocontrol Sci Technol* 34:915–920
- Paterson ID, Klein H, Muskett PC, Griffith TC, Mayonde S, Mofokeng K, Mnqeta Z, Venter N (2021) Biological control of *cactaceae* in South Africa. *Afr Entomol* 29:713–734
- Paynter Q (2024) Prioritizing candidate agents for the biological control of weeds. *Biol Control* 188:105396
- Paynter Q, Paterson ID, Kwong RM (2020) Predicting non-target impacts. *Curr Opin Insect Sci* 38:79–83
- Poveda-martínez D, Aguirre MB, Logarzo G, Hight SD, Triapitsyn S, Diaz-sotero H, Diniz Vitorino M, Hasson E (2020) Species complex diversification by host plant use in an herbivorous insect: the source of Puerto Rican cactus mealybug pest and implications for biological control. *Ecol Evol* 10:10463–10480
- R Core Team (2024). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.rproject.org/>
- Reid MK, Paterson ID, Coetzee JA, Gettys LA, Hill MP (2023) Know thy enemy: investigating genetic contributions from putative parents of invasive *Nymphaea mexicana* hybrids in South Africa as part of efforts to develop biological control. *Biol Control* 184:105291
- Sheppard AW (2003) Prioritising agents based on predicted efficacy: beyond the lottery approach. In: Spafford Jacob H, Briese DT (eds) Improving the selection, testing and evaluation of weed biological control agents, Technical Series No. 7. CRC for Australian Weed Management, Glen Osmond, pp 11–21
- Sutton GF, Canavan K, Day MD, Paterson ID (2021) Field-based ecological studies to assess prospective biological control agents for invasive alien plants: an example from giant rat's tail grass. *J Appl Ecol* 58:1043–1054

- Sutton GF, Klein H, Paterson ID (2018) Evaluating the efficacy of *Hypogeococcus* sp. as a biological control agent of the cactaceous weed *Cereus jamaru* in South Africa. *Biocontrol* 63:493–503
- TAG-BCAW Manual (2021) Technical advisory group for biological control agents of weeds manual. USDA, Washington, DC, USA. Available from: <https://www.aphis.usda.gov/sites/default/files/tag-bcaw-manual.pdf>
- van Dam AR (2013) Species delimitation of the cochineal insects (*Dactylopius*) using molecular phylogenetics and morphology. PhD thesis, University of California
- van Klinken RD, Raghu S (2006) A scientific approach to agent selection. *Aust J Entomol* 45:253–258
- Williams HE, Madire LG (2008) Biology, host range and varietal preference of the leaf-feeding geometrid, *Leptostales ignifera*, a potential biocontrol agent for *Lantana camara* in South Africa, under laboratory conditions. *BioControl* 53:957–969
- Zachariades C (2021) A catalogue of natural enemies of invasive alien plants in South Africa: classical biological control agents considered, released and established, exotic natural enemies present in the field, and bioherbicides. *Afr Entomol* 29:1077–1142

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

T. C. Griffith has interests in the biological control of invasive plants and insects and conducted her recent post-doctoral research on the host specificity and pre-release efficacy of potential biocontrol agents for the control of *Trichocereus spachianus*, an invasive cactus in South Africa.

M. B. Aguirre specializes in applied entomology and ecology, with emphasis on the use of natural enemies for the biological control of insect pests and the study of insect–plant interactions.

G. F. Sutton is a senior research entomologist at the Centre for Biological Control at Rhodes University, South Africa. His research primarily deals with the prioritisation and development of novel weed biological control agents, with particular emphasis on the biological control of invasive grasses.

L. Chari has interests in native range surveys and ecological interactions between prospective agents and their plant hosts. Previously, as a post-doctoral researcher, he investigated biological control strategies to manage weeds. Currently, he explores community ecology dynamics in freshwater ecosystems, with an interest to apply this expertise to biological control contexts.

I. D. Paterson is a professor at Rhodes University, South Africa where he is a research scientist at the Centre for Biological Control. His research focus is biological control of invasive alien plants.