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Biological control of torch cactus in South Africa: Finding a suitable agent for a non-native weed with an unknown indigenous distribution.

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Abstract

Trichocereus spachianus is an invasive cactus species in South Africa and poses challenges as a target for biological control due to confusion around its taxonomy and origin. Adapted to arid environments, this cactus is of particular concern in dry savannah and Karoo biomes, where its invasion of rangelands reduces grazing capacity for both indigenous wildlife and livestock. While previous records indicate that *T. spachianus* is indigenous to Argentina, recent field surveys have failed to verify its presence. Determining the origin of the target weed was important in developing a biological control programme since it enables the collection of potential agents directly from native populations of the target plant. Successful biological control programmes against cactus species in South Africa have often involved utilising both new associations and oligophagous insects, made possible because of the lack of indigenous and valued cacti in the region. Lack of *T. spachianus* locations in the native distribution, meant direct collection of insects from the target weed was not possible. Efforts to find biological control agents were focused on new associations between closely related cacti and their oligophagous herbivores. Suitability of multiple *Hypogeococcus* (mealybug) entities and a cochineal species, *Dactylopius confertus*, were investigated for their efficacy on various South African weedy cactus species, including *T. spachianus*. Findings revealed that none of the *Hypogeococcus* entities (species or lineages) were effective biological control agents, but *D. confertus* demonstrated potential as a biological control agent with a relatively high fecundity and survival rate on *T. spachianus*. Efficacy trials indicated that *D. confertus* could reach population densities sufficient to cause mortality of *T. spachianus* plants. Limited host specificity trials revealed that *D. confertus* was suitably host specific for release in South Africa, provided host specificity testing was conducted on additional plants. Approval and release of *D. confertus* has the potential to reduce the invasiveness of this damaging cactus in a sustainable and environmentally friendly manner.

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List of Abbreviations

%	Percentage
°	Degree(s)
′	Minute(s)
″	Second(s)
AICc	Akaike’s Information criterion corrected for small sample sizes
AUD	Australian dollar
CAM	Crassulacean Acid Metabolism
CLMM	Cumulative link mixed model
cm	Centimetre
E	East
ERH	Enemy Release Hypothesis
GLM	Generalised linear model
GLMM	Generalised linear mixed model
h	Hour
Ha	Hectare
HAD	Host-associated genomic differentiation
HSI	Host Suitability Index
USA	United States of America
USD	United States dollar
LRT	Likelihood Ratio Test
NEM:BA	National Environmental Management: Biodiversity Act (2004)
NZ$	New Zealand dollar
PREA	Pre-release efficacy assessment
SAPIA	Southern African Plant Invaders Atlas
S	South
Spp.	Species

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Chapter One
General Introduction

1.1. Cactaceae

The Family Cactaceae is native to the New World, with representative species being found from Patagonia (southern Argentina) to the southwestern parts of Canada (Novoa *et al.*, 2015b). No native cactus is found in the Old World except for one species of epiphytic cactus, *Rhipsalis baccifera* (J. S. Miller) Stearn (Caryophyllales: Cactaceae) (mistletoe cactus) (Oulo *et al.*, 2020). in Europe, eight *Rhipsalis* species in tropical Africa and one in Sri Lanka (Britton & Rose, 1919). The origin of *Rhipsalis* species found outside the New World is a topic of debate. The possibility that they may have been transported by migratory birds from the New World raises questions about their native or introduced status (Wallace & Gibson, 2002; Rebman & Pinkava, 2001). Many cactus species have been introduced by human activity outside their native distribution and many become naturalised or invasive (Novoa *et al.*, 2015a, 2015b). Cacti introductions have occurred for various reasons, such as horticultural purposes, drought-tolerant crops and for hedging (Novoa *et al.*, 2015a, 2015b; Witt *et al.*, 2017).

Cacti play an important role in agriculture worldwide, particularly in Mexico, USA, Spain, Italy, and northern Africa. These crops have various uses, including medicinal, fruit production, vegetable crops, and as livestock feed (Shetty *et al.*, 2012). Extracts from *Lophophora williamsii* (Lemaire) Coulter, have been used to stimulate the central nervous system and regulate blood pressure (Shetty *et al.*, 2012). The extract from *Opuntia ficus-indica* (L.) Miller (Caryophyllales: Cactaceae) fruit is a traditional remedy for burn wounds, indigestion, and fluid retention (oedema), as well as a popular commercial edible fruit. Fruit of other cactus species like *Hylocereus undatus* (Haw.) Britton & Rose (dragon fruit) and *Myrtillocactus geometrizans* (Mart. ex. Pfeiff.) (garambullos) are also widely consumed. *Stenocereus griseus* (Haw.) Buxbaum (pitaya) has been used in southern Mexico's diet since pre-colonial times. Spineless *Opuntia* plantations for nopalito (a vegetable made from young stems) production is grown in Mexico and Texas, USA (Shetty *et al.*, 2012). Cactus is also widely used as livestock feed in many parts of the introduced range (e.g., Algeria and

Tunisia), as well as the indigenous distribution (e.g., Brazil and Mexico), especially during dry seasons (Nefzaoui, 2010). *Opuntia* spp. are important crops for subsistence farmers in semi-arid areas as they are easy to grow, drought-tolerant, and palatable. The cladodes, a modified stem in certain plants that functions like a leaf, of *Opuntia* spp. can be fed fresh or dried to livestock, providing a supplementary feed source during droughts (Nefzaoui, 2010).

Although cacti are important agricultural crops, the horticulture trade is also one of the main contributors to the widespread global introduction of cacti. The horticultural industry has gained significant momentum (Novoa *et al.*, 2015b) with an annual revenue in the USA exceeding 11 billion USD, with cactus trade alone contributing about 3.7 million USD (Novoa *et al.*, 2015a). The growth of this industry is driven by the growing demand by breeders to create new cultivars and collectors to acquire unique ornamental plants from various parts of the world. The majority of traded plants are not native to their target markets. Non-native, or alien, species are plants introduced to areas outside their natural range, usually through human intervention, either intentionally or accidentally (Vilà *et al.*, 2011). Many non-native plants that are globally traded do not have adverse environmental effects, and often remain in their intended locations (Niemiera & Holle, 2009). Plants that possess desirable characteristics, such as drought tolerance, high germination, and fast growth rates, are frequently chosen as ornamental plants. While these traits make cactus plants popular in the trade, they can also increase the likelihood of certain species becoming invasive, as they are already adapted to the new environment, allowing them to rapidly establish and propagate. Most growth forms of invasive cacti, including angled, cylindrical, flattened-padded, or sprawling, can also grow vegetatively, further increasing their invasive potential (Novoa *et al.*, 2015b).

The spread of Cactaceae around the world has been facilitated by their beneficial attributes, but there are also negative consequences associated with these introductions and several species of this family are now considered some of the most important alien species worldwide. Approximately 57 invasive cactus species are distributed across 22 countries (Novoa *et al.*, 2015b). Alien plants that become established in their new environment and reproduce without human assistance can become invasive, resulting in the overabundance of the plant in the introduced range and negative impacts to native species, ecosystems, and even human and animal welfare (Vilà *et al.*, 2011). Invasions can cause a decline in biodiversity, compete for resources like water, light, and oxygen, disrupt nutrient cycling, and harm ecosystem services (Coetzee *et al.*, 2007; Gooden *et al.*, 2009; Hejda *et al.*, 2009; Walters *et al.*, 2011; Vilà *et al.*, 2011; Paterson *et al.*, 2011; Wilson *et al.*, 2017).

1.1.1. Negative impacts of cacti

Invasive cacti have negative impacts, both environmentally and socially. Their suitability to arid environments and ability to survive in areas prone to drought make them particularly effective invaders in dry areas. Invasion by some species may start with low numbers at only a few locations before rapidly increasing in numbers, a phenomenon known as lag phase (Aikio *et al.*, 2010; Crooks *et al.*, 1999). Many cactus species have a long lag phase, lasting over 50 years, and often go unnoticed until the species becomes a problem (Wilson *et al.*, 2017). Cacti can spread vegetatively, shortening the lag time, quickly leading to dense, invasive patches (Wilson *et al.*, 2017).

The invasions of cacti can be harmful to native wildlife in the invaded areas. For example, *Pereskia aculeata* Miller (Caryophyllales: Cactaceae), an invasive cactus vine, is a threat to the indigenous forests in the subtropical and tropical eastern coastal regions in South Africa. The cactus covers and kills the native plants, even causing large indigenous trees to break from the weight of the heavy vines (Paterson *et al.*, 2011). *Opuntia stricta* (Haw.) Haworth (Caryophyllales: Cactaceae) is another example of a cactus invasion that has negative impacts. *Opuntia stricta*, native to North America, mainly invades savannas and arid grasslands, and is considered a transformer species (Witt *et al.*, 2017). Transformer species are defined as invasive plants that alter the character, condition, form, or nature of ecosystems on a significant scale compared to the size of the ecosystem itself (Richardson *et al.*, 2000). The introduction of *O. stricta* resulted in a decrease of native biodiversity in Madagascar, including grasses, herbs, and trees, as it inhibits their growth and regeneration (Larsson, 2004).

The introduction of invasive cacti has caused numerous negative impacts on livestock, small animals, and people (Walters *et al.*, 2011; Witt *et al.*, 2017). *Opuntia* species not only have large spines, but they also contain spine-like hairs called glochids that can be found on the cladodes and fruit. Studies in Kenya and Madagascar have shown that livestock that consume the fruit of *O. stricta* can experience negative health impacts such as weight loss and potential death due to the presence of glochids in their lips, mouths, and intestines. Milk production of female animals was also decreased in areas invaded by *O. stricta* (Larsson, 2004; Shakleton *et al.*, 2017; Witt *et al.*, 2017). Ecological and economic impacts are well documented, but it is equally important to consider the social impact of cactus invasions. For example, a study by Shakleton *et al.*, (2017) in Laikipia County, Kenya found that peoples' livelihoods were impacted as they could no longer sell tripe because of the presence of glochids in animals' intestines (Shakleton *et al.*, 2017). Other negative impacts experienced by residents included restricted movement to water sources, homesteads, and grazing lands, loss of medicinal plants, and skin and eye irritation from contact with glochids. In some cases, land was abandoned due to the severity of cactus invasions (Witt *et al.*, 2017). Similarly, in Madagascar, the encroachment of *O. stricta* invasions on roads and villages restricted villagers' movement (Larsson, 2004).

Australia has the largest number of invasive cactus species, 39 in total (Novoa *et al.*, 2015b; Novoa *et al.*, 2019), and their invasions have several negative effects. Some cactus species have spread extensively in Australia, such as *Opuntia aurantiaca* Lindley (Caryophyllales: Cactaceae), jointed cactus, which covers an area of 200,000 ha in New South Wales, and *Opuntia robusta* Wendl. (Caryophyllales: Cactaceae), which has invaded an area of 35,000 ha in South Australia. These invasions incur costs for control and management, as well as veterinary expenses for livestock injuries caused by cactus spines. The grazing industry also experienced a decrease in production (Lloyd & Reeves, 2014). Invasions limit access to water sources for livestock and lead to the loss of biodiversity and harm to wildlife. For example, in Western Australia, bats have been impaled on spines of *Cylindropuntia fulgida* var. *mamillata* (A. Schott ex Engelm.) Backeberg (Caryophyllales: Cactaceae) (Lloyd & Reeves, 2014).

In South Africa, cactus invasions are prevalent, the country has the second-highest number of invasive cactus species globally (35) (Novoa *et al.*, 2015b; Novoa *et al.*, 2019) and they pose a threat to the dry inland areas of the country. Before control measures were taken, *O. ficus-indica* was spread over 900,000 ha, with 500,000 ha of dense infestations leading to vast areas of land being deemed unsuitable for agricultural practices (Zimmermann & Moran, 1991). *Opuntia cespitosa* Raf. (Caryophyllales: Cactaceae) invades arid grasslands and savannas, posing a threat to native plants of conservation concern and indirectly impacting livestock by reducing grazing land with its dense, low-growing mats that cover vegetation (O'Connor & van Wilgen, 2020). The dense glochids on its cladodes can negatively impact wildlife and livestock. *Cylindropuntia pallida* (Rose) F.M. Knuth (Caryophyllales: Cactaceae) is found in the arid regions of the Karoo and Kalahari and causes dense, extremely spiny infestations that pose a direct threat to wildlife and livestock and reduce available grazing areas (Paterson *et al.*, 2021b).

In South Africa, *Opuntia stricta* also invaded the Kruger National Park (KNP), which is considered a flagship wildlife reserve. One of the major threats to the park's native biodiversity is invasive plant species (Foxcroft & Freitag-Ronaldson, 2007). The first record of *O. stricta* was reported in the vicinity of Skukuza in the KNP in 1953. By 1980, the invasive cactus had taken over an area of roughly 1000 hectares, and by 1990, this had grown to almost double that size (Lotter & Hoffmann, 1998). The invaded area has since reached 66 000 ha making *O. stricta* one of the most widespread invasive plants in KNP (Foxcroft *et al.*, 2004). Robertson *et al.* (2011) studied the effects of these invasions on spider and beetle diversity, as arthropods play a crucial role in regulating many key processes essential to ecosystem functioning (Wilson, 1987). The study found that while *O. stricta* invasions did not affect spider diversity, the beetle population in invaded areas was significantly different from uninvaded areas, potentially leading to further consequences within the ecosystem.

Opuntia aurantiaca, native to South America, is another invasive species in South Africa. This low-growing, many-jointed plant can cause harm to livestock due to its detachable joints that can become embedded in the animal's fur and skin (Shakleton *et al.*, 2007). Large infestations of this cactus leads to reduced grazing lands. Residents of Tidbury village in the Kat River valley report that besides posing a threat to livestock with potentially lethal injuries, the plant also endangers barefoot children playing in the fields (Shakleton *et al.*, 2007). The negative effects of cactus invasions are severe, and many landowners try removing them or limit their spread. These efforts can require significant investments of both time and money and often require ongoing management.

1.1.2. Control methods

Mechanical removal is a method used to manage cactus invasions, which involves physically removing the plants using equipment like spades or bulldozers. The removed plants must be treated properly to avoid regrowth, such as soaking them in water or drying and burning them (Novoa *et al.*, 2019). This technique can be costly and time-consuming, especially for large infestations, and requires thorough cleaning of equipment to prevent the dispersal of seeds or fragments. Because cacti can spread both sexually and vegetatively, areas where cacti have been removed need to be monitored regularly for several years to prevent resprouting of the plants (Novoa *et al.*, 2019). Mechanical removal is only suitable for small-scale cactus invasions, given the cost and time it entails.

The use of herbicides for chemical control is also a method employed in cactus population management. However, the application of herbicides can be limited by country-specific regulations and restrictions. For instance, some chemicals used in South Africa and Australia are banned in Europe and cannot be used near water sources or in protected areas (Novoa *et al.*, 2019). Foliar spray of herbicides may not be as effective on cacti as other plants, due to a thick waxy layer that forms part of the stem cuticle and the habit of closing the stomata during the day due to Crassulacean Acid Metabolism (CAM) photosynthesis which inhibits the entry of the herbicide into the plant (Novoa *et al.*, 2019). Ensuring that the entire plant is thoroughly sprayed during treatment can inadvertently result in the native flora being sprayed as well. Injection of the herbicide into the cactus stem may reduce non-target effects from foliar spray and quickly spread to all parts of the plant to ensure complete coverage. However, access to the cactus stems may be limited by spines and smaller branches (Novoa *et al.*, 2019).

Chemical control may not always be an option for some cactus species, due to their unique growth form and other limitations. For example, *P. aculeata*, which grows intertwined with native vegetation, is not suitable for chemical control as the foliar spray treatment will have non-target impacts by killing native plants. Additionally, injecting these plants can be challenging, as they often grow in inaccessible areas such as cliffs or the canopy of trees (Paterson *et al.*, 2011; Muskett *et al.*, 2020). Herbicides are ineffective against certain cactus species such as *Harrisia* species and *O. cespitosa*, as these chemicals cannot target the large and persistent underground tubers that allow the plants to rapidly regrow (Klein, 1999). Furthermore, the ability of cacti to reproduce vegetatively means that any missed fragments will grow back, requiring frequent and sustained follow-up treatments, particularly for species that easily fragment, such as *O. aurantiaca* (Paterson *et al.*, 2011).

Both mechanical and chemical control methods are not sustainable in the long-term and continuous follow-up treatments are required. Biological control has proven to be effective against several cactus species and is considered the most viable solution for reducing cactus invasion density and curbing further spread.

1.2. Principles of biological control

Plants in their native habitats are subject to top-down control from natural enemies such as herbivorous insects. One hypothesis that might explain how non-native plants can become invasive is the Enemy Release Hypothesis (ERH) (Kean & Crawley, 2002). The introduction of a plant into a new area often leads to the absence of specialised insects, which typically regulate plant populations in their native range. As a result, the introduced plant escapes herbivore pressure which results in a competitive advantage (Allen *et al.*, 2014). For example, *Acacia longifolia* (Andr.) Willd (Fabales: Fabaceae), native to Australia, is an aggressive invader in South Africa, while *Chrysanthemoides monilifera* (L.) T. Norl (Asterales: Asteraceae), native to South Africa, is invasive in Australia. The invasiveness of these species in their introduced ranges is believed to be a result of their abundant soil seed banks (Weiss, 1987). A study comparing the seed banks of both species in their native and introduced ranges found that *C. monilifera's* seed bank was 30 times greater in Australia than in South Africa, and *A. longifolia's* seed bank was 500 times greater in South Africa compared to its native range in Australia (Weiss, 1987). The high seed bank numbers in the introduced ranges were attributed to the absence of seed predators. Pieterse & Cairns (1988) also studied the factors affecting reproductive success of *A. longifolia* in both South Africa and Australia. Factors such as pollination failure, resource deficiency, seed predation, and seed development failure due to genetic defects were investigated. The findings suggested that seed predation played a significant role in influencing the number of viable seeds in both Australia and South Africa, accounting for 34% of non-viable seeds recorded in Australia but almost no seed predation was detected in South Africa. This lack of predation led to a large soil seed bank of *A. longifolia* in its invaded range in South Africa (Pieterse & Cairns, 1988). The same was found for *A. longifolia* invasions in Portugal, with pre-dispersal seed predation being 12% higher in the native range compared to the introduced range (Correia *et al.*, 2016). Biological control aims to address the release from natural enemies by introducing host-specific insects from the native range of the invasive plant to the introduced range thus introducing top-down control.

Introducing a host-specific biological control agent reduces the possibility of causing indirect harm to the native biota. A host-specific herbivore is one that can only feed and complete its life cycle on a limited range of host plants, usually one or a few closely related species (Zimmermann *et al.*, 2004). If the agent is successful, the introduction will result in a significant reduction in the invasive plant populations, causing a subsequent decrease in the control agent population as the target plant is the only source of food available (Briese, 2000). Over time, the populations of both the agent and the plant will continue to fluctuate, but if the agent is effective, the plant populations will be permanently reduced, limiting the negative impacts of the invasion (Zachariades *et al.*, 2017; Hoffmann *et al.*, 2019). Biological control has been considered one of the most effective methods for controlling invasive plants because the released agent becomes a permanent part of the ecosystem, often leading to permanent control. The long-term sustainability of biological control compared to other control methods therefore contributes to its success (McFadyen, 1998; McQueen *et al.*, 2001).

The efficacy of numerous biological control programmes highlights the significance of top-down regulation by natural enemies in controlling host plant populations

(McFadyen, 1998). In addition to its efficacy, biological control is recognised as an environmentally friendly, cost-effective, and safe control method (McFadyen, 1998; Moran *et al.*, 2013). Nevertheless, the safety and potential for direct and indirect non-target effects on native plants remain a concern in the implementation of biological control (Hinz *et al.*, 2020). The fear of non-target effects has resulted in a decrease in support and utilisation of biocontrol in some countries, particularly Europe and the USA (Moran *et al.*, 2013). Despite this, biological control of weeds using invertebrate and pathogen agents, has a strong record of safety with less than 1% of all agents released globally resulting in direct non-target effects (Paynter *et al.*, 2004; Suckling & Sforza, 2014; Moran & Hoffmann, 2015; Hinz *et al.*, 2019). Critiques of the dangers linked to the biological control of invasive plants might be deceptive as they arise from unusual agent introduction histories that have occurred (Downey & Paterson, 2016).

Some negative impacts have been recorded from agents that were either not intentionally introduced or released at a time when the predicted non-target effects were considered less important than they are today (Delfosse, 2005; Fowler *et al.*, 2000; Moran & Hoffmann, 2015). Four examples often cited as non-target effects associated with biocontrol include *Chrysolina hyperici* Forster (Coleoptera: Chrysomelidae), *Rhinocyllus conicus* Frölich (Coleoptera: Curculionidae), *Larinus carlinae* Olivier (= *L. planus* Fabricius) (Coleoptera: Curculionidae), and *Cactoblastis cactorum* Berg. (Lepidoptera: Pyralidae) (Fowler *et al.*, 2000; Hinz *et al.*, 2020). All of which were released when possible negative impacts to native species by biocontrol agents was not considered as important as it is now and the benefit of releasing them was considered more important than the risks associated with their release (Delfosse, 2005; Downey & Paterson, 2016). *Chrysolina hyperici*, initially introduced to combat *Hypericum perforatum* L. (Malpighiales: Hypericaceae) (St. John's wort) in New Zealand, inadvertently targeted a non-target plant, *Hypericum androsaemum* L. (Malpighiales: Hypericaceae) (Julien & Griffiths, 1998). This non-target feeding was anticipated during host specificity trials. However, the impact it has on this non-target species is deemed negligible in terms of the damage inflicted. Initially released to control invasive *Carduus* thistles in North America, *R. conicus* inadvertently impacted native thistles as well. *Larinus carlinae* was released to control *Cirsium arvense* (L.) Scop. (Asterales: Asteraceae) but its unintended consequence has had a notable impact on the seed production of an endangered thistle species, *Cirsium pitcher* [Torr.]

Torrey & Gray (Asterales: Asteraceae), commonly referred to as Pitcher's thistle (Warneke *et al.*, 2020). Despite their unintended effects on non-target species, both *R. conicus* and *L. carlinae* are regarded as beneficial biological control agents. In contrast, *C. cactorum* is classified only as a pest in the USA and has become invasive in several regions, including the Florida Keys and southwestern USA (Zimmermann *et al.*, 2004; Jezorek *et al.*, 2012). *Cactoblastis cactorum* spread to Florida and the USA after its introduction in the Caribbean (Bennett & Habeck, 1992; Pemberton & Liu, 2007). Because adults have a high dispersal capacity it has the potential to spread further and eventually reach Mexico threatening the large industries based on *Opuntia* there (Zimmermann *et al.*, 2000; Holt & Hochberg, 2001). The moths spread into southern USA and Mexico also threatens the more than 60 endemic species known from these areas (Zimmermann *et al.*, 2004). While the release of *C. cactorum* in the Caribbean was a mistake, it is feeding only on the plants it was originally predicted to target, so there has been no change in the predicted host range of the species (Fowler *et al.*, 2000). It is important to avoid the misconception that the host range of these insects was incorrectly predicted by host specificity testing or that the host range has changed (Arnett & Louda, 2002; Hinz *et al.*, 2019)

Considering all potential risks associated with introducing biological agents is important to maintain the safety of biological control, as the release of an agent is irreversible and could result in significant negative impacts. Risk assessments that include weighing the risks of releasing the agent against the risks of not acting and allowing the target weed to continue to spread should be conducted (Shaw *et al.*, 2011; Downey & Paterson, 2016). Pre-release studies can assess potential risks associated with the release of a particular agent by predicting non-target hosts for the agents (van Klinken, 1999). Host-specificity tests have become more rigorous and are far more likely to predict potential non-target effects than in the past (Paynter *et al.*, 2015, 2018, 2020; Paynter & Teulon, 2019). Only agents that are suitably host-specific to the target weed and do not pose a threat to closely related, indigenous, or commercially valuable plant species are approved for release thus ensuring the safety of biological control (Briese, 2000). Consideration should extend beyond the country of release to include the entire region the agent may spread to, as it is unlikely to be confined by political boundaries (Pratt & Center, 2012).

1.3. Developing a biological control programme

The development of a new biological control programme may take several years to complete. Determining the taxonomy of the biological control target is important as an incorrect identification of the pest can result in a misidentification of its native range, where effective natural enemies are expected to be located (De Moraes, 1987). Once the taxonomy is established, surveys are conducted in the target weed's native range to investigate host-specific insects associated with the plant. These native range surveys are followed by assessments of host specificity and potential impact (Schroeder *et al.*, 1996), usually conducted in quarantine in the plant's introduced distribution.

Before an agent is released into the field, host specificity testing must be conducted. The level of host specificity required for each agent is determined based on the composition of the native flora in the introduced distribution and the presence of closely related species that may be utilised for commercial purposes, such as crops (van Klinken & Edwards, 2002; Zimmermann *et al.*, 2004). The host specificity trials in quarantine include both choice and no-choice trials (Schaffner, 2001). No-choice trials provide a highly conservative estimate of the fundamental host range, i.e., the widest possible range of hosts that the insect can feed on. Choice trials, on the other hand, offer a means to provide a more pragmatic estimate of the likely host range, which is valuable for understanding the potential feeding preferences of the insect in the field. Choice tests are often conducted using the test plants that were accepted during the no-choice trials and provide more insight into the plants that the insect would accept under more natural conditions (Schaffner, 2001).

The development of an appropriate host specificity procedure involves several key considerations. Firstly, it is necessary to determine the most appropriate life stage of the insect to undergo host specificity testing as different stages may exhibit different feeding behaviours and host plant preferences. Secondly, the important part of the plant to be tested must also be determined, with consideration given to whether entire plants or only a portion of the plant are necessary for the test. Lastly, the decision must be made regarding which plants should undergo testing, including whether closely related species to the target plant should be the only focus or if plants from less closely related families to the target plant, with economic importance should also be tested (van Klinken, 1999).

Test host plant selection is fundamental to the success of host specificity testing. Biological control programmes followed the method proposed by Wapshere (1974); the centrifugal phylogenetic method which entails a sequence of test plants, starting with the most closely related plant species and moving to more distantly related species until the full host range of the agent is determined. Closely related species are more likely to be accepted as hosts by the agents as the physical and chemical properties of these are likely to be more similar to the target plant than distantly related species. Although this method is a solid foundation for plant selection, more and more research is being done into factors than can be included to better predict non-target effects (Paynter *et al.*, 2020). Factors such as genetic relatedness and taxonomic relatedness as well as the biogeographic overlap of the related plants have been proposed to be included in test plant selection (Briese *et al.*, 2002; Briese, 2003). Research into the importance of olfactory and visual cues in host plant selection has also been suggested (Park *et al.*, 2018). Secondary metabolites amongst potential host plants may be more important than phylogenetic relationships in the selection of host plants by agents (Kergoat *et al.*, 2005; Rapo *et al.*, 2019; Barrett *et al.*, 2021; Wheeler *et al.*, 2021). Test plants with similar volatiles could be included during host specificity trials to help predict potential non-target effects. Although there is always room for improvement in research, following the standardised host specificity procedures will continue to ensure the safety of biological control programmes.

The results of host specificity testing can result in false positives or false negatives that can be caused by several factors. False positives occur when a host plant is attacked in the test, even though the insect would not target that plant in the field after release. Conversely, false negatives may arise when there is no attack on a plant species recorded in the test, but the insect will attack the plant after it is released (Marohasy, 1998; Heard, 1999). The interpretation of no-choice trials must be done with care, as they are very conservative and may result in the rejection of agents that are safe due to false positives. Under no choice experiments an agent might feed on a plant that it would otherwise avoid in its natural habitat, even in the absence of its primary host plant. Choice trials may also lead to false positives or negative results. Some insects use olfactory cues to locate hosts, and in quarantine settings, decreased air movement can lead to a mix of volatiles from non-host plants with host plants. Consequently, insects might land on and attempt to feed on hosts that they are unlikely to be attracted to under natural field conditions (Macrohasy, 1998). For example, The Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae), is attracted to the volatiles produced by its host plant. Thiery & Visser (1986) conducted a wind tunnel study and found that the odour of non-host plants can inhibit the upwind responses of both inexperienced and experienced female beetles towards the odour of their host plant. False positives can result when volatiles from the host plant are absorbed onto the surfaces of test plants. This can cause insects to respond positively to the odour, leading them to accept non-hosts for feeding or oviposition (Thiery & Visser, 1986; Heard, 1999; van Driesche & Murray, 2004; Sutton *et al.*, 2017).

However, host specificity serves as just one filter in the prioritisation of agents. The process of identifying the most suitable agent for release can be challenging, with no assurance that the released agents will establish or effectively control the target species (McClay & Balciunas, 2005). Another important filter for prioritisation, therefore, is the potential efficacy of the agent. While there are concerns about the added cost of pre-release efficacy assessments in screening biological control agents, using pre-release efficacy assessments (PREAs) as an initial filter for prioritising agents can enhance cost-effectiveness compared to relying solely on host-specificity tests (McClay & Balciunas, 2005). Despite arguments against the predictability of an agent's efficacy, certain criteria must be met before an agent is released. Plant and insect parameters are assessed to evaluate the impact of the potential agent on the plant. This allows for the identification of the most damaging potential agent, or combination of potential agents, to be chosen for release (Balciunas, 2004; McClay & Balcianus, 2005).

The development and implementation of biological control agents play a crucial role in managing and mitigating the impact of invasive species. By identifying and introducing effective and safe agents, successful invasion management can be achieved while minimising harm to non-target species and the environment. This approach offers a sustainable and environmentally friendly alternative to conventional control methods, contributing to the preservation of biodiversity and the restoration of ecosystems.

1.3.1. Biological control of cactus in South Africa

Cacti have become prevalent invasive species in South Africa and the use of biological control against them has had a high success rate compared to other invasive terrestrial plants (Moran & Zimmermann, 1991; Paterson *et al.*, 2011). Approximately 35% of cacti control programmes have resulted in complete control, i.e., no other control measures are needed to reduce the weed populations to acceptable levels (Hoffman, 1995; Klein, 2011), compared to an average of 23% for other terrestrial plants (Moran *et al.*, 2021). Most successful cactus biological control programmes in South Africa utilise either cochineal insects (*Dactylopius* species) or the galling mealybug (*Hypogeococcus* sp. (Hemiptera: Pseudococcidae)) as agents (Table 1.1).

One of the most successful and well documented control programmes in South Africa is that implemented against *O. ficus-indica*, commonly known as prickly pear. *Opuntia ficus-indica* is native to Mexico and was introduced into South Africa more than 250 years ago. This species invaded areas mostly in the Eastern Cape Province and the Karoo where it became one of the most important weeds in South Africa. Several agents have been released against *O. ficus-indica* over the years, including *C. cactorum*, the cochineal *Dactylopius opuntiae* (Cockerell) (Hemiptera: Dactylopiidae), as well as two borer beetles, *Metamasius spinolae* Gylh. (Coleoptera: Curculionidae) and *Archlagocheirus funestus* Thomson (Coleoptera: Cerambycidae). *Dactylopius opuntiae* was collected and imported into South Africa from Australia where it has successfully controlled *O. ficus-indica*. Through the introduction of a biological control programme against *O. ficus-indica* the original invaded area (900 000 ha) has been reduced by 90% and now covers roughly 100 000 ha (Zimmermann & Moran, 1991). Among the biological control agents, *D. opuntia*e and *C. cactorum* were the most significant in reducing *O. ficus-indica* populations, whereas the other agents have played a minor role on a more localised geographical scale (Zimmermann & Moran, 1991).

Another problematic cactus in South Africa is *O. stricta* which also used to invade large areas in Australia but a successful biological control programme using *D. opuntiae* resulted in it being under complete control (Paterson *et al*., 2021). Initially, the moth *C. cactorum* was released against *O. stricta* in South Africa but resulted in very limited success (Hoffmann *et al.* 2020). *Cactoblastis cactorum* had been repeatedly credited for the success of the biological control programme against *O. stricta* in Australia and was therefore expected that this insect, on its own, would be able to control the *O. stricta* population (Hoffmann *et al.* 2020). During the 1920s and 1930s when large numbers of *C. cactorum* eggs were distributed on *C. cactorum* in Australia the *O. stricta* plants were indeed damaged by the moth, however regrowth from surviving stems was a problem (Raghu & Walton, 2007). *Dactylopius opuntiae* was abundant at this time as well and was therefore able to colonise, suppress and kill much of the regrowth (Hoffmann *et al.,* 2020). *Dactylopius opuntiae* is believed to have played the most significant role in controlling *O. stricta* in Australia (Hoffmann *et al.,* 2020). After the success of the biological control programme in Australia, the *D. opuntiae* already released against *O. ficus-indica* in South Africa was then redistributed on to *O. stricta* in hopes of replicating the same success.

Dactylopius opuntiae, that was redistributed from *O. ficus-indica* onto *O. stricta* only resulted in limited damage to the *O. stricta* plants in South Africa. It was later realised that *D. opuntiae* comprised several lineages and that the lineages released against the *O. stricta* and *O. ficus-indica* in Australia were different (Hoffmann *et al*., 2020). Therefore, the lineage released against *O. stricta* in South Africa was in fact the 'ficus' lineage. This then led to the collection of the correct lineage which had been successful in Australia, *D. opuntiae* 'stricta' (Hoffmann *et al*., 2020) and once released in South Africa, the *O. stricta* populations experienced a 90% decrease in density in the warmer north of the country (Paterson *et al.* 2011; Hill *et al.,* 2020). Both *Opuntia* species are currently under substantial control, meaning that the introduction of a biological control agent resulting in a significant decrease in the area, density, biomass, and rate of spread of the weed.

Table 1.1: Invasive cacti, their biological control agents, and level of control in South Africa. Only cacti that are controlled either by *Dactylopius* or *Hypogeococcus* species or lineages are listed (Paterson *et al.*, 2021b; Moran *et al.*, 2021).

Cactus	**Control agent**	**Status of control**
Opuntia aurantiaca	*Dactylopius austrinus*	Substantial
Opuntia monacantha	*Dactylopius ceylonicus*	Complete
Opuntia engelmannii	*Dactylopius opuntiae* '*stricta*'	Negligible
Opuntia humifusa	*Dactylopius opuntiae* '*stricta*'	Complete
Opuntia stricta	*Dactylopius opuntiae* '*stricta*'	Substantial
Opuntia ficus-indica	*Dactylopius opuntiae* 'ficus'	Substantial
Opuntia tomentosus	*Dactylopius opuntiae* 'ficus'	Substantial
Cylindropuntia fuligida	*Dactylopius tomentosus* 'cholla'	Complete
Cylindropuntia imbricata	*Dactylopius tomentosus* 'imbricata'	Substantial
Cylindropuntia leptocaulis	*Dactylopius tomentosus* 'imbricata'	Complete
Cereus jamacaru	*Hypogeococcus sp.*	Complete
Harrisia martinii	*Hypogeococcus sp.*	Complete
Harrisia balansae	*Hypogeococcus sp.*	Only recently released
Harrisia pomanensis	*Hypogeococcus sp.*	Substantial
Harrisia tortuosa	*Hypogeococcus sp.*	Substantial

The lack of native Cactaceae species in South Africa played a significant role in the success of biological control programmes against cacti, allowing for the use of oligophagous agents (insects that incorporate several species into their host range) that feed exclusively on cacti without risk of non-target effects (Hoffman, 1995; Klein, 2011). Including oligophagous insects allowed the selection and study of a larger pool of potential agents and the possibility of investigating new associations for biological control programmes. A new association occurs when an agent that has not coevolved with the target weed in its native range is used for biological control (Hokkanen & Sailer, 1985; Hokkanen & Pimental, 1989). The utilisation of new associations is restricted to situations where oligophagous agents are suitable. The success of biological control programmes with species of *Hypogeococcus* and *Dactylopius* is attributed to the insect's adaptation to the unique characteristics of Cactaceae species, resulting in a feeding preference only for plants within the Cactaceae family (Wilson *et al.*, 2017; Paterson *et al.*, 2021b).

1.4. *Dactylopius*

Cochineal, *Dactylopius* spp., have been used for centuries as a natural dye. The insects produce a defensive compound, carminic acid, a deep red anthraquinone that produces a vibrant red colour when extracted (Eisner *et al.*, 1980; Pérez-Guerra & Kosztarab, 1992). Dye produced from farmed *Dactylopius coccus* Costa (Hemiptera: Dactylopiidae) has been used for wool, cotton, paint, and cosmetics (Nobel, 2002). Today, cochineal is still used as a natural dye in some industries, particularly in the food and cosmetics industries where there is a demand for natural dyes (Borges *et al.*, 2012). They are also used as a biological control agent for several invasive Cactaceae and are very effective at controlling their target weeds. Dactylopiidae feed exclusively on Cactaceae and are endemic to the Neotropics, with a distribution of five species in North America and an additional five species in South America (Rodríguez *et al.*, 2001) (Table 1.2), with *D. coccus*, a domesticated species, present in both these regions.

Table 1.2: *Dactylopius* species that are found in North and South America and their recorded host plants.

Dactylopius species	**Host**	**Indigenous distribution**	**References**
Dactylopius confusus	*Cylindropuntia imbricata*, *Cylindropuntia leptocaulis*, *Cylindropuntia tunicata*, *Cylindropuntia kleiniae*, *Opuntia ficus-indica*, *Opuntia fuliginosa*, *Opuntia hyptiacantha*, *Opuntia jaliscana*, *Opuntia phaeacantha*, *Opuntia pubescens*, *Opuntia spinulifera*, *Opuntia streptacantha*	North America	Mann, 1969; Chávez-Moreno *et al*., 2011
Dactylopius opuntiae	*Cylindropuntia imbricata*, *Cylindropuntia tunicata*, *Nopalea cochenillifera*, *Nopalea karwinskiana*, *Opuntia atropes*, *Opuntia fuliginosa*, *Opuntia hyptiacantha*, *Opuntia jaliscana*, *Opuntia joconostle*, *Opuntia leucotricha*, *Opuntia macdougaliana*, *Opuntia megacantha*, *Opuntia phaeacantha,* *Opuntia robusta*	North America	Mann, 1969; Chávez-Moreno *et al*., 2011
Dactylopius tomentosus	*Cylindropuntia acanthocarpa*, *Cylindropuntia bigelovii*, *Cylindropuntia leptocaulis*, *Cylindropuntia tunicata*, *Mammillaria karwinskiana*, *Cylindropuntia atropes* = velutina	North America	Mathenge *et al.,* 2009a; Chávez-Moreno *et al*., 2011
Dactylopius gracilipilus	*Corynopuntia*	North America	van Dam & May, 2012

Dactylopius coccus	*Opuntia ficus-indica* *Nopalea cochenillifera*, *Opuntia atropes*, *Opuntia crassa*, *Opuntia fulginosa*, *Opuntia hyptiacantha*, *Opuntia jaliscana*, *Opuntia pilifera*, *Opuntia robusta*, *Opuntia steptacantha*, *Opuntia tomentosa*, *Opuntia undulata*	North and South America	Mann, 1969; Chávez-Moreno *et al.*,2011.
Dactylopius ceylonicus	*Opuntia quimilo*, *Opuntia bonaerensis* = elata, *Opuntia discolor*, *Opuntia salmiana*, *Opuntia monacantha*	South America	Mann, 1969
Dactylopius salmianus	*Opuntia salmiana*	South America	Pérez-Guerra & Kosztarab, 1992
Dactylopius zimmermanni	*Tephrocactus ovatus*, *Maihueniopsis ovata*, *Cereus aethiops*, *Maihuenia patagonica,* *Maihueniopsis darwinii*	South America	Claps & de Haro, 2001
Dactylopius confertus	*Cleistocactus baumannii,* *Echinopsis leucantha,* *Cereus aethiops,* *Denmoza rhodocantha,* *Gymnocalycium monvillei*, *Harrisia tortuosa,* *Pilocereus sp.,* *Trichocereus candicans*	South America	Claps & de Haro, 2001

1.4.1. Biology of cochineal insects

Different life stages of the insect aggregate and live on the surface of their cactus host, covered in a secretion of waxy filaments for protection (Figure 1.1a). Female insects remain sessile in these aggregations, while adult males have wings (Figure 1.1b). Females lay their eggs within the waxy coverings and the first instars, known as crawlers (Figure 1.1c) disperse on and between host plants (Volchansky *et al.*, 1999). Cochineal are sap sucking insects and feed on all aerial parts of their cactus host with a preference for young growth and developing fruit.

Figure 1.1: Life stages of *Dactylopius*; a) female cochineal covered in the waxy filaments on a piece of cactus, b) male cochineal c) cochineal crawler (Photo credit: David Taylor).

After finding a suitable host, female cochineal lay numerous eggs that can hatch within minutes to several days, depending on the species of cochineal. Some female crawlers may settle near the parent colony within 24 to 48 hours with others feeding in various locations before settling. Once a suitable location is chosen, they insert their proboscis deep in the cactus tissue and remain sedentary. Females cannot be removed from their settled area without damaging their delicate proboscis. Male crawlers freely move around (removing and inserting their proboscises as often as needed) and may change their feeding positions a few times within a 24-hour period. When males reach the pupation stage, they move into a female colony where they spin a cocoon and complete their last pre-adult stage. The male emerges fully developed in its winged form (Figure 1.1b) (Mann, 1969). Crawlers live up to 10 days without food and are easily picked up by gusts of wind, making this the most common and important form of dispersal (Mann, 1969).

The cochineal crawlers prefer to settle on shaded plants or choose to settle on the underside of the host plant for protection against direct sunlight. Settling on the underside of a cladode also provides protection from rain which washes away the woolly protective layer and can dislodge cochineal from the plant. Once the population reaches large numbers of insects, the infested cladode shrivels up and falls off the plant. Populations of cochineal on developing fruit cause premature ripening, which also causes the fruit to fall to the ground before the seeds ripen (Mann, 1969).

1.4.2. Control programmes using *Dactylopius* species

Cochineal insects have been successful agents in biological control programs against invasive cactus. Four cochineal species have been used successfully for biological control programmes against at least 15 invasive cacti globally (Paterson *et al.*, 2019, Winston *et al.*, 2023), including South Africa, Australia, Namibia, India and Kenya (Moran & Cabby, 1979; Hoffmann *et al.*, 2002; Paterson *et al.*, 2019; Hill *et al.*, 2020; Witt *et al.*, 2020). All four species of *Dactylopius* have been released in South Africa (Table 1.1), with some species comprising multiple host-specific intraspecific lineages (sometimes referred to as biotypes). Several *Dactylopius* species consist of separate genetically and/or behaviourally distinct groups that exhibit varying degrees of host specificity (Volchansky *et al.*, 1999; Mathenge *et al.*, 2009a, 2010a, b; Jones *et al.*, 2015a; Mathenge *et al.*, 2015). Accurately identifying different *Dactylopius* species and lineages is essential for choosing the most efficient biological control agents for Cactaceae, as they can cause varying degrees of damage to the target plants (van Steenderen *et al.*, 2021). Various lineage of *Dactylopius tomentosus* (Lamarck) (Hemiptera: Dactylopiidae) are used to control several *Cylindropuntia* spp. that are invasive in Australia. Host specificity and efficacy testing revealed that the lineage displayed different host specificity, host preference and performance across the *Cylindropuntia* spp. (Jones *et al.*, 2014). The most effective lineage of *D. tomentosus* needs to be matched to the targeted *Cylindropuntia* species to optimise biological control programmes. During efficacy trials *D. tomentosus* '*imbricata*' lineage had the biggest impact on its host, *Cylindropuntia imbricata* (Haw.) Knuth (Caryophyllales: Cactaceae), compared to the other hosts and *D. tomentosus 'cholla'* lineage had a wide host range but with *C. fulgida var. mamillata* being its preferred host. Furthermore, both these lineages experienced high fecundity when placed on the correct host plants

(Jones *et al.*, 2015a). The use of cochineal as biological control agents is discussed further in Chapter 4.

Cochineal insects have been invaluable for many control programmes against cacti with particular emphasis on *Opuntia* species. Most species of cochineal are restricted to the Opuntioidea, but there are lots of cactus species that are problematic outside of this group of cacti and various other agents have been implemented against these cacti, including the galling mealybug genus, *Hypogeococcus* (Pseudococcidae).

1.5. *Hypogeococcus*

Hypogeococcus species, commonly known as mealybugs, have a similar appearance and life history to cochineal. Most species feed exclusively on cactus, with a particular preference for organ-pipe cacti within the subtribe Cereanae, except for one lineage of *Hypogeococcus pungens* Granara de Willink (Hemiptera: Pseudococcidae) (*H. pungens sensu stricto*) which is associated with Amaranthaceae hosts (Aguirre *et al.*, 2016; Poveda-Martínez *et al.*, 2019; 2020; 2022).

1.5.1. Biology of *Hypogeococcus*

Female and male *Hypogeococcus* display differences in terms of appearance and life cycle. Adult females are round, plump, and light pink in colour with minuscule legs (Figure 1.2a). Once they settle, they become mostly sessile and produce a woolly mass of waxen threads for protection They generally reside within distorted stem tips or growth tips that are actively growing, feeding on their cactus host by inserting their long, thin proboscis to extract sap from the plant. Adult males are also pink in colour but capable of mobility due to their possession of two semi-transparent wings (Figure 1.2b).

Figure 1.2: Images displaying life stages of *Hypogeococcus* sp.; a) female without the waxy protective covering, b) male, c) crawlers and females covered in the waxy protective layer (Photo credit: David Taylor).

Females lay their eggs individually into the waxy secretion, and the eggs hatch within 20 minutes of oviposition (Klein, 2002). Once hatched, the newly emerged nymphs are equipped with functional legs to move around (Fig1.2c). They typically move to the top of the cactus where they are dispersed by wind (Klein, 2002). If they land on another suitable host plant, they gather at the base of a spine along with other crawlers. As soon as they settle, the crawlers start feeding on the plant sap by inserting their piercing mouthparts into the cactus. Female nymphs, once settled, will remain in that location for the rest of their life and start producing wax as soon as they begin feeding. After about a month, the females reach sexual maturity and, upon fertilisation by a male, will lay eggs approximately three weeks later (Klein, 2002). On average, gravid females produce two to four eggs per day and have a lifespan of approximately 50-90 days. Male nymphs, on the other hand spin a cocoon before emerging as sexually mature adults after around a month. The adult males, which do not feed, fly in search of a female to mate with and have a lifespan of only a few days as adults (Klein, 2002).

In their native range, mealybug populations are regulated by a range of natural enemies, including predatory coccinellids and encyrtid (*Anagyrus* sp.), signiphorid (*Signiphora* sp.) and cecidomyiid parasitoids (Whittaker, 2022). These natural enemies maintain low populations of the mealybugs, preventing them from causing extensive harm to cacti. However, when introduced to areas free from these pressures, mealybugs can rapidly increase in population size and become effective biological control agents or invasive alien pests. This has been observed in regions like Puerto Rico, and possibly Central and North America, where a South American species in the species complex previously known as *H. pungens* was accidentally introduced without its co-evolved natural enemies and is becoming a threat to native cactus diversity (Poveda-Martínez *et al.*, 2019). The escape from natural enemies may also partially explain the success of *Hypogeococcus* sp. as a biological control agent for cactus weeds in Australia and South Africa (McFadyen, 2012; Paterson *et al.*, 2021b).

Identifying *Hypogeococcus* species can be challenging due to their morphological similarities, making incorrect identification a possibility. For example, the *Hypogeococcus* that was introduced in Australia and South Africa was initially misidentified as *H. pungens* and later as *Hypogeococcus festerianus* (Lizer y Trelles) (Hemiptera: Pseudococcidae). A genetic study revealed that it was an undescribed species within a *Hypogeococcus* species complex (Aguirre *et al.*, 2016). In its native range, the true *H. pungens* (now referred to as *H. pungens sensu stricto*) is associated with Amaranthaceae species. Misidentifying biological control agents could have a significant impact on biological control programmes if the misidentified insects have a different host range from the original released species. In this case, although there was a misidentification, post-release evaluations showed that the *Hypogeococcus* sp. only feeds on Cactaceae (Aguire *et al.*, 2016). If researchers had collected and released the true *H. pungens sensu stricto* as a biological control agent, it could have had unintended consequences on native Amaranthaceae species in the introduced range.

1.5.2. *Hypogeococcus* in cactus biological control

Currently, only one species of *Hypogeococcus,* the undescribed species within the *H. pungens* species complex, is employed as a biological control agent worldwide, and it has proven highly effective in suppressing cactus weeds (Ezeh *et al.*, 2023). As the species is undescribed, it is referred to as *Hypogeococcus* sp. in this thesis. This insect is oligophagous and feeds on various species of cactus within the subfamily Cactoideae, thus eliminating the need to introduce any other lineages or species.

The insects gather on all parts of the plant that are actively growing and their presence results in the deformation of fresh growth, including the development of swollen and spiky formations at the stem tips (Paterson *et al.*, 2011). Although *Hypogeococcus* spp. do not produce true galls their infestation results in meristematic gall-like growths that provide a protected environment for the insect to feed, develop, and reproduce. For this study these will be referred to as galls. The colonisation of mealybugs on a host plant is a sign of successful establishment and in most cases will ultimately lead to the death of the infested plant. The speed at which the plant dies after being infested may vary based on factors such as the age and size of the plant. Younger plants are likely to die before they reach maturity, while older, more established plants may take a few years to die (Paterson *et al.,* 2011). Fruit production is significantly impacted by the presence of *Hypogeococcus*, as they tend to congregate on developing flowers. If new plants develop close to an infected one, they are likely to become infested quickly, thus reducing the spread of cactus infestations in the area. However, manual dispersal can be used to aid the spread of *Hypogeococcus*, as their crawlers are only dispersed by wind at a slow and unpredictable pace. This is done by transferring pieces of infested plants to healthy ones (Klein, 1999).

Hypogeococcus sp. was introduced in Australia to control *Harrisia martinii* (Labour.) Britton (Caryophyllales: Cactaceae), *Harrisia tortuosa* (J. Forbes ex Otto & A. Dietr.) Britton & Rose (Caryophyllales: Cactaceae), and *Harrisia bonplandii* (Parmentier) Britton and Rose (Caryophyllales: Cactaceae) (Houston & Elder 2019; Paterson *et al.*, 2021b; Ezeh *et al.*, 2023). The mealybugs quickly established themselves and caused significant damage to the weed populations, leading to the successful control of all three target weeds. The insect was also introduced against *Acanthocereus teragonus* (L.) Hummelinck (Caryophyllales: Cactaceae) in Australia, but only achieved partial control (Aguirre *et al.*, 2016). This is believed to be due to the slow growth and low abundance of spine clusters of *A. teragonus* compared with other target weeds, making insect population more vulnerable to predators, and preventing rapid population growth (McFadyen, 2012). *Hypogeococcus* sp. has also been introduced in South Africa and is now a control agent for several cacti, including *H. martinii*, *Cereus jamacaru* De Candolle (Caryophyllales: Cactaceae), *Harrisia pomanensis* (F.A.C. Weber ex K. Schum.) Britton & Rose (Caryophyllales: Cactaceae), *Harrisia balansae* (K. Schum.) N.P. Taylor & Zappi (Caryophyllales: Cactaceae), and *H. tortuosa* (Paterson *et al.*, 2011; Paterson *et al.*, 2021b). Like cochineal, *Hypogeococcus* consists of multiple species and lineages, and it is possible that different ones may be more effective as biological control agents against specific cactus species. However, at present only one *Hypogeococcus* species and lineage is used as a biological control agent globally.

Despite successful biological control programmes implemented against several cacti species, certain species, such as *Trichocereus spachianus* (Riccob.) (Caryophyllales: Cactaceae), still lack efficient biological control agents in South Africa. Considering the success of past biological control programmes against other cacti, this approach is likely to be the best solution for managing *T. spachianus* in South Africa.

1.6. *Trichocereus spachianus* (Torch cactus)

The Andean distribution of *Trichocereus* spans from Ecuador through Peru, Chile and southeastern Argentina to the Atlantic coast. They have big, attractive conical flowers that are usually white (Figure 1.3a), but some can be red. These flowers generally measure between 15 to 30 cm, and their receptacle is covered in hairs (Albesiano & Kiesling, 2012). These columnar cacti have sturdy, ribbed stems that may branch from the base or have a discernible trunk (Figure 1.3b). Certain Trichocereus species may also exhibit a creeping growth form.

The taxonomy and systematics of *Trichocereus* have been problematic, and acceptance of the genus has been questioned. Before the twentieth century, *Trichocereus* species were classified as part of the collective genus *Cereus*. Berger (1905) created several *Cereus* subgenera, including *Trichocereus* which consisted of 14 species. A few years later, Riccobono (1909) elevated *Trichocereus* to generic level, including only two species in this new genus: *T. macrogonus* (Salm-Dyck) Riccob. and *T. spachianus* (Lem.) Riccob. Britton and Rose (1902) then expanded the genus *Trichocereus* to include a total of 19 species and designated *Cereus macrogonus* as the type species.

Many years later, Friedrich (1974) included *Trichocereus* under *Echinopsis*. However, this change was disputed because although *Echinopsis* and *Trichocereus* are closely related most species can be assigned to one or the other genera based on some morphological characteristics. *Trichocereus* species have cylindrical stems, receptacles are wide, conical, with dense hairs on receptacular areoles, and scales on the receptacle are generally numerous and are closely spaced or overlapping. Conversely *Echinopsis* species have globular stems, hairs on the receptacular areoles are sparse and the scales on the receptacle are mostly few, with considerable space separating them. A phylogenetic study further supported that *Echinopsis* comprises of several divergent lineages with *Trichocereus* being one of them (Schlumpberger & Renner, 2012). 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 *Trichocereus* (Roberto Kiesling, 2023, pers. comms., Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET)). Even with the name change this cactus remains in the subtribe Trichocereinae indicating that the name change does not impact host specificity testing. Therefore, for the purpose of this thesis, this cactus will continue to be referred to as *Trichocereus spachianus*.

The identification of the South African *T. spachianus* population has faced various challenges including taxonomic confusion regarding the genus and species, due to previous misidentifications, as well as confusion surrounding its native range. *Trichocereus spachianus* is believed to have originated from northwestern Argentina and southern Bolivia. In the past, it has been referred to as *Echinopsis spachiana* (Lem.) H. Friedrich & G.D. Rowley and *Echinopsis schickendantzii* F.A.C. Weber (Caryophyllales: Cactaceae) due to the ambiguity surrounding its taxonomic classification. A study by Schlumpberger & Renner (2012) showed that *T. spachianus* does indeed fall within the genus *Trichocereus* not *Echinopsis*.

Figure 1.3: Target host plant, *Trichocereus spachianus* a) flower and b) close up of a stem. (Photo-credit: Tamzin Griffith).

The origin of *T. spachianus* introduced to South Africa is unknown but believed to have been introduced as an ornamental plant due to its attractive large white flowers. *Trichocereus spachianus* is a multi-stemmed shrub, growing between 1 and 2.5 meters in height, capable of regenerating from broken pieces and forming dense patches that can displace native flora and fauna. Invasions in rangelands can result in direct harm to livestock and socio-economic impacts on farmers due to reduced grazing areas. This species has been documented at over 250 sites in South Africa, primarily in the Karoo and dry savannah biomes (Southern African Plant Invaders Atlas (SAPIA)) (Figure 1.4). It is also present in dry regions of Europe, where it has become invasive in some areas and is one of the most widely cited species within the *Trichocereus* genus in regions such as northern and southern Italy, Sicily, Valencia, and Catalonia (Gómez *et al.*, 2013; Aymerich & Sáez, 2019).

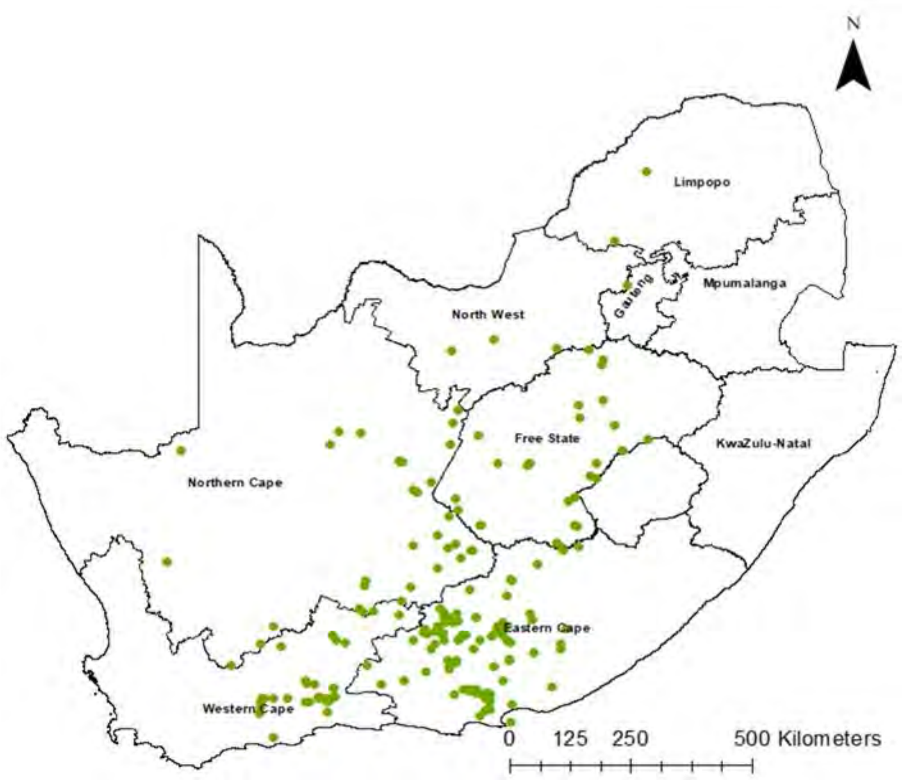

Figure 1.4: Distribution of *Trichocereus spachianus* in South Africa in 2019 (Southern African Plant Invaders Atlas database).

While *T. spachianus* is thought to have originated in northwestern Argentina and southern Bolivia, the plant is either exceptionally rare or does not exist as a similar species to those identified as *T. spachianus* in Europe and South Africa. There are few records of this plant in its native range and none of these records have been verified. Surveys conducted in northern Argentina did not result in any records of *T. spachianus* (L. Varone 2019, pers. comms, FuEDEI). The type specimen used to describe this species was a cultivated plant and the latest review of the genus used herbarium specimens and one cultivated plant from Europe to describe the distribution of the species (Kiesling, 1978; Albesiano, 2015). None of the localities used to describe the indigenous distribution of *T. spachianus* in these studies were confirmed to be naturally occurring populations (Paterson *et al.* 2021b). The *T. spachianus* name may have been mistakenly given to *Trichocereus thelegonoides* (F.A.C. Weber) Britton & Rose (Caryophyllales: Cactaceae), which is a cactus clearly distinguishable from *T. spachianus* due to its horizontal ribs that create the appearance of protruded areoles. The plant that is morphologically most like the cactus in South Africa is *Trichocereus lamphrochlorus* (Lem.) Britton & Rose (Caryophyllales: Cactaceae). Taxonomic uncertainties are further complicated by creating and using herbarium voucher specimens for Cactaceae as the specimens are usually unrecognisable from the living plant that they were taken from (Novoa *et al.*, 2019b). *Trichocereus spachianus* may be a hybrid of similar species, or modification through horticultural trade. It is possible that no plants like the invasive plants of *T. spachianus* exist in natural populations in the native range. Alternatively, the plant is extremely rare or possibly extinct in its native range. Despite multiple surveys and efforts to locate indigenous populations of *T. spachianus*, none have been found.

Closely related cacti to *T. spachianus* have been effectively controlled using biological control. If a closely related weed species has been successfully controlled through biological control, it indicates a high likelihood that the target weed will also be a good target for biological control (Paterson *et al.*, 2021a). This prediction is based on the assumption that plants with similar evolutionary backgrounds share comparable plant traits, leading to analogous plant-herbivore interactions and host-range patterns. Under this consideration, *T. spachianus* is a good candidate for biological control. However, the development of cactus biological control programmes can be impeded by taxonomic uncertainties (Paterson *et al.*, 2021b). These taxonomic issues can especially hinder efforts involving the collection of control agents and testing their host specificity. The lack of wild *T. spachianus* populations poses difficulties for the implementation of biological control measures, as it prevents the direct collection of host-specific agents from the indigenous population. However, cacti have been successfully controlled using new associations (Klein *et al.*, 2020); the use of an agent that has not coevolved with the target plant (Hokkanen & Sailer, 1985; Hokkanen & Pimental, 1989). Therefore, investigating the biological control potential of insect agents collected from other closely related cactus species in the suspected indigenous distribution is warranted.

1.7. Research Aims

Trichocereus spachianus is a declared weed in South Africa according to the Alien and Invasive Species Regulations under the National Environmental Management: Biodiversity Act (2004) (NEM:BA), which constitutes the South African legislative framework addressing invasive species (Department of Environmental Affairs 2016). Currently, there are no successful management programmes implemented against this cactus. Given its classification as an invasive weed, *T. spachianus* has become a prioritised weed target to limit the negative impacts associated with its establishment and spread (Henderson & Wilson, 2017).

Biological control programmes against Cactaceae have been successful in South Africa and it is therefore a promising strategy to control *T. spachianus* invasions in the country. This research aimed to investigate several possible biological control agents that may be implemented against *T. spachianus* in South Africa. Agents are considered successful if found to be suitably damaging to the target and host specific. Therefore, the predicted efficacy and host specificity of potential agents were investigated. In the context of this study, agent efficacy was measured as the insect's ability to establish on growing cactus hosts and cause observable damage and/or plant mortality. The insects' potential efficacy on the target weed was investigated for a previously released and established biological control agent for other invasive cacti in South Africa. If this agent proved suitable, redistributing an agent previously released on another target weed offered the benefits of reduced costs and time required to develop a new biological control programme, given that their host specificity is known. Several additional South American *Hypogeococcus* lineages and a cochineal lineage were identified as potential biological control agents and also investigated to determine their predicted efficacy on South African populations of *T. spachianus*. New agents found to have a high enough impact on *T. spachianus* plants were subjected to host specificity testing. Combining efficacy testing with host specificity allowed for the development of a safe and effective biological control programme against *T. spachianus*.

Chapter Two

Potential for *Hypogeococcus* sp., a biological control agent for columnar cacti in South Africa, as an effective control against *Trichocereus spachianus*

2.1. Introduction

Herbivorous insects exhibit varying degrees of dietary specialisation. Selection of biological control agents is focused on finding and releasing host-specific (monophagous, specific to one species of host plant) insects to target specific weeds (Walters *et al.*, 2011; Rule & Hoffmann, 2018). Oligophagous insects feed on several closely related plant species and have been utilised as biological control agents in certain situations (Mann, 1969; Paterson *et al.*, 2019). Approval for the release of oligophagous insects may be granted if their host range does not encompass any indigenous or valued plant species (Sutton *et al.*, 2019).

Using biological control agents with oligophagous feeding habits has proven effective and safe in managing invasive cacti (Hoffman, 1995; Klein, 2011). Cactaceae and the insects associated with them are endemic to the Americas. Therefore, insects that feed on multiple closely related species of cacti can be used in biological control programmes targeting cacti in regions outside of the Americas where no cactus species are indigenous, except for *R. baccifera* (Paterson *et al.*, 2011). *Cactoblastis cactorum* is an oligophagous insect that feeds on multiple cactus species and has played a role in the control of at least ten cactus species globally (Klein, 2011). The fact that *C. cactorum* is oligophagous has not posed any issues for the implementation of biological control against cactaceous weeds in the Old World, due to the lack of native Cactaceae in this region.

Only a single undescribed species of *Hypogeococcus,* referred to as *Hypogeococcus* sp., is currently used as a biological control agent against cactus species (Paterson *et al.*, 2021b). *Hypogeococcus* sp. has been released in South Africa, with varying success, against several different species of columnar cacti within the subtribe Cereanae (Klein, 2011; Paterson *et al.*, 2021b), including *H. martinii* (Moran & Zimmermann, 1991), *C. jamacaru* (Sutton *et al.*, 2018), *H. pomanensis* (Paterson *et al.*, 2019), *H. tortuosa* and *H. balansae* (Paterson *et al.*, 2021b).

Hypogeococcus sp. was initially released in Australia as a biological control agent against *H. martinii,* a sprawling shrub native to the Chaco region of Argentina and Paraguay (McFadyen, 2012). *Hypogeococcus* sp. was collected off several *Harrisia* spp. and *Cleistocactus baumannii* Lem. (Caryophyllales: Cactaceae) in Formosa Province, Argentina in 1972 where it was initially incorrectly described as *Pedronia festerianus* Lizer y Trelles (= *H. festerianus*) (McFayden, 2012). *Hypogeococcu*s sp. was one of several potential control agents for *H. martinii* that underwent host specificity tests in Argentina (McFadyen, 2012). The initial release of *Hypogeococcus* sp. took place in northern Queensland in 1975 and the insect established quickly. By 1978, it was widely established and caused significant damage to *H. martinii*. With no natural predators in Queensland, *Hypogeococcus* sp. populations rapidly grew to large numbers, depleting the plant's resources and causing plant death within three years (McFadyen, 2012). The efficacy of *Hypogeococcus* sp. as a biological control agent in central Queensland was verified in a recent post-release evaluation (Houston & Elder, 2019). *Harrisia martinii* infestations were reduced from between 25 and 30% to approximately 5% ground cover after about four years post-release. However, its efficacy as a control agent has been variable, with reduced control levels reported in southern Queensland compared to central Queensland (Tomley & McFadyen, 1984). A study conducted by Ezeh *et al.*, (2023) investigated whether the variable performance of this agent was attributed to the introduction of different *Hypogeococcus* species into Australia. Their findings suggest that all populations of this insect in Australia are the same species, rather suggesting that climate incompatibility might be a contributing factor to the insects' variable success (Ezeh *et al.*, 2023). Due to the success of this biological control programme in Australia, the insect was released into South Africa in 1983. *Harrisia martinii* is now considered under complete control in South Africa (Moran & Zimmermann, 1991a; Klein, 2011). *Hypogeococcus* sp. established on *H. martinii* was subsequently collected and distributed to other cactus species within its host range throughout the country (Moran & Zimmermann, 1991a; Klein, 2011).

Cereus jamacaru is the largest and only non-*Harrisia* species on which the biological control agent *Hypogeococcus* sp. has been released in South Africa. While the presence of *Hypogeococcus* sp. on this cactus results in a decline in fruiting relatively quickly, mortality of *C. jamacaru* plants takes longer due to their larger size when compared to smaller cacti species that are targeted by this agent. Nevertheless, it is under complete control in northern parts of South Africa where the insect has been established for an extended period (Sutton *et al.*, 2018). The insect reduces fruit production and effectively eliminates seedlings, leading to near-zero recruitment of new plants over a period of ten years (Sutton *et al.*, 2018). Further releases of *Hypogeococcus* sp. have been conducted in the Eastern Cape and KwaZulu-Natal regions with the aim of achieving similar levels of control as observed in northern South Africa (Paterson *et al.*, 2011; Sutton *et al.*, 2018; Paterson *et al.*, 2021b).

Harissia pomanensis, a more robust and sturdy cactus (Klein & Zimmermann, 2020) compared to the other *Harissia* species, is another target for biological control using *Hypogeococcus* sp.. Isolated infestations of *H. pomanensis* are scattered throughout South Africa (Mafanya *et al.*, 2017; Paterson *et al.*, 2019; Paterson *et al.*, 2021b). Although *Hypogeococcus* sp. was released on a large infestation of *H. pomanensis* in Alldays, Limpopo, in 2013, no mortality has been recorded to date (Paterson *et al.*, 2021b). However, plants no longer produce fruit due to *Hypogeococcus* sp. attack, and the spread of the population is reduced. Other releases have been made on *H. pomanensis* infestations in South Africa and similar impacts are predicted. It takes more years to see the effects after a release on *H. pomanensis* compared to *H. martinii* (Paterson *et al.*, 2021b). A fourth target weed for *Hypogeococcus* sp.*, Harissia balansae* also has scattered infestations across South Africa, with *Hypogeococcus* sp. released at three of them. Although the insect has established, it is too early to predict its impact (Paterson *et al.*, 2021b).

Developing a biological control agent is a costly and time-consuming process, despite the positive cost-benefit outcomes that biological control programmes can offer in the long-term (van Wilgen *et al.*, 2004). The average duration of screening and host specificity testing of a biological control agent in South Africa ranges from 5 to 9 years (Moran *et al.*, 2005). Cost of evaluating the host specificity and effectiveness of a novel biological control agent in New Zealand is estimated to be around NZ$ 364,500

(Ehlers *et al.*, 2020). Such expenses are a recurring feature across many biological control programmes, with an estimated cost of AUD 460,000 per novel agent in Australia in 1997 (McFadyen, 1998). Although the cost of developing a biological control agent may be lower compared to herbicide control, it may still be a significant expense relative to the budget for managing natural ecosystems (McFadyen, 1998).

Transfer programmes can reduce the time needed for the development of a biological control programme from about 10 years to 2.8 years (Moran *et al.*, 2005; Canavan *et al.*, 2021). A transfer programme uses an agent that has already been used successfully in another region usually against the same species or a closely related target weed (Doeleman 1989; Ehlers *et al.*, 2020; Paterson *et al.*, 2021a; Canavan *et al.*, 2021). Transfer programmes can also substantially reduce the costs of developing a biological control programme (Ehlers *et al.*, 2020). Using an agent that has already been studied or implemented as an agent elsewhere in New Zealand was estimated to save approximately NZ$ 226,000 per agent (Ehlers *et al.*, 2020). Evaluating outcomes of existing successful biological control programmes in other regions is also a strong indicator of potential success, provided climatic compatibility, of a biological control agent in the country of interest (Paterson *et al.*, 2021a). When conducting transfer projects, it remains important to assess the insects host range and efficacy, considering the potential differences in fauna in the new region. Nonetheless, the required efforts are typically considerably reduced, given that a significant portion of the necessary groundwork was completed in the original release country.

Initial stages of biological control against Cactaceae in South Africa were primarily focused on transfer projects, particularly on projects that had been successful in Australia (Zachariades *et al.*, 2017). The first intentional release of a biological control agent globally was an unintentional transfer programme (Lounsbury, 1915). *Dactylopius ceylonicus* Green (Hemiptera: Dactylopiidae) was originally introduced to India under the assumption that it was *D. coccus*, a species cultivated for production of carminic acid, used in red dye manufacturing. However, the insect caused substantial damage to *Opuntia monacantha* Haw. (Caryophyllales: Cactaceae), resulting in the collapse of the dye industry. Despite this setback, *D. ceylonicus* continued to be employed as a biological control agent for *O. monacantha* infestations in India and Sri Lanka (Zimmermann *et al.*, 2009). Based on its success in India, South

Africa introduced *D. ceylonicus* in 1913 to combat *O. monacantha* (Lounsbury, 1915; Zimmermann *et al.*, 2004; Paterson *et al.*, 2011). Australia also released *D. ceylonicus*, where it successfully established and provided effective control against *O. monacantha* (Winston *et al.*, 2014). Several other cochineals have also been part of transfer programmes. *Dactylopius opuntiae* 'ficus-indica' lineage was released for the control of *O. ficus-indica* in Australia, South Africa and Namibia. *Dactylopius tomentosus* 'cholla' lineage was shared between South Africa and Australia (Paterson *et al.*, 2019). Furthermore, the 'imbricata' cochineal was originally imported to South Africa from Australia (Zimmermann & Moran, 1991). Transfer programmes in South Africa have extended beyond Cactaceae to include plants like *Lantana camara* L. (Lamiales: Verbenaceae), *H. perforatum*, and *Parthenium hysterophorus* L. (Asteraceae: Heliantheae), had agents imported from their introduced range in Australia (Strathie *et al.*, 2021), *Tradescantia fluminensis* Vell. (Commelinales: Commelinaceae) had agents imported from their introduced range in New Zealand (Byrne *et al.*, 2021) and *Arundo donax* L. (Poales: Poaceae) had agents imported from their introduced range in the USA (Sutton *et al.*, 2021).

While certain transfer programmes may not require extensive host specificity testing, in cases where an agent is transferred to a different closely related weed species or lineage it is essential to determine their level of damage to warrant their release. Sheppard (2003) emphasised the importance of prioritising efficacy during biological control agent selection. Ineffective agents provided no benefits once released. Ineffective agents can reach high numbers without causing damage to the target weed, leading to unintended consequences for the ecosystem and possible negative interactions with future released biological control agents (Pearson & Callaway, 2005; Carvalheiro *et al.,* 2008). Testing the efficacy and potential impact of agents before release, along with host specificity testing, can minimise the use of ineffective agents (McClay & Balciunas, 2005). Therefore, the introduction of *Hypogeococcus* sp. onto *T. spachianus* should only be considered once it is proven that it will be suitably effective.

Using existing agents has the potential to be cost-effective and efficient, reducing the likelihood of unintended consequences. However, it is only appropriate to do so if there is evidence to suggest that these agents will be effective (Louda, 2000; Sheppard *et al.*, 2003; McClay & Balciunas, 2005; Paterson, 2010). Investigating the use of *Hypogeococcus* sp. as a biological control agent against *T. spachianus* prior to actively redistributing it to this new target weed is justified. Although *T. spachianus* belongs to the same subfamily as the cacti that are currently being controlled by *Hypogeococcus* sp. in South Africa, the effect of this insect on *T. spachianus* has never been investigated.

This chapter assessed the feasibility of using the cactus biological control agent, *Hypogeococcus* sp., as a biological control agent for *T. spachianus*. Suitability of *T. spachianus* as a host plant and the impact of *Hypogeococcus* sp. on *T. spachianus* was investigated. Comparison was made between impact to *T. spachianus* and impacts on other cactus weed species with known levels of biological control success in the field. These outcomes were used to predict whether *Hypogeococcus* sp. was likely to be sufficiently successful on *T. spachianus* to warrant its use as a biological control agent in South Africa.

2.2. Materials and Methods

Two experiments were conducted to evaluate the effectiveness of *Hypogeococcus* sp. as a biological control agent against *T. spachianus* in South Africa. The first experiment was conducted under laboratory conditions using cut pieces of cactus to measure insect performance as a measure of nymphal survival, days to egg production, fecundity, and percentage of insect coverage four months after introduction to each piece of cactus. These measurements determined the insect's performance on *T. spachianus* compared to its effectiveness on other cacti species that it controls in South Africa. The second experiment was conducted in a greenhouse using potted cactus plants to assess the effect of *Hypogeococcus* sp. on plant mortality, comparing the impact on *T. spachianus* to other known biological control targets of this agent (Table 2.1). Investigating both insect performance and plant mortality was important because, while it is necessary to determine the suitability of hosts for the insect, it is equally important to assess whether the insect's damage leads to any adverse effects on the plant's survival. The cactus species selected for comparison are currently controlled by *Hypogeococcus* sp. in South Africa (Paterson *et al.*, 2021b). While *Hypogeococcus* sp. has been released on various cactus species in South Africa, laboratory and greenhouse experiments were conducted to facilitate comparisons between the agent's performance and efficacy when placed on the target host *T. spachianus*, as opposed to other cactus hosts. The relative performance of the agent on plants it was known to control compared to the target weed can then be used to predict its impact in the field if it were to be used for the control of *T. spachianus*.

Table 2.1: Cactus species used in the experiments and their level of control from *Hypogeococcus* sp. in South Africa.

Cactus species	**Level of control**
Harrisia martinii	Complete
Harrisia pomanensis	Substantial
Cereus jamacaru	Complete
Harrisia balansae	Unknown (recent release)
Trichocereus spachianus	None, target weed

2.2.1. Biological material collection

The *Hypogeococcus* sp. 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). *Cereus jamacaru*, *H. balansae* and *H. martinii* were collected from a site between Makhanda and Southwell (33°28'36.8"S 26°38'53.2"E). *Cereus jamacaru* was collected just before the Ecca Pass towards Fort Beaufort (33°08'18.4"S 26°37'09.7"E). *Trichocereus spachianus* was collected from various populations within Makhanda (33°17'56.2"S 26°31'32.0"E; 33°18'18.6"S 26°31'03.2"E; 33°17'41.4"S 26°31'10.5"E) and *H. pomanensis* was collected along the road towards Graaff-Reinet just outside Kariega (33°38'13.01" S, 25°27'13.0" E). These cacti were used for both the laboratory and greenhouse experiments. All collected material was healthy and had no visible signs of *Hypogeococcus* sp. infestation.

2.2.2. Insect performance trials

Conducting experiments to record insect performance is important for evaluating the impact of a host plant on insect performance (Maw, 1976). Parameters that were measured included: nymphal survival (number of nymphs that survive on each piece of cactus), days to egg production, fecundity (number of nymphs produced by gravid females that developed on the cactus), and percentage of insect coverage four months after introduction to each piece of cactus (the percentage of surface area covered by *Hypogeococcus* sp.). Cactus pieces used were cut to 30 cm in length and left at room temperature for a week to form a dried layer over the cut side, avoiding mould forming from the excessive moisture.

Each piece of cut cactus was placed in a plastic container (19.5 cm × 27.5 cm × 9 cm) with holes (5 cm) covered with a fine mesh for ventilation while preventing insects from escaping. For *C. jamacaru*, plastic containers measuring 21.5 cm × 33.4 cm × 16.8 cm were used owing to its larger diameter. Gravid females from the Kariega *Hypogeococcus* sp. culture were collected, dewaxed using fine forceps and placed into Petri dishes (9 cm) which were then sealed with parafilm to ensure no nymphs could escape. Once nymphs hatched, 30 first instars (less than 24 h old) were carefully collected using a fine paint brush and placed onto the piece of cactus in the plastic container. The cacti were left for three days to allow settling of the nymphs before being checked every two days using a microscope to record the number of settled nymphs (indicated by white waxy filaments). After 14 days, all nymphs were considered either settled or dead, and the initial survival rate of the nymphs was determined. Cactus pieces were checked weekly for gravid females (identified by red egg-shaped masses in their bodies) which were collected and placed into sealed Petri dishes. The number of nymphs produced was recorded after 30 days to estimate relative fecundity when insects were reared on the different hosts. The experiment was conducted for a duration of four months, the period deemed appropriate for two complete life cycles of *Hypogeococcus* sp. (McFadyen, 2012) to occur, while avoiding potential degradation of the cactus pieces. After four months, the cactus pieces were assessed to determine the extent of infestation by *Hypogeococcus* sp.; an estimation of the percentage of each cactus piece covered by *Hypogeococcus* sp. was recorded. The protocol was followed using *T. spachianus*, *H. martinii, H. balansae*, *H.*

pomanensis and *C. jamacaru*. Five replicates, where a replicate was a piece of cactus, were conducted for each plant species. All insect performance trials were conducted under controlled conditions at 26°C and 12 h photoperiod (D12: L12).

2.2.2.1. Host suitability index (HSI)

A host suitability index adapted from Maw (1976) was used to identify and compare test plant species that were suitable *Hypogeococcus* sp. hosts. Host suitability of a plant cannot be determined by a single measure or parameter, so a combination of factors was taken into account to obtain a reliable indication of host suitability through the use of the equation:

$$Maw's\ host\ suitability\ index = \frac{pupal\ mass \times percentage\ pupation}{developmental\ time}$$

However, the index for this research was modified to cater to *Hypogeococcus* sp. since they do not undergo pupation. The modified index was employed:

$$Host\ suitability\ index(HSI) = \frac{fecundity \times percentage\ established}{developmental\ time}$$

Fecundity referred to the number of nymphs produced per females. The proportion of the initial 30 nymphs that settled on each cactus piece was recorded as the percentage of established nymphs, while the length of time taken by crawlers to reach maturity (when females started producing eggs) was recorded as the development duration in days. The HSI scores were interpreted as the higher the units, the greater the host suitability.

2.2.3. Potted plant trials

Plants were grown by planting a piece of cactus into a 20cm pot filled with garden soil. Potted plants were watered as needed. Cacti were grown for approximately 6 months, allowing sufficient time for roots to form and for the cactus to be actively growing during the experiments. Ten plants of each cactus species were grown. Individual cactus plants were placed inside a fine-meshed white breeding cage and infested with a *Hypogeococcus* sp. gall (approximately 2cm in diameter) by inserting a small wooden stake (about 20 cm) into the gall and then into the top of the cactus. This method is used in the field to distribute galls of *Hypogeococcus* sp. onto non-infested cactus plants (Klein, 2002). The galls were checked for predators, which were then removed before infesting the clean cactus plants. The top of the cactus was selected as the site of application, as it is typically the location of new growth and a rich food source for the *Hypogeococcus*. The cage prevented parasitoids and predators from impacting the infestation of *Hypogeococcus* sp. on each cactus species.

All treatments were run simultaneously, and plants were monitored weekly to determine the acceptance of each cactus as a host by the insects and the success of initial establishment. Establishment was successful if the nymphs settled and produced white waxy filaments and was unsuccessful if the nymphs did not settle (100% mortality of the nymphs). An ordinal scale of 1 to 6 (Figure 2.1) was used to record the population density of *Hypogeococcus* sp.. Once the establishment of *Hypogeococcus* sp. on each potted cactus was determined, monitoring was carried out every two weeks to assess the insect's population density. After a few months (5-6 months), the frequency of monitoring was reduced to once a month to track any potential plant mortality. Time to death for each cactus was recorded in months. Death was recorded as the point where no living tissue (green tissue) was observed on the cactus plant. Plant mortality was scored as a binary predictor variable, where alive = 1 and dead = 0 for every month over 15 months. Ten replicates and a control (cactus plants that were not infested with *Hypogeococcus* sp.) were conducted for each cactus species.

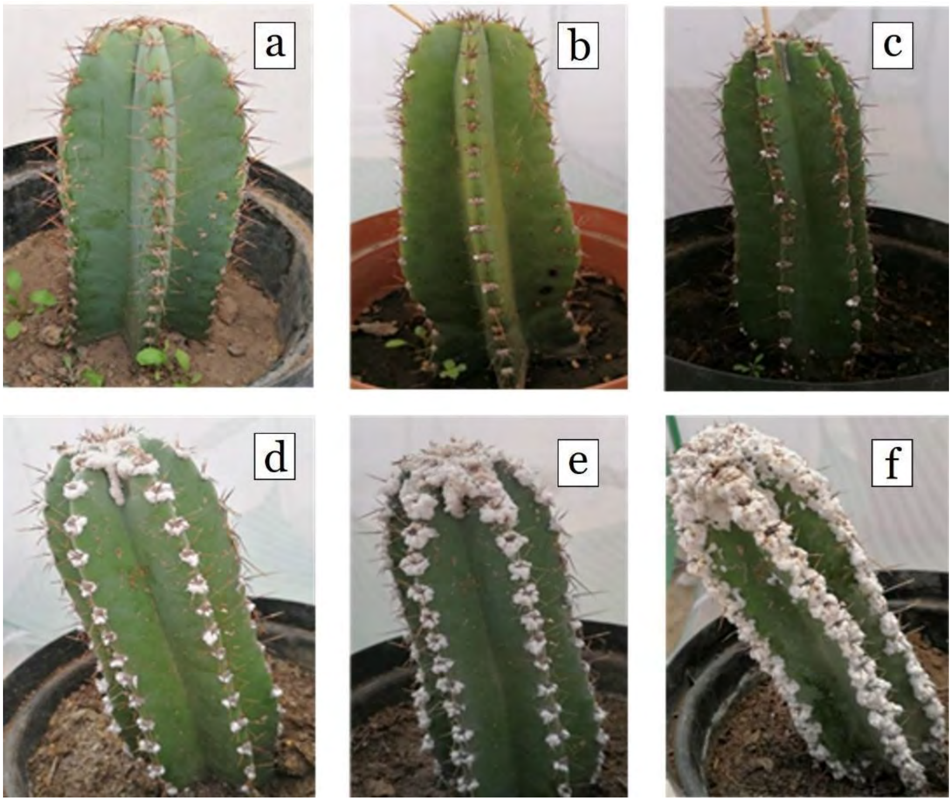

Figure 2.1: *Cereus jamacaru* infested plants representing the scale used to indicate levels of establishment of *Hypogeococcus* sp. establishment. a) Level 1 - no nymphs have settled and no white filaments (used to make the waxy protective layer) can be seen; b) Level 2 - small amounts of wax are visible on less than 20% of the spine bases; c) Level 3 - small amounts of wax is visible on 20 - 80% of the spine bases; d) Level 4 - large amounts of wax are visible on about 50% of the spine bases and small amounts on the other 50% of spine bases; e) Level 5 - all spine bases are infested and large amounts of wax are visible on >50% of the spine bases; f) Level 6 - The cactus begins to show signs of physical damage.

2.2.4. Statistical analyses

2.2.4.1. Insect performance trials

A generalised linear model (GLM) was used to assess whether initial nymph survival and the percentage of plant covered by *Hypogeococcus* sp. differed among plant species; *H. martinii*, *H. balansae*, *H. pomanensis, C. jamacaru* and *T. spachianus*. The GLM was specified using a binomial error distribution and a logit-link function. The potential effect of plant species on the time (in days) taken for nymphs to settle, the time required for female nymphs to reach maturity (measured as days until egg production) and the fecundity of mature females among host plant species, was assessed using a Poisson GLM, with a log-link function. The HSI of hosts for *Hypogeococcus* sp. among different cactus species were analysed using a Gaussian GLM, with an identity link function. For all the above analyses, fixed effect significance testing was performed using a Likelihood Ratio Test using the 'waldtest' function in the 'lmtest' R package (Zeileis & Hothorn, 2002). Where necessary, pairwise comparisons were performed using Wald's tests using the 'coeftest' function in the 'lmtest' R package (Zeileis & Hothorn, 2002). All comparisons were made using Eicker-Huber-White robust standard errors to account for heteroscedasticity between fixed effects groups (White, 1980). Statistical analyses were conducted using the statistical software R version 4.3.0. (R Core Team, 2023).

2.2.4.2. Potted plant trials

Hypogeococcus sp. coverage classes were treated as an ordinal variable with six levels (1, 2, 3, 4, 5 and 6). A cumulative link mixed model (CLMM) to assess how cactus species influenced the extent to which *Hypogeococcus* sp. populations covered the cactus was used. CLMM coefficients were estimated using the adaptive Gauss-Hermite quadrature method (Naylor & Smith, 1982). Rep ID was specified as a random intercept term to account for multiple samples being taken from the same plant. 95% confidence intervals for the CLMM coefficients were obtained by profiling the likelihood function. The CLMM was run using the 'ordinal' R package (Christensen, 2022). The assumption of proportional odds required for CLMM's was assessed using the 'nominal_test' function from the 'ordinal' R package (Christensen, 2022). The proportional odds assumption was not met for the week variable ($\chi^2 = 274.76$, df = 5, $P < 0.001$). As such, a partial proportional odds model was specified, allowing the β coefficients for the weeks predictor to vary depending on the coverage rating transition, following Peterson and Harrel (1990). Likelihood ratio tests (LRT's) were used to test for fixed effect parameter significance using the 'anova' function in R (Chambers & Hastie, 1992).

Plant mortality was modelled as a linear function of two fixed effects variables, namely: (1) plant species to determine if the *Hypogeococcus* sp. impact varied depending on the host plant and (2) time since the start of the experiment in months to evaluate how plant mortality varied over time. An interaction term between plant species and time since the experiment began was included to evaluate whether plant mortality rates varied between plant species over time. The model was specified as a logistic general linear mixed model (GLMM), with a binomial error distribution and a logit link function. The model was specified using the bias-reduction method of Firth (1993) and pseudo-data representation methodology (Kosmidis, 2007) to account for complete separation of the response variable and ensure that the regression parameters and standard errors are always finite, unlike the standard maximum likelihood estimates under conditions of complete separation by removing the first-order bias term (Firth, 1993). A null model approach (Tredennick *et al.*, 2021) was used to assess the importance of each predictor variable by comparing the Akaike's Information criterion corrected for small sample sizes (AICc) between a saturated model and the null model without the focal predictor variable. A predictor variable was considered to significantly improve model fit if it decreased the AICc score by at least 2 AICc points for each additional degree of freedom included in the saturated model. All modelling was performed using the '*brglm*' R package (Kosmidis 2021), using R version 4.3.0. (R Core Team, 2023).

2.3. Results

2.3.1. Insect performance trials

2.3.1.1. Days to establishment

Plant species significantly influenced the time required for nymphs to establish (χ^2 = 566.76, df = 4, $P < 0.001$). Nymphs took an average of eight days to settle on *T. spachianus*, but they settled notably faster on three of the previous control target species; a difference of 3.6 days on *H. martinii* (β = -0.74, z-value = -0.16, $P < 0.001$), 4.4 days on *H. balansae* (β = -0.80, z-value = -0.13, $P < 0.001$) and 3.6 days on *C. jamacaru* (β = -0.60, z-value = -0.13, $P < 0.001$) (Figure 2.2). Although not statistically significant nymphs took a similar amount of time, averaging 8.2 days, to establish on *H. pomanensis* compared to *T. spachianus*.

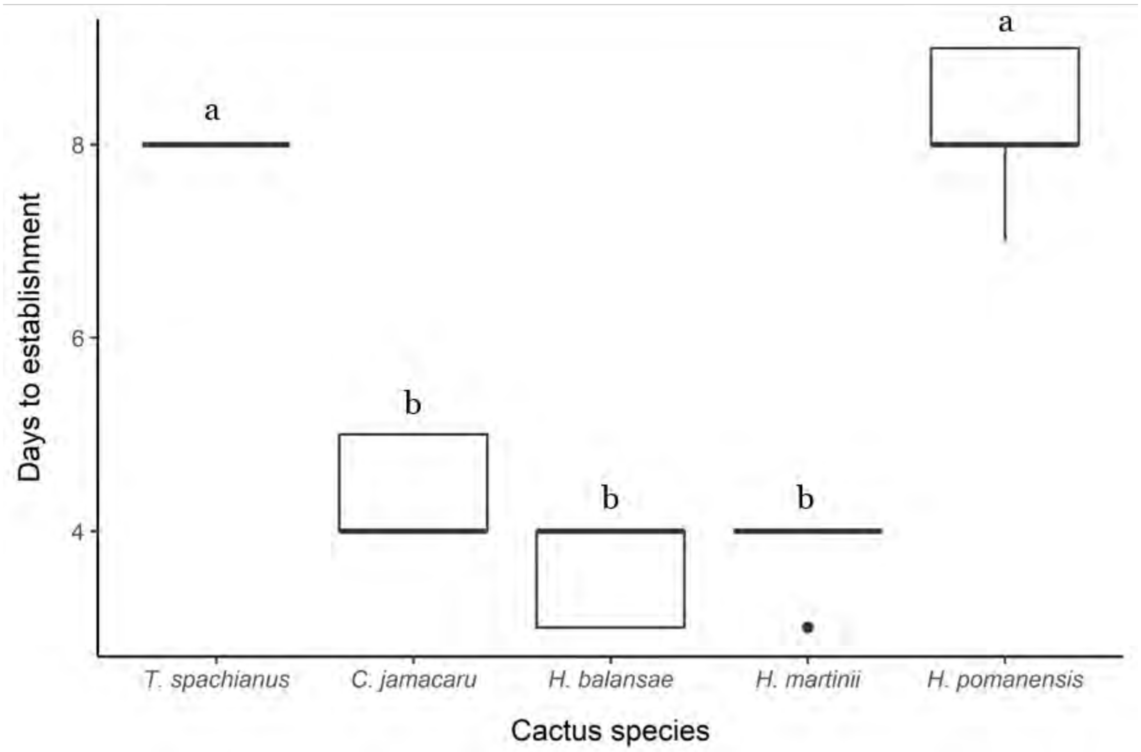

Figure 2.2: Median number of days for *Hypogeococcus* sp. nymphs to establish once placed onto each species of test cactus. A statistical difference between each cactus species, compared to only *T. spachianus*, was indicated by letters ($P < 0.05$). Whiskers represented the minimum and maximum values and the box the interquartile range. Outliers were represented by a closed circle.

2.3.1.2. Nymph survival

A statistically significant variation in nymph survival was identified among plant species ($\chi^2 = 57.15$, df = 4, $P < 0.001$). Compared to survival on *T. spachianus*, crawler survival rates were, on average, 50% higher on *H. martinii* ($\beta = 3.32$, z-value = 4.47, P =< 0.001), 52% higher on *H. balansae* ($\beta = 3.41$, z-value = 4.62, $P < 0.001$), and 42% higher on *C. jamacaru* ($\beta = 2.94$, z-value = 3.94, $P < 0.001$). In comparison to *T. spachianus*, nymph survival rates, although not statistically significant, demonstrated a slight 14% increase on *H. pomanensis* (Figure 2.3).

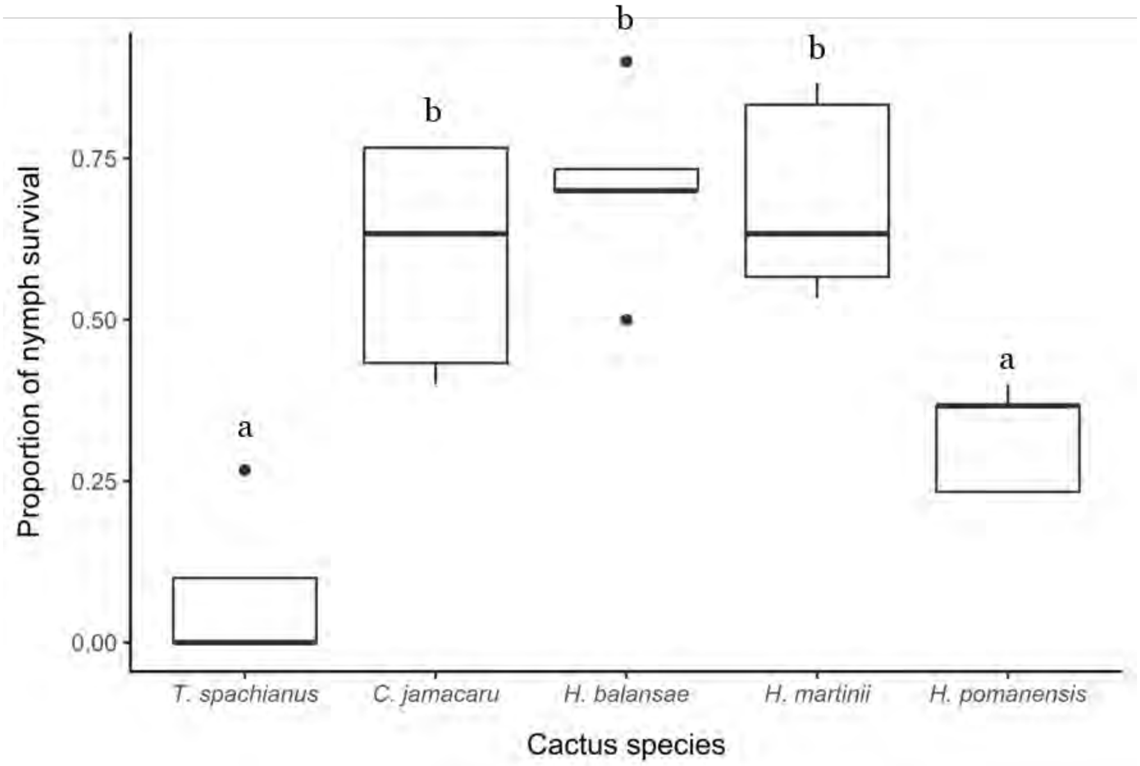

Figure 2.3: Median percentage of *Hypogeococcus* sp. nymphs that survived once placed onto each species of cactus. Whiskers represented the minimum and maximum values and the box the interquartile range. Outliers were represented by a closed circle. A statistical difference between each cactus species, compared to only *T. spachianus*, was indicated by letters ($P < 0.05$).

2.3.1.3. Days to maturity

Crawlers were not produced by female *Hypogeococcus* sp. on *H. pomanensis*. There was strong statistical evidence supporting the influence of other plant species on the time required for female *Hypogeococcus* sp. to reach maturity ($\chi^2 = 903.64$, df = 3, $P < 0.001$). Females took notably longer to mature on *T. spachianus*, with differences of 21.2 days compared to *H. martinii* ($\beta = -0.39$, *z*-value = -29.66, $P < 0.001$), 21 days compared to *H. balansae* ($\beta = -0.38$, *z*-value = -13.66, $P < 0.001$), and 13.4 days compared to *C. jamacaru* ($\beta = -0.23$, *z*-value = -8.51, $P < 0.001$) (Figure 2.4).

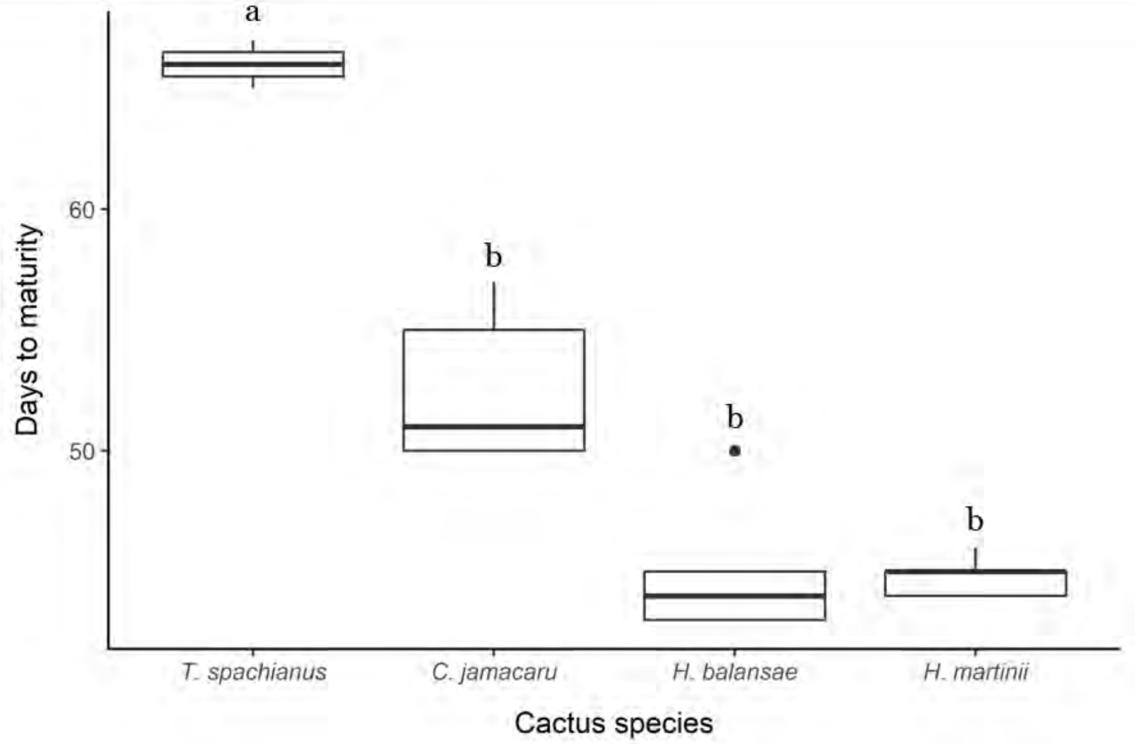

Figure 2.4: Median number of days taken for *Hypogeococcus* sp. nymphs to reach maturity on cacti hosts. Whiskers represented the minimum and maximum values and the box the interquartile range. Outliers were represented by a closed circle. A statistical difference between each cactus species, compared to only *T. spachianus*, was indicated by the letters ($P < 0.05$).

2.3.1.4. Fecundity

Despite multiple attempts, no fecundity was recorded for *Hypogeococcus* sp. on *H. pomanensis*. One explanation was that *Hypogeococcus* sp. can only live on the actively growing segments of this cactus and cut pieces of *H. pomanensis* did not exhibit the same level of growth as they did in the field.

Plant species had a significant impact on the fecundity of *Hypogeococcus* sp. (χ^2 = 10.14, df = 3, P = 0.017) (Figure 2.5). In comparison to the 35 nymphs produced by females on *T. spachianus*, roughly double the number of nymphs were produced by females on *C. jamacaru* (β = 1.74, z-value = 3.08, P = 0.002), *H. martinii* (β = 1.66, z-value = 2.95, P = 0.003) and *H. balansae* (β = 1.58, z-value = 2.79, P = 0.005) (Figure 2.5).

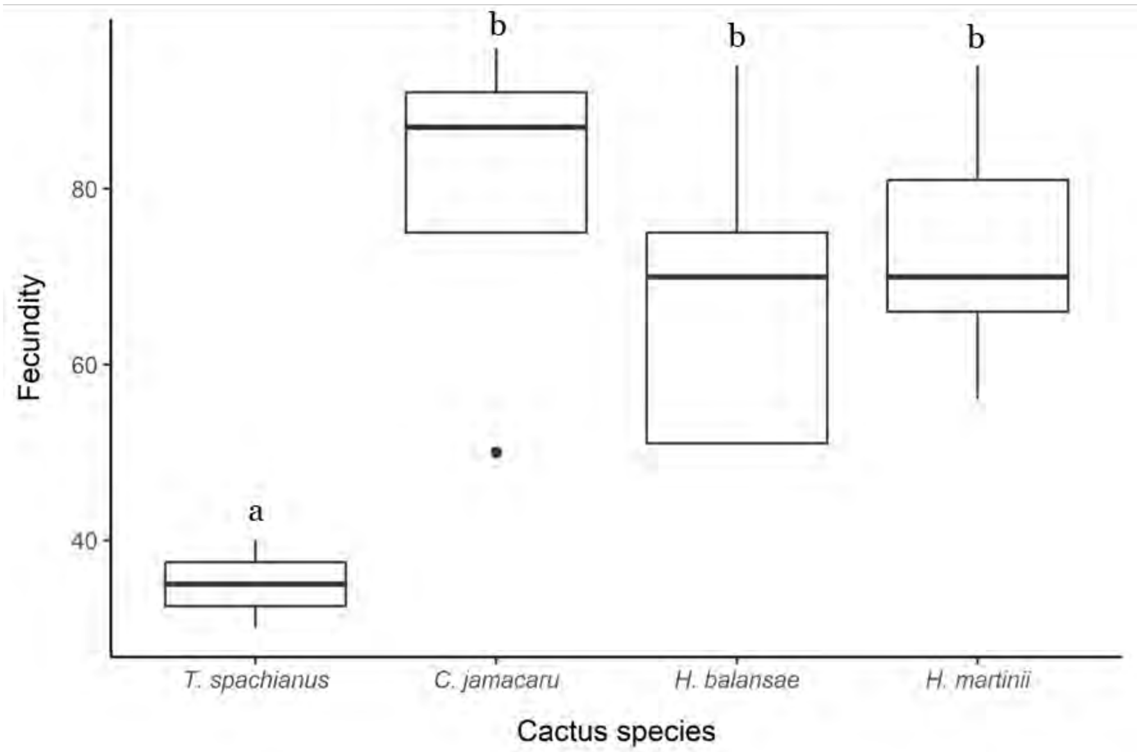

Figure 2.5: Median fecundity of female *Hypogeococcus* sp. on each cactus species. A statistical difference between each cactus species, compared to only *T. spachianus*, is indicated by letters ($P < 0.05$). Whiskers represent the minimum and maximum values and the box the interquartile range. Outliers are represented by a circle.

2.3.1.5. Percentage cover

The host plant species significantly influenced the percentage cover of the *Hypogeococcus* sp. populations ($\chi^2 = 22.59$, df = 4, $P < 0.001$). In particular, *Hypogeococcus* sp. populations covered *C. jamacaru* plants by 75% more compared to *T. spachianus* ($\beta = 2.94$, z-value = 3.94, $P < 0.001$) and on *H. martinii* plants, the insects covered an area 70% larger than that on *T. spachianus* ($\beta = 3.32$, z-value = 4.47, $P < 0.001$). Additionally, the insects occupied 65% more of the *H. balansae* plants compared to *T. spachianus* ($\beta = 3.41$, z-value = 4.62, $P < 0.001$) (Figure 2.6).

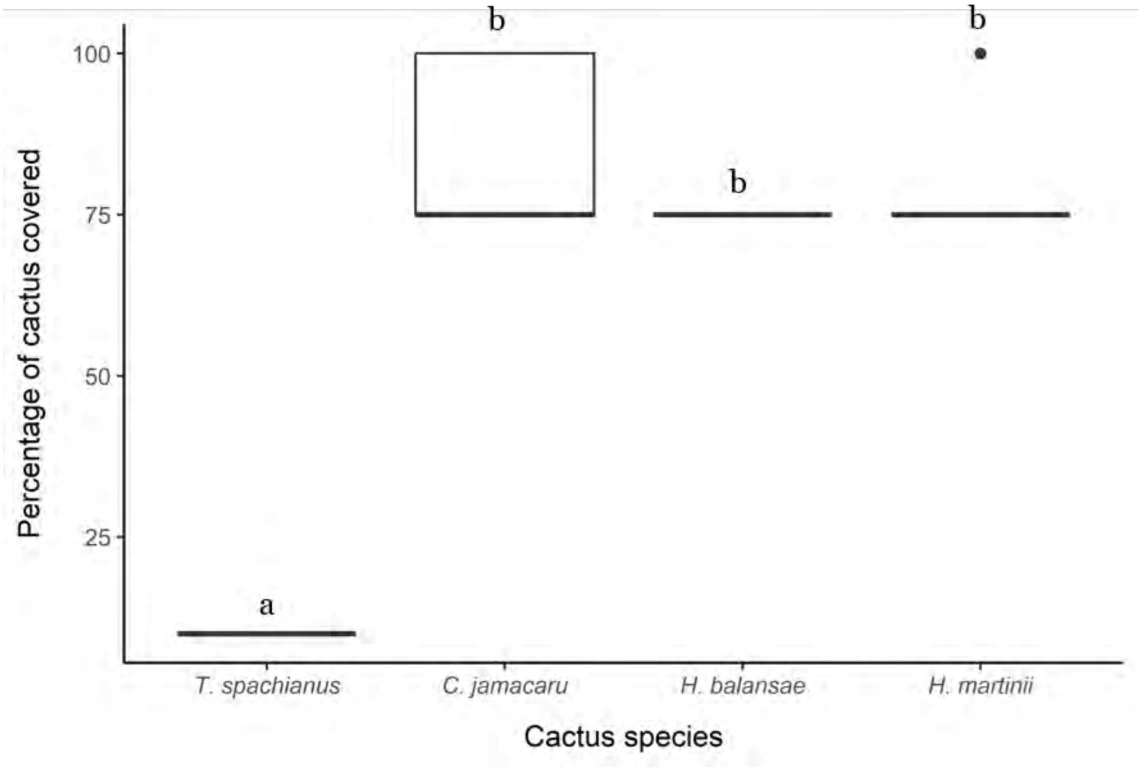

Figure 2.6: Median percentage cover of *Hypogeococcus* sp. on each cactus species four months after initial nymphs were introduced to the piece of cactus. The box represents the interquartile range. Outliers are represented by a circle. A statistical difference between each cactus species, compared to only *T. spachianus*, is indicated by letters ($P < 0.05$).

2.3.1.6. Host suitability index (HSI)

Plant species significantly influenced the suitability of the host (HSI) for *Hypogeococcus* sp. ($\chi^2 = 91.14$, df = 3, $P < 0.001$). In comparison to the HSI of *T. spachianus* (3.1), the HSI was 24.8 units higher for *C. jamacaru* ($\beta = 26.67$, z – value = 5.81, $P < 0.001$), 31.67 units higher for *H. martinii* ($\beta = 33.51$, z - value = 5.82, $P < 0.001$), and 29.7 units higher for *H. balansae* ($\beta = 31.54$, z - value = 5.27, $P < 0.001$) (Figure 2.7).

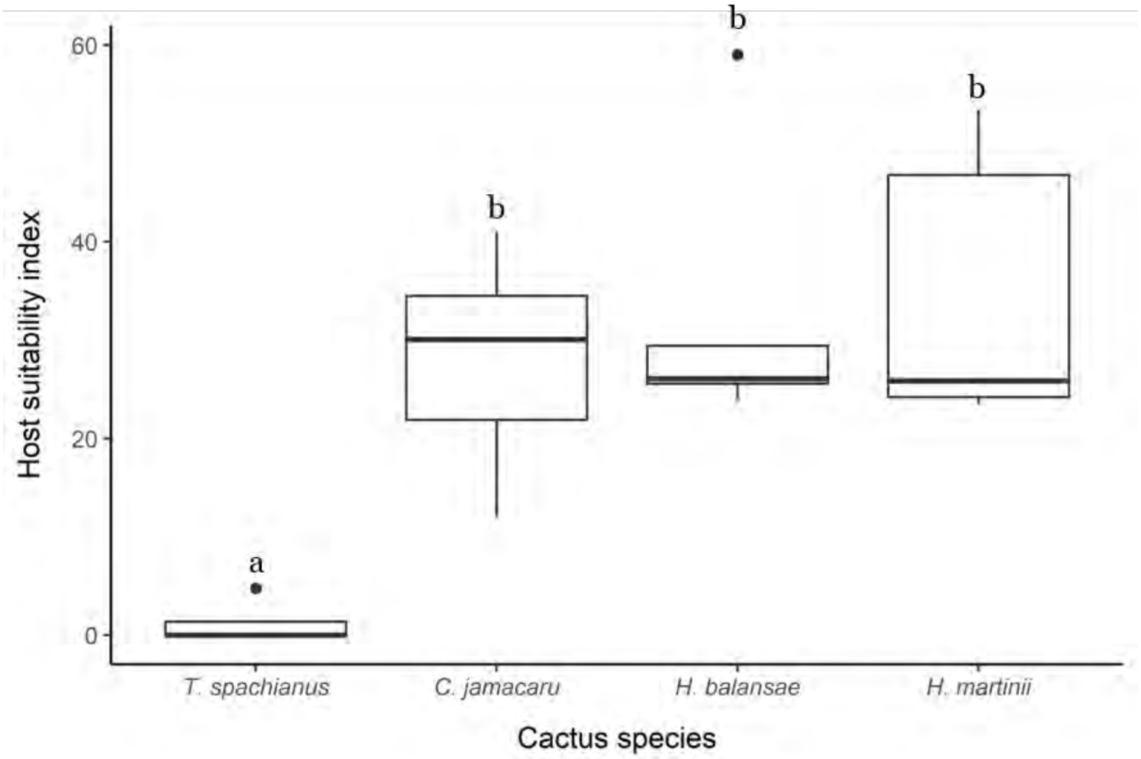

Figure 2.7: Median host suitability index values for *Hypogeococcus* sp. on each cactus species. A statistical difference between each cactus species, compared to only *T. spachianus*, is indicated by the letters ($P < 0.05$). Whiskers represent the minimum and maximum values and the box the interquartile range. Outliers are represented by a circle.

2.3.2. Potted plant trials

There was evidence for a statistically significant effect of the number of weeks on the *Hypogeococcus* sp. coverage score (χ^2 = 278.67, df= 682, P < 0.001) as well as a significant effect of the cactus species (χ^2 = 52.57, df = 682, P < 0.001). At the conclusion of the experiment, after 72 weeks, the likelihood of *Hypogeococcus* sp. attaining a coverage rating of 6 on either *T. spachianus* or *H. pomanensis* was exceedingly low, with probabilities of less than 1%. Specifically, the estimated probabilities were 0.0002% (95% profile CI: 0.00002% - 0.006%) for *T. spachianus* and 0.31% (95% profile CI: 0.03% - 3.2%) for *H. pomanensis*. The likelihood of *Hypogeococcus* sp. achieving a coverage rating of 6 for the other cactus species was much higher. The estimated probabilities for the other cactus species were 47% for *C. jamacaru* (95% profile CI:7.90 - 90.64%), 68% for *H. balansae* (95% profile CI: 17.26-95.49%) and 39% for *H. martinii* (95% profile CI: 5.92-86.80%) (Figure 2.8).

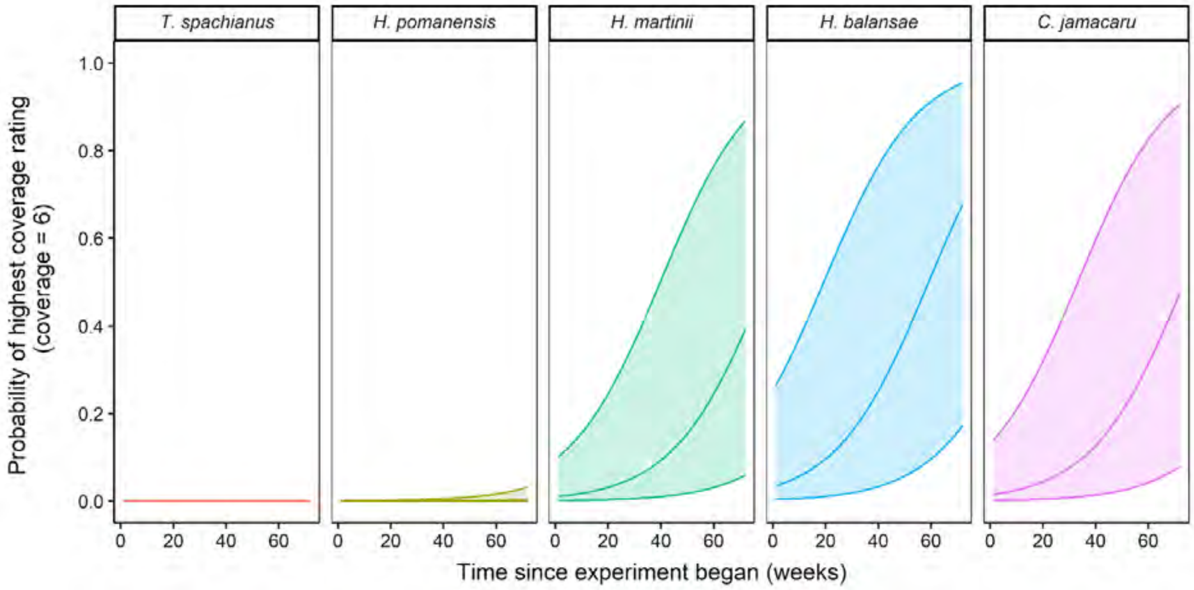

Figure 2.8: *Hypogeococcus* sp. density ratings on several cactus species. Lines showed the estimated marginal means, derived from fitted cumulative link mixed models, of the probability of cactus plants scoring in the highest *Hypogeococcus* sp. density rating (coverage = 6). Shaded bands indicated the 95% confidence interval of the mean.

There was strong statistical support for a significant interaction term between plant species and time since the start of the experiment improving model fit (global model: AIC_c = 381.00, df = 10; null model: AIC_c = 393.22, df = 6). This result indicated that plant mortality varied over time, however, the mortality rate varied between plant species (Figure 2.9). The most notable contrasts occurred between *C. jamacaru* and *T. spachianus*, whereby the probability that a *C. jamacaru* plant had died was over 50% by month 8 and 99% by the end of the experiment (month = 17), while none of the *T. spachianus* plants had died after week 17 (estimated probability of mortality was 1.9% with a 95% confidence interval of 0.01% - 25%).

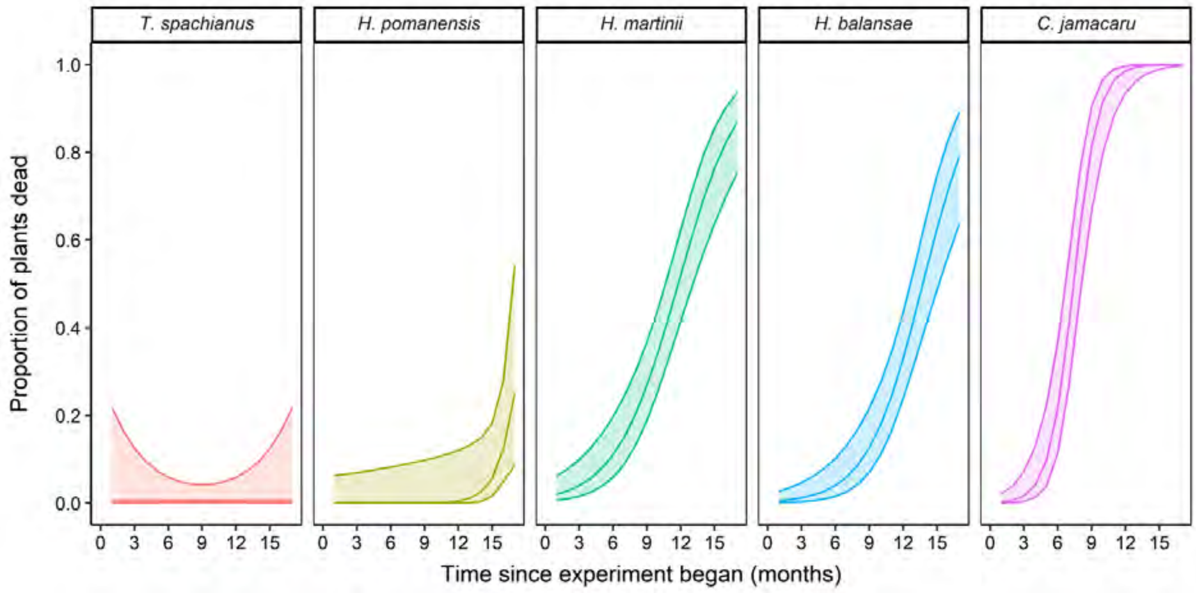

Figure 2.9: Marginal effects plots showing the effect of *Hypogeococcus* sp. on plant mortality. Lines showed the estimated marginal means, derived from a GLMM, of the probability of cactus plants being scored as alive (1) or dead (0). Shaded bands indicate the 95% confidence interval of the mean.

2.4. Discussion

Agents released and established against closely related target weeds can reduce the time and expenses associated with developing and implementing biological control agents for a new target. However, this study determined that the released *Hypogeococcus* sp. in South Africa was not a suitable biological control agent for *T. spachianus*. Low nymph survival, increased days to maturity and low fecundity were recorded for this insect on *T. spachianus*. The HSI score, which evaluated a combination of insect performance parameters, was low for *T. spachianus* compared to other cactus hosts. The observed low HSI score for *T. spachianus* indicated that any population growth of *Hypogeococcus* sp. on this cactus would be slow, given that high survival and fecundity were essential for a population to achieve high densities. The greenhouse experiment corroborated the laboratory outcomes and determined that *Hypogeococcus* sp. populations on *T. spachianus* potted plants never reached densities that caused plant mortality.

Results from both the greenhouse and laboratory experiments showed limited establishment of *Hypogeococcus* sp. on *T. spachianus*. On other cactus hosts, the highest levels of establishment were recorded on *H. martinii*, *C. jamacaru*, and *H. balansae* and a lower establishment recorded on *H. pomanensis*. Despite the initial establishment on some *T. spachianus* replicates, high mealybug mortality rates were observed in both the initial nymphs and the subsequent F1 generation, resulting in low population numbers. No impact on the target plant was recorded during these experiments, even when *Hypogeococcus* sp. settled on the cactus.

A field study on the impact of *Hypogeococcus* sp. on *C. jamacaru* (Sutton *et al.*, 2018) found a direct correlation between the increasing population density of *Hypogeococcus* sp. and the increased probability of mortality of *C. jamacaru* plants. Release of *Hypogeococcus* sp. into the field would likely not have an impact on *T. spachianus* populations, given the insects predicted slow population growth and failure to reach high densities. If any nymphs settled on *T. spachianus*, they often died a few days later, with dead insects congregated at the tips of the cactus. This suggested that other factors, such as physical or chemical defences, and nutritional requirements, play a role in their lack of *Hypogeococcus* sp. establishment on *T. spachianus* (Scriber & Slansky, 1981).

Redistributing *Hypogeococcus* sp. onto *T. spachianus* is not recommended as, although establishment levels may be low it is likely to establish at a few sites. This would be problematic as it would result in the establishment of an ineffective agent with little or no impact on the plant's population, which could hinder the establishment and success of any future biological control agents released against this cactus. If a future agent is released after *Hypogeococcus* sp. has been redistributed to *T. spachianus*, they may use similar resources from the host. Employing multiple agents that use similar resources from the target weed can lead to negative interactions (Crowe & Bourchier, 2007; Weyl & Hill, 2012). Studies have shown that sap-sucking insects, such as *Eccritotarsus catarinensis* (Carvalho) (Heteroptera: Miridae) and *Hypogeococcus* sp., can diminish the nutritional value of a plant, thereby reducing the food source and potential performance of other insects (Weyl & Hill, 2012).

Assessing an agent or combination of agent's potential effectiveness, or the ability to cause damage to the target weed, can be determined through efficacy experiments (McClay & Balciunas, 2005; Goolsby *et al*., 2009) or through field-based impact assessments in the indigenous distribution (Sutton *et al*., 2021). The potential success of a biological control agent can be estimated based on various factors like host-plant suitability, reproductive potential and voltinism (number of generations per year), which can be assessed before release. Determining which plant species are the most suitable hosts for potential biological control agents is a crucial aspect of the process. A host suitability index can be used to assess the relative suitability of different plant species as hosts for the biological control agents. The underlying assumption of the index is that insects feeding on more suitable host plants will have higher rates of survival, greater body mass, and shorter developmental times than those feeding on inferior host plants (Maw, 1976; Czypionka & Hill, 2007). By utilising the host suitability index, researchers can make informed decisions on which plant species to target with biological control agents to achieve the greatest level of control over invasive plants. However, it's crucial to note that successful biological control also relies on the ability of the agent to damage the target plant, ultimately reducing its fitness and ideally causing its death. Releasing an agent on a target weed that not only has a higher suitability but also effectively damages the plant will significantly contribute to the success of the biological control programme.

Hypogeococcus sp. is an effective biological control agent against the other cactus hosts used during these experiments. The high HSI scores recorded during the laboratory experiments likely contributed to the high infestations of *Hypogeococcus* sp. on *H. martinii*, *C. jamacaru*, and *H. balansae* in the greenhouse experiments, ultimately leading to the observed high levels of plant mortality. This mirrors the effects seen in the field, where high densities of *Hypogeococcus* sp. on *H. martinii* and *C. jamacaru* result in plant death (Klein, 2011; Sutton *et al.*, 2018). The implementation of *Hypogeococcus* sp. as a biological control agent on *H. balansae* is recent and the extent of its performance in the field is currently unknown but, based on the results from this greenhouse experiment and field observations, it is predicted that the use of *Hypogeococcus* sp. as a biological control agent against *H. balansae* will be successful.

The insect's behaviour differed on different hosts during both the laboratory and greenhouse experiments. As thigmotactic insects, *Hypogeococcus* usually gather on parts of the cactus that permit maximum contact with the plant surface, such as cracks, between buds, and under spines (McFadyen, 1979). On *H. martinii*, *H. balansae*, and *C. jamacaru*, *Hypogeococcus* sp. settled on all parts of the cactus. However, when established on *H. pomanensis*, the insects only settled at the tip of the cactus or on new growth or buds/roots. Although *Hypogeococcus* sp. established on *H. pomanensis*, initial crawler mortality was high, leading to lower initial population levels and slower population growth compared to the other cacti species. The relatively poor establishment of *Hypogeococcus* sp. on *H. pomanensis* was surprising. This slower population growth will require more time to reach a sufficient density for a noticeable mortality impact on the cactus. *Hypogeococcus* sp. is known to establish and reach high densities on this species in the field and a complete halt in fruiting was observed eight years after the release of *Hypogeococcus* sp. (Paterson *et al.*, 2021b). Despite being abundant for 10 years at this first release site (Alldays, Limpopo Province) no plant mortality has yet been recorded. Complete control of *H. pomanensis* in the field will be achieved eventually but will take longer than on other cacti such as *H. martinii* (Paterson *et al.*, 2021b). Given that *Hypogeococcus* sp. is known to take a substantial amount of time to have an effect on *H. pomanensis*, this likely explains why no mortality of *H. pomanensis* was observed for this experiment, as the experiment was conducted over a comparatively short duration (17 months). Although similar results in terms of establishment were found under greenhouse conditions for *Hypogeococcus* sp. on *H. pomanensis* and *T. spachianus,* the main mode of spread for *H. pomanensis* is through seed dispersal whereas *T. spachianus* relies primarily on asexual reproduction. Consequently, releasing *Hypogeococcus* sp. onto *H. pomanensis* can reduce the fruiting of the plant and therefore its spread, however, releasing *Hypogeococcus* sp. on *T. spachianus* will not reduce the population level or spread of this cactus.

In conclusion, there was not sufficient evidence to suggest that *Hypogeococcus* sp. would have a great enough impact on *T. spachianus* to warrant its use as a biological control agent and releasing *Hypogeococcus* sp. on *T. spachianus* could result in a disruption to future biological control efforts against this weed. Given the growing concern of *T. spachianus* in South Africa, the need for a new biological control agent was imperative. Further investigation of alternative agents was necessary and explored in the subsequent chapters of this thesis.

Chapter Three

Hypogeococcus species and lineages from Argentina as potential biological control agents for *Trichocereus spachianus*

3.1. Introduction

Hypogeococcus sp., currently used as a biological control agent in South Africa for several closely related cactus weeds, was determined to be a poor candidate against *T. spachianus* (Chapter 2). While *Hypogeococcus* sp. currently established in South Africa is not effective, several other species and lineages of *Hypogeococcus* are associated with closely related South American cactus species (McFadyen, 1979). Lineage refers to an insect population that is geographically separate or collected from another host in the insects' native range, resulting in the population adapting to its host (Paterson *et al.*, 2019). One or more of these alternative lineages in South America may prove to be effective against *T. spachianus* in South Africa. Due to the lack of indigenous populations of *T. spachianus* (Roberto Kiesling, 2018, pers. comms., Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET)), any potential *Hypogeococcus* species or lineages collected and tested as a biological control agent would constitute a new association interaction with South African *T. spachianus*.

Knowing the indigenous distribution of target plants is an important step in developing a biological control agent, but for some plants, the origin and indigenous distribution may be unknown (Paterson *et al.*, 2021a). For example, the origin of *Myriophyllum spicatum* L. (Haloragaceae), which exhibits an almost global distribution, is a subject of debate regarding its status as an alien or indigenous species in Africa (Weyl & Coetzee, 2014). Overcoming challenges associated with a weed targeted for biological control lacking indigenous populations and the unavailability of agents directly collected from indigenous plant populations can be addressed to some extent by implementing a new association biological control programme. When the target weeds origin is difficult to determine or unknown, investigating agents that are associated with closely related species (new association) may result in a successful agent being sourced (O'Connell *et al.*, 2012; Dennill & Hokkanen, 1990). For example, *C. cactorum*, a native species of Argentina that feeds on several *Opuntia* species, controlled *O. stricta*, a native North American species that *C. cactorum* had not encountered until it was released on this species in Australia (McFadyen, 1983) and the stem borer, *Megacyllene mellyi* Chevrolat (Coleoptera: Cerambycidae), that feeds on several *Baccharis* species, was collected from *Baccharis microdonta* de Candolle (Asterales: Asteraceae) for the biological control programme against *Baccharis halimifolia* L. (Asterales: Asteraceae) (Boldt, 1987). Both these agents were collected off closely related species to their target weed had a sufficiently broad host range which allowed them to utilise several species within the genus but were specific enough to still release into a new environment.

Most classical weed biological control programmes use old associations where a long evolutionary relationship between host plant and natural enemy has occurred, and where the agent is collected from populations of the target weed in the indigenous distribution (O'Connell *et al.*, 2012). Due to the evolutionary associations with their host, these insects developed the adaptions required to overcome the plants defences for successful feeding and reproduction (Dennill & Hokkanen, 1990). Although old associations are the most common biological programmes for invasive species, new associations can be extremely successful in controlling targeted pests (Hokkanen & Pimental, 1984).

New associations for cactus biological control were successful in the past and could be a solution for *T. spachianus*. Most cactophagous insects are oligophagous; their host range includes several species in a genus and potentially two or more genera of Cactaceae as hosts in their native ranges (Goeden & Kok, 1986). While certain cactophagous insects may exhibit a somewhat narrower host range, feeding solely on species within a single genus, it's important to note that this genus can encompass hundreds of cactus species that the insect may feed on. Cactus biological control programmes often use agents that do not include the target weed as a host in their native range (Moran & Zimmermann, 1984). The agent is collected from a close relative with a distribution different from the target plant. Consequently, the insect is not present in the same area as the target plant, preventing it from being included in the insect's host range in the indigenous range. Due to the oligophagous nature of cactophagous insects, it has been suggested that searches for biological control agents for cactus weeds should include the target weed and host plants that are not conspecific with the target weed (Moran & Zimmermann, 1984). Therefore, potential agents for cactus weeds can be found on alternate hosts.

Host-plants have a significant impact on the evolution and differentiation of insect populations (Antwi *et al.*, 2015). Various factors such as natural selection, genetic drift, mutation, and isolation, can result in populations of insects diverging genetically over time, whilst associated with their host plants (Antwi *et al.*, 2015; Mikheyev *et al.*, 2013). This phenomenon is referred to as host-associated genomic differentiation (HAD) (Bush, 1969; Abrahamson *et al.*, 2001). Host-associated genomic differentiation results in the emergence of distinct genetic variants within the population, referred to as lineages. These lineages may exhibit different ecological and evolutionary dynamics and display adaptations to various host plants (Bernays 1991; Antwi *et al.*, 2015). The significance of HAD in the diversification of insects is becoming widely appreciated (Stireman *et al.*, 2005; Barman *et al.*, 2012; Mikheyev *et al.*, 2013; Harrison *et al.*, 2021) and can be useful in biological control programmes to target specific host plants. Sessile insects, such as *Dactylopius* spp. (cochineal) and *Hypogeococcus*, are more likely to develop lineages compared to insects that are good dispersers (Paterson *et al.*, 2012). The sessile nature of cactophagous insects and their short generational times result in multiple generations on individual plants with little or no gene flow between populations (Karban, 1989). This, along with the rapid passing of genetic mutations through generations (Karban, 1989), and isolation by host plants, can lead to locally adapted populations (Hanks & Denno, 1994). This was observed in populations of *Pseudaulacaspis pentagona* Targioni (Hemiptera: Diaspididae), where there was a substantial increase in survival when these insects were raised on their host plant as opposed to novel trees (Hanks & Denno, 1994). As *Hypogeococcus* spp. in Argentina are found on different host plants, it is possible that each lineage of *Hypogeococcus* may have distinct characteristics, such as host preference, feeding behaviour, survival rate, and reproduction rate. Therefore, it is important to consider each lineage as a separate entity when assessing their potential as biological control agents to ensure the most effective insect is released.

The use of multiple lineages as biological control agents can be a highly effective strategy for controlling different cactus species (Volchansky *et al.*, 1999, Mathenge *et al.*, 2009b, Jones *et al.*, 2015a). The impact on the host plant species depends on the combination of agent and host plant (Mathenge *et al.*, 2010a), with impacts being most significant when the 'appropriate' lineage is associated with its preferred cactus host (Hoffmann, 2004; Paterson *et al.*, 2019; Klein *et al.*, 2020). Dactylopidae (cochineal insects), are important agents in cactus biological control and have a diverse range of hosts, with the majority of the host species being limited to the Family Opuntioideae. The Family Dactylopidae encompasses several oligophagous lineages, some of which are employed in controlling multiple target weeds and other lineages which are more host specific. Lineages of *D. tomentosus* are all restricted to *Cylindropuntia* spp. and various lineages were investigated for the control of several *Cylindropuntia* spp. that are invasive in Australia. Host specificity and efficacy trials showed that the lineages, although all restricted to *Cylindropuntia*, all differed slightly in host preferences (Jones *et al.*, 2015a, 2015b). *Dactylopius tomentosus* 'cholla' lineage had the widest host range and showed preference for *C. fulgida*, *Cylindropuntia tunicata* (Lehm.) Knuth (Caryophyllales: Cactaceae), *C. imbricata* and *Cylindropuntia kleiniae* (D. C.) Knuth (Caryophyllales: Cactaceae), the 'imbricata' lineage showed a slightly narrower host range with preference for *C. imbricata* and *C. kleiniae* and the 'rosea' lineage showed the smallest host range with preference only for *C. kleiniae* (Jones *et al.*, 2015a). Additional lineages were tested for their potential as biological control agents against the same cacti and revealed that *D. tomentosus* 'acanthocarpa × echinocarpa' lineage had its greatest impact on *C. tunicate* (Grawin), the *D. tomentosus* 'acanthocarpa' lineage was most damaging on *Cylindropuntia rosea* De Candolle (Caryophyllales: Cactaceae) (Lorne Station), while the *D. tomentosus* 'cylindropuntia sp.' lineage was most damaging on *C. rosea* (Grawin) (Jones *et al.*, 2015b). The multiple lineages of *D. tomentosus* and their divergent host preferences demonstrated the importance of testing all lineages to determine their impacts on various hosts to obtain the best match of lineage and host plant. Since *Hypogeococcus* spp. are cactus specialists and have a similar life history as cochineal (sedentary and low dispersal), there is a possibility that *Hypogeococcus* spp. contain comparable numbers and/or diversity of lineages as cochineal. lineages, given that it is a cactus specialist and has a similar life history to cochineal.

Given the cactophagous nature and past success in cactus biological control programmes of *Hypogeococcus* sp. against close relatives of *T. spachianus*, *Hypogeococcus* species and lineages were selected to assess their biological control potential against T. *spachianus* in South Africa (Paterson *et al.*, 2021b). Three species are found in South America, *H. festerianus*, *Hypogeococcus spinosus* Ferris (Hemiptera: Pseudococcidae) and *H. pungens* (McFadyen, 1979). Recent molecular genetics studies on *H. pungens* has divided this species into a species complex of two distinct clades: *H. pungens sensu stricto* and 3-4 undescribed species (Aguirre *et al.*, 2016; Poveda-Martínez *et al.*, 2019, 2020, 2022). These *Hypogeococcus* species are all restricted to Cactaceae, with the exception of *H. pungens sensu stricto*, which is restricted to Amaranthaceae (Aguirre *et al.*, 2016). The utilisation of different cactus hosts among the lineages that fall within the undescribed species suggests that different lineages may have slightly different host ranges. All the cactophagous *Hypogeococcus* are restricted to hosts that fall within the same subfamily as *T. spachianus* (Cactoideae). However, a few of the lineages that form part of the species complex feed on *H. martinii*, *H. pomanensis*, and *Cleistocactus* spp., which are the most closely related to *T. spachianus* out of the host plants, as they form part of the same subtribe, Trichocereeae.

The aim of this chapter was to evaluate the suitability of several species and lineages of *Hypogeococcus* native to Argentina as biological control agents for *T. spachianus* in South Africa. This study looked at the nymph survival, developmental days and fecundity of each *Hypogeococcus* to assess the suitability of *T. spachianus* as a host for these insects. Determining the suitability of *T. spachianus* of these agents was used as an initial screening to select agents for host specificity testing.

3.2. Materials and Methods

3.2.1. Collection of insect material

Surveys for *Hypogeococcus* were conducted in the Northern parts of Argentina at coordinates previously surveyed by researchers at Fundación para el Estudio de Especies Invasivas (FuEDEI). Each area was searched for the cactus species known to be the host of the *Hypogeococcus* species or lineage. Once the cactus plants were found, they were evaluated for signs of *Hypogeococcus* infestation indicated by white waxy filaments produced by females on the plant surface. Infested cactus was cut using a machete and placed into a paper bag and sealed. Bags were labelled with the area, cactus species and which *Hypogeococcus* had been collected (Table 3.1). The amount of cactus and insects collected from each collection site varied depending on cactus and insect densities.

Table 3.1: Names, host plant information and location of all *Hypogeococcus* spp. and lineages collected in Argentina and imported into South Africa.

Hypogeococcus	**Host plant**	**Location of collection**	**Relationship to *T. spachianus***
Hypogeococcus festerianus	*Cereus aethiops*	Mendoza	Same tribe (Cereeae)
Hypogeococcus spinosus	*Neowerdermannia* sp.	Jujuy	Same subfamily (Cactoideae)
Hypogeococcus sp. (R9)	*Harrisia pomanensis*	Rd 9 Km 1515 Guemes, Salta	Same subtribe (Trichocereinae)
Hypogeococcus sp. (Alemania)	*Cleistocactus smaragdiflorus*	Alemania, Salta	Same subtribe (Trichocereinae)
Hypogeococcus sp. (Tucuman)	*Cleistocactus* spp.	Near Toma del Río Vipos, Tucumán	Same subtribe (Trichocereinae)
Hypogeococcus sp. (Catamarca)	*Cleistocactus* spp.	Catamarca	Same subtribe (Trichocereinae)
Hypogeococcus sp. (Lo Lapachos)	*Harrisia* sp.	Los Lapachos, Salta	Same subtribe (Trichocereinae)

Field collections were transported to the FuEDEI research laboratory in Hurlingham, Buenos Aires, Argentina. Due to the presence of numerous natural enemies of *Hypogeococcus* in the native range, predators and parasitoids were removed to prevent interference with the cultures. The main enemies of *Hypogeococcus* included species of beetles (Coleoptera: Coccinellidae) and a few species of Encyrtidae (Hymenoptera: Chalcidoidea) parasitoids (Triapitsyn *et al.*, 2014; 2016; 2018; 2020). To remove beetles, both larvae and adults were removed using forceps under a microscope. To ensure the absence of parasitoids, gravid females from field-collected cultures were placed on fresh, insect-free cactus pieces, and monitored for the emergence of any parasitoids. If detected, they were removed from the containers. Nymphs were monitored until no more parasitoids were apparent. Clean cultures were created for each population of *Hypogeococcus*, which consisted of two species and five lineages (Table 3.1). These clean cultures were imported into a South African quarantine.

3.2.2. Insect performance trials

Experiments were conducted in quarantine at Rhodes University using cut pieces of cactus, following the protocol described in Chapter 2.2.2. Parameters that were measured included: nymphal survival (number of nymphs that survive on each piece of cactus), days to egg production, fecundity (number of nymphs produced by gravid females that developed on the cactus), and percentage of insect coverage four months after introduction to each piece of cactus (the percentage of surface area covered by *Hypogeococcus* sp.). Each *Hypogeococcus* was evaluated on five cactus species, including *H. martinii*, *H. pomanensis*, *H. balansae*, *C. jamacaru*, and *T. spachianus*. Each cactus species was also scored as being a new association or old association, whereby an old association was defined as the test plant belonging to the same genus as the source plant population from which each *Hypogeococcus* population was originally collected on. This was subsequently used to assess whether the HSI score was affected by the nature of the association between the agent and the host plant.

3.2.3. Statistical analyses

3.2.3.1. Insect performance trials

A GLM was used to assess variations in initial nymph survival and the percentage of plant coverage by *Hypogeococcus* across different plant species, namely, *H. martinii*, *H. balansae*, *H. pomanensis*, *C. jamacaru*, and *T. spachianus*, as well as between *Hypogeococcus* species/ lineages. For initial nymph survival and plant coverage, the GLM was specified with a binomial distribution and a logit link function. To investigate the impact of plant species on the time taken for nymphs to settle, the duration for female nymphs to reach maturity (measured in days until egg production), and the fecundity of mature females among host plant species, a Poisson GLM with a log link function was used.

3.2.3.2. HSI

The HSI for *Hypogeococcus* species/ lineages among different cactus species was analysed using a GLM. For this analysis, the GLM was specified with a Gaussian distribution and an identity link function. To evaluate the potential effect of new vs old associations between *Hypogeococcus* population performance, a linear mixed model (LMM) was used. The HSI was specified as a continuous numeric response variable and each cactus species was scored as being a new association or old association. The LMM was specified assuming a gaussian error distribution and an identity link function using the '*glmmTMB*' R package (Brooks *et al.*, 2017). Plant species was specified as a random intercept term (Bolker *et al.*, 2009). Significant differences in HSI between new vs old associations were assessed with a Likelihood Ratio Test (LRT) using the 'car' R package (Fox & Weisberg, 2019).

To explore potential interaction effects for all the above analyses, an interaction term was introduced into the model. Specifically, the interaction term between cactus species and *Hypogeococcus* was included in the GLM formula to assess how the relationship between the response variable and cactus species varied based on the *Hypogeococcus* present. For all the above analyses, fixed effect significance testing was performed using a Likelihood Ratio Test using the 'waldtest' function in the 'lmtest' R package (Zeileis & Hothorn, 2002). Where necessary, pairwise comparisons were performed using Wald's tests using the 'coeftest' function in the 'lmtest' R package (Zeileis & Hothorn, 2002). All comparisons were made using Eicker-Huber-White robust standard errors to account for heteroscedasticity between fixed effects groups (White, 1980). Statistical analyses were conducted using the statistical software R ver. 4.2.2, (R Core Team, 2023).

3.3. Results

3.3.1. Insect performance trials

All populations of *Hypogeococcus* were imported successfully into South Africa and into the quarantine facility at Rhodes University. The cultures were sustained for all populations, with the exception of *H. spinosis*, as all female *H. spinosis* individuals died before producing any offspring on the imported plants. Maintaining this species in quarantine conditions is challenging, as this species is possibly adapted to a very specific, cooler climate compared to the other *Hypogeococcus* (B. Aguirre 2019, pers. comms, FuEDEI). Establishment and performance trials for the remaining *Hypogeococcus* populations were carried out successfully.

3.3.1.1. Days to establishment

The time taken for nymphs to establish was significantly influenced by the interaction between cactus species and species/ lineage of *Hypogeococcus* ($\chi^2 = 97.41$, df = 18, $P < 0.0001$) (Figure 3.1). The Alemania lineage did not establish on *T. spachianus*, but on average took 5 days to establish on *C. jamacaru*, 3 days on *H. balansae*, 4 days to establish on *H. martinii*, and 6 days to establish on *H. pomanensis*. The Catamarca lineage did not establish on *T. spachianus* but took around 5 days to establish on *C. jamacaru* and *H. balansae*, and 6 days on *H. martinii* and *H. pomanensis*. Comparatively, the Del Rio lineage settled 2 days faster on *C. jamacaru*, 1 day faster on average for *H. balansae*, *H. martinii* (z-value = -9.102, $P < 0.0001$), and *H. pomanensis* in contrast to the 6 days taken to settle on *T. spachianus*. The Los Lapachos lineage, which took 7 days to settle on *T. spachianus*, settled 1 day faster on *C. jamacaru*, 3 days faster on *H. balansae* (z-value = -3.17, $P = 0.006$), and *H. pomanensis* (z-value = -3.19, $P = 0.0056$), and 2 days faster on *H. martinii* (z-value = -3.78, $P = 0.0006$). The R9 lineage, which took 10 days to establish on *T. spachianus*, settled 4 days faster on *C. jamacaru* (z-value = -3.37, $P = 0.003$), 5 days faster on *H. balansae* (z-value = -41.40, $P < 0.0001$), *H. martinii* (z-value = -8.31, $P < 0.0001$), and 2 days faster on *H. pomanensis* (z-value = -27.87, $P < 0.0001$). *Hypogeococcus festerianus* did not establish on *T. spachianus*, but the nymphs took 4 days on average to settle on *C. jamacaru* and *H. martinii*, and 5 days to settle on *H. balansae* and *H. pomanensis*.

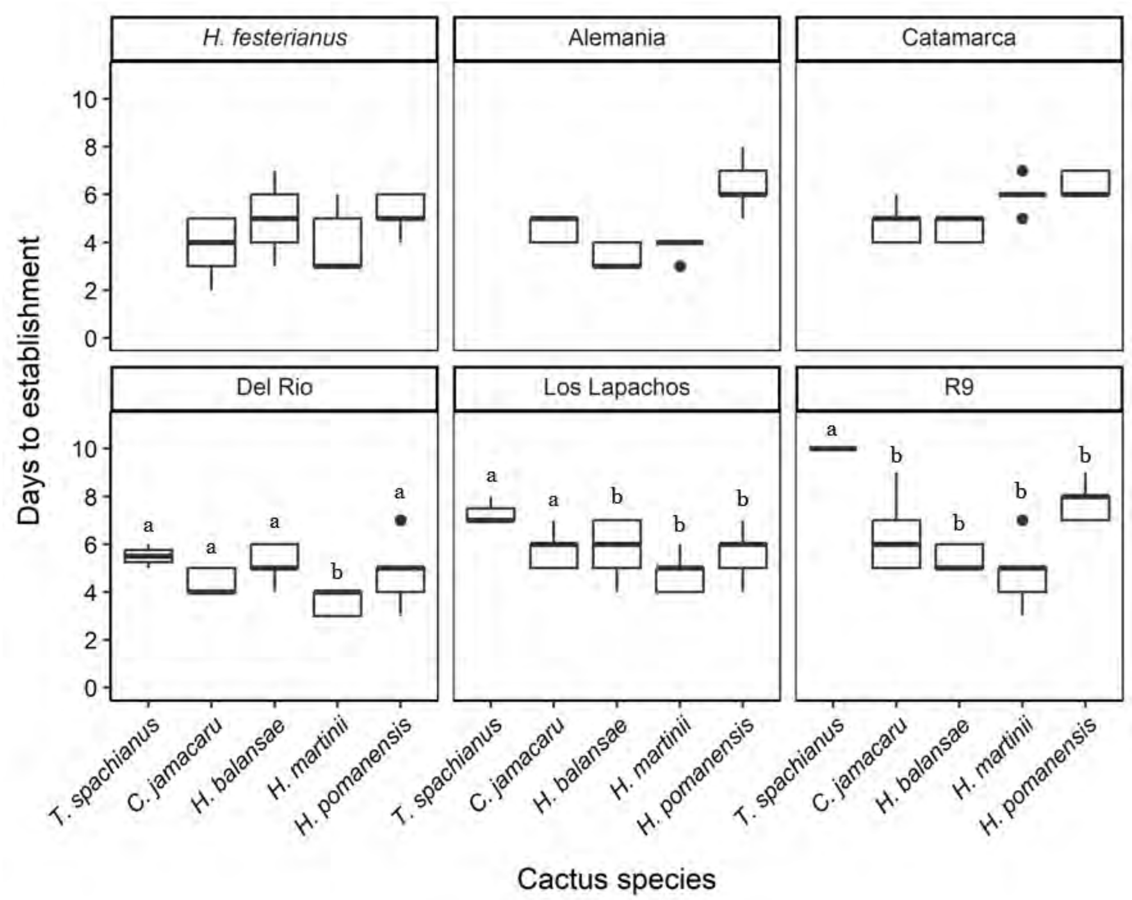

Figure 3.1: The median duration, in days, for nymphs from each *Hypogeococcus* species/lineage to settle on various cactus species. Whiskers represent the minimum and maximum values and the box the interquartile range. Outliers are represented by a circle. For lineages where statistical analyses were possible a statistical difference between each cactus species, compared to only *T. spachianus*, is indicated letters ($P <$ 0.05).

3.3.1.2. Survival

There was a significant interaction between cactus species and *Hypogeococcus* species/ lineage on nymph survival ($\chi^2 = 1982.6$, df = 20, $P < 0.001$) (Figure 3.2). Compared to their survival rates on *T. spachianus* (20%), the Alemania lineage exhibited a 78% increased survival on *C. jamacaru* (*z*-value = 5.29, $P < 0.0001$), 66% higher survival on *H. martinii* (*z*-value = 5.68, $P < 0.0001$), 75% higher on *H. balansae* (*z*-value = 5.29, $P < 0.001$), and 64% higher on *H. pomanensis* (*z*-value = 4.29, $P = 0.0001$). The Catamarca lineage, which had 0% survival on *T. spachianus*, showed significantly higher survival rates on other cactus species: 51% survival rate on *C. jamacaru* (*z*-value = 18.69, $P < 0.0001$), 83% survival rate on *H. balansae* (*z*-value = 25.29, $P < 0.001$), 57% survival on *H. martinii* (*z*-value = 26.70, $P < 0.0001$), and 37% survival on *H. pomanensis* (*z*-value = 22.66, $P < 0.0001$). Similarly, the Del Rio lineage exhibited 73% higher survival on *C. jamacaru* (*z*-value = 3.98, $P = 0.0003$), 71% higher on *H. balansae* (*z*-value = 4.00, $P = 0.003$), 65% higher on *H. martinii* (*z*-value = 3.93, $P = 0.0003$), and 41% higher on *H. pomanensis* (*z*-value = 3.14, $P = 0.0068$) compared to their 7% survival rates on *T. spachianus*. *Hypogeococcus festerianus* had 0% survival on *T. spachianus* but exhibited substantially higher survival rates on other cactus species: 87% survival rate on *C. jamacaru* (*z*-value = 22.87, $P < 0.0001$), 84% on *H. balansae* (*z*-value = 23.37, $P < 0.001$), 74% on *H. martinii* (*z*-value = 23.22, $P < 0.0001$), and 54% on *H. pomanensis* (*z*-value = 20.66, $P < 0.0001$) compared to *T. spachianus*. The Los Lapachos lineage, which had an 11% survival rate on *T. spachianus*, displayed a 32% higher survival rate on *C. jamacaru*, 66% higher on *H. balansae* (*z*-value = 5.83, $P < 0.001$), 61% higher on *H. martinii* (*z*-value = 5.31, $P < 0.0001$), and 12% higher on *H. pomanensis*. The R9 lineage had an 8% survival rate on *T. spachianus* but showed higher survival rates on other cactus species: 65% higher on *C. jamacaru* (*z*-value = 3.65, $P = 0.001$), 79% higher on *H. balansae* (*z*-value = 4.58, $P < 0.001$), 65% higher on *H. martinii* (*z*-value = 6.52, $P < 0.0001$), and 40% higher on *H. pomanensis* (*z*-value = 3.25, $P = 0.0046$).

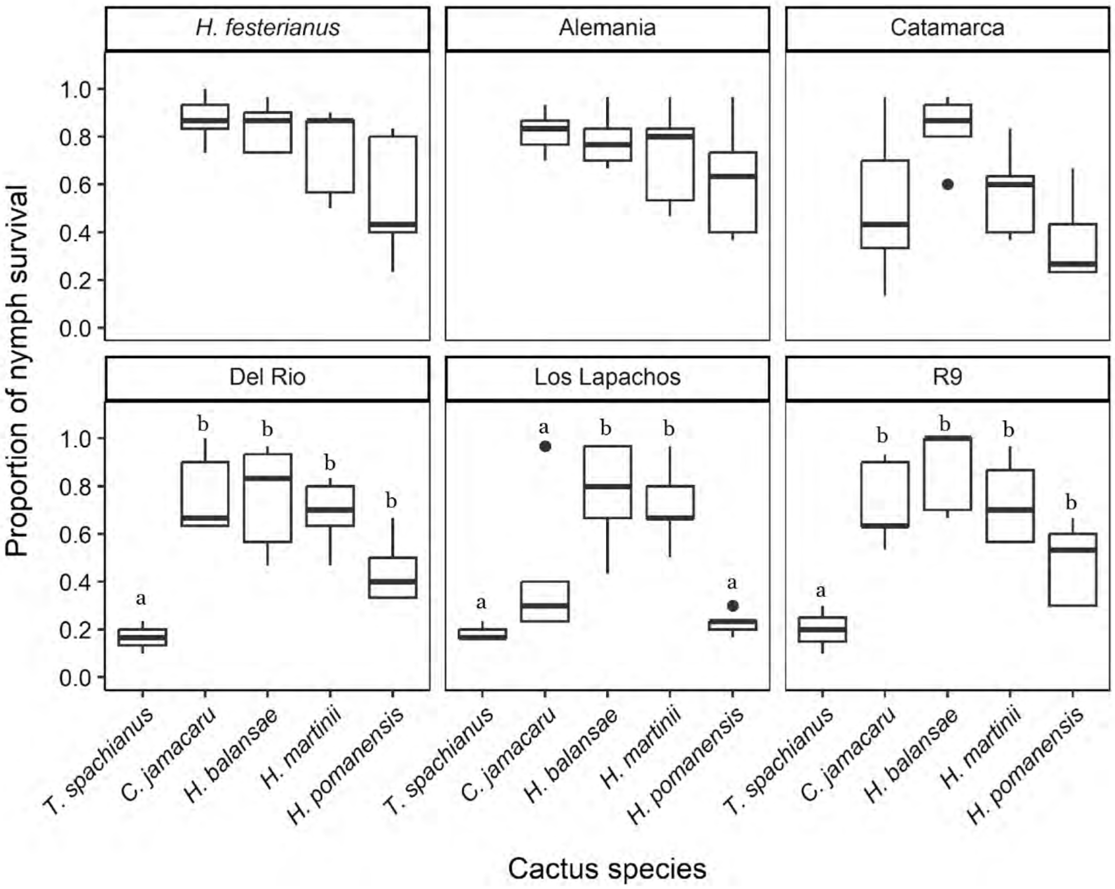

Figure 3.2: Initial median survival of the 30 nymphs from each *Hypogeococcus* populations on each cactus species. Whiskers represent the minimum and maximum values and the box the interquartile range. Outliers are represented by a circle. For lineages where statistical analyses were possible a statistical difference between each cactus species, compared to only *T. spachianus*, is indicated letters (P < 0.05).

3.3.1.3. Development days

The duration for female nymphs to reach maturity was significantly impacted by the interaction between cactus species and the *Hypogeococcus* species/lineage (χ^2 = 1827.7, df = 17, P < 0.0001) (Figure 3.3). The Alemania lineage failed to establish on *T. spachianus*, taking 46 days to develop on *C. jamacaru*, 47 days on *H. martinii*, 41 days on *H. balansae*, and 40 days on *H. pomanensis*. Similarly, the Catamarca lineage did not establish on *T. spachianus* but took 52 days to develop on *C. jamacaru*, 56 days on *H. martinii*, 34 days on *H. balansae*, and 60 days on *H. pomanensis*. Del Rio nymphs, which typically took 60 days to develop on *T. spachianus*, exhibited faster development when on the other hosts, taking 14 days less on *C. jamacaru* (*z*-value = -7.63, *P* < 0.0001), 11 days less on *H. balansae* (*z*-value = -21.19, *P* < 0.001), 17 days less on *H. martinii* (*z*-value = -8.25, *P* < 0.0001), and *H. pomanensis* (*z*-value = -3.29, *P* = 0.004). The Los Lapachos lineage, which took 55 days to develop on *T. spachianus*, took on average 4 days longer to develop on *C. jamacaru* (*z*-value = 8.94, *P* < 0.0001) but developed 19 days faster on *H. balansae* (*z*-value = -16.92, *P* < 0.0001), 7 days faster on *H. martinii* (*z*-value = -9.85, *P* < 0.0001), and 11 days faster on *H. pomanensis* (*z*-value = -10.96, *P* < 0.0001). The R9 lineage nymphs, taking 60 days to develop on *T. spachianus*, showed faster development on the other cacti hosts, taking on average 27 days less on *C. jamacaru* (*z*-value = -21.08, *P* < 0.0001), 1 day less on *H. balansae* (*z*-value = -2.14, *P* = 0.123), 16 days less on *H. martinii* (*z*-value = -26.89, *P* < 0.0001), and 6 days less on *H. pomanensis*. *Hypogeococcus festerianus* did not establish on *T. spachianus*, but nymphs took an average of 61 days to develop on *C. jamacaru*, 37 days on *H. martinii*, 39 days on *H. balansae*, and 60 days on *H. pomanensis*.

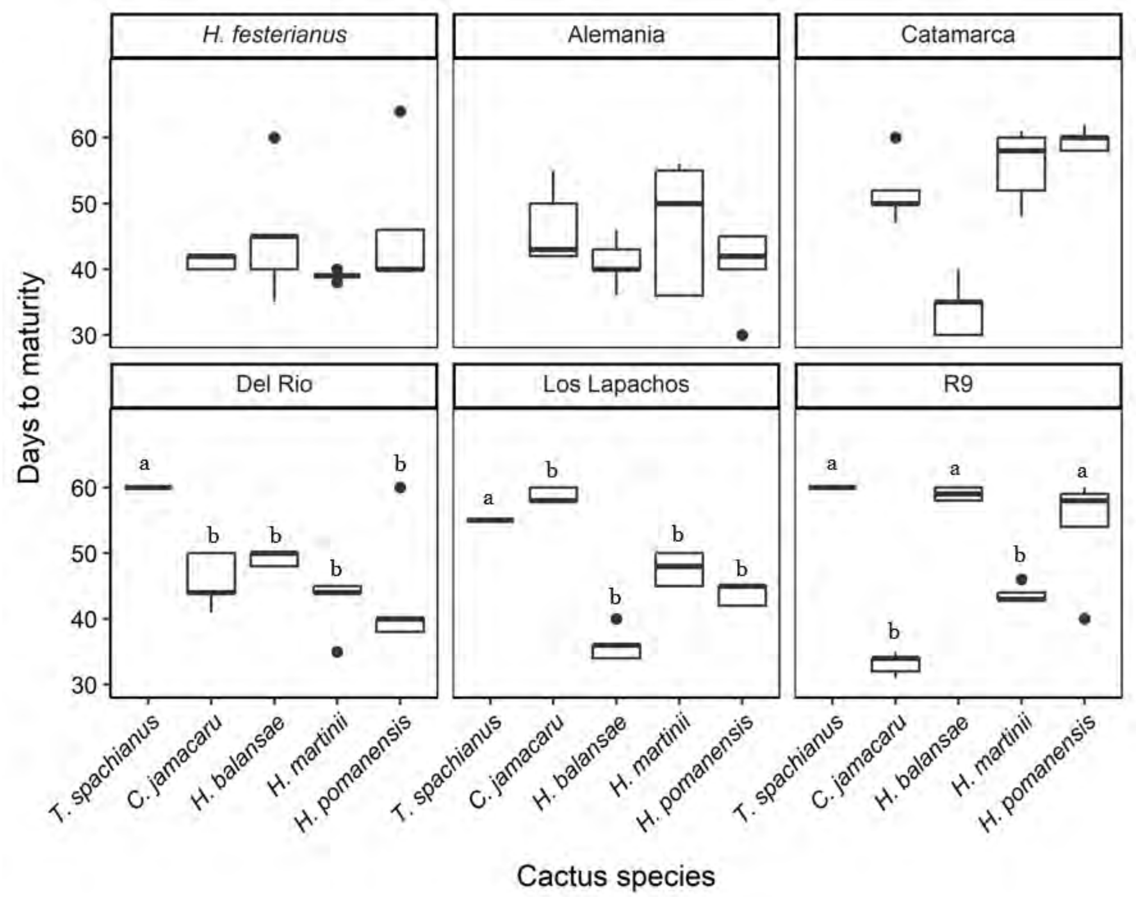

Figure 3.3: The median time, measured in days, taken by nymphs from each *Hypogeococcus* population to reach maturity on different cactus species. Whiskers represent the minimum and maximum values and the box the interquartile range. Outliers are represented by a circle. For lineages where statistical analyses were possible a statistical difference between each cactus species, compared to only *T. spachianus*, is indicated letters ($P < 0.05$).

3.3.1.4. Fecundity

A significant interaction effect between cactus species and *Hypogeococcus* species/lineage was observed in relation to fecundity ($\chi^2 = 728.49$, df = 17, $P < 0.0001$) (Figure 3.4). Due to the absence of establishment of the Alemania lineage on *T. spachianus*, no fecundity measurements were possible. However, Alemania lineage females produced an average of 68 nymphs on *C. jamacaru*, 46 nymphs on *H. martinii*, 92 nymphs on *H. balansae*, and 60 nymphs on *H. pomanensis*. Similarly, the Catamarca lineage did not establish on *T. spachianus*, leading to no nymph production on this plant. However, on other cacti, Catamarca females produced an average of 68 nymphs on *C. jamacaru*, 58 nymphs on *H. martinii*, 80 nymphs on *H. balansae*, and 55 nymphs on *H. pomanensis*. In contrast to the 1 nymph produced by Del Rio lineage females on *T. spachianus*, they produced 55 nymphs on *C. jamacaru* (z-value = 53.59, $P < 0.0001$), 59 nymphs on *H. balansae* (z-value = 50.55, $P < 0.0001$), 76 nymphs on *H. martinii* (z-value = 51.91, $P < 0.0001$), and 83 nymphs on *H. pomanensis* (z-value = 26.28, $P < 0.0001$). Los Lapachos lineage females, which produced an average of 40 nymphs on *T. spachianus*, showed increased nymph production on all other cacti, yielding 88 nymphs on *C. jamacaru* (z-value = 128.11, $P < 0.0001$), 76 nymphs on *H. balansae* (z-value = 14.06, $P < 0.0001$), 81 nymphs on *H. martinii* (z-value = 13.16, $P < 0.0001$), and 79 nymphs on *H. pomanensis* (z-value = 18.72, $P < 0.0001$). The R9 lineage females, which produced an average of 22 nymphs on *T. spachianus*, displayed higher nymph production on all other cacti, generating 52 nymphs on *C. jamacaru* (z-value = 4.51, $P < 0.0001$), 79 nymphs on *H. balansae* (z-value = 7.68, $P < 0.0001$), 40 nymphs on *H. martinii* (z-value = 3.91, $P = 0.0004$), and 56 nymphs on *H. pomanensis* (z-value = 4.28, $P = 0.0001$). *Hypogeococcus festerianus* did not establish on *T. spachianus*, resulting in no nymph production on this plant. However, this lineage produced 61 nymphs on *C. jamacaru*, 37 nymphs on *H. martinii*, 39 nymphs on *H. balansae*, and 60 nymphs on *H. pomanensis*.

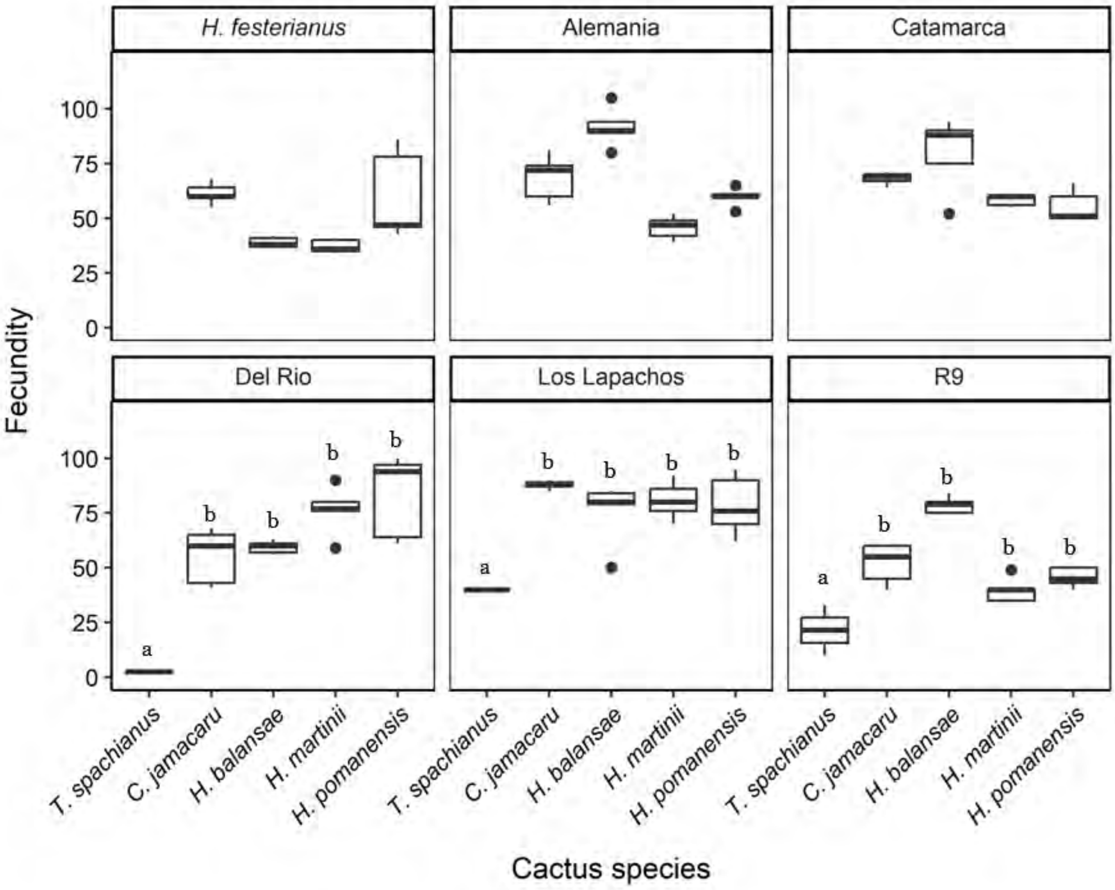

Figure 3.4: The median number of nymphs produced by females of each *Hypogeococcus* population on different cactus species. The interquartile range is depicted by the box, with whiskers indicating the minimum and maximum values. Outliers are denoted by circles. For lineages where statistical analyses were possible a statistical difference between each cactus species, compared to only *T. spachianus*, is indicated letters ($P < 0.05$).

3.3.1.5. Percentage of cover

There was a notable interaction effect between cactus species and *Hypogeococcus* species/lineage concerning the percentage of cactus covered by *Hypogeococcus* after four months ($\chi^2 = 1659.2$, $20 = 20$, $P < 0.0001$) (Figure 3.5). In the study, populations of Alemania lineage covered only 10% of *T. spachianus* plants, whereas they covered 40% more of *C. jamacaru* plants (z-value = 2.71, $P = 0.026$), 55% more of *H. balansae* (z-value = 4.93, $P < 0.0001$), and *H. martinii* (z-value = 4.87, $P < 0.0001$), and 20% higher on *H. pomanensis* (z-value = 3.99, $P = 0.0003$). The Catamarca lineage covered 0% of *T. spachianus* plants but 42% of *C. jamacaru* plants (z-value = 21.44, $P < 0.0001$), 65% of *H. balansae* plants (z-value = 23.50, $P < 0.0001$), 32% of *H. martinii* plants (z-value = 18.67, $P < 0.0001$), and 22% of *H. pomanensis* plants (z-value = 21.11, $P < 0.0001$). The Del Rio lineage covered 10% of *T. spachianus* plants, but its coverage significantly increased when placed on other cacti. This lineage covered 50% more of *C. jamacaru* plants (z-value = 5.67, $P < 0.0001$), 70% more of *H. balansae* plants (z-value = 6.93, $P < 0.0001$), 80% more of *H. martinii* plants (z-value = 9.33, $P < 0.0001$), and 45% more of *H. pomanensis* plants (z-value = 5.04, $P < 0.0001$) compared to *T. spachianus*. Similarly, the Los Lapachos lineage covered an average of 10% of *T. spachianus* plants but they cover 3% more of *C. jamacaru* plants, 60% more of *H. balansae* plants (z-value = 7.18, $P < 0.001$), 25% more of *H. martinii* plants (z-value = 5.07, $P < 0.0001$), and 6% more of *H. pomanensis* plants. Meanwhile, the R9 lineage covered 20% of *T. spachianus* plants on average but covered 30% more of *C. jamacaru* plants (z-value = 3.93, $P = 0.0003$), 45% more of *H. balansae* plants (z-value = 7.17, $P < 0.001$), and *H. martinii* plants (z-value = 6.23, $P < 0.0001$), and 10% more of *H. pomanensis* plants (z-value = 5.68, $P < 0.0001$). *Hypogeococcus festerianus* populations covered 0% of *T. spachianus* plants, but covered significantly more of the other cacti hosts, they covered 70% more of *C. jamacaru* plants (z-value = 19.75, $P < 0.0001$),60% more of *H. martinii* plants (z-value = 21.90, $P < 0.0001$), 70% of *H. balansae* plants (z-value = 20.61, $P < 0.0001$), and 27% more of *H. pomanensis* plants (z-value = 18.75, $P < 0.0001$).

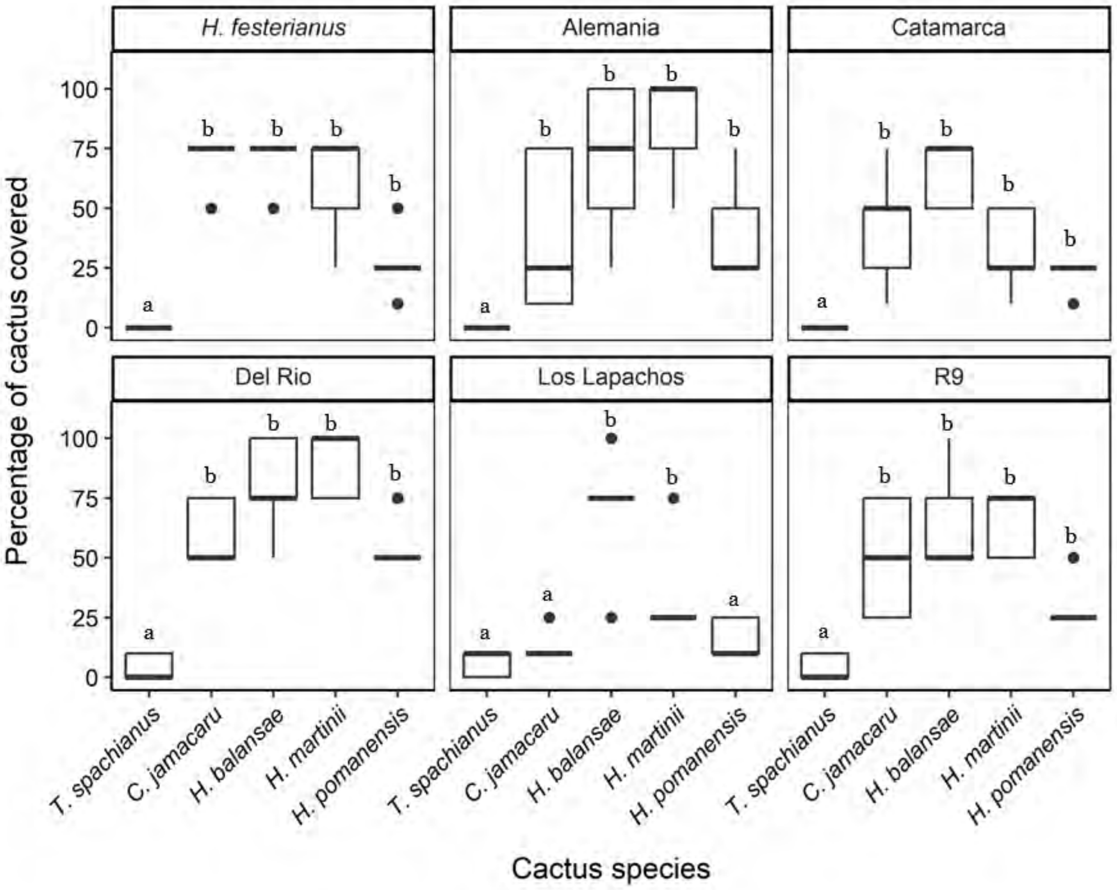

Figure 3.5: Median percentage cover of each *Hypogeococcus* population on five different cactus species. Whiskers represent the minimum and maximum values and the box the interquartile range. Outliers are represented by a circle. For lineages where statistical analyses were possible a statistical difference between each cactus species, compared to only *T. spachianus*, is indicated letters (P < 0.05).

3.3.1.6. Host suitability index (HSI)

Where the *Hypogeococcus* species/ lineage did not establish on a cactus host an HSI score of 0 was assigned for the analyses. A significant interaction between cactus species and *Hypogeococcus* species/lineage was found in relation to the host suitability index (χ^2 = 225.05, df = 20, *P*<0.0001) (Figure 3.6). The Alemania lineage had an HSI value of 0 when associated with *T. spachianus*, increasing significantly on other hosts: by 108 units on *C. jamacaru* (*z*-value = 7.16, *P* < 0.0001), 116 units on *H. balansae* (*z*-value = 43.41, *P* < 0.0001), 63 units on *H. martinii* (*z*-value = 13.00, *P* < 0.0001), and 40 units on *H. pomanensis* (*z*-value = 17.30, *P* < 0.0001). The Catamarca lineage had a HSI score of 0 on *T. spachianus*, which increased by 70 units on *C. jamacaru* (*z*-value = 3.72, *P* = 0.0012), 202 units on *H. balansae* (*z*-value = 13.52, *P* < 0.0001), 59 units on *H. martinii* (*z*-value = 12.43, *P* < 0.0001), and 35 units on *H. pomanensis* (*z*-value = 3.99, *P* = 0.0005). The Del Rio lineage had an HSI value of 0.3 on *T. spachianus*, increasing by 90 units on *C. jamacaru* (*z*-value = 21.48, *P* < 0.0001) and *H. balansae* (*z*-value = 7.22, *P* < 0.0001), 122 units on *H. martinii* (*z*-value = 21.27, *P* < 0.0001), and 84 units on *H. pomanensis* (*z*-value = 10.54, *P* < 0.0001). Los Lapachos had an HSI score of 17 on *T. spachianus*, rising by 47 units on *C. jamacaru* (*z*-value = 3.57, *P* = 0.002), 140 units on *H. balansae* (*z*-value = 8.20, *P* < 0.001), 106 units on *H. martinii* (*z*-value = 17.70, *P* < 0.0001), and 23 units on *H. pomanensis* (*z*-value = 9.90, *P* < 0.0001). The R9 lineage had an HSI score of 9 on *T. spachianus*, increasing by 104 units on *C. jamacaru* (*z*-value = 7.34, *P* < 0.001), 111 units on *H. balansae* (*z*-value = 7.85 *P* < 0.001), 58 units on *H. martinii* (*z*-value = 7.14, *P* < 0.0001), and 27 units on *H. pomanensis* (*z*-value = 3.26, *P* = 0.006). *Hypogeococcus festerianus* had an HSI score of 0 on *T. spachianus*, which increased significantly to 130 units on *C. jamacaru* (*z*-value = 14.06, *P* < 0.0001), 76 units on *H. balansae* (*z*-value = 42.40, *P* < 0.0001), 71 units on *H. martinii* (*z*-value = 5.99, *P* < 0.0001), and 75 units on *H. pomanensis* (*z*-value = 6.22, *P* < 0.0001).

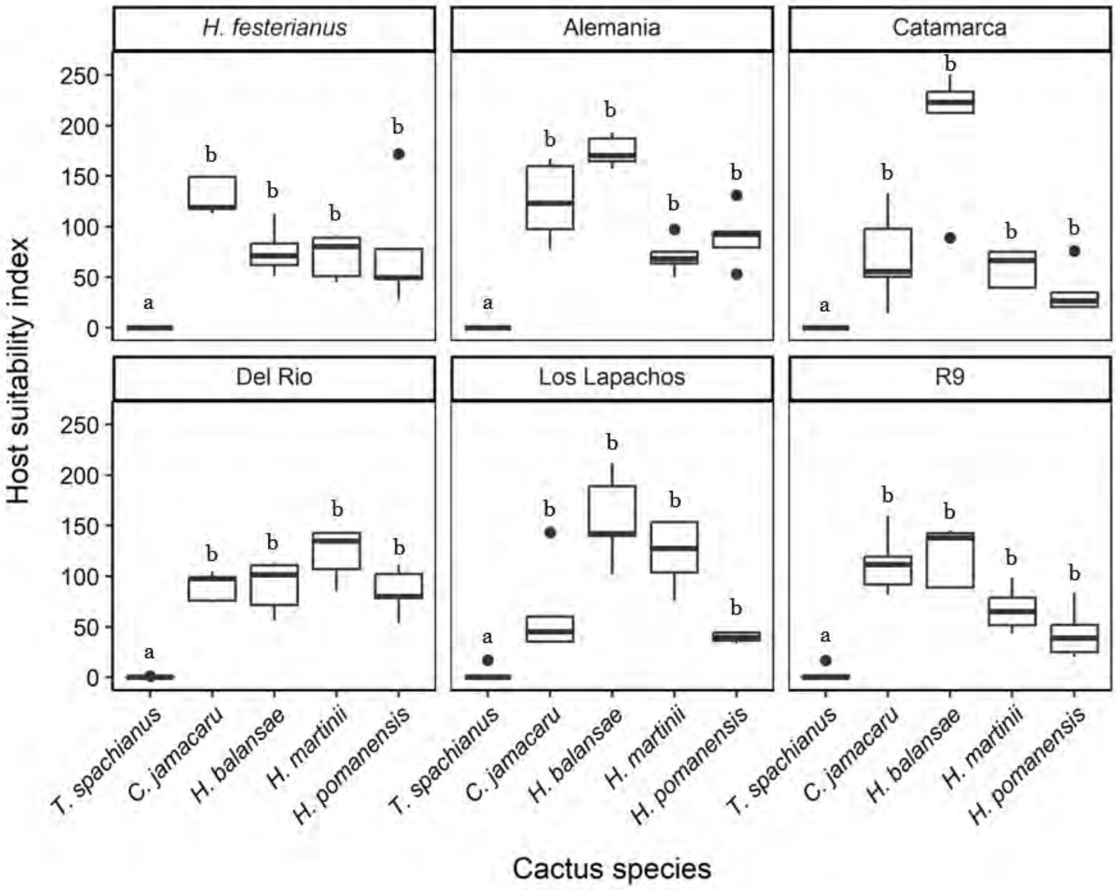

Figure 3.6: The median suitability of various cactus species as hosts for each *Hypogeococcus* population. The interquartile range is shown by the box, with whiskers indicating the minimum and maximum values. Outliers are denoted by circles. For lineages where statistical analyses were possible a statistical difference between each cactus species, compared to only *T. spachianus*, is indicated letters ($P <$ 0.05).

There was statistical support for significantly higher HSI scores for old associations between *Hypogeococcus* and their cactus hosts for both the *H. festerianus* ($\chi^2 = 5.35$, df = 1, $P = 0.021$) and *Hypogeococcus* sp. lineages ($\chi^2 = 3.79$, df = 1, $P = 0.050$) (Figure 3.7). Indeed, the HSI scores were 75 ± 32 units higher for old associations in the *H. festerianus* lineage, and 13 ± 7 units higher for old associations in the *Hypogeococcus* sp. lineage. While not considered statistically significant, HSI scores were also 73 ± 38 units higher for old associations in the Los Lapachos lineage. In contrast, there was no evidence for a statistically significant difference in HSI scores between new vs old associations in the R9 lineage.

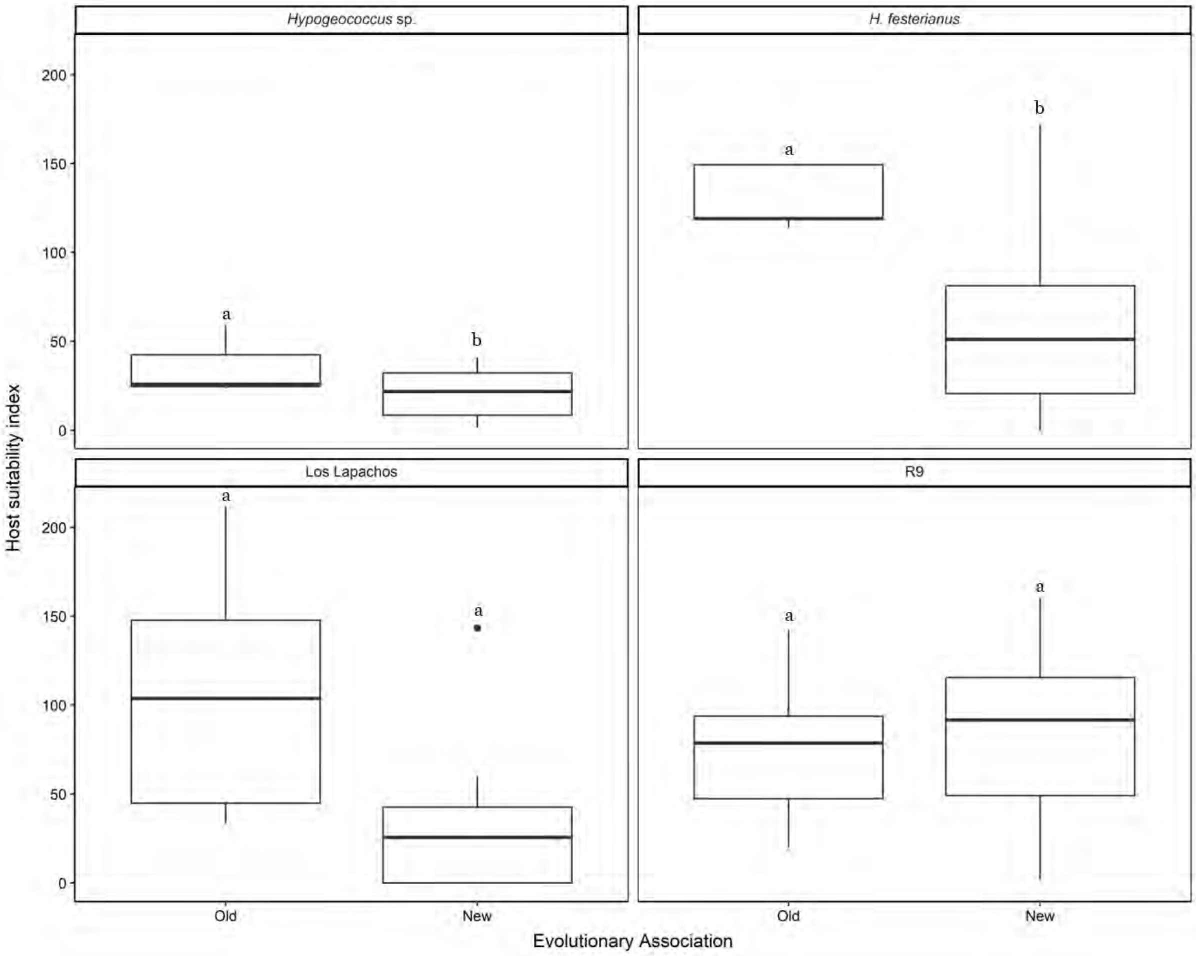

Figure 3.7: Median host suitability of *Trichocereus spachianus* and *Harrisia pomanensis* for *Hypogeococcus* species/lineages showing the evolutionary association between host suitability index and new and old host plant associations for several *Hypogeococcus*. The interquartile range is shown by the box, with whiskers indicating the minimum and maximum values. Outliers are denoted by circles. Significant figures are indicated by different letters ($P < 0.05$).

3.4. Discussion

This research determined whether any of the collected *Hypogeococcus* species/ lineages included *T. spachianus* in their host range, warranting further study. Testing the *Hypogeococcus* imported from Argentina, and previous laboratory trials using *Hypogeococcus* sp. from South Africa, indicated that *Hypogeococcus* spp. were not suitable agents against *T. spachianus* and none of the lineages should be prioritised for further testing. The *Hypogeococcus* species and lineages exhibited slightly different host ranges in their native distribution. Therefore, it was plausible that one of the various lineages collected from Argentina could incorporate *T. spachianus* into their host range, unlike the *Hypogeococcus* sp. already released in South Africa. Unfortunately, none of these *Hypogeococcus* were considered the 'appropriate' match for *T. spachianus* as none incorporated this cactus into their host range. Even if found to be host specific enough for release in South Africa, it is not advised to release ineffective agents that are unlikely to reduce a weed's population (Denoth *et al.*, 2002). The release of such ineffective agents can also have unintended consequences on other parts of the ecosystem, as they become integrated within it (Carvalheiro *et al.*, 2008), as discussed in Chapter 2.

Cactus species within the same genera as the host from which the insects were collected were classified as old associations, while cacti in other genera were deemed new associations. Although not all lineages and species of tested *Hypogeococcus* species/ lineages were examined for old associations, the HSI value reflected the influence of the closeness between the cactus species and the insect's original host. The HSI value demonstrated an increase for old associations between *Hypogeococcus* species/ lineages and their plant hosts. *Trichocereus spachianus* was categorised as a new association for all the tested insects. Several contributing factors, including plant chemical defences, structural attributes, nutrient availability, and insect feeding biology, may have contributed to the observed inability of *Hypogeococcus* species/ lineages to feed on *T. spachianus*.

Overall, *Hypogeococcus* species/ lineages exhibited a preference for feeding on the fresh growth of cacti. Nitrogen, an important nutrient for insect development, is typically more abundant in new growth compared to older plant tissues (Quintero &

Bowers, 2012), rendering the new growth more nutritionally favourable for the insects. While *Hypogeococcus* species generally preferred new growth, they would also feed on older parts of certain cacti. Notably, they avoided feeding on the older sections of *H. pomanensis* and *T. spachianus*, suggesting that there were potential differences in these cacti that deterred insect feeding on parts other than new growth.

Plants employ various defence mechanisms to mitigate herbivory, encompassing both chemical and structural strategies. The variability in anti-herbivore defence expression is significantly influenced by plant development (Ochoa-López *et al.*, 2015), with insects demonstrating a preference for feeding on younger plants (Barton & Hanley, 2013). Young seedlings, or new growth, are considered weakly defended by structural traits (Barton & Hanley, 2013), allowing mealybugs to feed on *H. pomanensis* and *T. spachianus* when cuticle thickness was decreased in younger plant parts. In the field, *Hypogeococcus* sp. selectively feeds at the tips of *H. pomanensis*, where new growth occurs, forming galls. This behaviour might be attributed to a thicker cuticle in *H. pomanensis* and *T. spachianus*, especially in new growth. Research conducted on sorghum (*Sorghum bicolor* (L.) Moench (Poales: Poaceae)) plants resistant to sorghum aphid, *Melanaphis sorghi* (Theobald) (Hemiptera: Aphididae), revealed a potential association between aphid resistance and leaf thickness. Resistant plants exhibited a notable increase in leaf thickness, approximately 9% more than susceptible plants (Triplett *et al.*, 2023). Like *Hypogeococcus* species/lineages, aphids possess piercing mouthparts. Consequently, like the insect resistant sorghum plants, the thicker cuticle of *T. spachianus* and *H. pomanensis* may have augmented its resistance to this specific type of insect feeding. However, it likely represented only one facet preventing a sustained population of these insects on *T. spachianus*, given that *Hypogeococcus* species/lineages fed on growing *H. pomanensis* plants in South Africa and their indigenous range, but not on *T. spachianus* (Chapter 2). This implied the existence of other distinctions between these two plants that contributed to the observed behaviour.

Secondary metabolites play a major role against insect herbivory (War *et al.*, 2020) and Cactaceae have a broad array of secondary metabolites (allelochemicals) (Hasson *et al.*, 2009). Typical allelochemicals of some columnar cacti include terpenoids that serve as feeding deterrents and isoquinoline alkaloids that inhibit neurotransmission

(Corio *et al.*, 2013; Soto *et al.*, 2014). The chemistry (presence of toxic compounds and nutritional sufficiency) of cactus hosts is one of the major determinants of host-plant specificity for cactophilic *Drosophila*, which have higher fitness when reared on *Opuntia* spp. compared to columnar cactus species, like *Trichocereus terscheckii* (Parmentier) Britton & Rose (Caryophyllales: Cactaceae) (Corio *et al.*, 2013). Unlike *Opuntia* spp., columnar cacti produce toxic compounds such as alkaloids, medium-chain fatty acids, sterol diols, and triterpene glycosides. For example, cacti in the genus *Trichocereus* contain alkaloids such as candicine and trichocereine in *Trichocereus candicans* (Gillies ex Salm-Dyck) Britton & Rose (Caryophyllales: Cactaceae) and *T. terscheckii*, respectively (Soto *et al.*, 2014). *Hypogeococcus* spp. and other species that feed on columnar cacti have potentially developed mechanisms to overcome these allelochemicals that are present in these cacti. In contrast to the other cactus species used in this study, there is no evidence of coevolution between *Hypogeococcus* and *T. spachianus*. Consequently, there is a possibility that these insects have not developed the needed adaptations to effectively counter the defensive secondary metabolites present in *T. spachianus*. This absence of coevolutionary adaptations may hinder the insects' ability to successfully feed on and develop on *T. spachianus*.

Coevolution between hosts and insects may have increased significance for galling insects, especially given their sessile nature and the need to maintain a population on a host plant for an extended period. In the case of galling insects like *Hypogeococcus* spp., sustaining a population on the host requires effective suppression or adaptation to the plant's defences (Borges, 2018). However, plants will continue to develop new defence mechanisms that the galling insects will need to overcome creating an evolutionary arms race between insect and host (Oates *et al.*, 2016). Galling requires an intricate relationship between the plant and its enemy suggesting that coevolution between plant and insect may be important. Therefore, new associations may not be as advantageous as established, older associations for insects that demonstrate galling behaviour like *Hypogeococcus* spp..

The results of this study indicated that the tested *Hypogeococcus* species/ lineages are not promising agents for controlling the invasive cactus *T. spachianus*. The potential negative implications associated with releasing these insects also suggested that it would be more beneficial to focus resources on identifying a new, more effective agent.

Previous cacti biological control programmes have been successful due to the use of cochineal insects. While most cochineal species primarily target *Opuntia* species, *Dactylopius confertus* De Lotto (Hemiptera: Dactylopiidae) has been found to include species of *Echinopsis*, *Lobivia*, and *Trichocereus* into their host range in their native range, Argentina (Jujuy and Catamarca) (van Dam, 2013). Given the close relationship between *Lobivia, Echinopsis*, and *Trichocereus* (Albesiano, 2015), *D. confertus* may also target *T. spachianus* established in South Africa. As *D. confertus* has been known to target columnar cacti, further research will be conducted in Chapter 4 to examine the potential of using it as a biological control agent against *T. spachianus* in South Africa.

Chapter Four

Potential of the cochineal insect *Dactylopius confertus* as a biological control agent against *Trichocereus spachianus* in South Africa

4.1. Introduction

The existing *Hypogeococcus* sp. previously released in South Africa and multiple Argentine *Hypogeococcus* lineages and species representing new associations were tested in Chapter 2 and 3, respectively. No *Hypogeococcus* species or lineages were suitable as candidates for a biological control programme against *T. spachianus*. Cochineals (*Dactylopius* spp.) have been effectively used to control several cactus species, making them potential candidates for controlling this cactus (Paterson *et al.*, 2021b). Cochineals are a diverse group of insects found in both North and South America, with five species in North America and five species in South America (Rodríguez *et al.*, 2001). Presently, four species of cochineal are utilised as biological control agents in South Africa and Australia against species of *Opuntia* and *Cylindropuntia* (Zachariades, 2018). Some *Dactylopius* spp. comprise multiple lineages that are host-specific and target particular cacti (Paterson *et al.*, 2021b). Considering cochineal lineages as potential biological control agents of *T. spachianus* warranted further investigation.

The first cactus biological control programme in South Africa, implemented in 1913, was against *O. monacantha* using a cochineal insect, *D. ceylonicus* (Lounsbury, 1915). The release of *D. ceylonicus* decreased populations of *O. monacantha* to negligible levels over a relatively short period of time, and this level of control has been sustained until present (Paterson *et al.*, 2021). Since this first cactus biological control intervention, several *Dactylopius* species and lineages have been released as biological control agents against several invasive cactus species. These include *Dactylopius austrinus* De Lotto (Hemiptera: Dactylopiidae) against *O. aurantiaca*, *D. opuntiae* lineages against *Opuntia engelmanii* Salm-Dyck (Caryophyllales: Cactaceae), *O. ficus-indica*, *Opuntia humifisa* Raf. (Caryophyllales: Cactaceae), *O. stricta* and *O.*

tomentosus, as well as *D. tomentosus* Lamarck (Hemiptera: Dactylopiidae) lineages released against various *Cylindropuntia* species; *C. fuligida*, *C. imbricata*, *Cylindropuntia leptocaulis* (D. C.) Knuth (Caryophyllales: Cactaceae) and *C. pallida* (Paterson *et al.*, 2021b).

Using cochineal lineages and species, these biological control agents achieved substantial success in effectively managing their targeted host plants in South Africa, resulting in varying levels of reduction in the density, biomass, and area of each cactus invasion (Paterson *et al.*, 2021b; Moran *et al.*, 2021). Effectiveness of biological control programmes using cochineal as an agent depends on using the appropriate cochineal specific to each targeted host plant. Genetics must be used to identify the lineages and ensure the correct ones are being implemented because the lineages are morphologically indistinguishable from one another (van Steenderen *et al.*, 2021). Like *Hypogeococcus* sp., cochineal are also poor dispersers, therefore active distribution is usually required (Hill *et al.*, 2021).

Following the success of using *D. opuntiae* to control *O. ficus-indica* and *O. stricta* in Australia the insect was then released into South Africa against the same two Opuntia spp. (Zimmermann & Moran, 1991). However limited damage occurred to target plants in South Africa. It was later realised that *D. opuntiae* was comprised of several lineages and that the lineages released against the *O. stricta* and *O. ficus-indica* in Australia were different; the lineage released against *O. stricta* in South Africa was in fact the 'ficus' lineage (Hoffmann *et al.*, 2020). Collection and release of the correct lineage which had been successful in Australia, *D. opuntiae* 'stricta' (Hoffmann *et al.*, 2020), caused a 90% decrease in *O. stricta* population density in South Africa (Paterson *et al.*, 2011; Hill *et al.,* 2020). Both *Opuntia* species are currently under substantial control due to biological control agents significantly decreasing the target weeds area of invasion, density, biomass, and rate of spread (Moran *et al.*, 2021).

Although native to Argentina, *D. confertus* is present in Namibia where it uses *H. pomanensis* as a marginal host. It is considered a marginal host because populations are maintained at a low abundance and do not appear to be particularly damaging to the plant in the field. If *D. confertus* performs better on *T. spachianus* compared to *H. pomanensis* then it has the potential to be an effective biological control agent. How it was introduced to Namibia remains unknown, but it is thought that it might have occurred accidentally, possibly through the cactus horticulture trade. The insect has never been recorded or released in South Africa and appears to be isolated to the area around Windhoek, the capital city of Namibia. *Dactylopius confertus* has not been purposely used as a biological control agent anywhere in the world, but it is known to feed on species closely related to *Trichocereus* and on at least one species within this genus (Claps & de Haro, 2001). Recorded cactus host plants for *D. confertus* in Argentina include *C. baumannii*, *Echinopsis leucantha* (Gillies ex Salm-Dyck) Walp. (Caryophyllales: Cactaceae), *Cereus aethiops* Haw. (Caryophyllales: Cactaceae), *Denmoza rhodocantha* (Salm-Dyck) Britton & Rose (Caryophyllales: Cactaceae), *Gymnocalycium monvillei* (Lem.) Britton & Rose (Caryophyllales: Cactaceae), *H. tortuosa*, *Pilocereus* spp., and *T. candicans* (Claps & de Haro, 2001). *Trichocereus spachianus* is not recorded as a host for *D. confertus*. However, this may be because of the apparent absence of plants in Argentina, rather than its lack of suitability as a host. Therefore, *D. confertus* may have potential as a biological control agent for *T. spachianus* in South Africa.

There are various methods for prioritising candidate agents during pre-release assessments. One approach involves conducting initial host specificity trials on all potential agents and then pre-release efficacy assessments (PREA) if any agents are determined to be sufficiently host specific (McClay & Balciunas, 2005). If the results of the efficacy assessment demonstrate that the agents are effective, they can be requested for release in the field. Alternatively, as proposed in this thesis, efficacy trials were conducted first, where all potential agents undergo a PREA. If the results of this assessment were positive, that agent undergoes host specificity testing. If agents were found to be suitably host specific, an application for release can be compiled and submitted to the relevant authorities. Both approaches can be effective for prioritising candidate agents for release. However, using agent efficacy as an initial screening method can be advantageous, particularly for agents known to have a highly restricted host range, such as *Dactylopius* spp., all of which are likely suitably host specific for release in Africa. If there are multiple potential agent species or lineages known for high PREA, it would be more effective to focus host specificity testing on the most damaging lineage among them, rather than conducting host specificity assessments for all.

The effectiveness of a biological control agent does not negate the need for thorough host specificity testing (Harris, 1973). Evaluating host specificity is considered the key tool for safe weed biological control. Emphasising agent host specificity can result in concern of the efficacy of an agent being overlooked (Balciunas, 2004, McClay & Balciunas, 2005). The host range of most cactophagous insects is limited to Cactaceae, and as there are no native cacti in South Africa, most cactophagous insect should be suitably host specific for release in South Africa. Evaluating an agent's ability to effectively control the target weed prior to release is an important step in determining the overall success of a biological control programme. Agent selection is often regarded as the "holy grail" of biological control (McFadyen, 1998) not just a desire of practitioners to make optimal choices, but also recognising the inherent difficulty in establishing a flawless selection system (Sheppard, 2003). Investigating an agent’s efficacy is improved by incorporating field comparisons. As was performed in the previous research, Chapter 2.2.3 on assessing the suitability of *Hypogeococcus* sp. as a biological control agent, agent performance on the target plant was compared to performance on plants with known levels of success in the field (Chapter 2.2.3). This method improved the predictability of comparing an agents’ laboratory results with the agent's success in the field.

This chapter aimed to conduct pre-release efficacy studies under quarantine conditions to assess the suitability of *D. confertus* as a prospective biological control agent for *T. spachianus*. Initial efforts involved conducting host suitability and efficacy trials to predict the likelihood that an agent was suitably damaging to the target weed, followed by preliminary host specificity testing. Emphasis was placed in identifying an agent that was both effective and host-specific, a crucial prerequisite for establishing a safe and effective biological control programme.

4.2. Materials and Methods

4.2.1. Collection of biological material

Once the correct permits were obtained, *D. confertus* was collected just outside Windhoek, Namibia (22°35'14.4"S 17°06'22.6"E) on *H. pomanensis* plants. Pieces of *H. pomanensis* with *D. confertus* present were collected and sealed in cardboard boxes. The pieces of cactus and insect were then imported following the correct regulations into quarantine at Rhodes University.

South African cut sections of *T. spachianus* stems were collected from various populations within Makhanda (33°17'56.2"S 26°31'32.0"E; 33°18'18.6"S 26°31'03.2"E; 33°17'41.4"S 26°31'10.5"E) and *H. pomanensis* was collected along the road towards Graaff-Reinet just outside Kariega (33°38'13.01" S, 25°27'13.0" E). Only *T. spachianus* and *H. pomanensis* were used to investigate and compare how *D. confertus* performed on *T. spachianus* compared to its confirmed marginal host in Namibia, *H. pomanensis*.

Host specificity test plants were collected at various sites. *Opuntia elata* and *O. stricta* plants were sourced from the plants that are kept growing at Rhodes University (33°18'36.8"S 26°31'04.6"E). *Opuntia ficus-indica* plants were collected at Bunt kraal (33°16'59.8"S 26°29'19.6"E). *Rhipsalis baccifera* was collected from Port St Johns (31°35'55.2"S 29°32'00.9"E), *Crassula aborescens* (Mill.) Willd plants were bought from Sunnyside nursery in Makhanda, *Carpobrotus deliciosus* (L. Bolus) L. Bolus plants were collected from Rhodes University and *Portulacaria afra* (L.) Jacq was collected from the Kariega mass rearing facility (33°42'47.3"S 25°26'08.9"E).

4.2.2. Insect performance trials and host suitability index

A quarantine study was conducted to determine the suitability of South African *T. spachianus* and *H. pomanensis* as hosts of Namibian *D. confertus*. Female weight and fecundity, duration for crawlers to settle, and initial crawler survival were recorded for each cactus piece. Five replicates of cut stems were set up for each cactus species. Females were weighed once they reached maturity. Fecundity referred to the number of crawlers produced by each female. Length of time taken by crawlers to attain maturity was recorded as the development duration in days. Initial crawler survival was measured as the percentage of the initial 30 crawlers that successfully settled on each cactus piece. Insect parameters were used to determine a Host Suitability Index (HSI) for each of the two cactus hosts. The HSI score was determined using the same equation as used in Chapter 2.2.2.1. The HSI score determined the suitability of South African *T. spachianus* and *H. pomanensis* as hosts for *D. confertus*.

4.2.3. Efficacy trials

The performance of *D. confertus* on potted plants of *T. spachianus* and *H. pomanensis* was assessed as an initial prioritisation filter in considering the insect as a biological control agent. Five replicates of entire plants of *T. spachianus* and *H. pomanensis* were grown in quarantine and each plant inoculated with 100 *D. confertus* crawlers. Plants were monitored bi-weekly to document the number of "colonies" on each plant. A colony was identified by the presence of a female, indicated by the waxy protective layer she produced. Plants were monitored for signs of plant chlorosis and plant tissue necrosis, indicative of cochineal feeding, which eventually leads to plant mortality (Jones *et al.*, 2015). Plant mortality was recorded for each plant.

4.2.4. Host specificity

Host specificity testing was conducted to assess the suitability of *D. confertus* for release in South Africa. Selected host specificity test plants followed the methodology proposed by Briese (2003), relying on degrees of phylogenetic separation. The monophyletic suborder, Portulacineae (or Cactineae), in the order Caryophyllales, contains the Cactaceae family and closely related families such as Portulacaceae, Basellaceae, Didieriaceae, Halophytaceae, Talinaceae, Montiaceae, and Anacampserotaceae (Nyffeler & Eggli, 2010; Ocampo & Columbus 2010). Test plants within the family Cactaceae and belonging to the same subtribe (Trichocereinae) were the closest relatives to *T. spachianus*, included *H. martinii*, *H. pomanensis*, and *H. balansae*. *Cereus jamacaru* shares the same tribe (Cereeae) as *T. spachianus*, while *Opuntia* spp. belonged to the Opuntieae tribe within Cactaceae, signifying a more distant relationship compared to other non-native cactus species in relation to *T. spachianus*. Testing the potential use of these cactus species, which have biological control agents implemented against them, was important to determine whether concerns would arise from agent-agent interactions, especially given that these species, being the most closely related to *T. spachianus*, were the ones on which *D. confertus* was most likely to feed. O*puntia ficus-indica* was chosen for its economic importance as fruit and animal feed in South Africa (Shakleton *et al.*, 2011). *Rhipsalis baccifera* was tested as it is the only possible native old world cactus species, and thus the most closely related indigenous species in South Africa (Nyffeler & Eggli 2010). *Portulacaria afra*, native to South Africa, belongs to the Didieriaceae family, making it the closest non-Cactaceae test plant family to *T. spachianus*. In parts of South Africa's Eastern and Western Cape Provinces, *P. afra* forms a significant component of the vegetation in semi-arid areas, especially in the Eastern Little Karoo (Bruyns *et al.*, 2014). This distribution overlapped with the regions where *T. spachianus* invades. *Carpobrotus deliciosus*, also indigenous to South Africa, is part of the Aizoaceae family, which falls within the same order as Cactaceae but is more distantly related compared to the previously mentioned test plant species (Novoa *et al.*, 2023). Included as a test plant with a more distant relationship, *C. arborescens* is indigenous to South Africa and belongs to the Crassulaceae family, which falls under the order Saxifragales.

Potted plants of all test species were grown and placed in cages to prevent crawler movement between them, with five replicates for each plant. Fifteen *D. confertus* crawlers were inoculated onto each plant, and after two weeks, the number of established crawlers was recorded. The established crawlers were monitored until maturity, and mature females were weighed and placed in a petri dish. The crawlers produced by each female was then recorded. Tests on control plants were included to ensure that damage seen on the plants was caused by *D. confertus*. These insect parameters were then used to calculate the HSI for all plant species using the protocol explained in Chapter 2.2.2.1. All test plants were included in this analysis to clearly show the host range of *D. confertus*.

4.2.5. Statistical analyses

4.2.5.1. Insect performance trials and host suitability index

A generalised linear model (GLM) was used to assess variations in the number of days taken for crawlers to establish and initial crawler survival. The GLM for crawler establishment was specified with a Poisson distribution and a log link function, and the GLM for crawler survival was specified with a binomial distribution and a logit link function. Effect of host plant developmental days (measured in days until egg production) and weight and fecundity of adult *D. confertus* females on *H. pomanensis* and *T. spachianus* were determined using generalised linear mixed models (GLMMs) specified using a Poisson distribution with a log link function and a gaussian distribution with an identity link function, respectively. The suitability (based on survival, duration of development, and fecundity) of each host plant for *D. confertus* was analysed using a GLM which was specified using a gaussian distribution with an identity link function.

4.2.5.2. Efficacy trials

Colony size was modelled as a linear function of two fixed effects variables, (1) plant species to determine if the *D. confertus* colony sizes varied depending whether they were reared on *T. spachianus* or *H. pomanensis* and (2) time since the start of the experiment in weeks to evaluate how colony size varied over time. An interaction term between plant species and time since the experiment began was included to evaluate whether the rate of colony development varied between the two plant species over time. A random intercept term was used for each replicate to account for repeated measurements from the same replicate over time. The model was specified as a GLMM, with a negative binomial distribution. Significant differences in the established colony sizes between *H. pomanensis* and *T. spachianus* were assessed with a Likelihood Ratio Test (LRT) using the 'car' R package (Fox & Weisberg, 2019). Post hoc comparisons were performed to investigate whether colony sizes differed at 8, 18, 28 and 38 weeks between the two cactus species using the 'emmeans' R package (Lenth, 2022).

To evaluate the effect of *D. confertus* on the mortality of two cactus species, *T. spachianus* and *H. pomanensis*, a logistic general linear mixed model (GLMM) was used (Bolker *et al.*, 2009). Plant mortality was scored as a Bernoulli variable (alive or dead) every two weeks for 50 weeks. Plant mortality was modelled as a function of two fixed effects variables, namely: (1) plant species to determine if mortality varied depending on the host plant and (2) time since the start of the experiment in weeks to evaluate how plant mortality varied over time. An interaction term between plant species and time since the experiment began was included to evaluate whether plant mortality rates varied between plant species over time. A random intercept term was included for each replicate to account for the repeated measurements taken from the same plant replicate over time (Bolker *et al*., 2009). The model was specified with a binomial error distribution and a logit link function. Likelihood ratio tests (LRT's) were used to test for fixed effect parameter significance using the 'Anova' function in R (Fox & Weisberg, 2019).

4.2.5.3. Host specificity

Only hosts plants that had *D. confertus* successfully settled on were included in the statistical analysis. The analysis excluded *C. jamacaru* due to the success of only one replicate, making statistical analysis unfeasible for this cactus species. A GLM was used to assess variations in the number of crawlers that settled on each cactus host. The GLM specified with a Poisson distribution and a log link function. To determine if cactus species had an effect on female *D. confertus* size and fecundity GLMMs were used. The GLMMs were specified using a gaussian distribution with an identity link function for assessing weight, and a Poisson distribution with a log link function for fecundity. To conduct pairwise comparisons, post hoc tests were conducted using the 'emmeans' R package. A GLM was used to determine whether the cactus hosts effected the HSI of *D. confertus*. The GLM was specified using a Gaussian distribution and an identity link function. Significant differences in the HSI between all plant species was assessed with a Likelihood Ratio Test (LRT) using the 'car' R package (Fox & Weisberg, 2019). A post hoc comparison was performed using the 'emmeans' R package (Lenth, 2022). Statistical analyses were conducted using the statistical software R ver. 4.2.2, (R Core Team, 2023).

4.3. Results

4.3.1. Insect performance trials and host suitability index

Dactylopius confertus successfully established and produced crawlers on both *H. pomanensis* and *T. spachianus*.

4.3.1.1. Days to establishment and survival

The number of days taken for crawlers to settle was not significantly affected by the host species ($\chi^2 = 0.57$, df = 1, $P = 0.452$) (Figure 4.1). Cactus species had a significant effect on the initial survival of crawlers. Crawlers had a 46% higher survival rate of *T. spachianus* compared to *H. pomanensis* ($\chi^2 = 13.96$, df = 1, $P = 0.00019$) (Figure 4.2).

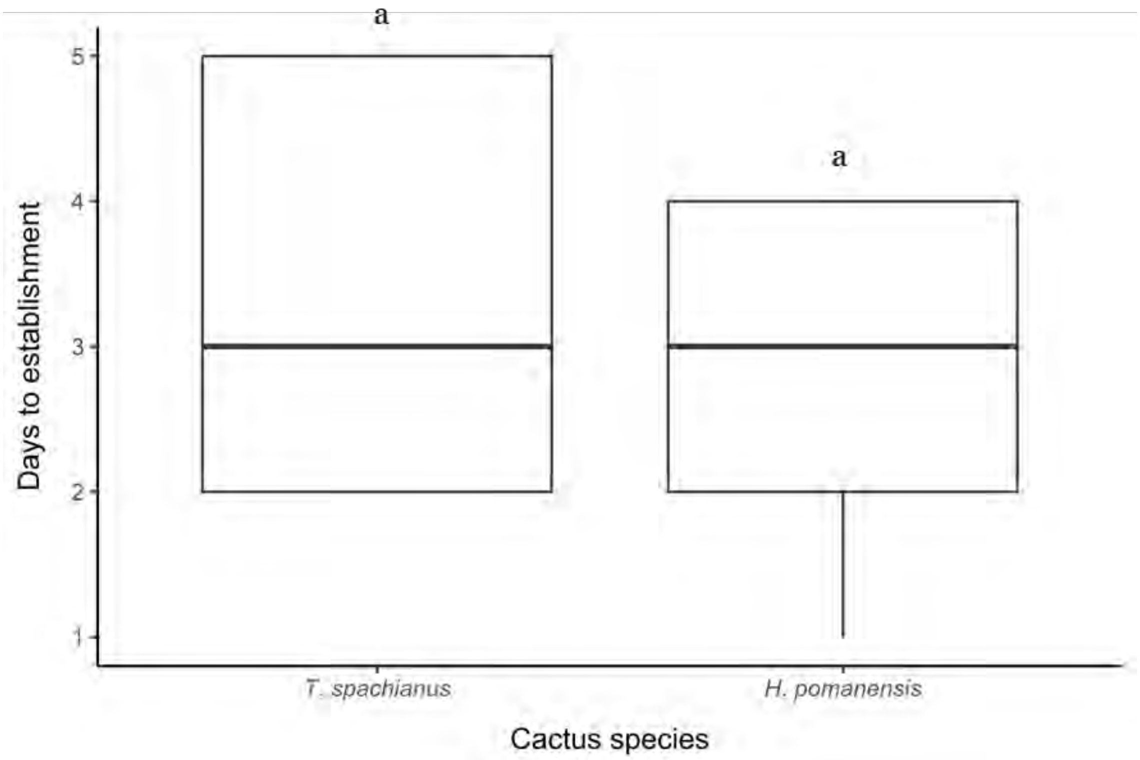

Figure 4.1: The median time it took for crawlers of *Dactylopius confertus* to establish on two South African non-native cactus species. The box represents the interquartile range. Significant differences indicated by different letters ($P < 0.05$).

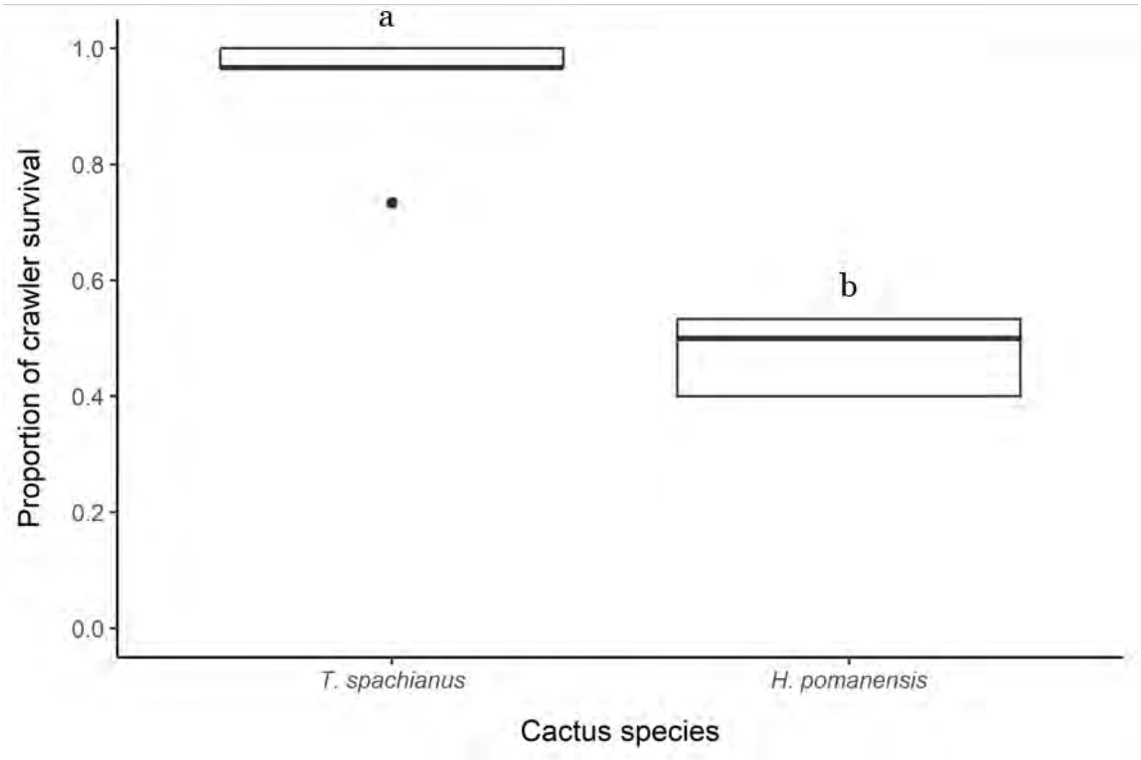

Figure 4.2: Median initial survival of 30 *Dactylopius confertus* crawlers on two South African non-native cactus species. The box represents the interquartile range. Outliers were represented by a closed circle. Significant differences were indicated by different letters ($P < 0.05$).

4.3.1.2. Developmental time

While not significantly different, developmental time of *D. confertus* on *T. spachianus* was 4.5 days less than development time on *H. pomanensis* ($\chi^2 = 1.46$, df = 11, P = 0.2268) (Figure 4.3).

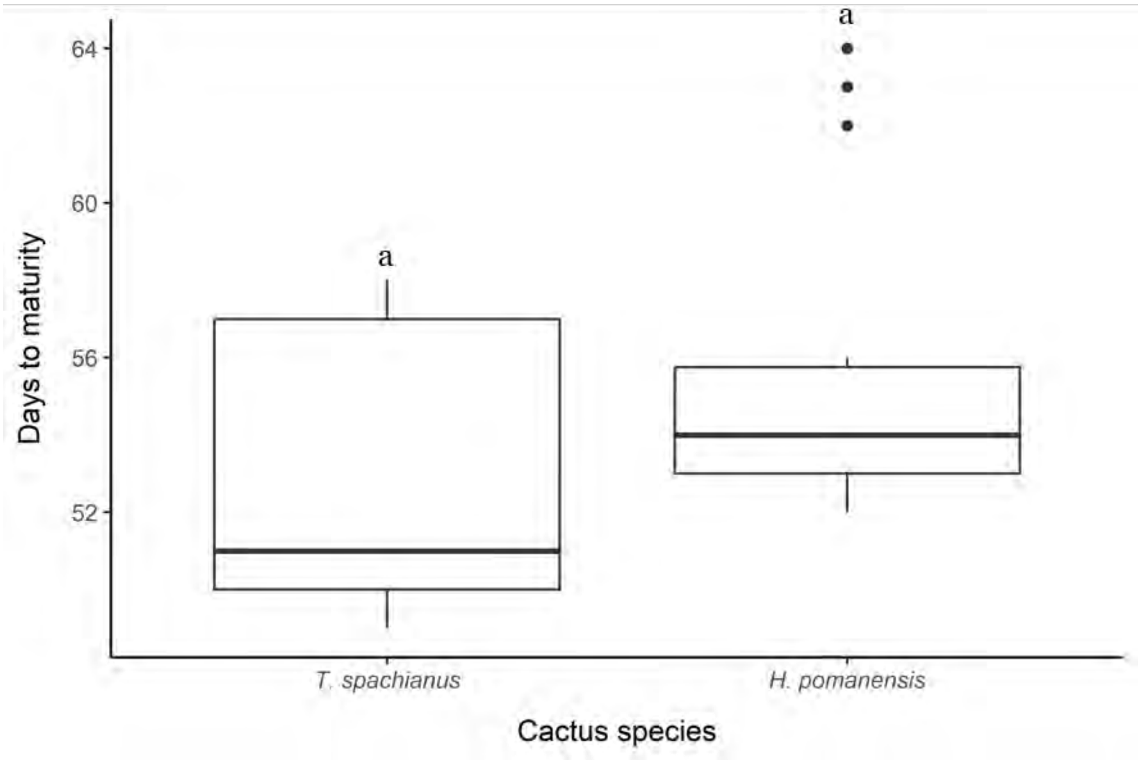

Figure 4.3: Median duration of development (from day 1 crawler to maturity) of *Dactylopius confertus* on two South African cactus species. The box represented the interquartile range. The whiskers represented the lowest and highest values that were not outliers. Outliers were represented by a closed circle. Significant differences were indicated by different letters ($P < 0.05$).

4.3.1.3. Weight and fecundity

Although no statistically significant difference was found, females were 0.00089g heavier when feeding on *H. pomanensis* compared to *T. spachianus* ($\chi^2 = 3.533$, df = 1, $P = 0.06$) (Figure 4.4). No statistically significant difference was found for fecundity either, however females produced on average 14 less crawlers when on *T. spachianus* compared to when on *H. pomanensis* ($\chi^2 = 2.341$, df = 1, $P = 0.126$) (Figure 4.5).

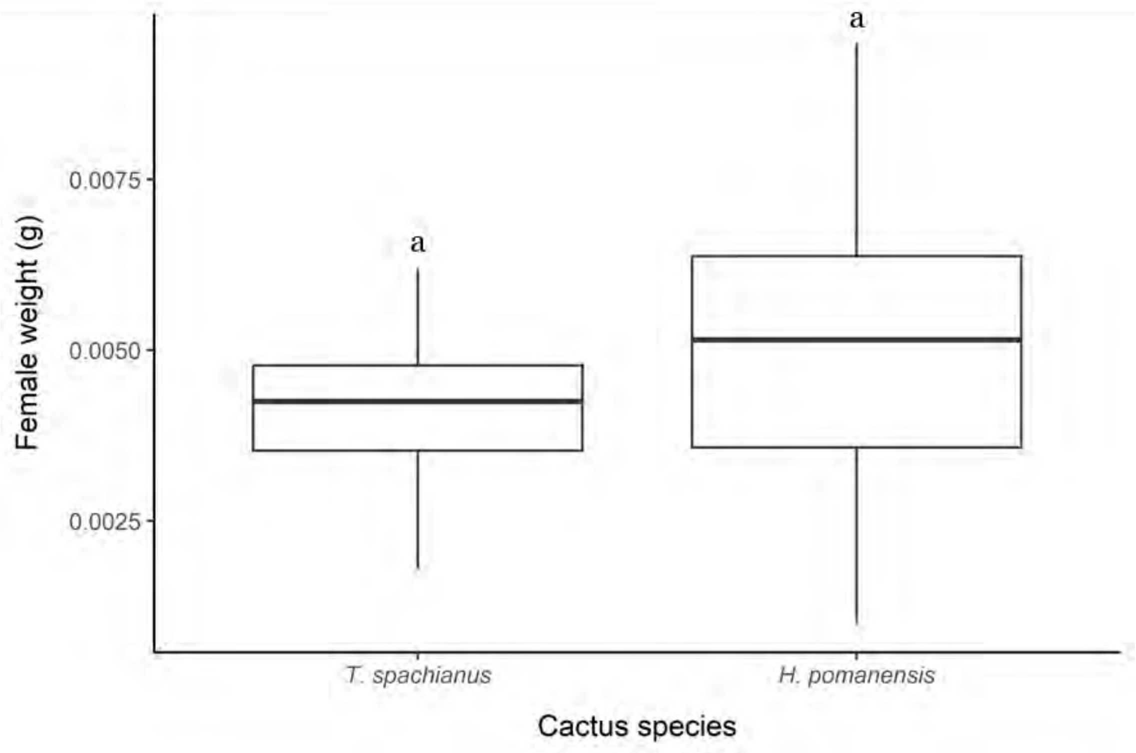

Figure 4.4: The median weight of females *Dactylopius confertus* on *Harrisia pomanensis* and *Trichocereus spachianus*. The box represented the interquartile range. The whiskers represented the lowest and highest values that are not outliers. Significant differences were indicated by different letters ($P < 0.05$).

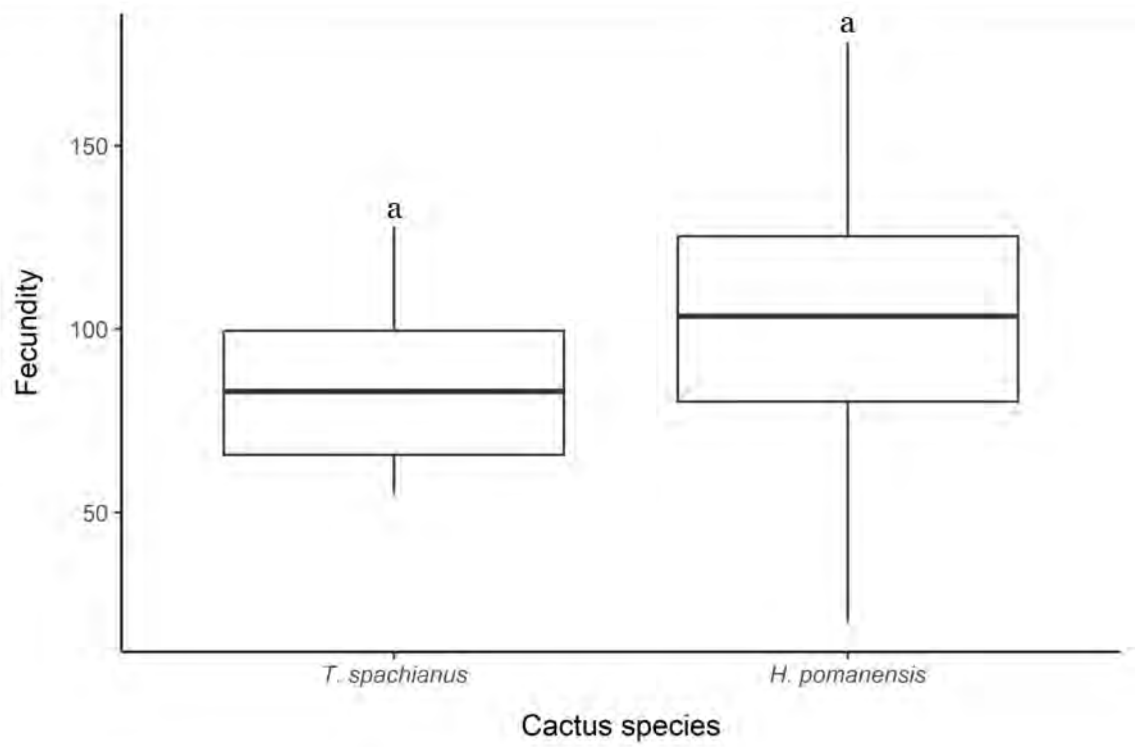

Figure 4.5: Median fecundity of *Dactylopius confertus* on two South African non-native cactus species. The box represented the interquartile range. The whiskers represented the lowest and highest values that were not outliers. Significant differences were indicated by different letters ($P < 0.05$).

4.3.1.4. HSI

The type of cactus species significantly affected the suitability of the host for *D. confertus*. The HSI score for *T. spachianus* was on average 56 units higher than *H. pomanensis* ($\chi^2 = 11.98$, df = 1, $P = 0.0005$) (Figure 4.6).

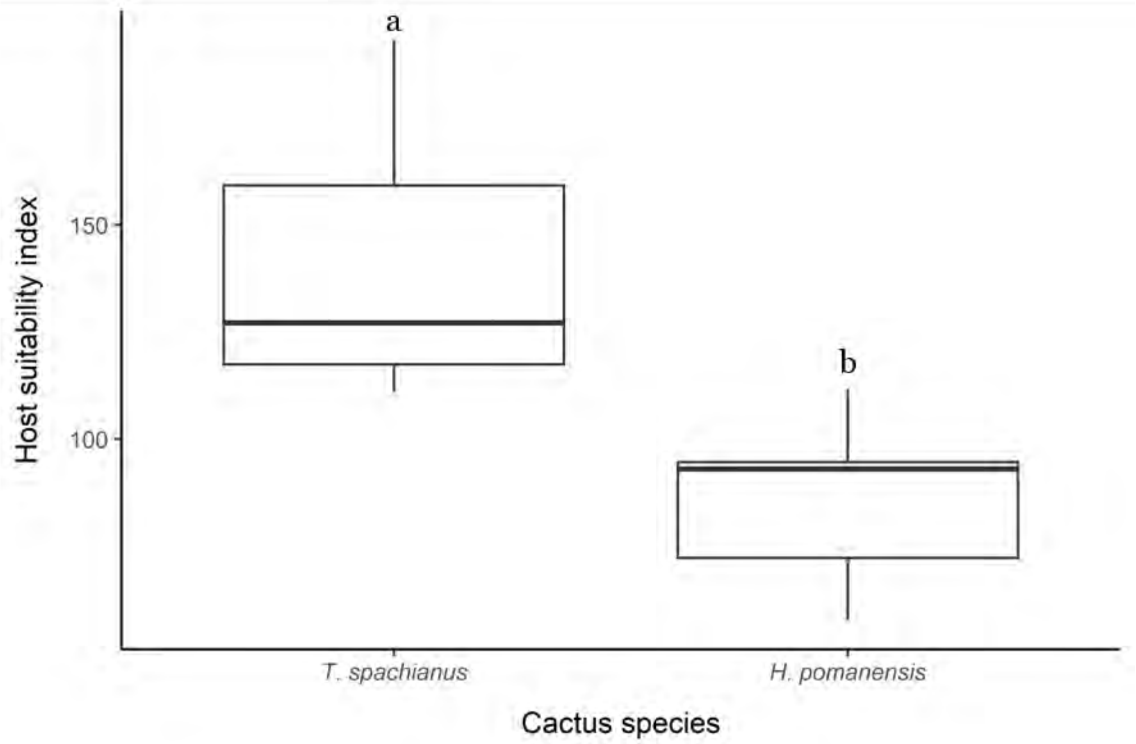

Figure 4.6: Median host suitability index of *Trichocereus spachianus* and *Harrisia pomanensis* as hosts for *Dactylopius confertus*. The box represented the interquartile range. The whiskers represented the lowest and highest values that were not outliers. Significant differences were indicated by different letters ($P < 0.05$).

4.3.2. Efficacy

Presence of *D. confertus* on both *H. pomanensis* and *T. spachianus* resulted in plant chlorosis and plant tissue necrosis which eventually led to plant death, indicating that *D. confertus* infestations do cause damage to both cactus species under quarantine conditions. There was no statistically significant interaction between cactus species and weeks on *D. confertus* colony size ($\chi^2 = 1.955$, df = 1, $P = 0.1620$). This result indicates that the rate at which the colonies increased over time was not statistically different between the two cactus species (Figure 4.7). Despite the lack of a statistically significant interaction term, comparing the two species counts at different intervals show some evidence for a difference in cochineal counts between cactus species, but only near the end of the experiment. As such, the lack of a statistically significant interaction term may be a statistical artefact driven by the low replications for the two cactus species tested (Gelman *et al.*, 2007). For example, at week eight and week 18 there was no statistically significant difference, but at week 8 counts were approximately 50% lower on *H. pomanensis* than *T. spachianus* and 54% lower at week 18. However, a statistically significant difference was seen from week 28 where colonies were 58% lower on *H. pomanensis* (β = -0.862, z-value = -2.039, *P*-value = 0.041) and 62% lower in week 38 (β = -0.954, *z*-value = -2.193, *P*-value = 0.028).

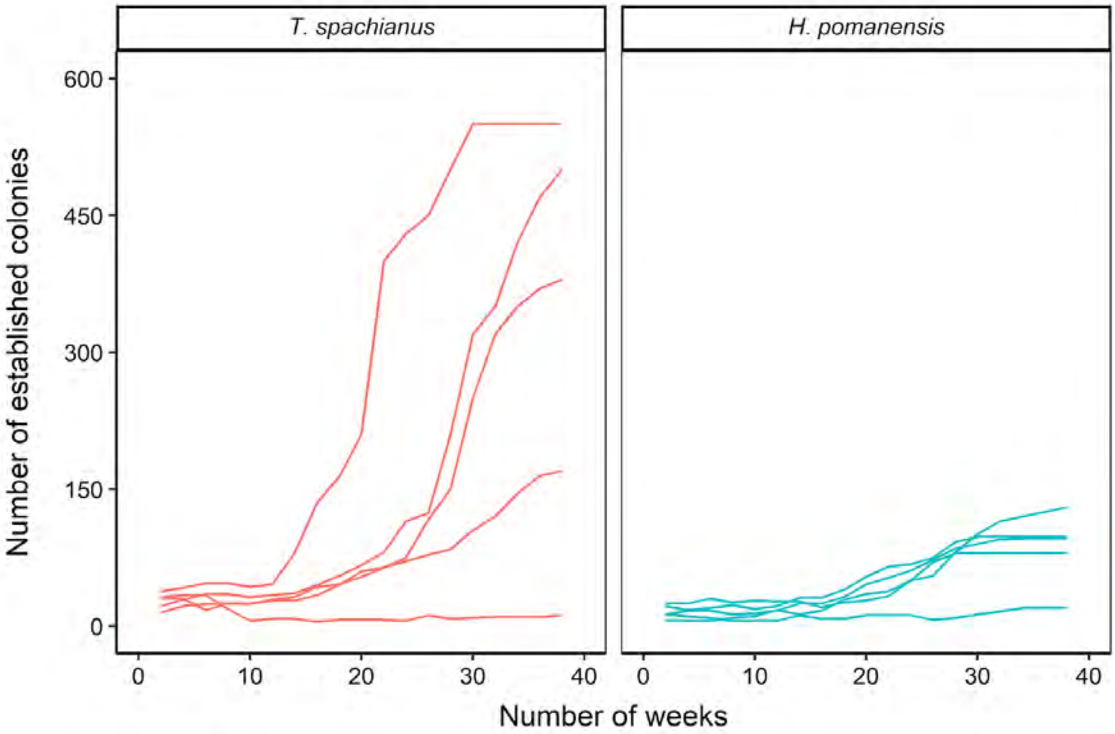

Figure 4.7: Number of established *D*actylopius *confertus* colonies over 40 weeks on two South African non-native cactus species.

There was no statistically significant interaction between cactus species and weeks on plant mortality (χ^2 = 1.705, df = 1, P = 0.191) (Figure 4.8). This result indicates that the mortality rate of cactus plants was not significantly different between the two species. Thus, the presence of *D. confertus* causes plant mortality over a similar period for both cactus species.

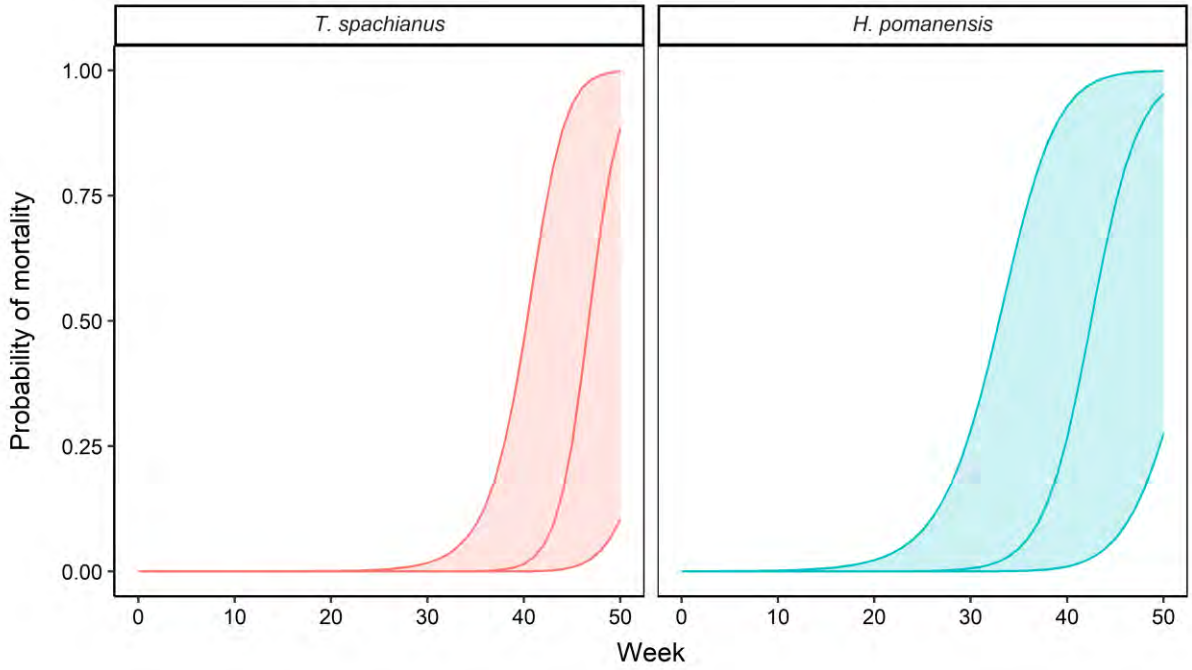

Figure 4.8: The probability of *Dactylopius confertus* infestations causing the death of *Harrisia pomanensis* and *Trichocereus spachianus*.

4.3.2.1. Host specificity

Crawlers of *D. confertus* settled on *T. spachianus*, *H. pomanensis*, *H. balansae*, *H. martinii*, *C. jamacaru,* but did not settle on any Opuntia spp. or on any of the indigenous test plant species (Figure 4.9). Therefore, host specificity differences were analysed using only the cactus hosts where *D. confertus* successfully settled (except for *C. jamacaru,* excluded because *D. confertus* survived on only one replicate).

Number of crawlers that settled on each host was significantly affected by cactus species ($\chi^2 = 230.97$, df = 11, $P < 0.0001$) (Figure 4.9). Compared to the zero crawlers that settled, an average of 6 more crawlers settled on *T. spachianus* compared to *O. stricta* ($\beta = 0.000001$, *z*-value = -25.22, $P < 0.0001$), *O. elata* ($\beta = 0.000001$, z-value = -25.22, $P < 0.0001$), *O. ficus-indica* ($\beta = 0.000001$, *z*-value = -25.22, $P < 0.0001$), *C. arborescens* ($\beta = 0.00001$, *z*-value = -25.22, $P < 0.0001$), *R. baccifera* ($\beta = 0.00001$, *z*-value = -25.22, $P < 0.0001$), *C. deliciosus* ($\beta = 0.00001$, *z*-value = -25.22, $P < 0.0001$) and *P. afra* ($\beta = 0.00001$, *z*-value = -25.22, $P < 0.0001$). Although not statistically significant, on average one more crawler settled on *T. spachianus* compared to *H.*

pomanensis. *T. spachianus* had three and two more crawlers settling compared to *H. martinii* (β = 21.91, *z*-value = -4.089, P = 0.0005) and *H. balansae*, respectively.

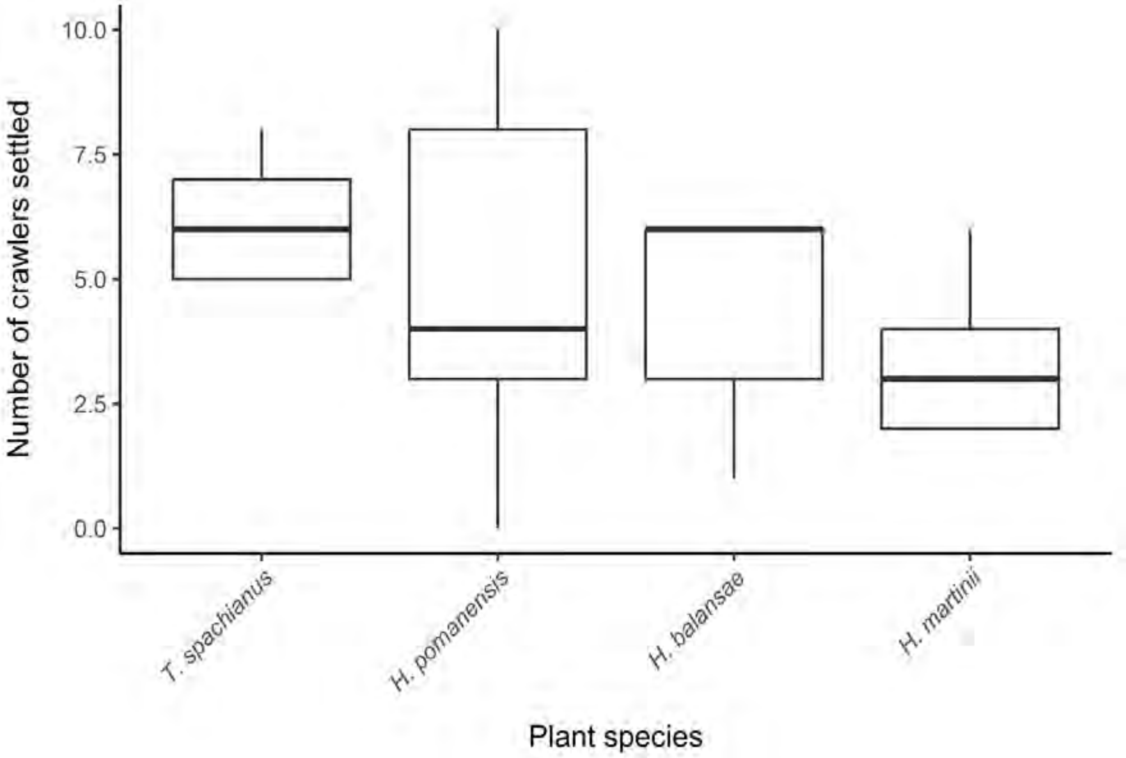

Figure 4.9: Median initial survival of 15 *Dactylopius confertus* crawlers on each cactus species. The box represents the interquartile range. The whiskers represent the lowest and highest values that are not outliers. Significant differences are indicated by different letters ($P < 0.05$).

The weight of female *D. confertus* was significantly influenced by the host plant (χ^2 = 9.84, df = 3, P = 0.02) (Figure 4.10). On average, females exhibited a weight increase of 0.0008g when on *T. spachianus* compared to being on *H. martinii* (β = - 0.0004.78, *z*-value = -2.707, P = 0.23) and 0.00048g more compared to *H. balansae* (β = -0.0084, *z*-value = -1.756, P = 0.028). However, there was no significant difference in the size of females on *T. spachianus* and *H. pomanensis* (β = 0.00002, *z*-value = 0.068, P = 1.00).

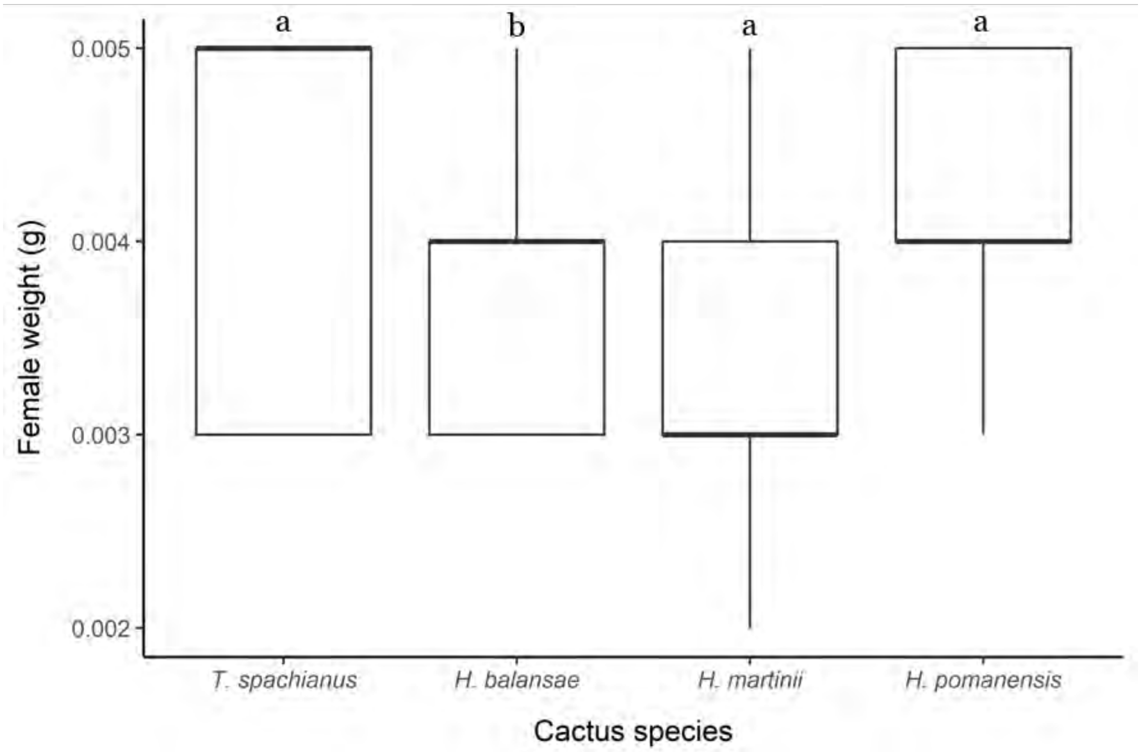

Figure 4.10: Median weight of female *Dactylopius confertus* on each cactus species. The box represents the interquartile range. Significant differences are indicated by different letters ($P < 0.05$).

The fecundity of female *D. confertus* was also significantly influenced by the host plant ($\chi^2 = 23.002$, df = 3, $P < 0.0001$) (Figure 4.11). On average, females produced only 4 more crawlers on *T. spachianus* compared to *H. pomanensis*, 21 more crawlers on *T. spachianus* compared to *H. martinii* ($\beta = -0.209$, z-value = -2.934, $P = 0.0100$), and 31 more crawlers on *T. spachianus* compared to when on *H. balansae* ($\beta = -0.296$, z-value = -4.349, $P <.0001$).

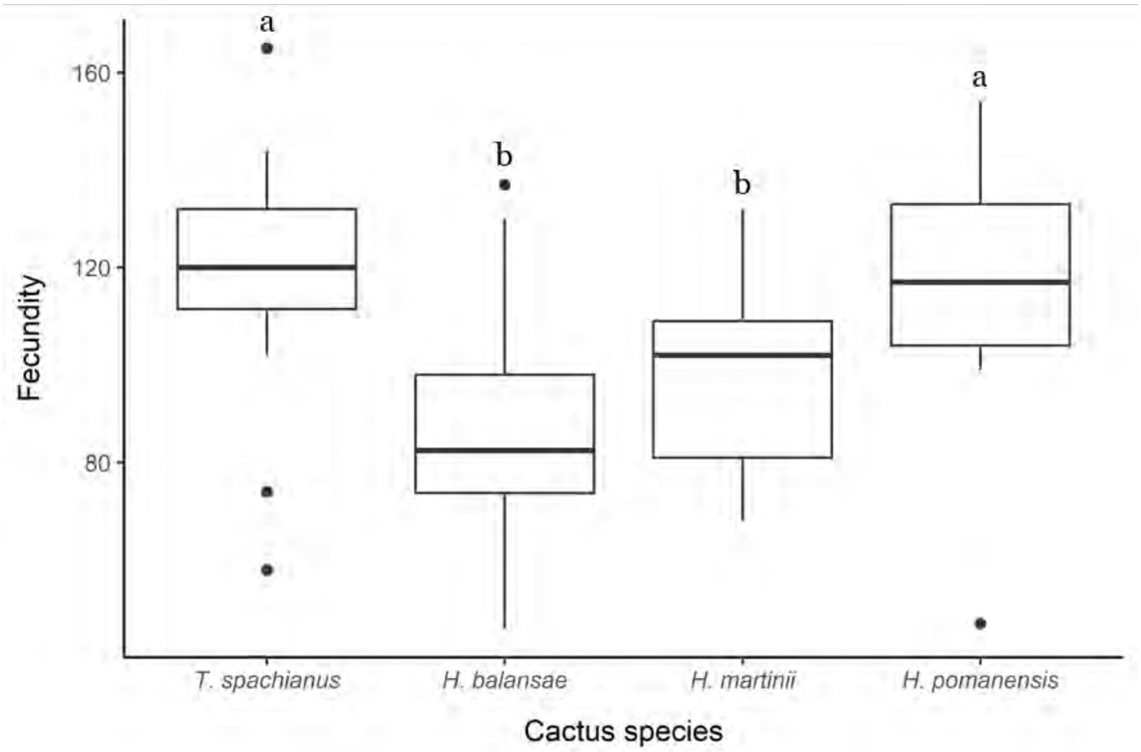

Figure 4.11: Median number of crawlers (fecundity) of *Dactylopius confertus* females on each cactus species. The box represents the interquartile range. Outliers are represented by a circle. Significant differences are indicated by different letters ($P < 0.05$).

Host plant exhibited a statistically significant impact on the HSI score ($\chi^2 = 689$, df = 11, $P < 0.0001$) (Figure 4.12). In comparison to the HSI score of 142 on *T. spachianus*, the HSI score was zero for several plant species, including *C. jamacaru* ($\beta = -141.59$, z-value = -10.33, $P < 0.0001$), *O. stricta* ($\beta = -141.59$, z-value = -10.33, $P < 0.0001$), *O. elata* ($\beta = -141.59$, z-value = -10.33, $P < 0.0001$), *O. ficus-indica*, *O. elata*, *C. arborescens*, *R. baccifera* ($\beta = -141.59$, z-value = -10.33, $P < 0.0001$), *C. deliciosus* ($\beta = -141.59$, z-value = -10.33, $P < 0.0001$), and *P. afra* ($\beta = -141.59$, z-value = -10.33, $P < 0.0001$). *Harrisia balansae* showed an HSI score that was 105 units lower than *T. spachianus* ($\beta = 104.46$, z-value = -7.55, $P < 0.0001$), while *H. martinii* exhibited an HSI score 119 units lower than *T. spachianus* ($\beta = -118.54$, z-value = -7.68, $P < 0.0001$) and *H. pomanensis* had a HSI score of 56 units lower than *T. spachianus* ($\beta = -55.67$, z-value = -3.46, $P = 0.0005$).

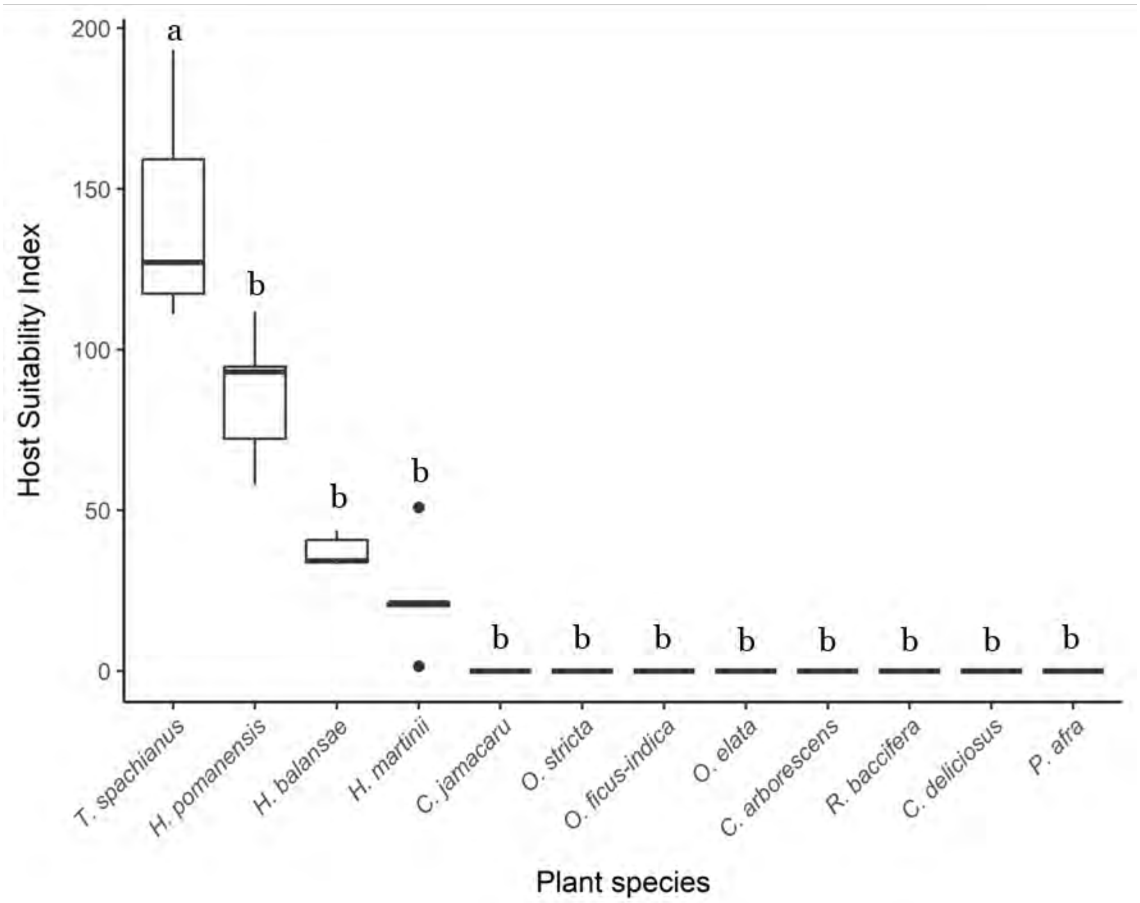

Figure 4.12: Suitability of several plant species as a host for *Dactylopius confertus*. The box represents the interquartile range. The whiskers represent the lowest and highest values that are not outliers. Outliers are represented by the circles. Significant differences are indicated by different letters ($P < 0.05$).

4.4. Discussion

Important information was determined on the suitability of *D. confertus* as a potential biological control agent for *T. spachianus* in South Africa. Several *Dactylopius* species and lineages were successfully used for past South African biological control programmes against cacti, however, *D. confertus* was not so its potential as an agent against *T. spachianus* was unknown. Results identified that *D. confertus* had a higher host suitability index (HSI) on *T. spachianus* compared to one of its known host plants, *H. pomanensis*. Although differences in several parameters were similar for *D. confertus* on both cactus species, higher survival and evidence from the number of established colonies on the potted plants suggested that *D. confertus* would be more damaging on *T. spachianus* than *H. pomanensis*. High densities of *D. confertus* on *T. spachianus* caused plant mortality. Similar studies by Sutton *et al.*, (2018) demonstrated a correlation between the density of *Hypogeococcus* sp. and the likelihood of the insect causing mortality to its host plant and Jones *et al.*, (2023) who found that high cover and density of cochineal resulted in higher plant mortality.

Pre-Release Efficacy Assessments (PREAs) could be conducted under quarantine conditions and/or under field conditions in the target plants indigenous distribution. Due to the lack of wild indigenous populations of *T. spachianus*, a PREA was conducted under laboratory and quarantine conditions in South Africa. Conducting experiments under quarantine conditions was an important tool for investigating effectiveness of an agent without external factors such as climate and natural enemies. However, investigating an insect's potential efficacy in the host's indigenous distribution would be advantageous by more accurately representing the insect's performance under field conditions. Country of origin PREAs may reduce the cost of the biological control programme if only agents that have demonstrated a potential to be effective were imported into the country where quarantine host specificity testing was conducted. Sutton *et al.*, (2021) proposed a field-based approach to better assess the potential host-specificity and efficacy of potential biological control agents. They suggest conducting extensive field assessments to determine the realised host range, which is influenced by ecological and environmental factors, and select insects with acceptable levels of host-specificity and damage potential before importing agents into quarantine for further testing. Implementation of field-based assessments, if feasible, contribute to the process of evaluating potential agents for further testing by providing valuable insights into their host range, efficacy, and interactions (Sutton *et al.*, 2021). Conducting country of origin field studies for this research was impossible due to the absence of *T. spachianus* plants in their indigenous distribution. However, using *H. pomanensis* as a comparative host plant addressed the challenges of extrapolating quarantine results to field results. Given that *D. confertus* infestations caused mortality of *T. spachianus* and *H. pomanensis* under quarantine conditions and the ability of *D. confertus* to establish and cause damage on *H. pomanensis* under Namibian field conditions, increases the likelihood that this insect will establish and cause sufficient damage on *T. spachianus* in its invaded range.

This research demonstrated that PREAs can serve as an initial step in prioritising agents for cactus biological control programmes, especially where a weed has few close relatives in the invaded distribution. Focusing on efficacy for initial screening could prove advantageous for agents that are very likely to be host specific. For example, the Brazilian spiderwort *T. fluminensis* in South Africa has few native relatives, reducing the potential for non-target effects (Burns *et al.*, 2011; Byrne *et al.*, 2021). By utilising PREAs as a first step, resources are optimised by concentrating host specificity tests on effective biological control agents.

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. Although a wider test plant list needs to be investigated, the preliminary host specificity done in this study suggests that *D. confertus* will be suitably host specific for release in South Africa. 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* feeds on several cactus species, provided its host plants are not native to South Africa, surrounding countries or are economically important plants, insects may still be approved for release (Klein, 2002; Klein & Zimmermann, 2020). Feeding on cacti other than the target plant does not raise concerns regarding the host specificity of *D. confertus*, given that all these plants are classified as weeds in South Africa (Paterson et al., 2021b). *Dactylopius confertus* did not feed on any of the economically important or indigenous test plants. Future host specificity testing should incorporate more replicates of the tested cactus species and broaden the test plant list.

One of the concerns about releasing a new agent as discussed in Chapter 2, is the potential for agent-agent interactions once a new agent is released into the introduced range (Milbrath & Nechols, 2014). In Namibia both *Hypogeococcus* sp. and *D. confertus* use *H. pomanensis* as a host. Although, *D. confertus* is found to feed on other cacti that *Hypogeococcus* sp. feeds on, it is unlikely that *D. confertus* will have a negative effect on *Hypogeococcus* sp. establishment as *Hypogeococcus* sp. is already widely established and due to its effectiveness as an agent, kills its hosts relatively quickly.

Cochineal and *Hypogeococcus* sp. target different parts of the cactus and have different feeding mechanisms. Consequently, if both insects establish on the same plant, direct competition is unlikely, however, there may be competition for the same nutrient resources. Plant-mediated indirect interactions can occur between insects that are spatially isolated on the same plant, where the feeding or other activities of an insect can trigger a change in the plant, subsequently influencing other species that feed on the same plant (Milbrath & Nechols, 2014). *Hypogeococcus* sp. is also unlikely to use *T. spachianus* as a host and is not expected to be present on any *T. spachianus* plants, thus, the probability of these insects' sharing hosts in the field is unlikely. Several *Dactylopius* species and lineages are currently implemented as biological control agents in South Africa, but because *D. confertus* has a different host range (Claps & de Haro, 2001), it is unlikely that this species will interact with any of the other *Dactylopius* species and so the potential for disruption of these well-established agents is low.

When using multiple lineages of a species in the same introduced distribution there is a risk of hybridisation between the new lineages and the already established lineage in the area. Hybridisation could result in the offspring preferring one or the other host plant (Craig *et al.*, 2001; Dambroski *et al.*, 2005) or a shift in host plant entirely (Hoffmann *et al.*, 2002). Hybridisation has been studied among cochineal species, and it has been shown that depending on the dominance of specific genes, hybrid offspring react differently on their parents' host plants (Mathenge *et al.*, 2010b). *Dactylopius* *confertus* is a distinct species from the previously released cochineal species in South Africa (van Steenderen *et al.*, 2021). Given this differentiation, coupled with their different host ranges, the likelihood of hybridisation is very low.

This study indicated that *D. confertus* showed promise as a candidate for a biological control programme targeting *T. spachianus*. The insect readily accepted *T. spachianus* as a host, females successfully reproduced on this cactus, and a relatively high suitability index was recorded. *Dactylopius confertus* attained high population densities on potted *T. spachianus* plants, causing plant mortality and was the most damaging of the potential agents tested against *T. spachianus*. Preliminary host specificity results were encouraging, suggesting that this insect was sufficiently host specific for release in South Africa.

Chapter Five
General Discussion

5.1. Thesis overview

Trichocereus spachianus is an increasing concern as an invasive plant in South Africa, and there is a need for a control programme (Paterson *et al.*, 2021a). Implementing biological control is challenging as there are no wild populations of *T. spachianus* in its indigenous range on which to search for potentially host specific insects. New association biological control was used to evaluate non-coevolved agents on *T. spachianus*. *Hypogeococcus* species/ lineages and *D. confertus* were gathered from cactus species closely related to *T. spachianus* and subjected to investigation.

This thesis emphasised the significance of conducting efficacy assessments before releasing new agents against Cactaceae to avoid introducing ineffective agents, even if they were host-specific enough for release. *Hypogeococcus* sp. previously released and established in South Africa and five different *Hypogeococcus* species/ lineages collected in the indigenous distribution of Argentine *T. spachianus* were able to form viable populations on a range of cactus species within the genera *Harrisia* and *Cereus*, but not on the new association species *T. spachianus*.

In quarantine trials, *D. confertus* showed higher suitability for *T. spachianus* compared to its host, *H. pomanensis*, and a restricted host range. All *Dactylopius* spp. are expected to be sufficiently host-specific for release in Africa (De Lotto, 1974; Gunn, 1979), and extended host-specificity testing will be required to ensure there are not unintended risks to non-target plants (Ewel *et al.*, 1999; Louda *et al.*, 2003). *Dactylopius confertus* infestations also resulted in the mortality of potted *T. spachianus* plants.

5.2. Identifying the origin of *Trichocereus spachianus*: challenges and solutions

Developing a successful biological control programme for invasive weed species requires a thorough understanding of their taxonomy, indigenous distribution, and origin (Gaskin *et al.*, 2011). Despite being technically challenging and time-consuming, exploring the indigenous range of these plants is an essential aspect of this process as it allows for the investigation of natural enemies associated with the species, making it imperative to know the weed's precise origin (Paterson *et al.*, 2021a). Research begins with literature reports and herbarium records worldwide to locate the weed's origin, while molecular methods can provide a more precise resolution regarding the origin of the weed population and any genotypes present in the introduced range (Gaskin *et al.*, 2011; Goolsby *et al.*, 2006). If the indigenous distribution of the plant is known, molecular characterisation can determine the origin of the invasive weed or population within the indigenous distribution (Paterson *et al.*, 2009; Barker *et al.*, 2015; Gaskin, 2023). As there are no indigenous populations of *T spachianus* the prospects of identifying a genetically matched candidate agent are very low.

In some cases, determining the indigenous distribution and origin of a weed can be a challenging task, especially when the weed is widespread or a horticultural variety/hybrid (Paterson *et al.*, 2009; Paterson *et al.*, 2021a). For example, *P. aculeata* is an invasive cactus weed in South Africa with a disjointed indigenous distribution in South America where the wild populations exhibit morphological differences. The origin of the weed in South Africa and Australia could not be determined solely based on morphological characteristics as it displayed characteristics from both populations in the indigenous range. Identifying the population from which the invasive plant originates may be crucial for a successful biological control programme since different populations may be susceptible to different biological control agents (Paterson *et al.*, 2009). Genetic analysis was conducted to compare the South African population to the two indigenous populations, and the results suggested that the South African weed population likely originates from the southern population of the indigenous distribution. However, the South African population was unexpectedly found to be genetically distinct from the indigenous populations, indicating that the weed population may also be a descendant of a horticultural variety of the plant. This highlights the difficulty in determining the origin of a weed, as *P. aculeata* has been cultivated as a garden plant in England since 1696. *Trichocereus spachianus* is extensively cultivated in the horticultural trade, and there is a possibility that it is now a different species, distinct from its parent species. The classification of this species is further complicated by its initial description from a cultivated plant in a botanical garden, outside of its recorded indigenous distribution.

It is also possible that no wild populations of *T. spachianus* were found in its indigenous distribution because the plant has become extinct in the wild and only exists as a cultivated species, mainly in areas where it has become invasive. Invasive plants that have no known indigenous distribution but continue to exist in cultivation can pose a challenge for biological control efforts. While cultivation can help preserve the genetic diversity of a plant species, it can also make it difficult to find suitable biological control agents. This is because cultivated plants may have been altered extensively over time, and no longer resemble their parent species. The South African populations of *T. spachianus* may have undergone selective breeding or hybridisation, resulting in a deviation from the wild populations. This divergence from the indigenous distribution could have contributed to the species' invasiveness in the introduced range (Ellstrand & Schierenbeck, 2000). Compared to their indigenous distribution, introduced plants generally exhibit lower genetic diversity due to limited introduction of genotypes. Hybrids in weed populations can arise from crossbreeding with native plants in the introduced region or through cultivation. These hybrids can contribute to greater genetic diversity and the emergence of new phenotypes (Ellstrand & Schierenbeck, 2000). In cases where the origin of the target weed is unknown or difficult to determine; or when the invasive population may comprise several hybrids, such as with *T. spachianus*, investigating agents associated with closely related species (new associations) could help mitigate these challenges.

5.3. New associations in biological control

The success for new associations is based on the target weed has not coevolving with the agent and lacking specific insect defensive mechanisms, allowing the agent to cause more damage to the weed (Hokkanen & Sailer, 1985). In contrast, a new association plant may develop unfamiliar defences to the insect that prevents feeding and/or survival.

New associations are common in biological control programmes against Cactaceae. Performance of *C. cactorum* was investigated on a South American host, *Opuntia megapotamica* Arechav. (Caryophyllales: Cactaceae), that had coevolved with the insect and a North American host, O. *ficus-indica,* that was "naive" to *C. cactorum* but is established in the insect's indigenous range (Varone *et al.*, 2012). The study compared the performance of *C. cactorum* populations collected from each of these hosts. *Cactoblastis cactorum* from *O. ficus-indica* had higher performance on its original host compared to insects on *O. megapotamica*, suggesting that this population had potentially lost its evolutionary traits that had allowed it to overcome the coevolved plant defences. However, the *C. cactorum* population that was collected off the native host, *O. megapotamica* still showed increased performance on *O. ficus-indica* (Varone *et al.*, 2012). Studies have shown that *C. cactorum* experiences three generations annually on its new association hosts, *O. ficus-indica* and *O. stricta*, in contrast to the two generations it experiences on its original hosts, *Opuntia quimilo* K. Schum. (Caryophyllales: Cactaceae) and *O. megapotamica* (Varone *et al.*, 2019). Some plant species that have not coevolved with certain herbivores have not developed defence mechanisms to defend against herbivory, creating defence free space which favours the new herbivore (Woodard *et al.*, 2012). North American *Opuntia* have evolved to defend themselves against their native enemy, *Melitara prodenialis* Walke (Lepidoptera: Pyralidae), a cactophagous moth with similar feeding habits to *C. cactorum*. Woodard *et al.*, (2012) showed that the *Opuntia* defended themselves to a higher degree when exposed to *M. prodenialis* compared to when they were exposed to *C. cactorum*.

Introducing *D. confertus* as a biological control agent against *T. spachianus* in South Africa would represent a new association between the insect and its host; host plants of *D. confertus* are closely related to *T. spachianus,* but *T. spachianus* is not recorded as a host plant species. Managing *T. spachianus* with *D. confertus* is similar to the biological control programme addressing *C. fulgida* var. *mamillata*. Forma *monstrosa* Coult ('boxing-glove cactus') with *D. tomentosa 'cholla'* (Klein *et al.*, 2020). *Dactylopius tomentosa 'cholla'* was collected from a closely related cactus species in an isolated part of Mexico where it had never been exposed to the target cactus, even though *C. fulgida* var. *mamillata* is common in other parts of Mexico (Klein *et al.*, 2020).

5.4. Release safety

To reduce the potential for biological control agents becoming pests outside their introduced range, it is essential to release only those agents that are effective and suitably host specificity for that region. It is also important to consider the entire region and the dispersal abilities of the agents before their release, as agents are not constrained within political borders (Pratt & Center, 2012; Langa *et al.*, 2020). Insects that are good dispersers are more likely to cross borders compared to insects that are sessile and poorer dispersers. Introducing an agent in a country where the target insect or plant is perceived as a pest could have adverse effects on a neighbouring country where the same plant might be viewed as beneficial (Paterson & Witt, 2022).

Additionally, it is important to understand the differences between a pest and a biological control agent. A pest insect is a species that causes harm or damage to crops, ecosystems, or other important plants, often leading to economic losses or ecological imbalances. On the other hand, a biological control agent is a living organism intentionally introduced to control the population of pest species. Although some biological control agents may become pests in areas where they have unintentionally been released, pest insects and biological control agents are often seen as the same entities. For example, all cochineals are frequently viewed as a singular entity, and similarly, *Hypogeococcus* is often treated as a single entity, despite the existence of various distinct species and lineages. To avoid confusion, it is important to clarify and potentially assign distinct names to these different species and lineages. Enhancing our understanding of their taxonomies is an important step to avoid the misconception that biological control agents are pests. Furthermore, acknowledging if and when an agent does become a pest is important so that the situation can be studied and learned from to further improve future biological control programmes.

Both *Hypogeococcus* sp. and cochineal species have proven to be highly effective biological control agents against cacti when employed for this purpose. They are just as effective in reducing cactus populations when used as biological control agents as they are when they are pests. While there is always a concern about the unintended consequences of releasing any biological control agent into a new area, the host-specificity of these agents and the absence of native cacti in South Africa make it highly unlikely that *D. confertus* would cause any unintended harm once released. Furthermore, *D. confertus* host range does not include the cacti that are cultivated in sub-Saharan Africa (*Opuntia* spp.), reducing concerns regarding its dispersal and potential pest status in the region. Nevertheless, more host specificity tests will still be conducted to ensure the safety of releasing this agent in South Africa and to prevent any non-target attacks on commercially important species of Cactaceae.

5.5. Host suitability during pre-release assessments

Although individual insect performance parameters may not indicate the suitability of a host, the high initial survival and fecundity of *D. confertus* on *T. spachianus* was a good indication that *D. confertus* could reach high population densities once released into the field. Agents that produce abundant offspring are more likely to extend their geographical range in the introduced area by natural dispersal (McClay & Balciunas, 2005). The success of a biological control agent depends on its ability to cause mortality or reduce the fitness of its host, along with other key life-history characteristics such as fecundity, number of generations, and dispersal ability. Additionally, significant environmental factors like host-plant suitability, climatic conditions, and the presence of natural enemies in the release area can also impact the agent's abundance and range (McClay & Balciunas, 2005).

Using the Host Suitability Index (HSI) approach in this research identified several important aspects of potential control agents/ lineages for biological control of *T. spachianus* in South Africa. Having evaluated the HSI of all potential *Hypogeococcus* species/ lineages and *D. confertus*, it was determined that *D. confertus* was the agent with the highest suitability for targeting *T. spachianus*, thus establishing it as the most suitable agent for controlling *T. spachianus* in South Africa. Determining the HSI of potential biological control agents can aid in the selection of potential biological control agents for invasive plant species by identifying insects that have a high suitability for a specific weed, allowing researchers to narrow down the list of potential agents and focus their efforts on those that have the highest chances of success. Selecting biological control agents that are better suited to the target weed can improve the success of biological control programmes as insects that have a higher preference and performance on the target weed are more likely to feed and reproduce on that plant, which can lead to a reduction in the plant's population over time. On the other hand, an insect that has a low HSI on the target weed may not be an effective biological control agent.

In the past, the HSI has been utilised as a tool to assess agents potential as biological control agents. For example, Hill and Hulley (1995) employed the HSI as a tool to assess the potential of a leaf-feeding tortoise beetle ((*Gratiana spadicea*) Klug (Chrysomelidae: Cassidinae)) as a biological control agent for *Solanum sisymbriifolium* Lam. (Solanales: Solanaceae), an invasive non-native plant in South Africa. They recorded the percentage of insects that pupated, mean pupal mass and duration of development of *G. spadicea* on several *Solanum* species. The results suggested that *G. spadicea* had a higher suitability for *S. sisymbriifolium* compared to the other *Solanum* species. Due to the insect's high rate of increase, long-lived adults that are relatively mobile, several generations per year, and high per capita feeding rate, coupled with the host specificity results and the high suitability of *S. sisymbriifolium*, *G. spadicea* was deemed a potential candidate for biological control of *S. sisymbriifolium*. As a result, this collective data was utilised to support the release of this agent in South Africa. The weed's invasiveness has been only minimally impacted by the introduction of *G. spadicea* (Moran *et al.*, 2020) however, this is thought to be due to climatic incompatibility (Cowie *et al.*, 2018).

Host suitability can also be used to determine agents that may not be suitable candidates for a particular invasive species. To assess the suitability of the tortoise beetle, *Charidotis pygmaea* Klug (Coleoptera: Chrysomelidae), as a potential biological control agent for the invasive weed, *L. camara* in South Africa, its host range was investigated under quarantine laboratory conditions (Williams, 2008). The insect was tested on a variety of *Lantana* species and only minor differences in the original parameters (development time, percentage of pupation, and pupal weight) were observed during the comparison. The use of HSI, which looked at these parameters in combination, enabled clear differentiation between the hosts. The outcomes showed that the indigenous, African *Lantana rugosa* Thunb. (Lamiales: Verbenaceae), had the highest suitability and that *L. camara* and the commercially grown, *Lantana montevidensis* Briq. (Lamiales: Verbenaceae) had a similar suitability index (Williams, 2008). These findings led to the recommendation that releasing this insect for biological control of *L. camara* in any African countries would not be advisable. This decision was made because the potential benefits of releasing the insect do not outweigh the possible risks posed to horticulture, and most importantly, to environmental conservation efforts in Africa (Williams, 2008).

5.6. The way forward with *T. spachianus* in South Africa

Dactylopius confertus exhibited promise as a biological control agent of *T. spachianus*. Quarantine efficacy trials determined that *D. confertus* can reach densities that cause mortality of *T. spachianus* plants. Host specificity trials revealed a limited host range, but additional replication and test plant species need to be evaluated to fully reveal the insects host specificity. If these future trials prove satisfactory, this insect should be considered for release in South Africa. Pre-release assessments will provide important information about whether *D. confertus* can effectively curb the invasiveness of *T. spachianus*. As with all biological control programmes, pre- and post-release monitoring will be important to evaluate the establishment and impact of *D. confertus* on the target and non-target weeds. Comparing pre- and post-release evaluations provide valuable insights for assessing the feasibility of exploring additional new association programmes. *Trichocereus spachianus* is one of the very few biological control targeted weeds without an indigenous range or known origin, a challenging obstacle. Overcoming this challenge, suggests that the effort invested was justified. Releasing *D. confertus* has the potential of successfully controlling a weed in a sustainable and environmentally friendly way with significant benefits to agricultural productivity and indigenous biodiversity.

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