Post-Release Evaluation and Thermal Physiology of the Pereskia Stem-Wilter, *Catorhintha schaffneri* (Coreidae), a New Biological Control Agent for *Pereskia aculeata* (Cactaceae)

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Abstract

*Catorhintha schaffneri* Brailovsky and Garcia (Hemiptera: Coreidae) is a biological control agent that was recently accepted for release in South Africa to control *Pereskia aculeata* Miller (Cactaceae), an invasive creeping cactus. The aim of this thesis was to conduct post-release research to ensure that *C. schaffneri* is utilised to its full potential. To achieve this aim, and focus release efforts, the thermal physiology of *C. schaffneri* was investigated to predict where in South Africa the agent is most likely to establish. These predictions were then tested by releasing the agent at field sites with a wide variety of climatic conditions and evaluating establishment success.

When invasive plants invade a wide distribution, made up of areas with different climatic conditions, biological control agents may not establish or be effective throughout the invaded distribution. According to the thermal physiology of *C. schaffneri*, it is most likely to establish and become effective in the subtropical region of South Africa, along the coast of KwaZulu-Natal. Cold winters, or generally low year-round temperatures, may limit establishment in the more temperate areas of South Africa in the Eastern and Western Cape as well as inland in the Highveld region. These predictions can be used to focus release efforts to climatically suitable regions and stop releases in areas where *C. schaffneri* cannot survive.

Predictions based on thermal physiology may not account for all of the variables which affect establishment. To account for other variables, the establishment of *C. schaffneri* was tested using closely monitored field release studies. During these studies the effect of other variables such as; microclimate temperature, humidity, precipitation, plant quality and release strategy were considered. Low humidity, precipitation and plant quality appear to affect the establishment of *C. schaffneri* in the subtropical areas of South Africa. The experiment was conducted during a period of drought, and this may have resulted in lower establishment rates. The most successful release strategy for *C. schaffneri* was the use of multiple, small releases.
rather than single releases of the same number of individuals. The field based study was therefore able to improve the biological control of *P. aculeata* by increasing the chance that each release of *C. schaffneri* results in establishment.

The post-release studies presented in this thesis will increase the impact of *C. schaffneri* by focussing release efforts to climatically suitable sites, releasing at appropriate times of year and releasing the agent in a manner that increases establishment success. Post-release studies, such as those presented here, can make biological control programmes more efficient and effective.
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Chapter 1

General introduction

Alien species can be considered as casual, naturalised or invasive depending on their effect on the environment they are found in (Richardson et al. 2000). Invasive plants are known for their ability to spread and dominate natural vegetation, causing significant damage to natural and agricultural ecosystems (Cronk and Fuller 1995; Richardson et al. 2000). Their ability to spread uncontrollably and often form dense monocultures can cause a lasting effect on ecosystems, making invasive plants a threat to biodiversity and the world’s most significant economic and environmental pests (Cronk and Fuller 1995; McFadyen 1998). Pimentel et al. (2005) reported that US$120 billion was lost per year because of invasive plants in the United States, and that 42 % of the species on the endangered or threatened list were there because of invasive species. In 2008, it was estimated that alien plant invasions in South Africa cost the country about US$50 billion annually (De Lange and Van Wilgen 2010). *Pereskia aculeata* Miller (Cactaceae) is an invasive alien plant which has spread into seven out of South Africa’s nine provinces (Henderson 1995). It reduces native biodiversity and causes negative effects on ecosystem functioning (Paterson et al. 2011b). Mechanical and chemical control do not effectively control *P. aculeata* and the first biological control agent released on it, *Phenrica guerini* Bechyné (Coleoptera: Chrysomelidae), did not result in acceptable levels of control (Paterson et al. 2011a, 2011b). For this reason, a new biological control agent, *Catorhintha schaffneri* Brailovsky and Garcia (Hemiptera: Coreidae), was accepted for release in South Africa in 2014 (Paterson et al. 2014b).

The aim of this study was to conduct an initial post-release evaluation of *C. schaffneri* in South Africa in order to focus the release efforts and utilise the new agent to its full potential. The thermal tolerance of *C. schaffneri* was investigated in both laboratory and field
experiments to determine where in the country was climatically suitable for release, and a release effort experiment was used to determine how many individuals and releases are likely to result in establishment. The overall aim of the study was to use post-release evaluation data to improve the implementation of the biological control agent *C. schaffneri* on *P. aculeata*.

This chapter is a general introduction into invasive alien plants, biological control of weeds, and the two key study organisms, *P. aculeata* and *C. schaffneri*. The chapter concludes by outlining the aims and structure of each chapter in this thesis.

### 1.1 Impacts of invasive alien plants

Humans have become more mobile than any other organism and have facilitated the breakdown of biogeographic barriers, directly or indirectly, spreading new species into areas where they have not been found before (D’Antonio and Vitousek 1992). The effects of these alien species on novel environments are often underestimated, far reaching and permanent (D’Antonio and Vitousek 1992). In 1997, it was estimated that 10 million ha of South Africa had been invaded by the 180 invasive species that had been mapped (Richardson and Van Wilgen 2004). By 2010 these estimates had gone up to about 20 million ha; therefore 16% of the country was considered to be invaded by 360 invasive plant species (Kotzé et al. 2010). The estimated area in South Africa covered by invasive alien weeds therefore doubled in thirteen years, and, considering that the nature of these invasions makes them difficult to quantify, this may still be an underestimation.

It is important to understand how invasive plants and invaded ecosystems interact to correctly treat the problem (Didham et al. 2005; MacDougall and Turkington 2005; Lindenmayer et al. 2015). While some invasive species drive ecosystem change (Lindenmayer et al. 2015), others, known as passengers, proliferate as a symptom of a disturbed ecosystem rather than the cause of it (MacDougall and Turkington 2005) and others are best described as
backseat drivers, because they take advantage of a disturbed ecosystem but then drive further change (Bauer 2012; Lindenmayer et al. 2015). In cases where the invasive itself is not the root cause of the problem, removing it does not necessarily allow the native plants to recover (MacDougall and Turkington 2005). Each invasive species, and the ecosystem that it is interacting with, needs to be studied to make sure the correct treatment programme is used (Lindenmayer et al. 2015).

There are a lot of problems caused by alien plants, such as a reduction in native biodiversity (Haddad et al. 2009; Paterson et al. 2011b), changes to nutrient cycling (Rothstein et al. 2004; Yelenik et al. 2004) and soil composition (Witkowski 1991; Yelenik et al. 2004; Parker and Schimel 2010), health hazards (Agarwal et al. 2008), a decrease in the amount of available water (Le Maitre et al. 2002) and changes to disturbance regimes such as natural fire regimes (D’Antonio and Vitousek 1992).

If an alien plant becomes dominant, it often displaces native plants, forming monocultures and reducing the plant diversity of an area (Tilman et al. 2001). This decrease in plant diversity has an effect on the biodiversity of the ecosystem as a whole, including the diversity of insects, reducing the productivity and stability of the area (Haddad et al. 2009). Changes to the richness and diversity of plants in an environment can cause trophic shifts, because invasive species can either be an added food source or they can outcompete other plants which were important food sources for herbivores (Haddad et al. 2009) or pollinators (Memmott and Waser 2002; Traveset and Richardson 2006). Invasive plants with vibrant inflorescences, especially those with long flowering seasons, such as *Lantana camara* L. (Verbenaceae) in South Africa, may reduce the seed set of native plant species by outcompeting the native plants for generalist pollinators (Traveset and Richardson 2006). Overall, a change in the plant diversity affects the organisms which are native to the environment, which in turn impacts the productivity and ecosystem services of the area.
All plant species use and release different nutrients in different concentrations (Witkowski 1991; Stock et al. 1995; Parker and Schimel 2010). They also decompose at different speeds which can have an effect on the nutrient cycle (Stock et al. 1995; Rothstein et al. 2004). The leaf litter of *Acacia saligna* (Labill.) H.L. Wendl. (Fabaceae), which is invasive in the South African Fynbos biome, increases the concentration of nitrogen in the soil, which can encourage a secondary invasion by the grass species *Ehrharta calycina* Sm. (Poaceae) (Yelenik et al. 2004). By changing the amount of available nutrients in the soil, invasive species can therefore have an impact on the species that grow even after they have been removed, with subsequent long-term effects on the ecosystem (Stock et al. 1995; Rothstein et al. 2004).

There are also species of weed which are noxious and dangerous to animal and human health. *Parthenium hysterophorus* L. (Asteraceae) is an example of such an invasive weed in South Africa which causes allergic contact dermatitis, creating a health hazard for humans and livestock (Ahmed et al. 1988; Agarwal et al. 2008). Another example of a noxious weed is Canada thistle (*Cirsium arvense* L. Asteraceae), which is poisonous to livestock (DiTomaso 2000; Ziska et al. 2004). Noxious weeds are very problematic for the people and animals that live around the infestations (Ahmed et al. 1988), and they are also difficult to manage because of the health implications for the people that work on them.

Many invasive species use large quantities of water (Le Maitre et al. 2002, 2015). This affects the amount of surface run off and therefore the water level in rivers and streams (Van Wyk 1986; Le Maitre et al. 2002, 2015). In water stressed countries like South Africa, where there are no native fast growing timber species, invasive acacia, pine and eucalypt species can capitalize on the unused water resources in fire prone areas because they grow fast enough to be less affected by the fire regime (Van Wyk 1986; Le Maitre et al. 2002). This reduction in surface run off, therefore negatively affects countries which are already water stressed (Le Maitre et al. 2002).
Invasive plants can dramatically disrupt fire regimes (Van Wilgen and Richardson 1985; Keeley 2002; Brooks et al. 2004). Natural ecosystems with fire regimes operate within a vulnerable balance (Keeley 2002; Brooks et al. 2004). These fire regimes are important for the reproduction of native plants and they also stop other plants from encroaching (Keeley 2002; Brooks et al. 2004). Some invasive plants have an effect on the fuel load which can affect characteristics of fire such as the frequency, intensity, extent, type and even the season that fires occur (Brooks et al. 2004). For example, *Hakea sericea* Schrad. (Proteaceae) and *A. saligna*, which both invade the Fynbos biome in South Africa, decrease the spread of fire by increasing the fuel and moisture load (Van Wilgen and Richardson 1985). Once the fire regime has been disrupted it can be very difficult to restore, resulting in long-term changes to the ecosystem (Brooks et al. 2004; Strayer et al. 2006).

1.2 What makes an alien plant become invasive?

Not all alien plants become invasive and many theories have been put forward to try and explain this (McFadyen 1998). Although it would be beneficial to find one theory that explains why invasions happen, it is more likely that none of the theories are mutually exclusive and some are just more important in specific cases. The traits which native plants possess have been selected over multiple generations to enhance their ability of to survive in specific environmental conditions, making them specialists of their particular environment (Blossey and Nötzold 1995; Moles et al. 2008). Alien plants will have evolved under the condition that they experienced in their native environment which would be a different set of environmental conditions to those in the invaded region (Moles et al. 2008). Even with high propagule pressure (Lockwood et al. 2005), environments all have a certain amount of resistance to invasion, but invasive species appear to be most likely to invade if one of the following four characteristics are found: (i) the environmental conditions of an area have changed in a way
that is beneficial for the invasive and detrimental for the native species, (ii) the invasive species is able to fill an unoccupied niche, (iii) has a novel trait (Callaway and Ridenour 2004), or (iv) a release from natural enemies has given the invasive species a competitive advantage (Blossey and Nötzold 1995; Keane and Crawley 2002; Moles et al. 2008).

Human mediated or natural disturbances, such as changes in climatic conditions or land degradation, can cause an environment to change too quickly for the native plants to evolve (Blossey and Nötzold 1995; Moles et al. 2008). Combining these disturbances with the fact that human mediated movement results in a constant stream of propagule pressure, can mean that plants which have evolved under different conditions are able to capitalise on the change in conditions, if the new conditions are similar to their native range, and outcompete native plants (Blossey and Nötzold 1995; Lockwood et al. 2005; Moles et al. 2008).

One theory for why species become invasive is known as the novel weapons hypothesis, which is the theory that plants which have a novel, beneficial trait are able to become invasive (Moles et al. 2008; Jeschke et al. 2012). The prime example of a novel trait are plant biochemicals used in allelopathy and their negative affect on the plants surrounding them (Callaway and Ridenour 2004). If an invasive plant produces biochemicals that do not normally occur in the invaded area, the plants which are native to that area are not adapted to them and therefore can be negatively affected (Callaway and Ridenour 2004). *Centaurea diffusa* Lamark (Asteraceae) has strong negative effects on the native grass species growing around it in North America, where it is invasive, due to root exudates which plants in its native range are unaffected by (Callaway and Aschehoug 2000). The novel weapons hypothesis is a well-supported theory with 77% of the 23 studies which had tested it by 2012 being in support of it (Jeschke et al. 2012) but not all invasive plants possess novel weapons so the theory does not apply to all invasive species.
Another theory is that invasive species become invasive by occupying a vacant niche. A vacant niche is an area of trait space which is vulnerable to infestation because no native plant is capable of occupying it (Moles et al. 2008). Australian *Acacia* species, for example, are able to take advantage of the nutrient-poor soils in the fynbos biome in South Africa through nitrogen fixation, which enables them to outcompete the native species (Le Maitre et al. 2002). When plants have specific traits which make them more successful than the native plants in certain areas, they can occupy a previously vacant niche (Moles et al. 2008).

The enemy release hypothesis (ERH) is the theoretical basis for biological control (Keane and Crawley 2002). It states that upon introduction into a new environment, populations of invasive species experience a reduction in regulation by specialist herbivores that are only present in the native distribution, allowing them to thrive and outcompete native species (Keane and Crawley 2002). This hypothesis relies on three key assumptions: (i) natural enemies regulate plant populations, (ii) enemies cause more damage to the species that they have evolved with than exotic species, and (iii) plants are able to exploit a reduction in enemies (Keane and Crawley 2002; Myers and Sarfraz 2017).

Support for the ERH has mainly been found in the success of biological control, the fact that plants tend to have a higher level of herbivorous damage in their native distributions and the fact that insect herbivores can influence the densities of host plants, but, the ERH is still criticised widely because it is believed that other factors could explain these results (Myers and Sarfraz 2017). Some plants have evolved strong defences against herbivore attack which can mean that in an enemy free space, they may not be able to exploit enemy release because the plant still allocates resources to these defences (Keane and Crawley 2002). Over a longer time period, well defended alien plants may evolve to take advantage of the release from enemies (Blossey and Nötzold 1995; Keane and Crawley 2002). If there are a lot of generalist enemies in the introduced range, the well defended species may have a competitive advantage (Keane
and Crawley 2002). When a species is not very well defended, it also tends to survive through high fecundity which is a beneficial trait for an invasive species (Keane and Crawley 2002). The successful control of invasive species through classical biological control has been cited as evidence of the ERH, but there are other reasons why biological control agents have been successful, especially considering that the biological control agents are also invasive species and therefore are also released from their natural enemies (Colautti et al. 2004).

Overall, invasive plants become invasive for different reasons and the theories for why invasions happen are usually not mutually exclusive. Theories which address invader-ecosystem interactions are more supported than those that focus on specific species or ecosystems, and it has been suggested that habitats should be studied to see if they are vulnerable to certain taxa and explained by certain theories (Jeschke et al. 2012). It is important to study each invasive and the environment it is invading individually to get the best results from control (Moles et al. 2008).

1.3 Control of invasive alien plants

Invasive plants need to be controlled to mitigate their negative effect on the productivity and stability of natural ecosystems. There are three main methods for controlling invasive plants, with varying success rates, often depending on the species. Besides biological control, there are two main control options used for alien invasive plants: mechanical and chemical control. Land use management has been integrated into control programmes such as livestock grazing (DiTomaso 2000) or even through controlled fires (Paynter and Flanagan 2004), but these have been used to a lesser extent, especially in natural ecosystems.
1.3.1 Mechanical and chemical control

Mechanical control involves physically removing the invasive weed, while chemical control uses herbicides to kill the plants. Chemical and mechanical control have achieved varying degrees of success and are often used in combination (Campbell 2015), but they have non-target effects on the native fauna and flora, become expensive and have been considered unsuccessful in the long-term for widespread alien invasive plants (Van Wilgen et al. 2012).

Mechanical control can include removal by hand or machine. Research has shown that it can be highly effective for eradicating small infestations when done intensively (Campbell 2015), but when put into practice, it is often not done in the necessary rigorous manner, resulting in a poor success rate (Van Wilgen et al. 2012). It can have serious non-target effects on the surrounding vegetation (Klein 1999; Center et al. 2013), and if the plant has a large seed bank (Richardson and Kluge 2008) or grows vegetatively (Klein 1999), the success of mechanical control can be very short lived (Van Wilgen et al. 2012). Invasive alien plants growing in areas which are difficult to access are difficult to control mechanically (Campbell 2015). The climbing and intertwining nature of vines makes them particularly hard to manually remove without causing serious damage to the native vegetation or leaving pieces behind to regrow (Forseth and Innis 2004; Center et al. 2013). For example, grazing was encouraged to control an invasive vine in the USA known as Kudzu (*Pueraria montana* Lour. Merr. Fabaceae), but because the plant spreads into trees and can root if in contact with the ground this control method was ineffective (Forseth and Innis 2004).

Herbicides have been effective as a control method for invasive plants (Reddy et al. 2007; Campbell 2015) but they can also have negative effects on the surrounding native vegetation (Center et al. 2013) and are often not considered a long-term solution (DiTomaso 2000). Many herbicides are not able to translocate through plant tissue, therefore plants which propagate vegetatively can regrow (Klein 1999). Below-ground storage tissue may also remain unaffected.
Another problem with chemical control is that when a single herbicide is used, the target weed can become resistant (DiTomaso 2000). Vines are also particularly difficult to control using herbicides because it is too difficult to target the vine without damaging the native plants with which it has become intertwined (Klein 1999). Herbicidal control for the vine *P. montana* was also ineffective because the species was able to asexually reproduce and therefore small fragments left behind re-established (Forseth and Innis 2004).

Chemical and mechanical control can be successful when targeting isolated patches of invasive weeds, especially when the plant has not spread extensively, but to be successful, the plant needs to be completely removed because neither mechanical nor chemical control are not self-regulating methods (Van Wilgen et al. 2012). Both methods often rely on multiple follow up visits which end up having a large economic cost (Cilliers 1991; Van Wilgen et al. 2012). Van Wilgen et al (2012) reported that over fifteen years, US$38.4 billion had been spent on trying to control invasive weeds in South Africa, and the invasive species had only declined in very few cases. Most of the money had been used for mechanical and chemical control while only around three percent of the US$ 38.4 billion went towards biological control (Van Wilgen et al. 2012). In many cases biological control is a more sustainable, long-term, environmentally safe and economically beneficial method (Van Wilgen et al. 2004, 2012).

1.3.2 Biological control

Classical biological control is when a natural enemy, known as a biological control agent, is intentionally and permanently released to control an alien invasive organism (McFadyen 1998). In weed biological control, the agent is usually either a pathogen (Charudattan and Dinoor 2000), mite or insect which is released to control an invasive alien plant (Hoffmann 1991). Biological control is a long-term, sustainable control method where the agent is able to
breed and continually repopulate infestations after establishment (Van Lenteren et al. 2003). Biological control has a history of success around the world (Winston et al. 2014) having started with the use of a cochineal insect on Opuntia monacantha Mill. (Cactaceae) in India in 1863 (Hoffmann 1991). The first time biological control was used in South Africa was in 1913 when Dactylopius ceylonicus Green (Hemiptera: Dactylopiidae) was released and successfully controlled O. monacantha (Hoffmann 1991).

South Africa has a long history of successful biological control programmes, specifically with water weeds, Cactaceae and Australian Acacia species, although successes have not been limited to these groups (Winston et al. 2014). Hoffmann (1995) classified success in biological control into three different categories: complete, substantial and negligible. ‘Complete’ success does not mean the weed is eradicated, but rather that no other control methods are needed at sites where the agent has established, ‘substantial’ control is when the use of other methods is reduced but they are still needed as a supplement to biological control, and ‘negligible’ is when there is no reduction in the need for other control methods (Hoffmann 1995).

Using biological control is not a control method with instant results, and in some cases 10-20 years are needed before the success or failure of an agent can be assessed (McFadyen 1998). Between 1863 and 2014, 810 agents were released worldwide to control 330 species of weed (Winston et al. 2014). Of the 347 agents that have been tested in South Africa, 119 have established on 85 species of invasive plant (Klein 2011, updated 2016: http://www.arc.agric.za/arc-ppri/Documents/ Target weed species in South Africa.pdf), 23 % of the invasive plants that agents have established on have been completely controlled and 38 % are under substantial biological control (Klein 2011; Moran et al. 2013). The long period of time that it usually takes for biological control agents to effectively control the targeted invasive plant suggests that many of the agents that have already been released could become damaging in the future (McFadyen 1998).
For biological control to be successful, there are a series of steps that must be taken to ensure that the right agents are selected (Van Klinken and Raghu 2006). Once biological control has been chosen as the best control method for an invasive weed, the next step is selecting an area to survey within the weeds’ native distribution, based on genetic and climatic matching, and then a survey for potential biological control agents can be carried out (McFadyen 1998). Potential agents are prioritised, and these agents are imported into quarantine to go through host-specificity testing, approval for release, mass-rearing, release and post-release monitoring procedures (McFadyen 1998).

Prioritising potential agents found during surveys is very important, because if done correctly, it can reduce wasted time and effort on biological control agents that would not be safe for release or would never establish and control the target plant, therefore making biological control more effective and economically efficient (Van Klinken and Raghu 2006; Paterson et al. 2014b). The procedure from selecting an appropriate agent to releasing it can take three years or longer which comes at a large economic cost and makes it important to eliminate insects that are unlikely to fit the criteria of a good biological control agent as early as possible (Van Klinken and Raghu 2006). Good biological control agents need to be host-specific, do sufficient damage to the right part of the plant and be able to reach high enough population densities in the field to cause damage (Van Klinken and Raghu 2006). The research and experiments needed to prioritize agents with these characteristics can be run concurrently and over a short space of time (Paterson et al. 2014a). Collecting detailed information about the agents found and any observations made during surveys in the native region can help with the prioritisation of agents. For example, conducting a survey of the surrounding plants and checking historical records can give an indication of whether the insect is host-specific or not (Van Klinken and Raghu 2006; Paterson et al. 2014b). The amount of damage that each insect inflicts on the target plant is a vital measure of how beneficial it will be as a biological control
agent and can be used to prioritise certain insects found during surveys (Harris 1973; Goeden 1983). It is also important to assess whether the potential agent can reach high enough densities to impact the weed sufficiently, which can also be used to prioritize agents for biological control (Van Klinken and Raghu 2006).

To find each agent, the native range of the invasive plant is surveyed (McFadyen 1998). To select an area of the plant’s distribution to survey, it is important to survey genetically similar plants, especially when the target plant is known to have disjunct populations (McFadyen 1998; Gaskin et al. 2011). A natural enemy has the best chance of establishing on plants that are genetically similar to the ones it was collected on (Kniskern and Rausher 2001; Goolsby et al. 2006). This is because natural selection takes place through an interaction between the genotypes and the environment causing local adaptations to occur within the same species when they experience different environmental conditions (Kawecki and Ebert 2004). This means that if an agent is collected off a population which is distantly related to the invasive population, it may not be adapted to feed on the invasive population or it could have reduced fitness (Kawecki and Ebert 2004). It is also beneficial to choose an insect from an area with a similar climate to the country where it will be released, because this also increases the chances of establishment (Robertson et al. 2008). The agent will have evolved over multiple generations to suit the specific environmental conditions that it experiences in its native region, therefore a deviation from these conditions may result in the agent not establishing (Kniskern and Rausher 2001; Goolsby et al. 2006; Robertson et al. 2008).

Once an agent has been prioritised and transported into quarantine, the next step is host-specificity testing (McFadyen 1998), where the potential risk of non-target effects is assessed (Briese and Walker 2002). Releasing a biological control agent into a novel environment will always have some risk, therefore the risks need to be known and compared to the potential benefit of the new agent before a release is made (Briese and Walker 2002). The risk can vary
from the possibility of significant non-target damage to native plants, which can have wide spread, long-term and irreversible consequences, to a risk of some feeding but no development on native plants (Briese and Walker 2002). Often the risks that the biological control agent could pose are considered more important than the threat already being caused by the invasive weed, which can be detrimental to the end goal of restoring the native vegetation (Downey and Paterson 2016).

Test plant species are chosen for host-specificity testing based on their relation to the invasive plant (Briese and Walker 2002). If a natural enemy is able to expand onto other hosts, it is more likely to feed on plants that are genetically similar (Briese and Walker 2002; Goolsby et al. 2006). The fundamental host range of a species, which is genetically determined, includes all the plants that it can survive on, while the realised host range describes the plant species that it actually feeds on within a particular environment (Van Klinken and Edwards 2002). The most conservative test for host-specificity are no choice tests, which asses the fundamental host range of the agent by exposing it to a single species of plant (Syrett and Emberson 1997; Hill 1999; Fowler et al. 2012). These extreme conditions, which force the insect to feed on non-target plants, can result in host-specific insects feeding outside of their realised host range on species that they would never feed on under natural conditions, because the non-target plants are within their fundamental host range (Syrett and Emberson 1997; Briese 2005). In cases where the agent being tested has survived on plants other than the intended target of biological control, paired choice testing can be used to determine whether the agent would really damage native species when given a choice, which will show the agent’s realised host range (Syrett and Emberson 1997). Choice testing is when the insect is given the choice of two plants, which are usually the target plant and an alternative (Syrett & Emberson 1997; McFadyen 1998). However, these tests can also be misleading, and can result in false positive or false negative results, because it is sometimes difficult to set up paired choice experiments where the agent
can make a true choice between the plants (Fowler et al. 2012). If the plants are not in a similar condition, as the quality of potted plants may differ slightly, the choice can be compromised. The space restrictions in quarantine can also result in a build-up of plant volatiles which can cause an insect to oviposit or feed on an unsuitable host plant during paired or multiple-choice tests (Syrett and Emberson 1997). Most agents will rely on long distance cues in the field that cannot be simulated under quarantine conditions resulting in both false positive and negative results (Marohasy 1998; Heard 1999). Open field tests in the native range can help determine the realised host range of a biological control agent, but can be difficult and expensive (Clement and Cristofaro 1995; Briese and Walker 2002).

Of the 347 biological control agents that have been brought into South African quarantine facilities for host-specificity testing, 138 have been released, 40 are still under investigation and 71 have been rejected, indicating stringent protocol (Klein 2011, updated 2016: http://www.arc.agric.za/arc-ppri/Documents/ Target weed species in South Africa.pdf). The number of agents which have been rejected does appear to represent a small portion (21%) of the agents brought into quarantine, but the fact that agents are only brought into quarantine once they are prioritised during surveys in the native range may explain this. Over time, host-specificity testing has improved, because of the number of agents which have been tested, therefore reducing the possibility of non-target effects (Suckling and Sforza 2014).

All introduced alien organisms have the potential to cause negative non-target effects on the native fauna and flora (Simberloff 2012). The chance of direct effects being caused by biological control agents is negated by having strict host-specificity testing procedures (Fowler et al. 2012; Suckling and Sforza 2014). Biological control agents can cause indirect non-target effects by affecting species in other trophic levels, such as predators and parasitoids, but these impacts are difficult to predict (López-Núñez et al. 2017). Significant direct non-target impacts from biological control agents are very rare (Suckling and Sforza 2014). Suckling and Sforza
(2014) found that over the last 150 years, more than 99% of the 512 agents they reviewed were not known to have any significant adverse direct effects (Suckling and Sforza 2014). Where there was evidence of non-target effects, in most cases the agents caused minimal problems and left no lasting negative effects on the ecosystem (Suckling and Sforza 2014). *Cactoblastis cactorum* Berg. (Lepidoptera: Pyralidae) on native cacti in Mexico and the United States of America (Zimmermann et al. 2000), and *Rhinocyllus conicus* Frölich (Coleoptera: Curculionidae) on native thistles in Michigan in the United states of America as well as in Canada (Havens et al. 2012) are the biological control agents which have had the most significant non-target effects. However, *C. cactorum* was not intentionally released and should therefore be considered a phytosanitary issue, and although the non-target effects of *R. conicus* were expected, they were not considered a high priority at the time of release and neither agent would be accepted for release into the areas where they are problematic today (Pemberton 2000; Suckling and Sforza 2014; Downey and Paterson 2016).

Once a biological control agent has been released, post-release evaluations are an essential, yet often neglected, step in the process (Carson et al. 2008; Morin et al. 2009). Post-release surveys enable practitioners to monitor the released populations, improve the implementation of each agent so that it establishes and proliferates, and evaluate the agent, therefore improving the control of the invasive weed (Grevstad 2006). Detailed climatic studies can also be beneficial post-release because knowing the biological control agent’s thermal tolerances can stop practitioners from wasting resources by releasing agents at sites where they will not be able to survive (Coetzee et al. 2007). Different release techniques have also benefited the establishment of certain agents and by researching the best life stage and release method the chance of establishment can increase (Grevstad 1999b). Once established, the population is less vulnerable to extinction. This is because the population is made up of more individuals which lessens the potential for the entire population to be affected by predators. Established
populations are also made up of a range of developmental stages which reduces the chance that the entire population is killed during a single stochastic event, because each stage will have slightly different vulnerabilities, especially when the species has a sessile developmental stage (Hill et al. 2012; Blubaugh and Kaplan 2015).

It is not a simple task to evaluate the effect that an established biological control agent has had on an infestation because any perceived changes to the weed population need to be measured and then attributed to biological control rather than other factors that may influence weed population levels (Morin et al. 2009). The growth or suppression of the plants can be affected by the natural variability in biological systems and abiotic conditions (Morin et al. 2009).

It is common practice to evaluate the establishment of biological control agents post-release (Van Klinken et al. 2003; Cowie et al. 2016) but the effect that the release has had on the target plant is often not measured or reported (Carson et al. 2008). Out of the 119 biological control agents which had been released onto 58 invasive plants (Julien and Griffiths 1998), Carson et al. (2008) only recorded twenty biological control agents on ten invasive weeds where the damaged caused by these agents had been quantified (Blossey and Skinner 1999; Story et al. 2000, 2006; Dhileepan et al. 2000; Clark et al. 2001; Dhileepan 2001, 2007; Mico and Shay 2002; Lindgren 2003; Seasteadt et al. 2003; Landis et al. 2003; Lesica and Hanna 2004; McConnachie et al. 2004; Kok et al. 2004; Paynter 2005; Butler et al. 2006; Cornett et al. 2006; Grevstad 2006). There are also even fewer studies which actually quantify the effect that a reduction in the weed has on the invaded ecosystem, and whether recruitment of native species is possible, after successful biological control, which is vital (Barton et al. 2007; Clewley et al. 2012). The effect that each biological control agent has on controlling the invasive weed and the effect this has on the native biodiversity is neglected mainly due to the long-term nature of
the studies, especially considering that biological control agents can take up to twenty years to establish (McFadyen 1998).

Post-release evaluations are beneficial in many ways: they provide information that helps scientists improve the release strategy and utilisation of each agent (Grevstad 2006), they provide information for practitioners to judge whether additional agents are necessary (Dhileepan 2001), they can indicate if additional control methods need to be stopped or added (Cornett et al. 2006) and they enable scientists to collect important data to illustrate the benefits associated with biological control and justify further investments into biological control programmes (Butler et al. 2006; Story et al. 2006; Dhileepan 2007; Morin et al. 2009; Clewley et al. 2012). It is just as important to report the failures of biological control agents as the successes, because reported failures can stop future investments into programmes which are likely to fail (Carson et al. 2008).

1.4 *Pereskia aculeata* Miller (Cactaceae)

1.4.1 Biology and native distribution

In South Africa, there are 21 invasive species from the family Cactaceae being targeted for biological control, most of which are described as either cholla (characterised by large succulent pads) or columnar cacti (characterised by succulent tall stems) (Klein 2011, updated 2016: http://www.arc.agric.za/arc-ppri/Documents/ Target weed species in South Africa.pdf). The genus *Pereskia* Miller, however, has well-developed leaves and woody stems unlike typical cacti (Figure 1.1) (Leuenberger 1986; Nyffeler 2002; Edwards et al. 2005). There are seventeen species within the genus *Pereskia* (Edwards et al. 2005) and only *P. aculeata* is invasive in South Africa (Department of Environmental Affairs 2014). *Pereskia aculeata* is a primitive creeping cactus with paired, short, hooked thorns, which extend out to the side and backwards from below each leaf on young shoots, while the older woody stems have clusters
of long needle-like thorns (Figure 1.1 and Appendix A) (Leuenberger 1986; Edwards et al. 2005). It has a creamy white flower with yellow or purple stamens, and produces yellow fruit covered in small thorns when they are immature, but as the fruit ripens, they lose the thorns and turn a more orange colour (Leuenberger 1986; Henderson 1995). The leaves of *P. aculeata* are eaten in Brazil (Leuenberger 1986) but it has not become a popular food source in South Africa. It was moved around the world and into South Africa being placed in botanical gardens as an interesting primitive Cactaceae species (Leuenberger 1986). In South Africa, *P. aculeata* is popular as a hedgerow because of its growth form and large thorns which make it an effective barrier to livestock and people (De Beer 1988).

*Figure 1.1* *Pereskia aculeata* drawn by G. Condy & published in Henderson (1995), ARC-Plant Protection Research Institute, Pretoria.

*Pereskia aculeata* has a disjunct native distribution that is separated into two main areas (Figure 1.2) (Leuenberger 1986). The northern populations are found in the Caribbean, Central
America and southern Mexico, while the populations south of the equator are in southern and
south-eastern Brazil, southern Paraguay and northern Argentina (Figure 1.2) (Leuenberger
1986).

*Pereskia aculeata* also has morphological polymorphisms and the species includes a number
of variegated garden cultivars (Leuenberger 1986; Paterson et al. 2009). In the native range, *P.
aculeata* exhibits morphological polymorphisms between the northern and southern parts of its
distribution (Paterson et al. 2009). In the northern areas, *P. aculeata* generally exhibits
spineless fruits, weakly spiny receptacles, white stamen filaments and the leaves range in size
but are generally broader than those in the southern region (Leuenberger 1986; Paterson et al.
2009). While in the southern area of the native distribution *P. aculeata* tends to have spiny
fruits and receptacles, carmine-red stamen filaments and narrow leaves (Leuenberger 1986;
Paterson et al. 2009). The plants in South Africa exhibit characteristics from both areas,
therefore the morphological polymorphisms could not be used to determine where the South
African population originated from (Paterson et al. 2009).
The first record of *P. aculeata* in South Africa comes from McGibbon’s (1858) catalogue of plants in the Cape Town Botanical Gardens, but it was only declared a weed in South Africa in 1979 (Proclamation R35 of 1979), and it is still a declared weed under the most recently promulgated legislation, the National Environmental Management Biodiversity Act (NEMBA) (Department of Environmental Affairs 2014). It was initially brought into South Africa as a botanical specimen that was spread throughout the eastern parts of the country by humans because of its use as a hedge plant (Paterson et al. 2009). Not only is it now found in disturbed areas, but it produces viable seeds which are moved by birds and bats into pristine and remote areas (Campbell 1988). Currently *P. aculeata* is found in seven out of the nine provinces in
South Africa and is particularly dominant along the Eastern Cape coast and in Kwazulu-Natal, preferring forest and coastal regions (Figure 1.3) (Paterson et al. 2011b).

**Figure 1.3** The distribution of *Pereskia aculeata* in South Africa (Originally Drawn by L. Henderson (1995) SAPIA database, ARC-Plant Protection Research Institute).

1.4.2 Impact

*Pereskia aculeata* has been known to cover trees in natural and commercial forests to such an extent that it eventually kills them (Moran and Zimmermann 1991) (see Appendix B and C). To test the effect that *P. aculeata* has on native plant diversity, Paterson (2011a) compared the species richness and diversity between areas that had different densities of *P. aculeata*. Native plant diversity was significantly higher where there was less than 50% cover of *P. aculeata*, illustrating its negative effect on native biodiversity (Paterson et al. 2011a).
Pereskia aculeata infestations also caused a shift in the dominant functional plant group in native forests, favouring creepers or vines (Paterson et al. 2011a). This is likely to affect the ecosystem functioning of the forest and its ability to provide ecosystem services (Loreau et al. 2001; Paterson et al. 2011a). Pereskia aculeata is considered a driver of biodiversity loss rather than a symptom of disturbance (Paterson et al. 2011a).

1.4.3 Mechanical and chemical control

There are severe non-target effects when using chemical or mechanical control for P. aculeata, and its ability to reproduce asexually and regrow from small fragments makes both of these methods short-term solutions (Moran and Zimmermann 1991; Paterson et al. 2011a). Pereskia aculeata becomes intertwined with indigenous plants, so manual removal also removes or damages indigenous plant species, and if pieces of P. aculeata are left behind they can regrow (Paterson et al. 2011a). Triclopyr (butoxy ethyl ester; 480 g/l a.i.) is the registered herbicide for P. aculeata but it does not translocate through the plant tissue effectively, therefore unless the entire plant is covered it is able to repopulate an area from fragments left behind (Klein 1999). The limitations of chemical and mechanical methods suggest that biological control is the only viable option for controlling the weed (Moran and Zimmermann 1991; Paterson et al. 2011a).

1.4.4 Biological control

The first surveys for biological control agents for P. aculeata were conducted on an opportunistic basis while surveying for agents for other invasive alien plants (Paterson et al. 2014a). Several potential biological control agents were found during these initial surveys which took place from 1988-1999 (Klein 1999). The surveys resulted in three species which were brought into quarantine in South Africa: the leaf-mining moth Loxomorpha cambogialis
[Formally *Epipagis*] (Guenée) (Lepidoptera: Pyralidae), the stem-boring moth *Maracayia chlorisalis* (Walker) (Lepidoptera: Crambidae), and the flea beetle *Phenrica guerini* Bechyné (Coleoptera: Chrysomelidae) (Klein 1999, 2011). Another insect found on *P. aculeata* in Brazil was an unidentified cerambycid (Klein 1999) which was later identified as *Acanthodoxus machacalis* Martins & Monné (Coleoptera: Cerambycidae) (Paterson et al. 2014a).

*Loxomorpha cambogialis* developed into adults on several plants, including two beneficial species and several indigenous species, making its host range too wide for it to be released in South Africa (Klein 1999, 2011). *Maracayia chlorisalis* was very difficult to rear and eventually host-specificity trials were abandoned and the agent was shelved (Klein 1999, 2011). Host-specificity testing of *P. guerini* indicated that the species was monophagous, feeding only on *P. aculeata* and it was subsequently released in South Africa in 1991 (Klein 1999). *Phenrica guerini* established at very few sites and did not successfully control *P. aculeata* to acceptable levels (Paterson et al. 2009).

Between 2009 and 2014 a number of studies were conducted to prioritise new biological control agents for *P. aculeata* (Paterson et al. 2009, 2014a). The disjunct native distribution and morphological polymorphisms of *P. aculeata* in its native range made it important to establish the origin of the South African invasive populations of *P. aculeata* so that potential agents could be collected off genetically similar plants in the native distribution. Surveying genetically similar *P. aculeata* should ensure that the agents that were collected were suitably adapted to feed on the target plant (Paterson et al. 2009). Paterson et al. (2009) found that the South African population was closely related to the plants found in Rio de Janeiro and Santa Catarina State in Brazil. It was also found that there are low levels of genetic variability in the South African population, probably resulting from a single introduction or multiple introductions from the same source (Paterson et al. 2009). Climatic matching was also important because agents found on plants in a similar climate to the South African distribution
of *P. aculeata* would be more likely to establish and proliferate (Paterson et al. 2014a). Climatic matching indicated that the most promising region to survey would also be south-eastern Brazil (Paterson et al. 2014a).

In 2012, a survey of the *P. aculeata* populations in south-eastern Brazil found 15 natural enemies in association with *P. aculeata*, and five were prioritised (Paterson et al. 2014a). The criteria for prioritization included historical host range data, field host range and the agent’s mode of damage (Paterson et al. 2014a).

The five natural enemies which were prioritised were: the three stem boring species, *Acanthodoxus machacalis* Martins & Monné (Coleoptera: Cerambycidae), *Pereskiophaga brasiliensis* Anderson (Coleoptera: Curculionidae) and *Maracayia chlorisalis* Walker (Lepidoptera: Crambidae); a fruit galler *Asphondylia* sp. (Diptera: Cecidomyiidae); and a stem wilter, *C. schaffneri* (Paterson et al. 2014a). *Acanthodoxus machacalis* and *P. brasiliensis* damage the structural tissue of *P. aculeata*, feeding within the woody stems as larvae (Paterson et al. 2014a). *Maracayia chlorisalis* mines the young shoots of *P. aculeata*, usually killing them (Paterson et al. 2014a). *Asphondylia* sp. galls the ovaries of *P. aculeata*, resulting in reduced seed set (Paterson et al. 2014a). *Catorhintha schaffneri* feeds on the new growing shoots, causing them to rot and die (Paterson et al. 2014a). All five potential agents were collected in the appropriate region in terms of climatic and genotype matching of *P. aculeata* (Paterson et al. 2009, 2014a). The first of the prioritised agents to finish host-specificity testing was *C. schaffneri* due to its short life cycle, which enabled host-specificity testing to be completed in the shortest amount of time compared to the other prioritised agents (Paterson et al. 2014b).

### 1.5 *Catorhintha schaffneri* Brailovsky & Garcia (Coreidae)

The adult and nymphal stages of *C. schaffneri* are gregarious and feed on the new growing shoots of *P. aculeata*, resulting in significant damage and often resulting in the death of the
shoot tip (Paterson et al. 2014a) (see Appendix A). The insect was collected at two sites (27°08'08.51" S 48°31'55.16" W; 26°46'31.29" S 48°36'04.19" W) in Porto Belo, Santa Catarina Province, Brazil, in May 2012, and 23 individuals were transported into quarantine in South Africa (Figure 1.4) (Paterson et al. 2014a, 2014b). These 23 individuals are the only live specimens that have been imported into South Africa and the biological control agent population is therefore the progeny of these 23 individuals.

![Catorhintha schaffneri](image)

**Figure 1.4 Catorhintha schaffneri** Brailovsky & Garcia (From Brailovsky & Garcia 1987).

The impact of *C. schaffneri* on *P. aculeata* was tested under quarantine conditions (Paterson et al. 2014b). To quantify impact, plants were exposed to ten newly hatched nymphs for a ten day period and plant parameters were compared with control plants which were exposed to the same conditions, without the exposure to the insects (Paterson et al. 2014b). Shoot length and
the number of leaves were both significantly reduced for the plants exposed to *C. schaffneri* herbivory (Paterson et al. 2014b). It was thought that this level of impact should be able to reduce *P. aculeata*’s ability to spread into the canopies of trees, and the reduction in leaves may allow an increase in indigenous plant diversity as it would reduce the ability of *P. aculeata* to compete for light and space (Paterson et al. 2014b).

South Africa has no indigenous cacti besides *Rhipsalis baccifera* (J. Müller), Stern which has had its status as a native disputed (Paterson et al 2014b). This reduces the risk of non-target attack by cactophagous biological control agents on native plants in the country (Moran and Zimmermann 1991; Suckling and Sforza 2014; Paterson et al. 2014b). *Catorhintha schaffneri* was found to have a very restricted fundamental host range, with nymphs only reaching maturity on one other plant species, the closely related congener *Pereskia grandifolia* Haw. (Cactaceae) (Paterson et al. 2014b). On *P. aculeata*, 74% of the *C. schaffneri* developed into adults, while only 3% developed into adults on *P. grandifolia*. Therefore, it is unlikely that *P. grandifolia* would be included in the realised host range of *C. schaffneri* (Paterson et al. 2014b). *Pereskia grandifolia* is also the closest relative of *P. aculeata* and an alien plant in South Africa. Thus, despite its ability to complete its development on *P. grandiflora*, *C. schaffneri* was not considered a threat to South African biodiversity (Paterson et al. 2014b). *Catorhintha schaffneri* is therefore suitably host-specific (Paterson et al. 2014b), and in June 2014, permission to release *C. schaffneri* in South Africa was granted (Klein 2011, updated 2016: http://www.arc.agric.za/arc-ppri/Documents/ Target weed species in South Africa.pdf).

### 1.6 Aims and rationale

This project aimed to improve the control of *P. aculeata* by attempting to maximise the efficacy of the new biological control agent, *C. schaffneri*. This was achieved by determining
where and under what conditions the agent was most likely to establish and thrive, as well as how best to release the agent to maximise the chances of establishment.

Conducting research into the thermal tolerance of a biological control agent can help predict where the agent is most likely to establish and become effective (Van Klinken et al. 2003; Coetzee et al. 2007; Wang et al. 2009). The thermal tolerance of *C. schaffneri* was investigated in two ways: in the laboratory through thermal physiological studies (Chapter 2), and subsequent establishment experiments were then conducted in the field (Chapter 3). Although thermal physiology was the main focus of the research, other climatic factors besides temperature, were included when possible. A study to determine whether release effort would affect establishment success was also conducted (Chapter 3). The data from this study therefore inform implementation of how and where to release *C. schaffneri* in order to gain the maximum benefit from the new biological control agent.
Chapter 2

The thermal physiology of *Catorhintha schaffneri*

2.1 Introduction

Not all biological control agents have been successful. In South Africa only around 34% have established, and of those, only 27% have done extensive damage to the target plant species (Klein 2011, updated 2016: http://www.arc.agric.za/arc-ppri/Documents/ Target weed species in South Africa.pdf). Climate incompatibility is one of the main reasons why biological control agents fail to establish or control invasive plants (McClay 1996; Van Klinken et al. 2003; Coetzee et al. 2007). There are a few different ways to research the climate compatibility of a biological agent and doing so can be beneficial both pre- and post-release (McFadyen 1998). Pre-release climate-matching, done by comparing the climatic conditions in the intended region of introduction to the climatic conditions in the native range, is a general way to look at climatic suitability. A detailed assessment of the biological control agent’s thermal physiology, conducted in laboratory-based experiments on the agent, can give a clearer description of where it has the best chance of establishing within the intended range of introduction (Senaratne et al. 2008). *Catorhintha schaffneri* was collected from an area which was climatically matched to the invaded distribution of *P. aculeata* in South Africa (Paterson et al. 2014a), but, in South Africa *P. aculeata* has invaded different climatic zones. In the past, extensive effort and costs have gone into the mass-rearing and release of agents into areas where they are preordained to fail due to climate incompatibility (Hill and Olckers 2001; Coetzee et al. 2007). Through thermal physiology research, biological control agents can be used more efficiently and effectively.

Over time, species adapt to the climatic conditions that they experience and become specialised to the conditions in their native range (Robertson et al. 2008). Individuals with traits
that improve their fitness can breed and pass on their genes, allowing the species as a whole to evolve and become successful in its native range (Kawecki and Ebert 2004). Biological control agents will therefore have evolved under the climatic conditions in their native range, which can make survival in a new environment, under different conditions, difficult.

There are many examples where the differences between climatic conditions in the native and introduced ranges have affected the establishment of biological control agents (Byrne et al. 2002; Van Klinken et al. 2003; Coetzee et al. 2007). If an agent has not developed mechanisms to cope with extreme weather conditions such as drought, frost (Coetzee et al. 2007), short growing seasons (McCay 1996) or long/cold winter periods (McCay 1996; Coetzee et al. 2007), they are unlikely to survive in areas which experience these conditions. Agents are not just more likely to survive in an area with a similar climate, but they are also more likely to be effective if they come from areas with similar climates to the region of introduction (Kawecki and Ebert 2004; Robertson et al. 2008). If the intended introductory region has a slightly different climate and the agent survives, but only in low numbers, it may not do sufficient damage to the target weed. Any amount of climate incompatibility can therefore result in a biological control agent being ineffective (Byrne et al. 2003).

Natural selection and phenotypic plasticity are processes, or abilities, which can make it possible for an agent to establish in an area with a different climate to where it evolved (Kawecki and Ebert 2004; Chown et al. 2009). Natural selection can enable the species to adapt over multiple generations to better suit the new climate (Kawecki and Ebert 2004). For this to happen, advantageous traits need to be present within the released population (Kawecki and Ebert 2004). If individual insects carry these advantageous traits, which increase their ability to survive in the new environment, they are more likely to reproduce and slowly increase the frequency that the trait is found in individuals within the population (Kawecki and Ebert 2004). Natural selection would occur over multiple generations and would therefore take a long time,
depending on the agent’s life cycle and duration of development, causing a lag period which could be misinterpreted as failed establishment or low effectiveness (McFadyen 1998).

The ability of a gene to express more than one phenotype under different environmental conditions is called phenotypic plasticity (Chown et al. 2009). This ability could also help biological control agents survive under conditions to which they are not accustomed (Chown et al. 2009). Rapid phenotypic acclimation is specifically beneficial for biological control agents because it can take place within an insect’s lifetime (Chown and Terblanche 2006; Overgaard and Sørensen 2008). Phenotypic plasticity in genes associated with thermal tolerance would give biological control agents an advantage if they are released into different climatic conditions to the ones that they have adapted to (Overgaard and Sørensen 2008). Phenotypic plasticity of thermal tolerance in the invasive Medetarian fruit fly, *Ceratitis capitata* Wiedemann (Diptera: Tephritidae), increases its ability to invade different habitats, in comparison to the less successful invader, *Ceratitis rosa* Karsch (Diptera: Tephritidae), which was not able to respond to cold hardening as quickly (Nyamukondiwa et al. 2010).

The time that each biological control agent spends in quarantine could potentially affect the thermal physiology of the population (Bownes et al. 2010). Most quarantine facilities have constant environmental conditions, and over a long period of time this could potentially affect the biological control agent’s ability to cope with variable conditions once released (Taylor et al. 2011) and, if the agent has been subjected to stable conditions for a long period, traits which benefit variable conditions will not have been selected for within the population. Some agents have spent a very long time in quarantine, for example *Cornops aquaticum* Brüner (Orthoptera: Acrididae), which was in quarantine for twelve years (Bownes et al. 2010). Insects which experience variable conditions in their native region are more likely to evolve phenotypic plasticity (Klok and Chown 2003) which could suggest that under stable conditions in quarantine, this beneficial attribute may not be selected.
Before surveys for potential biological control agents were done for *P. aculeata*, climate-matching was used to prioritise areas in its native distribution which have a similar climate to the areas in South Africa which were infested (Paterson et al. 2014a). Climate-matching studies usually use the distribution of the plant, rather than the distribution of a potential biological control agent, because the distributions and identities of the potential agents are usually unknown (Paterson et al. 2014a). Climate-matching has prioritized successful biological control agents in the past, such as, the leaf-tier *Evippe* sp. #1 (Lepidoptera: Gelechiidae) which is now widely established and controlling mesquite (*Prosopis* spp. Leguminosae) in Australia, but another agent on *Prosopis, Prosopidopsylla flava* Burckhardt (Hemiptera: Psyllidae) which was native to the same area and also prioritised according to its climatic suitability was not as successful in the cooler areas due to a slight difference in climate as well as ant predation (Van Klinken et al. 2003). The climate-matching study on *P. aculeata*, along with other factors, such as genetic matching, damage and host-specificity, resulted in the prioritisation of *C. schaffneri* and four other potential biological control agents (Paterson et al. 2014b). Santa Catarina and Rio de Janeiro, Brazil, were both considered to be good climatic matches to the infested area of South Africa, and *C. schaffneri* was collected for testing in South Africa from Porto Belo, Santa Catarina (Paterson et al. 2014a).

In South Africa, *P. aculeata* invades a variety of climatic zones from the warm, subtropical areas in KwaZulu-Natal to the cooler, more temperate climate in the Eastern and Western Cape (Paterson et al. 2014a). Thermal physiology research on *C. schaffneri* would be beneficial so that practitioners can focus the release efforts to areas in South Africa where it is most likely to establish and do sufficient damage.

Climate-matching gives an idea of where the agent is more likely to survive but often does not provide sufficient detail. There are other more detailed methods and programmes used for researching the thermal physiology of biological control agents such as degree-day models or
research into the critical and lethal thermal limits of the agents. Insect development studies and degree-day modelling (Campbell et al. 1974), in conjunction with distribution modelling programs such as CLIMEX (Sutherst and Maywald 1985), or data collected from weather stations, can help by predicting the number of generations that an agent could potentially complete in certain places (Campbell et al. 1974; Byrne et al. 2003). These models have proven to be useful at explaining why specific biological control agents are able to establish at certain sites and fail at others (Byrne et al. 2003; Coetzee et al. 2007; Cowie et al. 2016). Thermal physiological studies were done for the biological control agent Eccritotarsus catarinensis (Carvalho) (Heteroptera: Miridae) on water hyacinth (Eichhornia crassipes (Mart.) Solms (Pontederiaceae)), and they were able explain why its distribution is limited to part of the plants distribution in South Africa (Coetzee et al. 2007).

While degree-day modelling investigates the long-term effects of climate on establishment success, extreme temperatures that are only experienced for short periods of time can also prevent biological control agents from establishing (Stewart et al. 1996; Coetzee et al. 2007). These effects can be investigated by testing the critical and lethal limits of the agent (Mitchell et al. 1993). The critical limits (critical maximum (CTMax) and critical minimum (CTMin)) are the temperatures at which the insect loses locomotory function, and are therefore behavioural limits (Mitchell et al. 1993). At these temperatures, insects can no longer feed or protect themselves from predators or actively avoid potentially dangerous situations, such as heavy rain, which could result in mortality (Mitchell et al. 1993; Coetzee et al. 2011).

The lethal limits (ULT50 and LLT50) are the temperatures that would result in death if the agent is exposed to them for a certain length of time, and are therefore physiological limits (Mitchell et al. 1993). It is difficult to say that an agent was exposed to a specific temperature for an exact length of time in the field because the agent can actively avoid the temperature or exists in a microclimate (Coetzee 2012). However, if the air temperature goes above or below
the lethal temperature it is still likely to cause physiological stress and negatively affect the agent (Ma et al. 2015). Determination of a degree-day model and the thermal limits of _C. schaffneri_ could improve the prospects of establishment for this agent in South Africa.

The thermal physiology of _C. schaffneri_ was determined by developing a degree-day model using the reduced major axis method (Ikemoto and Takai 2000) and calculating the number of generations that the agent could potentially complete using weather station data from around South Africa. The survival time and reproductive output of _C. schaffneri_ adults at different temperatures was also determined, as well as the critical thermal limits (CTMax and CTMin) and lethal temperatures (ULT$_{50}$ and LLT$_{50}$) of the adults and first instar nymphs. These data were combined to determine the effect of temperature on establishment success of _C. schaffneri_ on _P. aculeata_ at different locations across South Africa.

2.2 Methods

Adult and first instar nymph _C. schaffneri_ used for these experiments were collected from the culture maintained at the Waainek Research Facility at Rhodes University, Eastern Cape, South Africa, and the culture at the South African Sugarcane Research Institute (SASRI), KwaZulu-Natal, South Africa. Both cultures were reared in controlled environment rooms where the temperature was kept between 25 and 27 °C, on a 12L:12D light regime. Nymph and adult _C. schaffneri_ were fed on fresh, actively growing _P. aculeata_ shoots which were collected from infestations in the field or plants grown in greenhouses at each facility. A fine mist of water was sprayed into the cages about three times a day to increase humidity.
2.2.1 Degree-day model: lower developmental threshold (t), rate of development (K) and degree-day acclimation

The thermal requirements of *C. schaffneri* were investigated by developing a degree-day model. Approximately 30 to 40 *C. schaffneri* adults were placed in a cage with newly cut shoots of *P. aculeata* to mate and oviposit. After 24 hours, all egg batches were collected and placed into individual petri dishes. Five petri dishes, each containing an egg batch, were then placed into each of six controlled environment rooms set at six experimental temperatures of 16, 18, 20, 24, 28 and 29 °C to allow for development from egg to adult at controlled temperatures. The controlled environment rooms had a 12L:12D light regime. The egg batches were checked three times a day and the lid of each petri dish was sprayed with a fine mist of water twice a day to avoid desiccation. The number of eggs to successfully hatch at each temperature was recorded. Two iButtons were placed in petri dishes at each of the temperatures to confirm the temperature being experienced by the eggs was the temperature that the controlled environment room was set at.

On hatching, the neonates from each petri dish were equally divided into two closed cylindrical containers containing blocks of florist foam and freshly cut young shoots of *P. aculeata* to allow for feeding. The temperature in the containers was also confirmed using iButtons. There were therefore ten replicates at each temperature from at least 5 different egg batches. A new shoot of *P. aculeata* was added whenever necessary, to avoid any competition for food among neonates, and older shoots were removed when the nymphs had moved onto the newer food. The containers were checked daily and each moult was recorded until the final ecdysis into the adult stage. Each ecdysis was indicated by the presence of exuviae and usually all of the neonates would moult on the same day, but if not, the day when the most neonates moulted was recorded.
The average number of days that the insects took to develop from egg to adult was then calculated for each experimental temperature and the reduced major axis regression method was used to estimate the thermal constant (K) and developmental threshold (t) (Ikemoto and Takai 2000). The chosen method was adapted from Ikemoto and Takai (2000) and supported by Lui et al. (2002) as a reliable degree day modelling method. The thermal constant (K) is the effective cumulative temperature and the developmental threshold (t) is the temperature at which we assume no development is possible (Ikemoto and Takai 2000). K and t were then used to calculate the theoretical number of generations (the accumulated degree-days) that C. schaffneri could complete annually and through winter at the closest weather station to where C. schaffneri was collected in its native distribution (Tijucas, Santa Catarina, Brazil), and at 27 different locations around South Africa, where P. aculeata has been recorded, using the equation (Campbell et al. 1974):

\[ K = \sum \left( \frac{T_{max} + T_{min}}{2} - t \right) \]

(Where \( T_{min} \) (the daily minimum) < \( t \) (the developmental threshold), \( t \) was used)

Daily mean minimum (Tmin) and maximum (Tmax) temperature data for Tijucas was collected from the Centro de Informações de Recursos Ambientais e de Hidrometeorologia de Santa Catarina (EPIGRI CIRAM), while data for the South African sites was collected by the South African Weather Service (SAWS). The 27 South African weather station localities were chosen because of their proximity to known P. aculeata infestations, viz.: Riversdale, George, Paarl and Knysna from the Western Cape; Port Edward, East London, Port Alfred and Grahamstown from the Eastern Cape; Port Edward, Margate, Pietermaritzburg, Mtunzini, Richards Bay, Charters Creek, Mbazwana, Pennington South, Durban South, Virginia and Mandini from KwaZulu-Natal; Vereeniging, Johannesburg Botanical Gardens and Pretoria from Gauteng; Nelspruit and Graskop Aws from Mpumalanga; Hoedspruit and Polokwane from Limpopo; and Rustenburg from the North West Province.
Using the Geographic Information Systems (GIS) programme, ARCVIEW (Version 10.3, Environmental Systems Research Institute Inc. Redlands, CA, USA), a map was created from the mean annual accumulated degree-day data, showing the number of generations that *C. schaffneri* could complete annually at weather station based locations around South Africa. A second map was also created to show the potential number of generations that could be completed through the winter (April – August) using the Geographic Information Systems (GIS) programme, ARCVIEW (Version 10.3, Environmental Systems Research Institute Inc. Redlands, CA, USA).

2.2.2 Hatching, developmental success, longevity and reproductive output at different temperatures

The influence of temperature on hatching and developmental success, longevity and reproductive output were tested at the six different temperatures. The percentage of eggs which hatched at the six experimental temperatures were recorded and compared using a Kruskal-Wallis ANOVA and a post hoc multiple comparison of mean ranks, because the data were not normally distributed. The percentage of *C. schaffneri* which then developed into adults was also recorded and compared using a Kruskal-Wallis ANOVA and a post hoc multiple comparison of mean ranks.

Adult longevity was then investigated using the adults which had developed from hatching at the four temperatures (20, 24, 28 and 29 °C) where development to the adult stage had been successful. One male and one female were paired and placed into separate closed cylindrical containers with fresh shoots of *P. aculeata*. The number of days that each adult survived was recorded, and the containers were checked daily for batches of eggs, the number of which were recorded. The shoots of *P. aculeata* were replaced as often as necessary and the enclosure was sprayed with a mist of water daily to maintain humidity. This was replicated six times at each
temperature to determine longevity for twelve individual insects. The longevity and reproductive output of *C. schaffneri* at the four different temperatures were statistically analysed using Kruskal-Wallis ANOVAs and post hoc multiple comparison of mean ranks because the both the longevity and reproductive output data were not normally distributed. All statistical analyses throughout this chapter were conducted in Statistica (version 13).

### 2.2.3 Critical thermal limits and lethal temperatures

The critical limits (CTMin and CTMax) of *C. schaffneri* are the mean temperatures where the species loses its locomotory function (Mitchell et al. 1993). Both *C. schaffneri* nymphs (first instars, after they had eaten) and adults were tested for their critical thermal limits. The *C. schaffneri* used for CTMin, CTMax, ULT50 and LLT50 were all taken from the Waainek research facility where they were kept at between 24 and 25 °C and then acclimated at 25 °C with fresh food for 24 hours before testing. Other factors that can influence critical and lethal temperatures, such as weight, feeding and the stage of development, were controlled by using insects of the same size and age. To calculate the CTMin and CTMax, it was necessary to subject the insects to controlled temperature conditions in a controlled temperature water bath (model: Grant GP200 - R4 refrigerated water bath and TXF heating circulator). Ten adults were separated into air-filled 2 ml Eppendorf tubes, sealed with damp cotton wool and placed into a floating tray, made from a piece of high density foam. The floating tray held the tubes so that only their opening was above the water’s surface. This method was adapted from Mitchell et al. (1993) and Terblanche et al. (2007), because these methods are standard procedures which are generally considered as reliable. For both the CTMin and CTMax experiments, the experiment commenced when the water temperature was 20 °C. For the CTMin, the temperature was then cooled from 20 to 10 °C in ten minutes, where it was kept for two minutes, before it was decreased at a rate of 1 °C every four minutes (Terblanche et al. 2007). For the
CTMax, the insects were placed in the water bath at 20 °C and the temperature was then increased to 30 °C (Terblanche et al. 2007). The temperature was then increased by 1 °C every four minutes (Terblanche et al. 2007). The insects were assessed to see if they were still able to hold onto the side of the vials every time the water temperature decreased/increased by a degree Celsius. The temperature at which each insect could no longer adhere to the sides of the vial, thereby indicating a loss of locomotory function, was recorded and the insects were then removed from the experiment and placed into a polystyrene container with fresh *P. aculeata* shoots to recover. The agents were observed after an hour, 24 hours and then 48 hours to ensure that they had recovered. Each experiment was replicated three times, using different individuals each time. To calculate both critical thermal limits (the CTMin and CTMax), the mean critical temperature across all three replicates and the standard errors were calculated.

The upper (ULT<sub>50</sub>) and lower lethal limits (LLT<sub>50</sub>) are the temperatures at which 50 % of the population dies (Mitchell et al. 1993). As with the critical limits, both *C. schaffneri* adults and nymphs (first instars, after they had eaten) were tested for their lethal limits. Once again, a controlled temperature water bath was used to determine these limits for *C. schaffneri*. Because lower lethal temperatures are often below zero, the water bath was filled with 50 % propanol due to its lower freezing point. Ten insects were placed separately into air filled eppendorf tubes, which were sealed with damp cotton wool and placed in a floating tray. Before testing the lower lethal temperatures, they were initially placed in room temperature water for two minutes then a mixture of water and ice for two minutes and finally just ice for two minutes before being put into the water bath at the experimental temperature, starting at 2 °C, for two hours. The insects were exposed to each experimental temperature for 2 hours, after which they were placed into a container with *P. aculeata* shoots to allow them to self-right. This is the same protocol used by Pieterse et al. (2017). The survival of the insects was then checked after an hour, 24 hours and then 48 hours, and the number of insects that were alive was recorded.
This was repeated at a 1 °C lower temperature each time, with a new set of ten insects until none could self-right. For the upper lethal temperature (ULT₅₀, the experiment was conducted in the same way but the starting point was 28 °C, and the water temperature was increased at a rate of 2 °C per minute to the necessary testing temperature, 35 °C, where it was kept for the two-hour testing period. This process was repeated for both C. schaffneri adults and first instar nymphs for all four thermal thresholds (CTMin, CTMax, LLT₅₀ and ULT₅₀) to see if the different life stages were affected differently by temperature. The first instar nymphs were between one and two days old and had fed. The LLT₅₀ and ULT₅₀ were calculated using probit regression analysis.

The duration of time when agents would be exposed to the critical thermal limits in the field was investigated by counting the number of days when temperatures went below or above the thermal limits of C. schaffneri at each of the 27 weather stations, as well as at Tijucas, Santa Catarina for a descriptive comparison. The number of days when the temperature was recorded below the CTMin were shown at each of the 27 locations with a graph constructed in the Geographic Information Systems (GIS) programme, ARCVIEW (Version 10.3, Environmental Systems Research Institute Inc. Redlands, CA, USA). The absolute minimum temperature per day at each site was used.

2.3 Results

2.3.1 Degree-day model: lower developmental threshold (t), rate of development (K) and degree-day acclimation

To create a degree-day model, the development time of C. schaffneri at six experimental temperatures (16, 18, 20, 24, 27 and 29 °C) was assessed. Catorhintha schaffneri eggs did not hatch at 16 °C and only 10 % hatched at 18 °C, but none of the insects that hatched survived more than six days, therefore the data from both temperatures were removed from the analysis.
The developmental rates for *C. schaffneri* at the different experimental temperatures are shown in Table 2.1. The reduced major axis regression equation, which estimates the developmental threshold (t) and rate of development (K) was DT = 13.62x + 338.31 (R^2 = 0.99; P > 0.05), indicating that *C. schaffneri* requires 338 days to develop from egg to adult (K) at a threshold of 13.62 °C (t) (Figure 2.1).

**Table 2.1** Developmental rates (days ± SE) and the percentage of the total developmental time of *Catorhintha schaffneri* at each life stage, at six experimental temperatures.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Rearing Temperatures</th>
<th>Mean duration (days) ± SE, (n), percentage of total developmental time at indicated temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>16</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>16.60 ± 0.34</td>
<td>9.60 ± 0.27</td>
</tr>
<tr>
<td></td>
<td>(10) 31 %</td>
<td>(10) 30 %</td>
</tr>
<tr>
<td>Egg - 1st Instar</td>
<td>27.2 ± 0.35 (5)</td>
<td></td>
</tr>
<tr>
<td>1st - 2nd Instar</td>
<td>7.00 ± 0.21</td>
<td>4.20 ± 0.42</td>
</tr>
<tr>
<td></td>
<td>(10) 13 %</td>
<td>(10) 13 %</td>
</tr>
<tr>
<td>2nd - 3rd Instar</td>
<td>7.80 ± 0.32</td>
<td>6.10 ± 0.23</td>
</tr>
<tr>
<td></td>
<td>(10) 15 %</td>
<td>(10) 19 %</td>
</tr>
<tr>
<td>3rd - 4th Instar</td>
<td>8.70 ± 0.42</td>
<td>3.70 ± 0.37</td>
</tr>
<tr>
<td></td>
<td>(10) 17 %</td>
<td>(10) 30 %</td>
</tr>
<tr>
<td>4th Instar – Adult</td>
<td>12.80 ± 0.29</td>
<td>8.50 ± 0.22</td>
</tr>
<tr>
<td></td>
<td>(10) 24 %</td>
<td>(10) 27 %</td>
</tr>
</tbody>
</table>
Figure 2.1 Temperature dependent development of *Catorhintha schaffneri*, from egg to adult, using the reduced major axis regression method (Ikemoto and Takai 2000). Summation is the time (D) multiplied by the temperature (T).

The parameters from the reduced major axis regression method were used to calculate the potential number of generations that *C. schaffneri* should complete annually and through winter in its native distribution (Tijucas, Santa Catarina, Brazil), and at 27 sites in South Africa that have known *P. aculeata* infestations (Figures 2.2 and 2.3).

In the native range (Tijucas, Santa Catarina, Brazil) *C. schaffneri* should complete 8.04 annual generations and 2.74 generations through the winter (April – August). In South Africa, the number of generations that *C. schaffneri* could potentially complete ranged from 4.9 to 10.9 annually (Figure 2.2), and from 1.1 to 3.9 through winter (April-August) (Figure 2.3). The
highest temperatures were recorded at sites in KwaZulu-Natal, resulting in high numbers of potential generations, ranging from 7.5 to 10.9 annually, and 2.5 to 3.9 through winter (Figures 2.2 and 2.3). In the coastal region in the Eastern Cape, the number of generations ranged from 5.3 to 6.1 generations annually, but inland in Grahamstown, *C. schaffneri* had the potential to complete the fewest generations with 4.6 annually (Figure 2.2). During winter, the potential number of generations was between 1.1 and 1.9 generations in the Eastern Cape (Figure 2.3). In the Western Cape, *C. schaffneri* can potentially complete between 4.6 and 7.2 populations annually, and between 1.3 and 1.9 generations during winter (Figures 2.2 and 2.3). *Catorhintha schaffneri* could potentially complete 9.4 generations annually and 2.6 through winter in the North-West Province (Figures 2.2 and 2.3). In Gauteng, the sites have the potential for a high number of annual generations, 7.1 – 9, but fewer through winter, 2 – 2.4 (Figures 2.2 and 2.3). The two weather stations in Limpopo had the potential to complete between 7.3 and 10.9 generations annually and 2.3 and 3.5 generations during winter (Figures 2.2 and 2.3). Weather data were collected from two weather stations in Mpumalanga. One has the potential for *C. schaffneri* to complete a high number of generations, 7.7 annually and 2.3 through winter, while the other only has the potential for 4.9 generations annually and 1.4 through winter (Figures 2.2 and 2.3).
Figure 2.2 Number of potential generations that *Catorhintha schaffneri* can complete annually at different locations around South Africa. The number of generations were calculated using the reduced major axis regression model on the developmental rate of *C. schaffneri*, and minimum and maximum daily temperature data from weather stations near known *Pereskia aculeata* infestations.
2.3.2 Hatching, developmental success, longevity and reproductive output at different temperatures

Out of 10 batches of eggs which were placed at 16 °C, none hatched (Figure 2.4). At 18 °C, the average number of eggs that hatched was very low (10 % ± 0.44 (mean ± SE; n = 51)). The number increased at 20 °C where 72 % ± 2.20 of the eggs hatched (mean ± SE; n = 93). A similar number of eggs hatched at 24 °C with 73 % ± 4.24 (mean ± SE; n = 73). The highest hatching success was at 28 °C where 89 % ± 0.86 of the eggs hatched (mean ± SE; n = 55). At 29 °C the percentage of eggs that hatched decreased to 59 % ± 0.75 (mean ± SE; n = 54). There was a statistically significant difference (H (5, n = 30) = 20.712, P < 0.001) between the number
of eggs that hatched at the different temperatures with a significantly higher number of eggs hatched at 28 °C compared to 16 °C and 18 °C (Figure 2.4).

Figure 2.4 The median number (± 25th percentiles) of eggs to hatch at each of the six experimental temperatures. Letters indicate statistically significant differences.
Of the eggs that hatched, the highest percentage that completed development to the adult stage was at 29 °C where 30 % ± 0.13 developed (mean ± SE; n = 32) into adults, and the least developed into adults at 28 °C where only 16 % ± 0.08 developed (mean ± SE; n = 49) into adults. No insects developed into adults at 16 or 18 °C (Figure 2.5). Developmental success was only significantly higher (H (5, n = 30) = 21.332, \( P < 0.001 \)) at 20 and 24 °C compared to 16 and 18 °C where no eggs developed into adults (Figure 2.5).

![Figure 2.5](image.png)

**Figure 2.5** The median number (± 25th percentiles) of eggs which developed into adults at each temperature. Letters indicate statistically significant differences.
The lifespan of adult *C. schaffneri* significantly decreased with increasing temperature ($H = (2, n = 36) = 8.242, P = 0.016$) (Figure 2.6). Longevity was significantly lower for adults at 29 °C than at 28 or 24 °C (Figure 2.6). The adults at 20 °C had lived for an average of $26.42 \pm 2.47$ days (mean ± SE; n=12) before a fault in the Controlled Environment room caused the temperature to rise temporarily and the experiment had to be cancelled. At 29 °C, on average the adults only lived for $7.67 \pm 0.99$ days (mean ± SE; n=12). At 27 °C they lived for an average of $11.75 \pm 2.68$ days (mean ± SE; n =12) and at 24 °C they lived for an average of $21.17 \pm 5.52$ days (mean ± SE; n=12).

![Figure 2.6](image)

**Figure 2.6** The median ($\pm 25^{th}$ percentiles) number of days that *Catorhintha schaffneri* adults survived at three different temperatures (24, 28 and 29 °C). Letters indicate statistically significant differences.
No mating was recorded and no eggs were oviposited by the adults at 20 °C before the experiment was compromised by the system malfunction. There was no significant difference between the numbers of egg batches that were oviposited at 24, 28 and 29 °C ($H = (2, n = 18) = 3.561, P = 0.169$) (Figure 2.7). Adult *C. schaffneri* oviposited the most egg batches per breeding pair at 24 °C, with an average of $3.5 \pm 1.82$ egg batches (mean ± SE; $n = 6$), and at 29 °C there were the fewest number oviposited, at only $0.17 \pm 0.13$ batches (mean ± SE; $n = 6$) of eggs per pair.

**Figure 2.7** The median (± 25th percentiles) number of egg batches oviposited per pair of *Catorhintha schaffneri* adults at three different temperatures. There were no significant differences between the number of eggs oviposited at each temperature. Letters indicate statistically significant differences.
2.3.3 Critical thermal limits and lethal temperatures

Adult *C. schaffneri* have a CTMax of 45.9 ± 0.15 °C (mean ± SE; n = 30) and a CTMin of 4.87 ± 0.13 °C (mean ± SE; n = 30). First instar nymphs of *C. schaffneri* have a slightly higher CTMax of 46.8 ± 0.21 °C (mean ± SE; n = 30) and a CTMin of 4.6 ± 0.26 °C (mean ± SE; n = 30).

The LLT_{50} for adult *C. schaffneri* was calculated as -7.45 ± 0.19 °C and its ULT_{50} was calculated as 43 °C. There were not enough points to run the probit regression analysis for the adult ULT_{50} because the adults were all alive at 42 °C and they all died at 44 °C, but at 43 °C, 50 % died therefore the ULT_{50} is reported without standard errors. For the *C. schaffneri* nymphs, the LLT_{50} was -7.10 ± 1.06 °C and the ULT_{50} was calculated as 44 ± 5.75 °C.

The number of days when the temperature went above or below the critical and lethal limits of the species will have caused increased stress to the organism resulting in a greater chance of temperature induced mortality (Liang 2014). Cooler temperatures in the evening may have enabled the insect to recover (Ma et al. 2015) but the effect that this would have on *C. schaffneri* is difficult to predict from these data. Temperatures just below the CTMin do not kill the agent but a significant number of days below the CTMin would cause stress to the agent, reducing the chance of it escaping predators or finding refuge from further decreases in temperature, thereby increasing the chances of cold induced mortality. A small number of days when the temperatures go above or below the critical or thermal limits may not drastically affect the species’ ability to establish, but the population might remain small which would make it less effective. In Tijucas where the agent was originally collected, the temperature did not go above or below the critical or lethal limits except for on one occasion when the temperature went below the critical minimum. This suggests that temperature stress for both hot and cold temperatures is limited in its native range.
Throughout 2015, none of the 27 weather station sites in South Africa experienced temperatures that went above the CTMax, but four sites did have maximum temperatures that were just below the ULT<sub>50</sub>: Paarl (42.6 °C), Pietermaritzburg (42.1 °C), Mtunzini (42.3 °C) and Richards Bay (42.7 °C) (Figure 2.8), suggesting that heat stress is limited in South Africa. Only one site on the coast of KwaZulu-Natal had any days when the temperature went below the CTMin, while inland in Pietermaritzburg, there were 21 days when temperatures went below the CTMin (Figures 2.8 and 2.9). The weather station in Rustenburg, North West province, recorded 30 days (non-continuously) when the temperatures went below the CTMin and the weather station in Polokwane, Limpopo, also recorded 23 days when the minimum was below the CTMin (Figure 2.9). All three weather stations in Gauteng recorded days when the minimum temperatures went below the CTMin, which would have a negative effect on the establishment of <i>C. schaffneri</i> (Figures 2.8 and 2.9). In the Eastern Cape, East London did not have any days where the minimum temperature was below 4.6 °C while Port Alfred had 9 days, Grahamstown had 15 days and Port Elizabeth recorded 7 days (Figure 2.9). In the Western Cape, Knysna had 3 days, George had 12 days, Riviersonderend had 22 days and Paarl recorded 24 days when the minimum temperature went below the CTMin (Figure 2.9).

The established distribution of <i>C. schaffneri</i> will likely be limited to the subtropical regions on the eastern coast of South Africa in KwaZulu-Natal and northern coastal areas of the Eastern Cape where warmer temperatures occur and the winters are mild. <i>Catorhintha schaffneri</i> is unlikely to establish in the Eastern and Western Cape due to generally cold temperatures and extreme winter temperatures and there is also less chance that it will establish inland in the Gauteng region of South Africa where temperatures become particularly cold through winter.
Figure 2.8 The minimum and maximum temperatures experienced at 27 weather stations. The ULT<sub>50</sub> (43 °C), LLT<sub>50</sub> (-7 °C), critical thermal maximum (46 °C) and critical thermal minimum (4.6 °C) for *Catorhintha schaffneri* adults are represented by the dashed lines.
Figure 2.9 The number of days when the temperature went below the minimum critical temperature (CTMin) for Catorhintha schaffneri adults at each of the weather stations.

2.4 Discussion

Once an agent has been accepted for release it is important to put sufficient effort into its establishment and proliferation to justify the costs incurred during pre-release research (McFadyen 1998). The distribution of P. aculeata in South Africa spans different climatic zones, therefore C. schaffneri may not adequately control the entire distribution of P. aculeata in the country, which makes it important to focus the release effort to certain areas. From a mass-rearing point of view, it is futile to rear an agent and then release it into climatic zones where it will not survive. This study predicted where C. schaffneri should successfully establish and control P. aculeata in the subtropical regions of South Africa, according to its thermal physiology. The results have shown that C. schaffneri should not be limited by high summer
temperatures because it has a high CTMin and ULT\textsubscript{50}, but cold winter temperatures and a high number of consecutive days when the temperature was recorded below its lower thermal limits (LLT\textsubscript{50}) may prevent its establishment in the more southern, temperate parts of the distribution of \textit{P. aculeata} in South Africa, including the Eastern and Western Cape. \textit{Pereskia aculeata} infestations are, to a large degree, most prevalent in the areas where \textit{C. schaffneri} should be able to establish, namely the subtropical region on the coast of KwaZulu-Natal (Henderson 1995; Paterson et al. 2014a).

Low temperatures in certain areas of South Africa have affected the establishment of biological control agents from subtropical areas (McClay 1996; Russel 2017). The distribution of \textit{Anthonomus santacruzi} Hustache (Coleoptera: Curculionidae), which was released to control \textit{Solanum mauritianum} Scop. (Solanaceae) and was originally collected from Argentina and Brazil and has similar critical minimum temperature (CTMin = 4.1 ± 0.2 °C) to \textit{C. schaffneri}, is restricted to the subtropical regions in South Africa (Byrne et al. 2002; Cowie et al. 2016). When agents are restricted to cooler areas of the introduced distribution, it can be beneficial to source new populations from the more temperate parts of the plants native distribution (Russell et al. 2017).

Very low temperatures, even for short periods of time, could affect agents with relatively high CTMins or high LLT\textsubscript{50}s, while consistently cold temperatures may affect the agent over time because development will be limited (Mitchell et al. 1993). When testing the effect that different temperatures had on the development and survival of \textit{C. schaffneri}, high mortality rates were recorded at either end of the temperature tolerance of the species, which is expected. This means \textit{C. schaffneri} would be less likely to establish in areas with consistently mild/cold temperatures, such as Knysna and George in the Western Cape and the species would also fail to establish in areas like Johannesburg in Gauteng, where temperatures are generally warm enough for the agent to develop through summer but bouts of very low temperatures in winter
would kill the population (Stewart et al. 1996; Coetzee et al. 2007). For example, *E. catarinensis*, one of the biological control agents on water hyacinth, cannot survive through the low winter temperatures in Gauteng (Coetzee et al. 2007). Augmenting the releases of *C. schaffneri* over the summer might be the only way to reduce the invasive weed in the colder areas of South Africa using *C. schaffneri*, but it is a difficult and expensive agent to mass-rear, therefore this may not be a feasible solution.

There was high variability in the biological data (section 2.3.2) on *C. schaffneri*, with relatively high mortality at all temperatures. Paterson *et al* (2014b) found similar results, so the variability in biological data was not unexpected. Variability could however be reduced by increasing the number of replicates, although this is unlikely to influence the overall outcomes of this study. In this study, the CTMax was higher than the ULT<sub>50</sub>. This result was unexpected and is difficult to interpret. Although it is counter-intuitive, it is not uncommon for arthropods to have a higher CTMax than ULT<sub>50</sub> and this has been recorded for other biological control agents (Mitchell et al. 1993; Prof John Terblanche (Stellenbosch University), Pers. Comm.; Prof J Coetzee (Rhodes University), Pers. Comm.). The use of additional methods, which use different duration x temperature regimes, such as those suggested by Chidawanyika and Terblanche (2011), could result in a better understanding of the upper thermal limits of these organisms.

Over time, *C. schaffneri* could adapt to the colder climate in certain areas but local adaptation would take a long time and initial establishment would need to be successful, even if it was only in low numbers (Robertson et al. 2008). If advantageous traits which enable some *C. schaffneri* individuals to survive in the new environment are present within the released population, it is possible that the insects would breed and pass on the beneficial genes so that eventually the population could become adapted to the cooler climate (Kawecki and Ebert 2004; Huey and Pascual 2009). The European fly, *Drosophila subobscura* Collin. (Diptera:
Drosophilidae), which is invasive in the USA, has displayed rapid evolution; within a decade it has adapted and successfully invaded areas with different climates (Huey and Pascual 2009). Areas where the climate is similar but not perfectly matched, such as East London in the Eastern Cape, where the number of potential generations are slightly low, might enable the agent to survive at low densities and slowly adapt. However, adaptation after release of an agent has not often been reported and would be unlikely, as usually biological control agents are released in small numbers that are vulnerable to extinction (McFadyen 1998; Grevstad 1999b), and the chance that sufficient well-adapted individuals would survive and breed would be low. Cold acclimation of *C. schaffneri* prior to releases in colder areas should be considered.

If an agent can express phenotypic plasticity, it may survive cooler temperatures than expected in the field, by adapting within its lifetime once released (Chown and Terblanche 2006; Overgaard and Sørensen 2008). Phenotypic plasticity is more likely to develop in agents that have adapted in areas where they would experience highly variable conditions because the agents will have developed the necessary mechanisms to survive though the unfavourable conditions that they experience (Klok and Chown 2003). Tropical and subtropical areas are unlikely to have wide variation in temperatures, therefore biological control agents from higher latitudes are more likely to develop phenotypic plasticity within genes which can help the agent survive unfavourable conditions (Chen and Kang 2004). So far however, an experiment on widespread and tropical *Drosophila* species did not indicate that phenotypic plasticity increased with latitude (Overgaard et al. 2011). *Catorhintha schaffneri* evolved in a subtropical area where the temperature did not range substantially which makes it unlikely that the species will have developed phenotypic plasticity that would help with survival during cold winters.

*Catorhintha schaffneri* was in quarantine for two years, allowing for the completion of about 30 generations before the first release was made (Paterson et al. 2014b), and is still mass-reared under consistent conditions which could change its thermal physiology and make it difficult
for the agent to establish in the variable conditions that it would be subjected to in the field (Taylor et al. 2011). This time spent in quarantine is shorter than other successful agents, such as *Gratiana spadicea* Klug (Coleoptera: Chrysomelidae), which has done extensive damage to the invasive plant *Solanum systimbrifolium* Lam. (Solanaceae), and was in quarantine for five years (Hill and Hulley 1995; Byrne et al. 2002). Considering the current physiological limits of *C. schaffneri* still fall within the range of temperatures that it would experience in its native distribution, it is unlikely that the thermal physiology of *C. schaffneri* has changed from the time that the agent has spent in quarantine. However, without testing the thermal physiology of *C. schaffneri* taken directly from their native distribution, it is difficult to conclusively say whether they have adapted to quarantine conditions.

The climate-matching study done by Paterson et al. (2014a) predicted that insects collected from the province of Santa Catarina in Brazil would be climatically suited to South Africa. Most of the sites where *P. aculeata* has been recorded are in KwaZulu-Natal, therefore the climate-matching program may have placed more emphasis on matching the native climate to the climate in KwaZulu-Natal. Climatic matching was successful in helping to choose an agent with the correct thermal physiology for the area in South Africa that has the most *P. aculeata*, but it could be necessary to select more agents from cooler parts of the plant’s native distribution to target the cold areas of South Africa where *P. aculeata* is found. This is also based purely on the thermal physiology of *C. schaffneri*, while in the field, other factors such as plant quality, and other climatic factors such as relative humidity and precipitation could affect establishment.

Theoretically, *C. schaffneri* should be able to successfully establish along the coast of KwaZulu-Natal and northern areas along the coast of the Eastern Cape, but its establishment might be limited in the areas within the distribution of *P. aculeata* which experience generally mild conditions or very cold winters, such as the Western and Eastern Cape. Releases of *C.
*schaffneri* should therefore be focused along the coast of KwaZulu-Natal and not done in the Western Cape, Eastern Cape or Gauteng, where it is unlikely to establish. There are however many other factors to take into account besides temperature, and extrapolation of laboratory-based results into the field can be difficult. Assessments of where the agent is capable of surviving in the field would therefore be beneficial.
Chapter 3

Post-release evaluation of Catorhintha schaffneri on Pereskia aculeata: assessing the effect of climate, plant quality and release effort on establishment in South Africa

3.1 Introduction

It is difficult to predict whether an introduced species, such as a biological control agent, will be capable of establishing populations in a new environment (Hight et al. 1995; Grevstad 1999b). Thermal physiology studies can estimate where agents will establish based on temperature, but there are many other variables which may affect establishment. The most direct way to confirm where an agent will establish is through experimental releases in the field. In this chapter, the predictions based on the thermal physiology experiment (Chapter 2) were tested in the field by releasing Catorhintha schaffneri at Pereskia aculeata sites with different climatic conditions, and then evaluating establishment and proliferation of these agent populations at each site. Other factors that might affect establishment were included in the assessment by the monitoring of microclimate temperature, humidity, precipitation and plant quality at each site. Additionally, the best release strategy was determined by testing the effect of release effort on establishment success.

Laboratory based thermal physiology studies indicated that C. schaffneri is likely to establish along the coast of KwaZulu-Natal in the subtropical areas of South Africa (Chapter 2). The agent is less likely to establish in the coastal regions of the Eastern and Western Cape, where lower temperatures may limit establishment because of slow development through the year and low survival through winter due to low winter temperatures (Chapter 2). Establishment at inland sites was also predicted to be less likely than at coastal sites, because even at inland sites with warm summer temperatures, the winter minima were very cold with
temperatures recorded below the developmental threshold and critical thermal minimum of *C. schaffneri* (Chapter 2).

While the potential for *C. schaffneri* to establish in South Africa is known (Chapter 2), it can be difficult to extrapolate laboratory-based studies, including thermal physiology and climate-matching studies, into the field (Van Klinken et al. 2003). For example, the distribution of *A. santacruzi*, the biological control agent for *S. mauritianum*, is limited by low relative humidity in some areas of South Africa where its thermal limits may otherwise have resulted in establishment (Cowie et al. 2016). Thus in addition to temperature incompatibility, biological control agents can be affected by other abiotic factors, such as microclimate temperatures (Coetzee 2012), humidity or precipitation (Cowie et al. 2016), and biotic factors, such as plant quality (Wheeler 2001; Uyi et al. 2016a), predation (Pratt et al. 2003), and release effort (Hopper and Roush 1993; Grevstad 1999b).

Microclimates and behavioural adaptations to climatic extremes can make it difficult to extrapolate thermal physiology studies from the laboratory into the field. An organism may actively avoid the negative effects of very high or low temperatures in the field by changing their behaviour, or avoiding temperature extremes by occupying favourable microclimates (Bogert 1949; Willott 1997; Coetzee 2012; Ma and Ma 2012). If the agent can escape unfavourable conditions in the field, which it would not have been able to do during laboratory testing, the impact of thermal limits on survival could be reduced. Experiencing temperatures at the edge of the limits could, however, still cause heat or cold induced stress to the agent.

Another factor which may affect agents in the field is relative humidity, which is often neglected in thermal physiology studies, but can explain why biological control agents do not establish in certain areas (Weissling and Giblin-Davis 1993; Byrne et al. 2002; Michaud and Grant 2005; Day et al. 2010; Cowie et al. 2016). Species from subtropical and tropical regions, such as *C. schaffneri*, are unlikely to establish and proliferate in areas with low humidity,
because they have evolved in climates with high relative humidity (Weissling and Giblin-Davis 1993). If an agent is sensitive to relative humidity, drought conditions/dry seasons could affect their establishment (Day et al. 2013; Cowie et al. 2016). While the effect of relative humidity on *C. schaffneri* was not tested in the laboratory, due to the difficulty that comes with testing an agent’s ability to survive droughts, it does need to be considered in the field following release. The effect of a drought is difficult to test under laboratory conditions because they can have different levels of severity and go on for an unknown length of time; directly and indirectly affecting the agent by damaging the health of the insect and the plant it feeds on (Michaud and Grant 2005; Rouault et al. 2006).

Host plant nutritional quality can also influence the establishment of biological control agents (Wheeler 2001; Uyi et al. 2016a). When a biological control agent feeds primarily on the new growth of the target plant, as is the case with *C. schaffneri*, it can be particularly difficult for the agent to establish when the plant is unhealthy, because there will be less new growth (Huberty and Denno 2004). Nitrogen, which is vital for growth (Mattson 1980; Uyi et al. 2016a), and water content, which may vary between seasons, have been reported to influence the survival and establishment of biological control agents (Wheeler 2001). Survival and developmental time of the Australian weevil, *Oxyops vitiosa* Pascoe (Coleoptera: Curculionidae), which develops by feeding on the new growth of the invasive tree *Melaleuca quinquenervia* (Cav.) Blake (Myrtaceae), increases when there is an increase in nitrogen and a decrease in the percentage dry mass of the plant (Wheeler 2001).

Seasonal changes in climate are likely to have an impact on the health of *P. aculeata*, with fewer good quality and actively growing shoots being present during both winter, when the photoperiod is short and temperatures are low, and dry seasons, when a lack of water may reduce growth and plant health. Plant health can therefore be affected by temperature and water availability, thereby indirectly affecting establishment (Wheeler 2001).
The quality of the invasive weed may vary between sites due to differences in climatic conditions or soil nutrients and quality. Plant quality at the time of release is therefore another important factor to consider when investigating factors that may affect establishment success (Uyi et al. 2016a).

Climatic conditions in the introduced range can result in failed establishment, but there are cases where agents did not establish in habitats that were considered to have suitable climatic conditions (Grevstad 1999b). Some studies have shown that the manner in which biological control agents are released can affect their ability to establish (Grevstad 1999b). Large release numbers increased the chances of *Galerucella calmariensis* L. (Coleoptera: Chrysomelidae) and *Galerucella pusilla* Duftschmid. (Coleoptera: Chrysomelidae) establishing on *Lythrum salicaria* L. (Lythraceae) in North America (Grevstad 1999a), yet *Stenopelmus rufinasus* Gyllenhal (Coleoptera: Curculionidae) successfully established on *Azolla filiculoides* Lam. (Azollaceae) and spread following releases of relatively few individuals in South Africa (McConnachie et al. 2004). In another case, multiple releases of 90 adult *Sericothrips staphylinus* Haliday (Thysanoptera: Thripidae), to control Gorse (*Ulex europaeus* L. (Fabaceae)) in New Zealand, improved establishment in comparison to a single large release of 1000 adults (Memmott et al. 1998). The number of individuals and releases will differ for each biological control agent, and each species will be affected differently by density dependent factors such as the Allee effect and demographic stochasticity, so these factors need to be considered on a case by case basis (Grevstad 1999b).

Allee effects cause a population to have a slower growth rate at low densities (Allee 1931). This happens when the species relies on large population densities for certain advantages such as finding mates or avoiding predation (Hopper and Roush 1993; Grevstad 1999b). The Allee effect is more significant for some species than others, particularly those that have not evolved mechanisms to overcome the problems associated with small populations (Kindvall et al. 1998;
Kuussaari et al. 2016). In biological control, species which are affected by the Allee effect are more likely to go extinct after a small release than others. The Allee effect could be intensified by the size of the release site of the target weed (Grevstad 1999a). If mate location is limited when the population is small, releases of a few individuals onto a very large site could affect establishment at the large site more than at smaller sites where the biological control agent will not be able to move far away from the rest of the population. Larger sites may therefore require releases of greater numbers of individuals to overcome the Allee effect.

Demographic stochasticity describes the way that population growth is randomly affected by variations in the birth rate, death rate, sex ratio and dispersal of the species (Wilfried and Bürger 1992). This can have implications for biological control when the released population comprises individuals of the same developmental stage after the release (Grevstad 1999b). Stochastic events are random events which can occur after a release and prevent the species from establishing. These can be natural events, such as fires, storms or predation; or human mediated disruption, such as manual removal of the target weed (Palmer et al. 1997). With smaller populations of individuals in the same developmental stage, there is far more chance that a large portion of the population will be preyed upon, will be unable to reproduce, or will succumb to extreme climatic events (Grevstad 1999b). Each developmental stage might be affected differently during a stochastic event because a specific developmental stage might be more vulnerable to certain conditions (Blubaugh and Kaplan 2015). For example, the relatively immobile carabid larvae, *Harpalus pennsylvanicus* Dej. (Coleoptera: Carabidae) which is a beneficial beetle species in agriculture, is particularly vulnerable to predation prior to pupation (Blubaugh and Kaplan 2015).

After a new biological control agent is released using a number of individuals at the same developmental stage, the population is likely to go through periods where it is made up of the same developmental stage for a long time. The population’s vulnerability would decrease as
the population became established, because established populations are likely to have representatives of all developmental stages present, therefore the entire population is unlikely to be made up of one particularly vulnerable developmental stage. Stochastic events can therefore cause small, recently released populations to go extinct even when the environmental conditions are favourable, while large, established populations are less vulnerable (Richter-Dyn and Goel 1972; Grevstad 1999b). There is less chance that demographic stochasticity will affect a single large release than a single small release, but multiple releases over a relatively short amount of time, taking into account the life cycle of the particular agent, are likely to be more effective at negating the impacts of stochastic events than any single release (Memmott et al. 2005).

The decisions about whether an agent has established or not can be difficult at the onset of a project, because the populations of the agent, in the field, can be very small. After the first release, low population densities can make it difficult to find agents, and this can make it seem that a biological control agent has failed to establish prematurely (Grevstad 1999a). Released populations often go through a lag period where agent population sizes remain very small for a long time, therefore it has been suggested that confirmation of success or failure is only possible up to twenty years after the first release (McFadyen 1998). This makes it important to consider the chance that the agent has established even if it was not found, especially at large or inaccessible sites.

In this chapter, environmental conditions and release techniques were tested during field releases to optimise the establishment of *C. schaffneri* in South Africa. Microclimate data from each site (rather than from weather stations because microclimate data are more exact) are considered and compared with the predictions from the laboratory-based study (Chapter 2). Other climatic factors besides temperature were also considered, such as precipitation, humidity, the plant quality at each site and the impact of release effort on successful
establishment. The aim of the study was to improve the implementation of *C. schaffneri* by investigating where, when and how this agent should be released.

### 3.2 Methods

3.2.1 The effect of site-specific variables on establishment (independent of release effort)

#### 3.2.1.1 Site description

Sixteen sites, spanning four different regions, each with different climate conditions, were chosen to test the effect of climatic factors and plant quality on the establishment of *C. schaffneri* (Figure 3.1). Four sites in each region were selected so that each site was further from the coast than the previous one, to sample as wide a variety of climates as possible (Figure 3.1). In the Western Cape, the site furthest inland was site 4 (11.5 km inland), and site 1 was closest to the coast (4 km inland) (Figure 3.1). The sites in the Eastern Cape have the largest distance between them with site 8 being 46.5 km inland and site 5 being 1.2 km inland (Figure 3.1). Two regions in KwaZulu-Natal where *P. aculeata* is most prevalent were selected. One was north of Durban, and the other south of Durban, and sites in the south were expected to have a slightly milder climate. In KwaZulu-Natal, the site furthest from the coast was site 16, which was 8.2 km inland, while site 9 in the south was 0.2 km inland (Figure 3.1). The altitude of the sites in the Western Cape ranged from 14 m (site 2) to 223 m (site 4) above sea level. In the Eastern Cape, the altitude of the sites ranged from 41 m (site 5) to 536 m (site 8) above sea level (Figure 3.1). South of Durban in KwaZulu-Natal, the altitude of the sites ranged from 52 m to 87 m above sea level, and in the north of Durban, the four sites ranged from 35 m to 93 m above sea level (Figure 3.1). For each site, the coordinates, area (m$^2$), distance from the coast (km), meters above sea level (m) and the area of each site that is inaccessible (m$^2$) are displayed in Table 3.1.
Figure 3.1 Site map of the sixteen sites used in the experiment; (1) illustrates the sites in the context of the whole of South Africa; (2) shows sites 1-4 in the Western Cape; (3) shows sites 5-8 in the Eastern Cape; (4) shows sites 9-12 in the south of Kwazulu-Natal; (5) shows sites 13-16 north of Durban in Kwazulu-Natal.
Table 3.1 Information on site location giving coordinates, description, area (m²), distance from the coast (km), meters above sea level (m) and the area of each site that is inaccessible (m²).

<table>
<thead>
<tr>
<th>Site</th>
<th>Province</th>
<th>Latitude</th>
<th>Longitude</th>
<th>Description</th>
<th>Area of Site</th>
<th>Distance from the Coast</th>
<th>Metres Above Sea Level</th>
<th>Area that is inaccessible</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Western Cape</td>
<td>34° 01' 78.2° S</td>
<td>22° 55' 10.2° E</td>
<td>Edge of pine plantation in sandy soil.</td>
<td>450 m²</td>
<td>3.6 km</td>
<td>167 m</td>
<td>180 m²</td>
</tr>
<tr>
<td>2</td>
<td>Western Cape</td>
<td>34° 01' 01.7° S</td>
<td>22° 59' 46.2° E</td>
<td>Roadside site on a dirt road. Frequently covered with fine dust.</td>
<td>276 m²</td>
<td>6.4 km</td>
<td>52 m</td>
<td>55 m²</td>
</tr>
<tr>
<td>3</td>
<td>Western Cape</td>
<td>33° 58' 01.1° S</td>
<td>22° 52' 89.3° E</td>
<td>Roadside site next to a quiet dirt road. Protected by Pine Trees.</td>
<td>310 m²</td>
<td>9.4 km</td>
<td>222 m</td>
<td>62 m²</td>
</tr>
<tr>
<td>4</td>
<td>Western Cape</td>
<td>33° 56' 66.1° S</td>
<td>22° 51' 79.7° E</td>
<td>Roadside site next to a ditch which is often filled with water.</td>
<td>160 m²</td>
<td>10.8 km</td>
<td>216 m</td>
<td>0 m²</td>
</tr>
<tr>
<td>5</td>
<td>Eastern Cape</td>
<td>33° 35' 53.41° S</td>
<td>26° 53' 16.28° E</td>
<td>Urban roadside site.</td>
<td>52 m²</td>
<td>1.3 km</td>
<td>40 m</td>
<td>0 m²</td>
</tr>
<tr>
<td>6</td>
<td>Eastern Cape</td>
<td>33° 30' 20.43° S</td>
<td>26° 50' 34.41° E</td>
<td>Roadside site next to a quiet dirt road, backing onto an empty field.</td>
<td>161 m²</td>
<td>12 km</td>
<td>231 m</td>
<td>0 m²</td>
</tr>
<tr>
<td>7</td>
<td>Eastern Cape</td>
<td>33° 20' 45.84° S</td>
<td>26° 40' 25.37° E</td>
<td>Roadside site, exposed to a busy tar road.</td>
<td>63 m²</td>
<td>35 km</td>
<td>457 m</td>
<td>18 m²</td>
</tr>
<tr>
<td>8</td>
<td>Eastern Cape</td>
<td>33° 18' 59.70° S</td>
<td>26° 32' 01.51° E</td>
<td>Below an urban bridge next to a flowing river.</td>
<td>200 m²</td>
<td>46.4 km</td>
<td>547 m</td>
<td>160 m²</td>
</tr>
<tr>
<td>9</td>
<td>KwaZulu-Natal</td>
<td>30° 01' 44.8° S</td>
<td>30° 54' 39.0° E</td>
<td>Exposed cliff facing the sea.</td>
<td>700 m²</td>
<td>0.14 km</td>
<td>50 m</td>
<td>490 m²</td>
</tr>
<tr>
<td>10</td>
<td>KwaZulu-Natal</td>
<td>30° 03' 58.5° S</td>
<td>30° 52' 14.0° E</td>
<td>Urban roadside site on an inland facing slope.</td>
<td>1232 m²</td>
<td>0.88 km</td>
<td>38 m</td>
<td>632 m²</td>
</tr>
<tr>
<td>11</td>
<td>KwaZulu-Natal</td>
<td>30° 02' 14.3° S</td>
<td>30° 52' 34.1° E</td>
<td>Urban roadside site in marginal land below tree cover.</td>
<td>70 m²</td>
<td>2.04 km</td>
<td>62 m</td>
<td>0 m²</td>
</tr>
<tr>
<td>12</td>
<td>KwaZulu-Natal</td>
<td>30° 16' 23.56° S</td>
<td>30° 41' 15.09° E</td>
<td>Roadside site in a valley next to a dirt road.</td>
<td>180 m²</td>
<td>6.72 km</td>
<td>61 m</td>
<td>126 m²</td>
</tr>
<tr>
<td>13</td>
<td>KwaZulu-Natal</td>
<td>29° 18' 00.2° E</td>
<td>31° 24' 54.4° E</td>
<td>Farmland, inland facing site on the edge of a small patch of semi-natural forest.</td>
<td>80 m²</td>
<td>1.22 km</td>
<td>90 m</td>
<td>0 m²</td>
</tr>
<tr>
<td>14</td>
<td>KwaZulu-Natal</td>
<td>29° 19' 21.6° S</td>
<td>31° 23' 01.2° E</td>
<td>Rural site in a small patch of semi-natural forest.</td>
<td>3600 m²</td>
<td>1.5 km</td>
<td>66 m</td>
<td>1080 m²</td>
</tr>
<tr>
<td>15</td>
<td>KwaZulu-Natal</td>
<td>29° 16' 17.4° S</td>
<td>31° 23' 33.3° E</td>
<td>Roadside site, exposed to busy traffic.</td>
<td>284 m²</td>
<td>5.05 km</td>
<td>66 m</td>
<td>0 m²</td>
</tr>
<tr>
<td>16</td>
<td>KwaZulu-Natal</td>
<td>29° 25' 38.1° S</td>
<td>31° 12' 16.8° E</td>
<td>Roadside next to a quiet dirt road and a dry riverbed.</td>
<td>468 m²</td>
<td>8 km</td>
<td>93 m</td>
<td>94 m²</td>
</tr>
</tbody>
</table>
3.2.1.2 Temperature

While most degree-day modelling studies use weather station data to determine potential establishment success, microclimate temperatures within a weed infestation may differ from weather station temperatures, which are often not in very close proximity to the release site. Using microclimate data gives a more detailed account of the temperature conditions at each site compared to weather station data, and may indicate reasons for unpredicted failed or successful establishment. Therefore, the effect of microclimate temperature on establishment was determined by placing a Thermochron iButton® (Model: DS1921K: -40 through to 85 °C) in a shaded area at each site. Each iButton was placed in a plastic pill container and attached to a tree, between one and four meters above the ground, using a PVC piping electrical fitting. The iButtons were set to record the temperature every two hours and replaced twice over the twenty-month period over which the experiment was run.

Using the thermal constant (K) and the developmental threshold (t) for *C. schaffneri*, which were calculated from laboratory-based thermal physiology studies (Chapter 2), the number of potential generations that *C. schaffneri* could complete annually and through winter were analysed from the iButton data recorded at each of the sixteen release sites. The thermal constant (K) and developmental threshold (t) were calculated using the reduced major axis regression method and information gathered on the developmental time of *C. schaffneri* at six experimental temperatures (Chapter 2) (Ikemoto and Takai 2000). To calculate the potential number of generations that *C. schaffneri* could complete at each site using the microclimate data, K and t were used in the equation (Campbell et al. 1974):

$$K = \sum \left( \frac{T_{max} + T_{min}}{2} - t \right)$$

The number of generations calculated from the iButton data were descriptively analysed and then compared to the number of generations predicted by the weather station data (Chapter 2), using a linear regression, to see if there was a difference between the predictions, and
potentially improve the efficiency of predictive studies. All statistical analyses throughout this chapter were conducted in Statistica (Version 13.2).

The minimum and maximum temperatures recorded at each site were also analysed descriptively and then compared to the minimum and maximum temperatures recorded at the nearest weather stations using linear regressions, for minimum and maximum temperatures separately, to see if there was a relationship between the temperature extremes recorded by the iButton in comparison to the microclimate data. A difference in temperature extremes could affect establishment chances, and assessing the effects of this may question the use of weather station data when predicting where an agent can establish.

The number of days where the minimum and maximum temperatures were recorded above/below the critical (CTMin and CTMax) and lethal limits (ULT50 and LLT50) at each site, according to the iButton data, were qualitatively assessed to explain failed/successful establishment at certain sites. If the agent experiences a few days when the temperatures go above or below the thermal limits of the species, it will cause heat or cold-induced stress and may limit establishment.

3.2.1.3 Humidity and precipitation

The level of relative humidity and the amount of precipitation could have a direct effect on C. schaffneri, but they could also have an indirect effect on C. schaffneri by affecting the health of P. aculeata. High relative humidity and precipitation was expected to be beneficial for C. schaffneri. Mean daily relative humidity and daily total precipitation recorded at the closest weather station to each site were obtained from the South African Weather Service (SAWS). Data were collected from a single weather station for the sites in the Western Cape, south of Durban and north of Durban. However, for the Eastern Cape sites, it was more accurate to use data from two weather stations because the sites were spread further apart, therefore data were
collected from a weather station in Grahamstown and one in Port Alfred. Additionally, precipitation and mean humidity data were collected from the Centro de Informações de Recursos Ambientais e de Hidrometeorologia de Santa Catarina (EPIGRI CIRAM) for Tijucas, Santa Catarina, which is the closest weather station to the site where *C. schaffneri* was originally collected.

The mean relative humidity per day between February 2015 and September 2016 was collected from the nearest weather station to each of the sites, and the native range (Tijucas, Santa Catarina) and separated into seasons, Summer (December – February), Autumn (March – May), Winter (June – August) and Spring (September – November), to limit the variability in the data for statistical analysis and therefore the data could be interrogated in more detail. The relative humidity from the five South African regions and the native region, were compared using a Kruskal-Wallis ANOVA and a multiple comparison of mean ranks for each season. The daily precipitation, also collected from SAWS, and recorded at the nearest weather station to each region, and the daily precipitation recorded from the weather station in Tijucas, Santa Catarina, were separated into the same seasons as humidity and also compared per season using a Kruskal-Wallis ANOVA and a multiple comparison of mean ranks because the distribution of the data did not fit the requirements for a one-way ANOVA.

3.2.1.4 Plant quality

Young, actively growing *P. aculeata* shoots are expected to be better quality food for *C. schaffneri* because they appear to have higher water content from their larger size and they are softer to the touch, while the older shoots are thin and tough and expected to be poor quality. To investigate the difference between the good and poor quality *P. aculeata* shoots, the mineral content of the good and poor quality shoots was compared. Ten samples of *P. aculeata* considered as good quality shoots and ten considered as poor quality shoots were collected
from field sites and tested by the Fertiliser Advisory Service at the South African Sugarcane Research Institute (SASRI). Each sample was made up of ten 60 cm terminal shoots of *P. aculeata*. Leaves were removed and the shoots were cut into small pieces before the Fertiliser Advisory Service dried and tested them for Potassium, Phosphorus, Calcium, Magnesium and Nitrogen levels, as these are all indicators of food quality. These data were used to confirm that the shoots considered as good quality on inspection were made up of a larger percentage of the minerals that *C. schaffneri* needs for survival and are thus better quality shoots for feeding. The mineral compositions for good compared to poor quality *P. aculeata* shoots were compared using a Mann-Whitney U test for each mineral because the data was not normally distributed.

Before each experimental release (section 3.2.1.6 below), a mean count of good quality *P. aculeata* shoots was determined by counting the number of good quality shoots in ten 1 m² quadrats which were placed randomly along a permanent transect at each site. Using a Kruskal-Wallis ANOVA and a post hoc multiple comparison of mean ranks, the numbers of new shoots during each release month (February, April and December 2015) were compared. This gave an indication of the quality of food available to *C. schaffneri* during the seasons when releases were made, which could help to indicate when releases should be made for the best chance of successful establishment. To compare the average number of shoots over time at each site, the numbers of good quality shoots, recorded seven times throughout the first year (2015) of the experiment; in February, March, April, May, September, October and December, from each site, were compared between sites using a Kruskal-Wallis ANOVA and a post hoc multiple comparison of mean ranks, because the distribution of the data did not fit the requirements for an ANOVA.
3.2.1.5 The effect of the inaccessible area of each site on search ability

The relationship between site inaccessibility and the number of *C. schaffneri* recovered was analysed. During the surveys, it became apparent that specific sites had areas which were inaccessible, which may have influenced the ability to find *C. schaffneri*. The size of each site was measured using a tape measure and the area of the site that was inaccessible was estimated using these measurements. Areas within sites were considered inaccessible if it was not possible to visually determine if *C. schaffneri* was on a shoot tip or if tips of *P. aculeata* had *C. schaffneri* damage. A linear regression of the size of the inaccessible area compared to the number of insects found at each site at the end of the experiment in December 2015 and February 2016 was conducted to determine the effect of site inaccessibility on the recovery rate of *C. schaffneri*. The *C. schaffneri* collection data from December 2015 and February 2016 were chosen because they were the surveys when *C. schaffneri* was found at the most sites.

3.2.1.6 Population census

Releases of 30 adult *C. schaffneri* (including both males and females) were made in February, April and December 2015. Releases were conducted at each site at the same time of year, thereby keeping the release effort at each site constant. The number, size and timing of each release was determined by the number of available adult *C. schaffneri* in mass-rearing facilities and it was believed that *C. schaffneri* could establish from a release of 30 adults because a release of 10 adults had resulted in initial establishment (Dr I Paterson (Rhodes University), Pers. comm.).

After each release, *C. schaffneri* was considered to have initially established if it was recorded at a site five weeks after the release or within the same summer as the release. Five weeks after the releases in February and April, and eight weeks after the release in December, a survey at each site assessed the *C. schaffneri* populations by counting the number of each
developmental stage of *C. schaffneri* found. Each site was searched for twenty minutes and binoculars were used to search the less accessible areas. *Catorhintha schaffneri* was considered to have established long-term when it was found at a site after a full winter period. A survey of each site was conducted in September 2015 and September 2016 to assess agent survival and establishment after winter.

To quantify the damage inflicted by *C. schaffneri* to *P. aculeata*, the numbers of damaged and undamaged *P. aculeata* shoots at each site were recorded in February and September 2016 in order to calculate a percentage of damaged shoot tips. Damage from *C. schaffneri* is very distinctive and cannot be confused with other forms of damage (see Appendix A).

### 3.2.2 The effect of release effort on establishment

This section aimed to determine the effect of release effort (number and size of releases) on the establishment of *C. schaffneri* in the field. Four release size treatments were used: small (30 adults), medium (60 adults) large (120 adults) and multiple small releases (30 adults released once a week for four weeks). To do this, it was necessary to release different size cohorts of the agent in a relatively small area, in comparison to the field release experiment above (section 3.2.1), to keep site-specific variables constant so that the agent was only affected by release effort in each area. Therefore, six areas were chosen where the four size cohort treatments were released within a relatively small area (Figure 3.2). Climatic variables were considered the same within each replicate but were different between replicates.

#### 3.2.2.1 Site description

Six groups of four sites were chosen to form six replicates for each treatment (Figure 3.2 and Table 3.2). The four sites which made up each group or replicate were all within 5 km to limit the variability between the conditions experienced at each site within a replicate. The four
treatments, as described above, are indicated on Figure 3.2. The different treatments were randomly assigned, using Microsoft Excel (2016), to the four sites within each group (Figure 3.2). All releases were conducted between February 2015 and early January 2016.
Figure 3.2 Site map of the six groups of four sites and the treatment implemented at each in the six different areas. Areas of main human settlement are shaded in green; (1) illustrates
where each of the six areas (replicates) were situated in the context of the whole of South Africa and it also indicates where the more detailed maps are, using the numbers associated with each map; (2) illustrates the sites in Grahamstown, (3) illustrates the sites in Bathurst, (4) illustrates the sites in Port Alfred, (5) illustrates the sites in Amanzimtoti, (6) illustrates the sites in The Bluff and (7) illustrates the sites in Desainagar.

Table 3.2 Information on site location, coordinates, the release treatment used, the total number of adults released, the number of releases made, area (m²), distance from the coast (km), meters above sea level (m) and the area of each site that was inaccessible (m²). Site accessibility was measured in the same way as it was in the first experiment to assess if it affected the ability to find C. schaffneri.

<table>
<thead>
<tr>
<th>Site</th>
<th>Area</th>
<th>Coordinates</th>
<th>Treatment</th>
<th>Number of Adults Released</th>
<th>Number of Releases</th>
<th>Area of Site</th>
<th>Area that is Inaccessible</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grahamstown Site 1</td>
<td>Eastern</td>
<td>33° 18' 59.70&quot; S 26° 32' 01.51&quot; E</td>
<td>Small</td>
<td>30</td>
<td>1</td>
<td>200 m²</td>
<td>100 m²</td>
</tr>
<tr>
<td>Grahamstown Site 2</td>
<td>Eastern</td>
<td>26°19'08.0 8°S</td>
<td>Medium</td>
<td>60</td>
<td>1</td>
<td>80 m²</td>
<td>0 m²</td>
</tr>
<tr>
<td>Grahamstown Site 3</td>
<td>Eastern</td>
<td>33°18'38.8 7°S</td>
<td>Large</td>
<td>120</td>
<td>1</td>
<td>157 m²</td>
<td>0 m²</td>
</tr>
<tr>
<td>Grahamstown Site 4</td>
<td>Eastern</td>
<td>33°18'53.7 6°E</td>
<td>Multiple</td>
<td>120</td>
<td>4</td>
<td>80 m²</td>
<td>0 m²</td>
</tr>
<tr>
<td>Bathurst Site 1</td>
<td>Eastern</td>
<td>33°30' 18.26&quot; S 26°51'02.91&quot; E</td>
<td>Large</td>
<td>120</td>
<td>1</td>
<td>77 m²</td>
<td>23 m²</td>
</tr>
<tr>
<td>Bathurst Site 2</td>
<td>Eastern</td>
<td>33°30'36.5 7°S</td>
<td>Medium</td>
<td>60</td>
<td>1</td>
<td>79 m²</td>
<td>40 m²</td>
</tr>
<tr>
<td>Bathurst Site 3</td>
<td>Eastern</td>
<td>33°30'15.1 6°S</td>
<td>Small</td>
<td>30</td>
<td>1</td>
<td>40 m²</td>
<td>4 m²</td>
</tr>
<tr>
<td>Bathurst Site 4</td>
<td>Eastern</td>
<td>33°29'51.6 2°S</td>
<td>Multiple</td>
<td>120</td>
<td>4</td>
<td>375 m²</td>
<td>338 m²</td>
</tr>
<tr>
<td>Port Alfred Site 1</td>
<td>Eastern</td>
<td>33°36' 06.53&quot; S 26°53' 31.81&quot; E</td>
<td>Large</td>
<td>120</td>
<td>1</td>
<td>50 m²</td>
<td>5 m²</td>
</tr>
<tr>
<td>Port Alfred Site 2</td>
<td>Eastern</td>
<td>33°35' 58.48&quot; S 26°53' 32.83&quot; E</td>
<td>Multiple</td>
<td>120</td>
<td>4</td>
<td>54 m²</td>
<td>0 m²</td>
</tr>
<tr>
<td>Port Alfred Site 3</td>
<td>Eastern</td>
<td>33°26' 56.20&quot; S 26°53' 11.10&quot; E</td>
<td>Medium</td>
<td>60</td>
<td>1</td>
<td>37 m²</td>
<td>15 m²</td>
</tr>
<tr>
<td>Port Alfred Site 4</td>
<td>Eastern</td>
<td>33°35' 53.13&quot; S 26°53' 11.10&quot; E</td>
<td>Small</td>
<td>30</td>
<td>1</td>
<td>27 m²</td>
<td>3 m²</td>
</tr>
<tr>
<td>Desainager Site 1</td>
<td>North</td>
<td>29°55'44.0 5°S 31°90.52&quot; E</td>
<td>Small</td>
<td>30</td>
<td>1</td>
<td>25 m²</td>
<td>0 m²</td>
</tr>
<tr>
<td>Desainager Site 2</td>
<td>North</td>
<td>29°37'5.85 8°S 31°95.56&quot; E</td>
<td>Medium</td>
<td>60</td>
<td>1</td>
<td>108 m²</td>
<td>18 m²</td>
</tr>
<tr>
<td>Desainager Site 3</td>
<td>North</td>
<td>29°36'53.7 8°S 31°914.34&quot; E</td>
<td>Multiple</td>
<td>120</td>
<td>4</td>
<td>375 m²</td>
<td>75 m²</td>
</tr>
</tbody>
</table>
3.2.2.2 Temperature

The effect of temperature was determined by recording temperatures with an iButton, placed at a central site within each group of sites (i.e. within each replicate). The numbers of potential generations annually and through winter for *C. schaffneri* at each group of sites were descriptively compared, as well as the number of days below and above the critical and thermal limits.

3.2.2.3 Population census

The most effective treatment in terms of initial (five weeks after release) and long-term establishment was determined by considering both damage and *C. schaffneri* as positive establishment. Each site was surveyed for damaged *P. aculeata* and numbers of *C. schaffneri* post winter in September 2016 to assess long-term establishment. The numbers of agents and damage found were quite low, therefore descriptive comparisons were conducted because the data did not fit the requirements for statistical analysis.
3.3.3 A qualitative assessment of abiotic factors (site-specific variables) and release effort on establishment of *Catorhintha schaffneri*.

The population survey data, (numbers of *C. schaffneri* counted during the surveys for initial and long-term establishment) from the first experiment (experiment 3.3.1) and the establishment data from the second experiment (experiment 3.3.2), were descriptively compared to temperature, climate, plant quality and site accessibility to assess the effect of each factor on establishment.

### 3.3 Results

3.3.1 The effect of site-specific variables on establishment (independent of release effort)

3.3.1.1 Temperature

The number of potential generations calculated using microclimate data predicted different numbers of generations in comparison to the number calculated from data collected at the nearest weather station (Figure 3.3). The microclimate data from Port Alfred (site 5: 9.48 generations) indicated that the release site had far greater potential for establishment than predicted when using the weather station data (5.3 generations, Chapter 2) (Figures 2.2 and 3.3, Table 3.3). Site 9 had more generations predicted by the microclimate data, while sites 1 to 4 in the Western Cape had fewer generations predicted by the microclimate data (Figure 3.3). There was a significant positive relationship between the number of potential annual generations calculated from weather station data and those that were calculated for each site using the microclimate data ($R^2 = 0.431$, $P = 0.006$) (Figure 3.4). This indicates that the weather station data was generally a good predictor of the microclimate data, although there were some important differences in what was predicted by the two methods at some sites.
Figure 3.3 Number of potential annual generations of *Catorhintha schaffneri* predicted by microclimate data at each of the sixteen sites. 1) Illustrates the sites in the context of the whole of South Africa, 2) sites 1 - 4 in the Western Cape, 3) Sites 5 - 8 in the Eastern Cape, 4) sites 9 - 12 in the south of Durban, KwaZulu-Natal and 5) sites 13 - 18 in the north of Durban, KwaZulu-Natal.
There were certain sites where the highest or lowest recorded temperature differed between years (Figure 3.5). Sites 1, 8, 9 and 14 all recorded temperatures above the ULT$_{50}$ during the first year, with the maximum temperature at sites 1 and 9 rising above the agent’s CTMax, while all sites had much lower maximum temperatures in the second year (Figure 3.5). At site 5 temperatures below the CTMin were also only recorded during the second year (Figure 3.5). There was no relationship between the maximum temperature recorded at each site and the maximum temperature recorded at the weather station nearest each site ($R^2 = 0.035, P = 0.490$), but there was a significant positive relationship between the minimum temperature recorded at each site and the minimum temperature recorded at the weather station closest to each site was found ($R^2 = 0.470, P = 0.003$) (Figure 3.6 and 3.7).
Figure 3.5 The maximum and minimum temperature experienced at each site, recorded using iButtons, (A) over the first year and (B) over the second year with the critical (CTMin and CTMax) and lethal limits (LLT$_{50}$ and ULT$_{50}$) for Catorhintha schaffneri indicated by the dashed lines, and the sites where long-term establishment occurred indicated by black dots.
Figure 3.6 Relationship between the maximum microclimate temperatures recorded at each site and the maximum temperatures recorded at the weather station closest to each site.
In its native range, *C. schaffneri* can complete 8.04 generations annually and 2.74 generations through winter (Chapter 2). *Catorhintha schaffneri* can complete the most generations in KwaZulu-Natal annually (between 7.86 and 12.48) and through winter (between 2.8 and 5.45) which is similar to or greater than the 2.74 generations that *C. schaffneri* would experience in its native range (Chapter 2) (Figure 3.3, Table 3.3). In the Eastern Cape, *C. schaffneri* can complete a relatively low number of annual generations (Between 5.01 and 5.62), compared to the other areas in South Africa and its native range, except at site 5 which is on the coast in Port Alfred (9.48 annual generations) (Figure 3.3, Table 3.3). Through winter in the Eastern Cape, *C. schaffneri* has the potential to complete a low number of generations (0.75 – 1.53) at all four sites (Figure 3.3, Table 3.3). The sites in the Western Cape had the
lowest number of potential generations (ranging between 3.70 and 5.30 annually and between 0.85 and 1.35 through winter), which is similar to the predictions made using weather station data in Chapter 2 (Figure 3.3, Table 3.3).

In both KwaZulu-Natal groups of sites, only two sites (9 and 14) had days when the temperature went above the ULT$_{50}$ (Table 3.3). Sites 5 and 6 in the Eastern Cape, which were both close to the coast, had no days when the temperature went above the thermal or critical temperature limits (Table 3.3). Site 7 had a single day when the temperature went below the CTMin and site 8 also had a day when the temperature went below the CTMin, as well as four days where the temperature was above the ULT$_{50}$ (Table 3.3). In the Western Cape, none of the sites recorded temperature below the LLT$_{50}$, but site 1 had 14 days where the temperature was above the LLT$_{50}$, and 11 days where the temperature was above the CTMax (Table 3.3). All four sites in the Western Cape also went below the CTMin (site 1: 23 days, site 2: 11 days, site 3: 7 days and site 4: 14 days) (Table 3.3). In its native range, *C. schaffneri* would have only experienced a single day when the temperature went below the CTMin and no days below/above the LLT$_{50}$, ULT$_{50}$ or the CTMax during the testing period (Chapter 2).
Table 3.3 The number of annual generations, the number of generations which can occur through winter (April – August), the number of days annually when temperatures were recorded below or above the ULT<sub>50</sub>, LLT<sub>50</sub>, CTMin and CTMax of *Catorhintha schaffneri* at each of the sixteen sites, as predicted by microclimate data.

<table>
<thead>
<tr>
<th>Sites</th>
<th>Number of annual generations</th>
<th>Number of generations in winter</th>
<th>Number of days below the LLT&lt;sub&gt;50&lt;/sub&gt;</th>
<th>Number of days above the ULT&lt;sub&gt;50&lt;/sub&gt;</th>
<th>Number of days below the CTMin</th>
<th>Number of days above the CTMax</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.30</td>
<td>1.35</td>
<td>0</td>
<td>14</td>
<td>23</td>
<td>11</td>
</tr>
<tr>
<td>2</td>
<td>5.08</td>
<td>0.85</td>
<td>0</td>
<td>0</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>3.70</td>
<td>0.86</td>
<td>0</td>
<td>0</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>4.60</td>
<td>1.2</td>
<td>0</td>
<td>0</td>
<td>14</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>9.48</td>
<td>1.53</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>5.62</td>
<td>1.45</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>5.62</td>
<td>1.13</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>5.01</td>
<td>0.75</td>
<td>0</td>
<td>4</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>12.48</td>
<td>5.45</td>
<td>0</td>
<td>20</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>10</td>
<td>9.28</td>
<td>3.15</td>
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<td>0</td>
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</tr>
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<td>11</td>
<td>8.91</td>
<td>3.31</td>
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<td>0</td>
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<td>0</td>
</tr>
<tr>
<td>12</td>
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<td>2.8</td>
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<td>0</td>
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</tr>
<tr>
<td>13</td>
<td>8.24</td>
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</tr>
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</tr>
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</tr>
<tr>
<td>16</td>
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<td>2.85</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

3.3.1.2. Humidity and precipitation

Tijucas, in the native range, had the highest mean annual humidity (91 %) while Knysna (82 %), Port Alfred (81 %) and Grahamstown (82 %) had slightly lower humidity levels. South Durban had lower humidity (74 %) and north Durban had the lowest annual mean humidity (67 %). There was a significant difference between the levels of relative humidity which occurred in certain regions during the different seasons. During the summer (February 2015 and December to February 2016), the native region had significantly higher humidity levels than all of the South African regions (Figure 3.8). Durban had significantly lower relative humidity to all other South African regions. South Durban had significantly lower relative humidity than all of the South African regions besides Port Alfred (Figure 3.8). Grahamstown
was significantly different to all of the other areas and Knysna was significantly different to all other South African areas besides Port Alfred ($H(5, n = 869) = 318.666, P < 0.001$) (Figure 3.8).

In Autumn (March – May 2015 and 2016), the native region of *C. schaffneri* also had significantly higher humidity levels than all of the South African areas (Figure 3.8). South Durban and north Durban had significantly lower humidity levels than all other areas but were not significantly different to each other (Figure 3.8). The relative humidity in Grahamstown and Port Alfred was not significantly different but Grahamstown had significantly higher humidity levels than Knysna, and Port Alfred had significantly lower humidity levels than Knysna ($H(5, n = 1100) = 396.888, P < 0.001$) (Figure 3.8).

During the winter (June – August 2015 and 2016), the native region had significantly higher humidity levels than all other regions (Figure 3.8). North and south Durban were significantly different to each other and had significantly lower humidity than all of the South African areas. Grahamstown, Port Alfred and Knysna were not significantly different to each other but did have significantly higher levels of relative humidity compared to north and south Durban ($H(5, n = 1093) = 287.761, P < 0.001$) (Figure 3.8).

The native region of *C. schaffneri* also had significantly higher relative humidity in the spring (September – November 2015 and September 2016) compared to all of the other regions in South Africa (Figure 3.8). South and north Durban, which were similar, had significantly lower relative humidity than Grahamstown, Port Alfred and Knysna, which had similar levels of relative humidity ($H(5, n = 707) = 223.8334, P < 0.001$) (Figure 3.8). During every season, all of the South African regions had significantly lower humidity than the native region and the regions in Durban on the whole had lower humidity than Grahamstown, Port Alfred and Knysna (Figure 3.8).
Figure 3.8 The median relative humidity (%) recorded from the weather stations in Knysna, Port Alfred, Grahamstown, south Durban, north Durban and the native range (Tijucas, Santa Catarina, Brazil) during the study period (February 2015 – September 2016) separated into seasons: A) Summer B) Autumn C) Winter D) Spring. Letters indicate statistically significant differences.

Tijucas had a higher mean precipitation of 183 mm per month in 2015 compared to the other areas that were tested in South Africa: Knysna (72 mm), Port Alfred (80 mm), Grahamstown (59 mm), south Durban (51 mm) and north Durban (63 mm). Tijucas (the native region) had significantly higher precipitation than all of the South African areas in Autumn ($H (5, n = 1093) = 49.119, P < 0.0001$) and Spring ($H (5, n = 713) = 43.012, P < 0.0001$), while the South African areas had similar amounts of precipitation (Figure 3.9). In the summer, Tijucas had
significantly higher precipitation than the other South African areas, while all of the South African areas, except for north and south Durban compared to Port Alfred, had similar precipitation ($H(5, n = 716) = 95.817, P < 0.0001$) (Figure 3.9). In winter, all of the regions had low precipitation but the native range did have significantly higher precipitation than north Durban, and north Durban had significantly higher precipitation than Knysna ($H(5, n = 1095) = 32.104, P < 0.0001$) (Figure 3.9).

**Figure 3.9** The mean (± SE) precipitation in each of the regions where releases were conducted, as well as the native range of *Catorhintha schaffneri* (Tijucas, Santa Catarina) for: A) Summer B) Autumn C) Winter D) Spring, over the study period (January 2015 – September 2016). The letters above the means indicate significant differences between the regions. Means and
standard error are presented although Kruskal-Wallis comparisons of the data used medians and percentiles, as the visual representation is clearer using means. Letters indicate statistically significant differences.
3.3.1.3 Plant quality

*Pereskia aculeata* shoots that were visually assessed as good quality had significantly higher percentages of Phosphorus ($U = 2.500, P < 0.001$), Potassium ($U = 0.000, P < 0.001$), Magnesium ($U = 7.500, P = 0.002$) and Nitrogen ($U = 0.000, P = 0.002$) than the shoots recognised as poor quality (Figure 3.10). There was no significant difference in Calcium levels between good and bad quality *P. aculeata* shoots ($U = 30.000, P = 0.141$) (Figure 3.10).

![Figure 3.10](image)

**Figure 3.10** The mean mineral composition (± SE) of good quality *Pereskia aculeata* shoots compared to bad quality shoots. Stars indicate statistically significant differences.
There was a statistically higher median number of good quality *P. aculeata* shoots recorded in the first summer (February 2015) than in the winter (May 2015) or in the second summer (December 2015) \((H = (2, n = 460) = 155.653, P < 0.001)\) (Figure 3.11).

**Figure 3.11** The median number of good quality *P. aculeata* shoots counted at each site in the first summer before the first release (February 2015), in winter (May 2015) and in the second summer (December 2015). Letters indicate statistically significant differences.
There was a significant difference between the median number of good quality *P. aculeata* shoots found at each site over the first year ($H = 15, n = 1110) = 233.018, P < 0.001$) (Figure 3.12). Sites with particularly high numbers of good quality shoots were sites 5, 11, 14 and 15, and the sites with particularly low numbers of good quality *P. aculeata* shoots were sites 1, 2, 3, 6, 7, 13 and 16 (Figure 3.12).

**Figure 3.12** The median number (± 25th percentile) of good quality *Pereskia aculeata* shoots found at each site over the study period. Long-term establishment took place at sites 5 and 10. Letters indicate statistically significant differences.
3.3.1.4 The effect of the inaccessible area of each site on search ability

There was no relationship between the size of the inaccessible area of a test site and the number of *C. schaffneri* recovered ($R^2 = 0.001$, $P = 0.922$), indicating that there was no relationship between the inaccessibility of the sites and the ability to find *C. schaffneri* (Figure 3.13).

![Figure 3.13](image)

**Figure 3.13** Relationship between the size of the inaccessible area at each site and the number of *Catorhintha schaffneri* found at the surveys in December 2015 and February 2016.

3.3.1.5 Population census

3.3.1.5 (a) Initial establishment

Initial establishment was found at sites 1, 3 and 4 in the Western Cape, sites 5, 7 and 8 in the Eastern Cape and sites 9 and 10 in KwaZulu-Natal. There were no insects found at sites 2, 6, 11, 12, 13 and 14 at any of the surveys over the twenty-month period, indicating that the
populations went extinct within five weeks after each of the three releases (Figure 3.14). The most *C. schaffneri* individuals were found at sites 5 and 10, in Port Alfred, Eastern Cape, and Amanzimtoti, south KwaZulu-Natal, respectively (Figure 3.14). Insects were found at these sites during every survey after the first release in February 2015 which explains the increase in *C. schaffneri* numbers over time because the populations were breeding (Figure 3.14). Only adults were released, therefore any nymphs found during surveys had hatched in the field (Figures 3.14 and 3.15).

*Catorhintha schaffneri* should have developed slower, and the adults should have survived longer, in colder areas (Chapter 2), such as in the Western Cape and inland in the Eastern Cape (Figure 3.3). There was only enough time for a single generation to have occurred before the return visit after the releases in February and April, therefore the adults found during these surveys were probably released adults, not second generation adults (Figure 3.14). However, it is possible that at sites 5, 9 and 10, where the summer was hotter, the adults found had hatched and developed in the field (Figures 3.3 and 3.14). Nymphs were found at sites 3 and 4 in the Western Cape after the release in February, indicating that the released adults had lived long enough to lay eggs, but *C. schaffneri* had not developed from egg to adult at these sites (Figure 3.14). The survey after the December release was 8 week’s post-release which means that there was enough time for second generation adults to develop in the field, and it would be unlikely that any of the released adults could have survived from the release until the sampling, therefore the adult *C. schaffneri* found at sites 1, 4, 7 and 8 were probably second generation (Figure 3.14).

After the February release, *C. schaffneri* initially established at five sites (Sites 3, 4, 5, 9 and 10) and continued to be found at sites 5 and 10 from that point onward (Figure 3.14). There were no new establishment sites after the April release, where *C. schaffneri* was only found at sites 5 and 10 (Figure 3.14). Therefore *C. schaffneri* was only found at the sites where it had
previously been found (Figure 3.14). Initial establishment then took place at four new sites after the December release (sites 1, 4, 7 and 8) (Figure 3.14). The chance of initial establishment was significantly more successful after releases in February compared to April or December ($H (2, n = 46) = 8.555, P = 0.013$).

Figure 3.14 The numbers of *Catorhintha schaffneri* adults and nymphs found at the survey after each of the releases in February, April and December. No *Catorhintha schaffneri* were found at any other sites after release.
3.3.1.5 (b) Long-term establishment

*Catorhintha schaffneri* were only found in low numbers at two sites (sites 5 and 10) after both winter periods during the study (Figure 3.15). At site 5, three adult *C. schaffneri* were found after the first winter and only one adult was found after the second winter (Figure 3.15). More adults and nymphs were found after the second winter at site 10 (Figure 3.15).

**Figure 3.15** The number of *Catorhintha schaffneri* nymphs and adults found in September (after winter) in 2015 and 2016. The biological control agent was not found at any of the other sites after winter.
3.3.1.5 (c) Damage

In February 2016, *C. schaffneri* had damaged 349 shoots (95 %) of the *P. aculeata* shoots at site 5, in Port Alfred, and it had damaged 185 (14 %) of the much larger site in Amanzimtoti, KwaZulu-Natal (Figure 3.16). After the winter period, the site in Port Alfred had less damaged (47 damaged – 13 % of the site) shoots than in February and there were lower numbers of *C. schaffneri*, while a similar amount (99 damaged shoots but 12 % of the site) of the Amanzimtoti site was damaged, compared to the damage recorded in February (Figures 3.15 and 3.16). The temperatures in Port Alfred were lower through winter than in Amanzimtoti (Table 3.3) which could have reduced *C. schaffneri* population numbers, enabling the plant to recover (Figure 3.16).
Figure 3.16 Number of the *Pereskia aculeata* shoots that were damaged by *Catorhintha schaffneri* in February 2016 after a year of establishment, compared to the amount damaged in September 2016 after the second winter period.

3.3.2 The effect of release effort on establishment

3.3.2.1 Temperature

The three groups of sites in the Eastern Cape (Grahamstown, Port Alfred and Bathurst) had a lower number of potential generations (between 5.93 – 6.27) than the three groups of sites in KwaZulu-Natal (between 9.78 - 10.45) (Table 3.4). In its native range, *C. schaffneri* could potentially complete 8.04 generations annually and 2.74 generations through winter, therefore Desainager, Amanzimtoti and The Bluff in KwaZulu-Natal lie within the same range. However, *C. schaffneri* might not establish long-term at the Eastern Cape sites (Grahamstown,
Bathurst and Port Alfred) due to the lower number of annual generations predicted at these sites (Table 3.4). None of the sites in KwaZulu-Natal had days when the temperature went above or below the thermal limits of *C. schaffneri* (Table 3.4). At the Port Alfred and Grahamstown sites in the Eastern Cape there were days when the temperature went below the CTMin, and Grahamstown had four days when the temperature went below the LLT$_{50}$ over the period that the sites were surveyed (Table 3.4).

**Table 3.4** The number of annual generations and the number of days over the surveyed period when temperatures, recorded on site using iButtons, were recorded above/below the ULT$_{50}$, LLT$_{50}$, CTMin and CTMax of *Catorhintha schaffneri* at each of the six areas.

<table>
<thead>
<tr>
<th>Area</th>
<th>Annual Generations</th>
<th>Generations through winter (April - August)</th>
<th>Number of days below the ULT$_{50}$</th>
<th>Number of days above the ULT$_{50}$</th>
<th>Number of days below the CTMin</th>
<th>Number of days above the CTMax</th>
</tr>
</thead>
<tbody>
<tr>
<td>Desainager</td>
<td>9.78</td>
<td>2.16</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Amanzimtoti</td>
<td>10.02</td>
<td>3.13</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>The Bluff</td>
<td>10.45</td>
<td>3.65</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Grahamstown</td>
<td>5.93</td>
<td>1.35</td>
<td>0</td>
<td>4</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Bathurst</td>
<td>6.11</td>
<td>2.24</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Port Alfred</td>
<td>6.27</td>
<td>2.08</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>
3.3.2.2 The effect of the inaccessible area of each site on search ability

The field sites were of different sizes and some had areas that were inaccessible (Table 3.2), but there was no relationship between the number of *C. schaffneri* individuals that were found and the size of the area that was inaccessible ($R^2 = 0.0086$, $P = 0.666$) (Figure 3.17).

![Graph showing relationship between size of inaccessible area and number of C. schaffneri found](image)

**Figure 3.17** Relationship between the size of the inaccessible area at each site and the number of *Catorhintha schaffneri* found five weeks after the first release at each site.
3.3.2.3 Population census

In September 2016, after the first winter, *C. schaffneri* had established at 67% of the multiple small release sites, but the populations were low and there was no evidence of *C. schaffneri* at any of the other sites (Figure 3.18). During the survey in September 2016 only one insect was found, at the multiple release site in Amanzimtoti, and damage was only found at multiple release sites, specifically the multiple release sites in Desainagar, The Bluff, Amanzimtoti and Bathurst (Figure 3.19).

![Graph showing percentage of establishment for different treatments](image)

*Figure 3.18* Percentage of the different treatments where insects or damage were found initially (during the summer, after release) and long-term (after winter).
Figure 3.19 The number of damaged *Pereskia aculeata* shoots at each of the sites after the winter in September 2016.

3.3.3 A qualitative assessment of abiotic factors (site-specific variables) and release effort on establishment of *Catorhintha schaffneri*

*Catorhintha schaffneri* survived for two years and through two winter periods at two out of the 16 sites (12.5 %): site 5 in Port Alfred in the Eastern Cape and site 10 in Amanzimtoti in the south of KwaZulu-Natal (Figures 3.1 and 3.15). Initial establishment occurred at sites 3, 4, 5, 9 and 10 after the first release in February 2015, with populations at sites 5 and 10 surviving through the winter (Figures 3.14 and 3.15). Initial establishment occurred at sites 1, 4, 7 and 8 after the release in December 2015 (Figure 3.14). There was also long-term establishment of
C. schaffneri found at four sites after one winter at the multiple release sites (Figures 3.18 and 3.19).

*Catorhintha schaffneri* initially established at site 1 in December 2015 but it did not establish in February 2015 when temperatures above the CTMax and ULT₅₀ of *C. schaffneri* were recorded, which may have inhibited establishment in the first summer (Figures 3.5, 3.14 and Table 3.3). After some initial establishment, cold temperatures may have stopped *C. schaffneri* from persisting at the sites in the Western Cape and inland in the Eastern Cape (Figures 3.3 and 3.14 and Table 3.3). The sites in the Western Cape and inland in the Eastern Cape only had the potential for *C. schaffneri* to complete a minimal number of generations through winter and recorded temperatures below the CTMin of *C. schaffneri* (Figures 3.3 and 3.5, Table 3.3).

Low relative humidity and precipitation may explain why most of the sites in the south, and none of the sites in the north, of KwaZulu-Natal had initial or long-term establishment (Figures 3.8, 3.9, 3.14 and 3.15). A population of *C. schaffneri* did establish at site 10 in the south of KwaZulu-Natal and this might be because the site was very sheltered in comparison to the other sites in the area, therefore it may have had higher humidity than the general area (Figure 3.15 and Table 3.1).

This study ran over 20 months which included the two winter seasons where *C. schaffneri* survived at sites 5 and 10 (Figure 3.15). At site 10, the population of *C. schaffneri* was larger after the second winter and nymphs were found as well as adults, while there were fewer *C. schaffneri* at site 5 after the second winter compared to the number of *C. schaffneri* found after the first winter (Figure 3.15). This may have occurred because during the second winter, site 5 experienced colder temperatures than it had in the first winter which could have affected the population negatively (Figure 3.5). Cold hardening *C. schaffneri* before releases are done at
sites where the temperatures slightly lower than ideal for the agent, such as site 5, might enable better establishment and long term survival (Overgaard et al. 2014).

The relationship between plant quality and establishment is not very clear and does not appear to have been a main contributing factor in this study. There were more good quality shoots at the sites before the February release and a greater number of sites had initial establishment in February which may suggest it is best to release a couple of months into Summer when the plant has grown new shoots (Figures 3.11 and 3.14). Both sites where *C. schaffneri* has established long-term (site 5 and site 10) had high numbers of good quality *P. aculeata* shoots on average, indicating that it may be important over the long-term (Figures 3.12 and 3.15). A single adult was found at site 7 after the release in December where no good quality *P. aculeata* shoots had been recorded, and *C. schaffneri* did not successfully establish initially or long-term at sites 11, 14 and 15 which had statistically higher numbers of good quality *P. aculeata* (Figures 3.12, 3.14 and 3.15).

Statistically, the size of the inaccessible area at each site accessibility does not affect search ability (Figure 3.13) but it is still possible that *C. schaffneri* was present at less accessible sites where it would have been more likely that the agent was not encountered during surveys.

Under ideal abiotic conditions, maximising release effort will ensure the greatest establishment success. Results have shown that although establishment is possible with small numbers (Figure 3.15), multiple releases of small numbers significantly increases chances of establishment (Figures 3.18 and 3.19).

A visual representation of this qualitative assessment is provided in Figure 3.20.
3.4 Discussion

Predicting if a biological control agent will establish based on laboratory experimentation can be difficult due to the variable field conditions that also need to be considered (Grevstad 1999b). *Catorhintha schaffneri* has established long-term and persisted through two winters at two sites in South Africa, and survived a single winter period at another four sites. Low cumulative temperatures which affect development, as well as rare extremely low winter
temperatures, which can stress or kill the agent, may explain why establishment failed in the Western Cape and inland in the Eastern Cape, as predicted by the laboratory work in Chapter 2. The results from this chapter, however, also indicate that high temperatures at exposed sites, relative humidity levels, as well as release effort contribute to establishment success, and explain why establishment in KwaZulu-Natal was limited even though the thermal physiology study did not predict this. These results illustrated how laboratory-based predictions are not always accurate representations of what happens in the field because they cannot assess every variable.

When using the thermal physiology of a biological control agent to predict where it can establish, temperature data are often gathered from weather stations (Coetzee 2012). Weather station data are usually a reliable estimate (Coetzee 2012) but do not give the exact temperatures that the agent experienced, because the insect may live within a microclimate, with different climatic conditions to those recorded at weather stations, or may actively avoid unfavourable conditions (Willott 1997). IButton temperature data can give a better estimate of the microclimate temperatures at specific sites but they do not take into account behavioural responses of the insect to extreme temperatures, so there could still be some degree of over-estimation. Coetzee (2012) found that the microclimate temperatures, recorded using iButtons placed in three different microclimates on water hyacinth, were not significantly different to the temperatures recorded by the nearest weather stations, but this may not be applicable for terrestrial ecosystems or plants with different growth forms. In this study, the iButtons were placed in a shaded area at each site, which is a good representation of the microclimate that *C. schaffneri* could be exposed to, but is not the microclimate where *C. schaffneri* is always found. Therefore, the microclimate data may not record the exact temperature being experienced by the agent but it does still give a more accurate estimate than weather station data. The microclimate data were able to show that the more exposed sites experienced different, often
more extreme, temperatures than those predicted from weather station data, and these temperatures may explain failed establishment in some cases. For example, temperatures above the thermal limits of \textit{C. schaffneri} were recorded at site 9 in KwaZulu-Natal and site 1 in the Western Cape, while the weather station data did not record any temperatures above the thermal limits at these sites. The microclimate minimum temperatures at site 5 in Port Alfred were higher than those recorded by the weather station, and were within the agent’s thermal range, indicating that the site was buffered from the cold, explaining why, though it was not predicted to establish based on the weather station data (Chapter 2), \textit{C. schaffneri} established at this site.

Incompatible humidity levels between an agent’s native and introduced range have affected the establishment of biological control agents in the past (Weissling and Giblin-Davis 1993; Michaud and Grant 2005; Cowie et al. 2016). KwaZulu-Natal had significantly lower relative humidity than the native range which could have affected establishment, even though the temperatures were within the correct range at most sites. In the north of Durban, relative humidity was lowest out of all of the areas and the amount of precipitation was also low, which may explain the lack of establishment at these sites. During the testing period, KwaZulu-Natal experienced a drought which may have caused the relative humidity to be unusually low. Droughts have been suggested as the reason why agents have failed to control other invasive plants in the past (Day et al. 2013). Establishment rates after the drought may improve with an increase in relative humidity and precipitation, but further investigation into the effect of humidity on \textit{C. schaffneri} survival should be conducted in the laboratory.

Herbivorous insects depend on the quality of the plant they feed on for survival, and poor food quality has negatively affected the establishment of some biological control agents (Wheeler 2001; Uyi et al. 2016b). Young, good quality \textit{P. aculeata} shoots have higher Nitrogen, Potassium, Phosphorus and Magnesium levels than old, poor quality \textit{P. aculeata} shoots, which suggests that good quality \textit{P. aculeata} shoots are more nutritious. Even though
good quality *P. aculeata* was more nutritious, the number of good quality *P. aculeata* shoots did not correlate with the initial establishment of *C. schaffneri*. Initial establishment was recorded at site 7 where no healthy *P. aculeata* shoots were recorded before the release was conducted. In some areas, plant quality may limit the growth of the population long-term, because *C. schaffneri* remains active through winter when *P. aculeata* would be expected to have limited growth due to colder temperatures and/or low rainfall. The different rainfall seasons may have also affected the agent, considering that the Eastern Cape has a bimodal rainfall pattern while KwaZulu-Natal has summer rainfall which may benefit growth of *P. aculeata* during the summer months. At the two sites where the agent has established long-term, the mean number of young *P. aculeata* shoots did remain high but these data only included one measurement during winter, therefore additional data on plant quality through winter and when precipitation is low could be beneficial.

Release strategies affect the establishment of biological control agents (Grevstad 1999a; Memmott et al. 2005). The number of insects released, the number of release events and the timing of releases can affect the chances of establishment (Grevstad 1999b). Release numbers are often dictated by the availability of the biological control agent in mass-rearing facilities, but knowledge of the optimal size and frequency of releases can enable the most efficient use of the available insects. Where some species of agent have been able to establish from a small release (McConnachie et al. 2004), others can only establish from a single large release or from multiple small releases (Grevstad 1999b). *Catorhintha schaffneri* appears to benefit from multiple small releases rather than a single release of the same number of individuals.

A species is most likely to benefit from one large release when it is affected by Allee effects, which occur when the population growth rate of a species is slower at low densities (Hopper and Roush 1993; Grevstad 1999a; Kuussaari et al. 2016). The initial field release trial, which investigated the effect of site-specific variables, indicated that establishment rates were low but
establishment was possible, even at large sites, when only a small number of individuals were released. Therefore, it is unlikely that Allee effects were an important factor in explaining the establishment success of *C. schaffneri*. Because the chance of *C. schaffneri* establishing increased when the same number of agents were released in a staggered manner over a relatively short period of time, demographic stochasticity or stochastic events probably have a larger effect on the chance of establishment than an Allee effect.

The original climate-matching study done by Paterson et al. (2014b) predicted that there was a high match between Santa Catarina, Brazil, where *C. schaffneri* was collected, and the South African distribution of *P. aculeata*. These results were partly correct in predicting that *C. schaffneri* could establish along the coast of KwaZulu-Natal, which is the area of South Africa where *P. aculeata* is most abundant, but the study did not predict that certain areas within the invaded distribution would not have a suitable climate for *C. schaffneri* (Paterson et al. 2014a, 2014b). Santa Catarina might be the most suitable climatically matched area to collect *C. schaffneri* from, but finding agents in the more temperate regions of the native range might be beneficial for controlling *P. aculeata* in the temperate regions of South Africa.

Temperature is not the only factor that contributes to establishment success (Hijmans et al. 2005) therefore, other climatic factors, such as precipitation, were analysed in the climate-matching study in 2014 (Paterson et al. 2014b). The climatic-matching study looked at the mean climatic factors over a few years but did not look at humidity specifically (Paterson et al. 2014a), therefore the study is unlikely to have considered seasonal changes caused by drought conditions which could not be predicted. The results presented in this study suggest that humidity and precipitation are factors reducing establishment success in KwaZulu-Natal, despite the range of temperatures falling within the thermal limits of *C. schaffneri*. Establishment rate was also higher when multiple releases were made, so it is possible that failure to establish was not only because of low rainfall and low relative humidity and could
be improved by using a new release strategy. The climatic-matching study done before the surveys (Paterson et al. 2014a) was therefore beneficial because it helped select an agent which should establish where the invasive plant is most prolific, but it was not able to show which areas within South Africa the species would not be effective in.

By assessing where *C. schaffneri* is most likely to establish and effectively control *P. aculeata* in South Africa, decisions can be made about the most appropriate areas to focus the release effort to, and how to effectively release *C. schaffneri* in a manner that encourages establishment. Release efforts for *C. schaffneri* should be focused along the southern coast of KwaZulu-Natal and the northern parts of the coast in the Eastern Cape (Chapter 2) where the climate is generally more favourable for the agent. Sites in northern KwaZulu-Natal are most likely too dry in terms of relative humidity. Multiple staggered releases should be made to give the agent the best chance of establishing at each site. The release should be made onto actively growing, good quality *P. aculeata* shoots at microsites within the infestation that are protected from extreme temperatures by the local topography. The agent should preferably be released after rains when relative humidity and plant quality are likely to be high. Hopefully establishment success in the warmer, northern areas of KwaZulu-Natal will increase after the drought has ended, as temperatures in this area are appropriate, but the conditions were too dry during the period of this study.
Chapter 4

General discussion

Biological control has become a well-supported method in invasive plant management because it is more environmentally sustainable and economically viable than other control options, such as manual and chemical control (McFadyen 1998). It is therefore important that biological control remains an environmentally friendly and cost effective practice. The environmentally friendly nature of the technique is illustrated through the excellent safety record of the biological control of weeds, and maintained through rigorous host range evaluations (Suckling and Sforza 2014). The long-term sustainability of biological control makes this method more cost effective than others (Van Wilgen et al. 2012), but this assumes that the implementation of biological control is conducted correctly and that agents are effective in controlling the target weeds. Post-release evaluations are therefore essential in order for implementation methods to be optimised and to evaluate the success of biological control agents, so that lessons from past successes and failures can be used to improve future biological control programmes and maintain biological control as a cost effective method (Morin et al. 2009). Post-release assessments of biological control agents are important for ensuring that the agents are used to their full potential, that unwarranted efforts are not put into a failing agent and sufficient effort is put into potentially successful agents (Carson et al. 2008).

*Catorhintha schaffneri* was prioritised for the control of *P. aculeata* because it was collected off genetically similar plants in a climatically-matched area, had a useful mode of damage and was likely to be host-specific (Paterson et al. 2014a). Host-specificity testing confirmed that the species has a very limited host range and is not a threat to native plants in South Africa, resulting in its release in South Africa in 2014 (Paterson et al. 2014b). In this study, detailed thermal physiology studies and field release surveys indicated that *C. schaffneri* should
successfully establish and contribute towards the control of *P. aculeata* in the subtropical region on the eastern coast of South Africa in KwaZulu-Natal and northern parts of the Eastern Cape, but may fail to establish permanently at higher latitudes in the temperate, southern areas in the Eastern and Western Cape of South Africa, where generally mild temperatures and cold winter temperatures will limit establishment (Chapters 2 and 3). In addition, the field release study showed that *C. schaffneri* had the best chance of establishing if released multiple times in small numbers (Chapter 3). Humidity and precipitation may also affect the establishment of the agent, which suggests the importance of carefully choosing a release season, by releasing in the summer after rains (Chapter 2 and 3). These post-release evaluations on *C. schaffneri* have therefore proven very useful by showing (1) that the agent can establish at some sites, (2) where to focus the release efforts in South Africa to reduce the amount of wasted effort from releasing elsewhere and (3) how to release the agent in the most effective way. The post-release evaluations conducted in this thesis have therefore contributed to improving the economic viability of the biological control programme against *P. aculeata* in South Africa and improved the chances that the programme against *P. aculeata* will be successful, by ensuring that *C. schaffneri* is used to its maximum potential. This chapter discusses the importance of post-release studies and how the knowledge acquired from these studies has contributed to biological control and the broader scientific field.

**4.1 The benefits of post-release evaluations**

Post-release evaluations of newly released biological control agents are important, yet often neglected, phases in a biological control programme (Morin et al. 2009). There are relatively more post-release studies which assess the factors affecting an agent’s establishment, such as the studies in this thesis, compared to studies which investigate the agent’s effect on the target weed or the affect which a reduction of the weed has on native biodiversity, which is the
ultimate goal of any biological control programme (Carson et al. 2008; Morin et al. 2009). The reason for the lack of studies that evaluate agent impact is that biological control often takes many years before it can be properly evaluated. Biological control agents can take up to twenty years to become effective, therefore it can take a long time before an agent causes sufficient damage to the invasive weed population, and even longer before positive outcomes for the surrounding biodiversity can be quantified (McFadyen 1998). During the period immediately following the first release of an agent, when the agent has been released but before it effectively controls the weed, it is vital to get the new agent established as effectively and economically as possible so that it can be utilized to its full capabilities, thereby ensuring resources are not wasted. The research done on each biological control agent at the onset of a new programme is beneficial because it can help focus the efforts of the project, and gives the agent the best chance of successfully establishing and controlling the invasive weed (Van Klinken et al. 2003).

4.1.1 The effect of climate on establishment

Invasive plants are known to invade areas with a range of different climates, and biological control agents are not always able to establish across the entire invaded distribution (Van Klinken et al. 2003; Coetzee et al. 2007). If an agent is predicted to fail in specific parts of the plant’s distribution, it not economically viable to spend money on mass-rearing and release for those areas. Therefore, research into the thermal physiology of biological control agents can decrease the amount of time and resources that are wasted (Byrne et al. 2003). The Highveld region of South Africa was found to be a climatically unsuitable habitat for the biological control agent *A. santacruzi* to establish on the invasive plant *S. mauritianum* after it successfully established in the more subtropical region along the eastern coast of South Africa (Cowie et al. 2016). The failed establishment of *A. santacruzi* in certain areas of South Africa
has also been attributed to low relative humidity, illustrating that release efforts for *A. santacruzi* should be concentrated in the subtropical regions, where humidity is higher and the temperature conditions less variable, not in the drier areas which experience cold winters (Cowie et al. 2016). The post-release work on *A. santacruzi* has therefore saved resources and effort by focusing the release efforts to areas where the agent can establish.

Biological control has had variable levels of success for water hyacinth in South Africa and although climate incompatibility was regarded as a possible reason for the limited establishment of the agents in South Africa by 2000, it was only confirmed in 2003 (Byrne et al. 2003; Coetzee et al. 2007) and during this time, agents were mass-reared and released in larger numbers (Hill and Olckers 2001) into areas where they were, in hindsight, likely to fail, indicating a misuse of resources and time. Investigations into the thermal physiology of the agent, as have now been performed on *C. schaffneri*, can therefore focus the efforts of each programme, making them more efficient and effective.

4.1.2 The effect of release effort on establishment

Researching the best way to release each biological control agent species is important, as mass-rearing can be an expensive and difficult process. As a result of the difficulties of mass-rearing there are often a limited number of individual available for releases. When there are only a limited number of agents available, it is important to use the release strategy with the best chance of resulting in establishment. If a biological control agent can establish after a release of small numbers, it is unnecessary to spend large amounts of money rearing and releasing large numbers of the agent. Releases can then be made across the country using small numbers without needing to focus on a small number of sites. For example, mass rearing and releases of *S. rufinasus*, the biological control agent for red water fern, in South Africa are now
unnecessary because it is highly successful, has dispersed unaided by humans, and if necessary, field collections can be made to release it into isolated areas (McConnachie et al. 2003).

Other biological control agents rely on large release numbers for establishment, for instance, \textit{G. calmariensis} and \textit{G. pusilla}, the biological control agents on \textit{L. salicaria}, where the chances of establishment increased with release number (Grevstad 1999a). With other biological control agents, the chance of establishment does not increase with the number of agents released, but rather the number of releases. The use of the biological control agent \textit{S. staphylinus}, which is a biological control agent on Gorse (\textit{Ulex europaeus}) in New Zealand, was improved by using multiple releases (Memmott et al. 1998) in the same way that \textit{C. schaffneri} has benefited from multiple releases. Post-release research into the most efficient release strategy, which has now been conducted on \textit{C. schaffneri}, can therefore improve the efficacy and reduce the cost of getting new agents established (Memmott et al. 1998; Grevstad 1999a).

4.2 Climate-matching and thermal physiology studies as prioritisation tools for biological control agents

The thermal physiology of \textit{C. schaffneri} has indicated that the agent may not permanently establish in the colder areas within the distribution of \textit{P. aculeata} in South Africa, but that it should successfully establish along the coast of KwaZulu-Natal and the northern area in the Eastern Cape, where the temperatures are more subtropical (Chapter 2). Investigating the specific thermal requirements of \textit{C. schaffneri} has focused release efforts on the areas within the invaded range of \textit{P. aculeata} where it is more likely to be effective.

Emphasis has been placed on choosing agents from climatically similar areas to the invaded region, which has proven beneficial in the past (Robertson et al. 2008). The research in this thesis has supported some of the benefits from the climate-matching study done to prioritise \textit{C.}
schaffneri (Paterson et al. 2014a), because the agent is climatically suited to the area where *P. aculeata* is most prolific in KwaZulu-Natal. The climate-matching study, however, did not indicate that *C. schaffneri* would not be climatically suited to certain areas or that humidity and precipitation may affect establishment. The climate-matching study conducted by Paterson et al. (2014a) used a mean range of climatic conditions, therefore the possible implications of drought conditions were not included which might be why the study did not predict limited establishment in northern KwaZulu-Natal. This may also suggest that establishment in northern KwaZulu-Natal could improve after the drought. Climate-matching can be an extremely beneficial tool for choosing better suited agents with the best chance of establishing, especially considering that it is not as expensive or time consuming as thermal physiology studies, but in this case, it was not entirely accurate and therefore results from future studies should be interpreted with caution. More detailed thermal physiology studies should be conducted post-release so that more accurate patterns of the likely distribution of the agent in the country of introduction can be determined.

4.3 The realised distribution of *Catorhintha schaffneri*

Field releases of *C. schaffneri* showed that there are more confounding variables affecting establishment than temperature alone (Chapter 3). Other climatic factors, namely low relative humidity and low precipitation, appear to have affected the establishment of *C. schaffneri* in KwaZulu-Natal (Chapter 3). The field releases were all done during a drought which was particularly evident in the humidity and precipitation records for KwaZulu-Natal, which were particularly low. The agent may establish more successfully once the drought has ended, but the effects of humidity on *C. schaffneri* need to be further researched in the laboratory. The results also indicated that the establishment success of *C. schaffneri* increases with multiple
releases of small numbers, which may indicate that demographic stochasticity or stochastic events affect the establishment of *C. schaffneri* (Chapter 3).

One important factor that was not included in this study, but may have an impact on establishment, is predation and parasitism. Predators and parasitoids have been cited as factors that may limit the efficacy and establishment of other biological control programmes (Pratt et al. 2003; Sebolt and Landis 2004; Ding and Blossey 2005; Hunt-Joshi et al. 2005). Native predatory mites suppress the populations of *Tetranychus limonius* (Dufor) (Trombidiformes: Tetranychidae), the biological control agent on Gorse *U. europaeus*, in the introduced range, thereby affecting the efficacy of the biological control agent (Pratt et al. 2003). During the post-release evaluations of *C. schaffneri*, predatory mites were found feeding on the nymphal stages intermittently in the field. No other predators or parasitoids were recorded and specific sites were not worse than others, in terms of the number of mites observed. Predators and parasites are therefore less likely to explain the lack of successful establishment for *C. schaffneri*, but at such an early stage post-release it is difficult to draw any definite conclusions.

This research will improve the biological control programme on *P. aculeata* by increasing the establishment success of *C. schaffneri* through carefully selecting when and how releases are made. This includes considering the season in which releases should be made, avoiding releases in periods of low rainfall and low humidity, and using a multiple release method which should result in an increased rate of establishment (Chapter 3). These releases must also be done in the subtropical areas of South Africa where *C. schaffneri* is most likely to establish. Releases were more successful when made during the summer, and at more protected sites where the humidity was assumed to be higher. It would therefore, be more effective to release *C. schaffneri* after rains in the summer, preferably early in the season (Chapter 3). An increase in the establishment of *C. schaffneri* should decrease the growth rate and spread of *P. aculeata*.
because at some of the sites where *C. schaffneri* has established (sites 5 and 10), it has inflicted significant damage to the target weed (Chapter 3).

4.6 Conclusion

Studies at the onset of a new biological control programme, such as this study, can improve the success of biological control agents and, through retrospective analysis, they can also help to improve future biological control programmes. Future biological control programmes will benefit by expecting and considering similar results when selecting new biological control agents. Studies like these should be considered for each new biological control agent at the onset of release to improve the efficacy of the agent’s establishment and contribute to the ultimate goal of controlling problematic invasive alien plants.
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Appendices

Appendix A Two *Catorhintha schaffneri* adults mating on a damaged shoot tip of *Pereskia aculeata*.
Appendix B Infestation of *Pereskia aculeata* where the invasive weed has covered the ground and two trees, outcompeting the native vegetation. Site 14, on Nonoti community land, near Zinkwazi, North of Durban, KwaZulu-Natal.
Appendix C *Pereskia aculeata* infestations in closer detail.