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Assessing the suitability of *Anthonomus morticinus* Clark (Coleoptera: Curculionidae) on *Solanum mauritianum* Scopoli (Solanaceae).

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(Master of Science: Biocontrol)

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22 September 2022

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Declaration

I, Vusumuzi Lucky Mkhomazi, declare that this dissertation is my own, unaided work. It is submitted for the degree Master of Science at the University of the Witwatersrand. This work has not been presented before for any degree or examination in any other University.



(Candidate's signature)

Date: 22 September 2022

Dedication

Dedicated to my late grandmother, Emma Sesana Nkosi, and to my late uncle, Themba Mkhomazi who both passed away during the course of this degree. Both of you have been supportive of my academic journey and I will never forget your positive words of motivation that kept me going.

"…You may not see the results right away, but keep going and eventually you will…"

-Emma Sesana Nkosi.

"... You are almost there …"

-Themba Mkhomazi.

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Abstract

Solanum mauritianum Scopoli (Solanaceae) is an invasive weed with detrimental impacts in South Africa. Since 1984, biocontrol efforts have been made to manage the weed, with only two agents, *Anthonomus santacruzi* Hustache (Coleoptera: Curculionidae), and *Gargaphia decoris* Drake (Tingidae) being released to date. These agents have a low impact on *S. mauritianum*, and hence there is a need for more candidate agents to be tested in order to improve the biocontrol of *S. mauritianum* in South Africa. *Anthonomus morticinus* Clark (Coleoptera: Curculionidae) was imported from Uruguay to assess its fecundity and longevity on *S. mauritianum*, its host specificity and risk analysis on solanaceous agricultural plants, as well as its thermal tolerance in terms of climate suitability. CLIMEX software was used to predict the potential of *A. morticinus* establishing in South Africa, by relating its cold tolerance to the minimum winter temperatures in regions where *S. mauritianum* is found in South Africa.

Anthonomus morticinus has a similar biology to *A. santacruzi*, with its larvae developing inside flowers and buds of *S. mauritianum*. From egg to adult, *A. morticinus* takes 19-30 days to complete its development at room temperature and relative humidity (T= 25.6 °C, RH= 49%). The larval phase takes about 18 days to complete, with pupation ranging from 4-7 days before an adult weevil emerges. The weevils live between 21 and 105 days, and each female produces 28 larvae on average.

It was found that *A. morticinus* exhibited non-target feeding only on *Solanum* species, and no feeding was observed on selected agricultural plants outside the genus, during no-choice tests. Oviposition and development by *A. morticinus* was found on four of the five tested *S. melongena* (eggplant) varieties used in no choice tests. In paired choice tests, feeding and oviposition was greater on *S. mauritianum* than all the other test plants. The non-target feeding risk was highest on *S. melongena* (black beauty variety), followed by *S. melongena* (little finger), *S. melongena* (violet moon), and *S. melongena* (black king). The non-target reproductive risk was lower in all the eggplant varieties (< 1%), with *S. melongena* (black king F1 hybrid) having no reproductive risk.

Anthonomus morticinus had a CT_{min} = 1.71 °C and was significantly more cold tolerant than *A. santacruzi* (CT_{min} = 4.93 °C). The CT_{min} of *A. morticinus* was lower than the low winter

temperatures experienced anywhere in South Africa where *S. mauritianum* is found, and the average CT_{min} of *A. santacruzi* (tested concurrently) was higher than the low winter temperatures of the Midlands and the Highveld *S. mauritianum* sites. In addition, *A. santacruzi* lethal limits (LT_{50} = -5.8 °C, n= 90) were relatively higher than *A. morticinus* (LT_{50} = -9.5 °C, n= 130).

Although more host specificity tests are required with *A. morticinus* on Solanaceae plants, the results, so far, are promising and suggest that *A. morticinus* may be a suitable, cold tolerant agent of *S. mauritianum.* The biological relationship of *A. morticinus* with *S. mauritianum* is similar to the one exhibited by *A. santacruzi* on *S. mauritianum*. Given that *A. morticinus* gets released, these two *Anthonomus spp.* may work effectively to improve the biocontrol of *S. mauritianum*, as *A. morticinus* should establish and overwinter further inland in South Africa than its congener.

Keywords: biocontrol, cold tolerant, establishment, fecundity, host specificity, invasive weed, non-target feeding risk, non-target reproductive risk, overwinter

Chapter 1: Problem statement, general introduction of invasive alien plants and rationale.

Problem statement

It is estimated that the alien tree, *Solanum mauritianum,* has invaded over 90 000 hectares of land across South Africa (Versfeld *et al*., 1998), and negatively impacts indigenous plants and people (Olckers, 2011). *Solanum mauritianum* replaces the lower canopy layers in natural ecosystems, transforming both their structure and function (Henderson, 2020). All parts of the tree are poisonous to humans and some animals, with the green berries being the most toxic, due to high alkaloid concentrations of solasodine (Henderson 2001; ISSG, 2006). The trichomes from *S. mauritianum* leaves can cause respiratory tract related illnesses (Olckers, 2011) and skin irritation to people (Henderson, 2001). The ripe yellow fruits may serve as hosts to several fruit fly species that are known pests of cultivated fruits (Olckers and Zimmerman, 1991; Copeland and Wharton, 2006). Agricultural sectors and conservation areas are threatened by the *S. mauritianum* invasion (Olckers, 2009), and Maluleke (2018) found that most Gauteng farmers were unaware of the weed in their farms, and some did not know what the weed is.

According to South Africa's National Environmental Management Biodiversity act (NE:MBA) legislation, *S. mauritianum* must be controlled because of its detrimental environmental effects (Klein *et al.*, 2011; Henderson 2020). However, *S. mauritianum* is one of the invasive alien plants also found to have negative social impacts, especially in the poorer municipalities of South Africa (Reynolds *et al*., 2020). *Solanum mauritianum* should be removed sustainably, using biocontrol as the primary management method, because successful biocontrol agents form self-sustaining populations on their host plants (Schwarzländer *et al.,* 2018). Although biocontrol efforts have been made in the past to manage the spread of *S. mauritianum* in South Africa (Cowie *et al.*, 2018; Olckers, 2009), there is a need for additional biocontrol agents, at sites where the released agents have failed to establish because of climate unsuitability (Cowie *et al*., 2016b; Venter *et al*., 2021).

General introduction

Invasive alien plants and their management techniques

One of the greatest threats to the natural biodiversity in any region is the invasion by alien plants (van Wilgen *et al*., 2001). Invasive alien plants (IAPs) were recorded over the past three and a half centuries in South Africa and are believed to have been introduced via European trade since 1652 (Potgieter *et al*.., 2020; Richardson *et al.*, 2020). Not only do the IAPs threaten South Africa's native biodiversity, but also its economy. Most invasive alien plants reduce the frequency of ecotourism, which is one of the important sources of income in South Africa (Henderson, 2020). High water loss to IAPs is the primary problem caused by their infestation (Le Maitre *et al*., 2016). According to the Working for Water programme (WfW), there are over 1400 known alien plant species in South Africa, which come with an annual cost of at least 2 billion ZAR for their clearance (van Wilgen *et al*., 2020). It is estimated that at least 15 billion ZAR has been spent on IAPs management since 1995 in South Africa (van Wilgen *et al*., 2020).

Invasive alien plants are weeds that are not native to the regions of introduction and can often spread faster than indigenous plants and therefore threaten many organisms (Wilson *et al.*, 2009). One of the hypotheses which explain the spread of IAPs is known as the enemy release hypothesis (ERH) (Roy *et al*., 2011). The ERH states that because IAPs are out of their native range they have escaped their natural enemies and are thus likely to be more competitive than the native plants of the region in which they have established (Roy *et al*., 2011). The lack of natural enemies often leads to their success (Hill *et al*., 2020a), leading to IAPs utilizing more resources such as water and minerals than native plants (Culliney, 2005).

Versfeld *et al*., (1998) assessed the distribution of the woody taxa of IAPs and found that 10 million hectares of South Africa's total landcover was invaded by 180 IAP species. Since 2006, the South African Plant Invader Atlas (SAPIA) has recorded an increase in the number of IAP taxa, which resulted in a total of 773 IAP taxa in 2016 (van Wilgen *et al*., 2020). The average cost of ecosystem services lost to IAPs every year was estimated to be 6.5 billion ZAR in South Africa, but this could have been 48.2 billion ZAR if there was no management of these plants (De Lange and Van Wilgen, 2010).

Invasive alien plants are either intentionally, or accidentally introduced (van Wilgen *et al*., 2001). The trade of ornamental plants, movement of organisms (including humans) all over the world and as stowaways in traded goods are the main drivers of IAP dispersal (Hill *et al.*, 2020b; Wilson *et al.,* 2009). The migration of humans regionally or globally increases the propagule pressure from IAPs, and hence the likelihood of their establishment (Wilson *et al.,* 2009). As a result, management techniques of IAPs must be implemented to reduce the spread of IAPs, which include chemical, mechanical, and biological control strategies (Culliney, 2005).

Eliminating IAPs using chemicals has non-target effects on native plant species and is expensive (Hill *et al*., 2020a). The physical removal of IAPs either by hand, tools or machinery is known as mechanical control (Culliney, 2005). This is often not a long-term solution, as IAPs resprout in cleared areas (Witkowski and Garner, 2008). In some cases, mechanical control is a potential health hazard to the workers, and it has to be implemented on multiple occasions in order to manage the IAPs, which is also expensive (Culliney, 2005). An alternative to the chemical and mechanical management of IAPs is known as biological control, commonly termed biocontrol (Schwarzländer *et al*., 2018).

Biological control of invasive alien plants

Biocontrol of IAPs uses living organisms including pathogens, mites and insects, termed biocontrol agents (BCAs), to suppress the growth, reproduction and spread of a target weed, ultimately reducing its abundance and negative impacts to manageable levels (Klein *et al.*, 2011; McFadyen, 1998). Biocontrol in South Africa commenced around 1913, when the first BCA, the cochineal insect, *Dactylopus ceylonicus* (Hemiptera: Dactylopiidae), was released against *Opuntia monacantha* Haw. (Cactaceae), known as the drooping prickly pear (van Wilgen *et al.,* 2020). Biocontrol involves the importation of natural enemies from their country of origin to the region invaded by IAPs, to reduce the detrimental impacts caused by the IAPs (Hill *et al*., 2020b). Ever since, biocontrol has continued in South Africa and is regarded as a long term, efficient technique in managing the spread of IAPs (van Wilgen *et al.,* 2020). Since that first biocontrol agent release in South Africa, 106 biocontrol agents have been released against 48 IAP species, placing South Africa third globally in biocontrol research (Moran *et al.,* 2013). There are an internationally agreed set of procedures that are needed for safe and successful

biocontrol to take place, the most important being the conducting of rigorous host specificity testing prior to the release of agents (McFayden, 1998; Weyl *et al*., 2020).

Host specificity of biological control agents

Host specificity tests take place in quarantine to assess the risks of non-target feeding by a potential BCA (Smith *et al*., 2019). Host specific BCAs should exhibit high host preference and suitability on target weeds, while posing non-significant risks to non-target plants (Olckers 2000, 2003). Taxonomically related plant species, to the target weed, are used in host specificity testing, to assess the behaviour and host preference of the potential agent on the target weed relative to the other plant species (Marohasy, 1998). This is done to estimate the field host plant range. Host specificity tests are essential in ensuring that the BCAs do not successfully survive and reproduce on non-target plants, which can either be native or commercially important plants (Olckers, 2003). All agents that exhibit high risks of non-target feeding during host specificity tests are rejected, as only host specific agents are permitted for release (Barratt *et al*., 2010; Olckers, 2003). The process of releasing BCAs takes several years of running such host specificity tests to ensure that the agent is safe for release (Olckers, 2003).

Klein *et al.,* (2011) noted that 21 % of the 48 IAPs with released BCAs were under complete control, which suggests that not all biocontrol programmes are successful. About 72 % of the top 18 worst taxa of IAPs in South Africa have BCAs released to manage their spread (Zachariades *et al*., 2017). Of these worst taxa, only 39 % are completely or substantially controlled (Zachariades 2018). The success of BCAs is affected by some of the ecological and environmental challenges experienced by the agents when released in the field (Cowie *et al*., 2018). Some are preyed on by other organisms which either results in limited success of the BCA on the target plant, or no establishment (Paynter *et al*., 2015). Despite these challenges, it was observed that the rate of spread of IAPs has been significantly reduced where the BCAs established successfully (Henderson and Wilson, 2017). Biocontrol was reported to be economically efficient relative to mechanical and chemical control; hence the support for biocontrol has grown significantly in South Africa (van Wilgen *et al*., 2004).

The South African government and various research organizations have been very supportive of weed biocontrol research in South Africa (Klein *et al.,* 2011; van Wilgen *et al.,* 2020). The

Working for Water (WfW) programme has been a great supporter, not only for biocontrol research funding, but also for the removal of IAPs, which are threatening the South Africa's biodiversity, by mechanical and chemical clearance (van Wilgen *et al*., 2004). The WfW programme encouraged the support of biocontrol as it increased the availability of ecosystem services by conserving the native plant species (van Wilgen *et al*., 2004). South Africa has an outstanding safety record in that no-significant non-target effects have been recorded since the release of the first BCA (Moran *et al.*, 2013), which indicates the efficacy of host specificity testing and thus the confidence in biocontrol by funding agencies (Klein *et al*., 2011).

Climate suitability of biocontrol agents

The success of BCAs can however be negatively affected by climate mismatches, where unfavourable climatic conditions such as temperature or humidity, limit the distribution of the released agent(s) (Harms *et al*., 2021). Therefore, assessing the potential of agent establishment using climate prediction models is important (Byrne *et al*., 2002; Cowie *et al*., 2016b). This allows scientists to predict the potential distribution, and success or failure of biocontrol agents when they are released in the field (Byrne *et al*., 2002; Cowie *et al*., 2016b). CLIMEX software has been used for some biocontrol research programmes to match the climate of the native range of biocontrol agents, to the distribution of their target weeds in invaded countries such as South Africa (Byrne *et al*., 2002; Cowie *et al*., 2016b) and Australia (Senaratne *et al*., 2008; Palmer and Senaratne, 2007). CLIMEX has also been used to select regions in the native range with suitable climate conditions that are similar to the invaded region to facilitate the collection of climatically adapted agents (Senaratne *et al*., 2006). Cowie *et al*., (2016b) used CLIMEX to assess the climate suitability of the biocontrol agent, *Anthonomus santacruzi* Hustache (Curculionidae), and found the agent to be incompatible with the unfavourable temperature and humidity conditions of the inland established regions of the target weed, *Solanum mauritianum* Scopoli (Solanaceae), in South Africa, which is the subject of this study.

Solanum mauritianum

Solanum mauritianum Scopoli (Solanaceae) is a South American tree that has invaded South Africa, with the earliest reports coming from the KwaZulu-Natal province (KZN) during the 1860s (Olckers and Zimmerman, 1991) when it was deliberately introduced as an ornamental

plant (Olckers, 2009). The tree became widespread around the 1930s, and it was listed as a critical invader on the National Weeds Act of 1937 (Richardson *et al.*, 2020). The biology of *S. mauritianum* is a major factor contributing to its invasion success (Witkowski and Garner, 2008).

The tree has leaves covered with trichomes and has lilac blue flowers that are present, and produces seeds, all year round (Olckers, 2009). *Solanum mauritianum* flowers are pollinated by generalist pollinators, especially bees, and are capable of self-pollination (Cowie *et al.*, 2018; Olckers, 2009). The flowers mature into green berries, each having up to 150 seeds (Olckers, 2011) that are dispersed by frugivores when the berries are ripe (Olckers, 2009; Witkowski and Garner, 2008). *Solanum mauritianum* trees can produce seeds within one year of germination and are also capable of vegetative reproduction (Olckers, 2011). For some *S. mauritianum* trees, seed production begins when the tree height is 1.5 m and each tree taller than 3 m can produce a maximum of 200 000 seeds annually (Olckers, 2011; Witkowski and Garner, 2008). *Solanum mauritianum* was found to have high soil seed bank densities of 47 to 554 seeds/m ^{2}, hence their high rate of invasiveness and the need for their immediate clearance (Witkowski and Garner, 2008).

The invasion of *S. mauritianum* is a global problem, and the weed has invaded regions of all continents, excluding Antarctica (Cowie *et al.*, 2018). Some of the islands in the Atlantic Ocean, Indian Ocean (Madagascar, Mauritius, and La Reunion) and Pacific Ocean (Fiji, Hawaii, and Tonga) are also invaded by *S. mauritianum* (Cowie *et al*., 2018; Olckers, 2009). In Africa, Kenya and South Africa are highly invaded by *S. mauritianum* relative to other African countries where *S. mauritianum* is present (Cowie *et al.*, 2018). *Solanum mauritianum* is abundant in the eastern, high-rainfall regions of South Africa, and South Africa considers *S. mauritianum* to be one of the top five invasive weeds in the summer rainfall region (Witkowski and Garner 2008). Although *S. mauritianum* was promoted as a nursery plant for forest regeneration in Australia, more recent evidence shows that *S. mauritianum* is an invasive tree with allelopathic properties (Olckers 2009). Since the 1980s, efforts of clearing and managing the spread of the weed were implemented (Olckers and Zimmerman, 1991).

The chemical and mechanical efforts on *S. mauritianum* were immediate but expensive and short-term, and therefore less effective than biological control (Olckers, 2011). Surveys

conducted in South America show high levels of insect herbivory on *S. mauritianum*, and hence a low seed output of the weed in its native range (Olckers, 2003). In South Africa, surveys showed that there was poor insect herbivory on *S. mauritianum* which resulted in high seed output (Olckers and Hulley, 1989). This led to the importation of South American insects for testing in quarantine for biocontrol of *S. mauritianum* (Cowie *et al*., 2018).

Biocontrol of *Solanum mauritianum* in South Africa

South Africa was the first country to initiate the biocontrol of *S. mauritanum* in 1984, after which fifteen candidate agents were imported from South America for host specificity testing in quarantine (Olckers, 1999). Twelve candidate agents were rejected due to high risks of non-target feeding on native *Solanum* plants and cultivated plants such as *S. melongena* (eggplant) and *S. tuberosum* (potato) (Olckers, 1999, 2003). Individuals of *Anthonomus morticinus* Clark (Coleoptera: Curculionidae) collected with *Anthonomus santacruzi* Hustache (Coleoptera: Curculionidae), during 1998 were destroyed to prevent the mixing and potential hybridization of these two *Anthonomus spp.* (Olckers, 1999). While the host specificity testing of *A. santacruzi* was ongoing during 1999, another agent *Gargaphia decoris* Drake (Tingidae) was released in that year (Cowie *et al.*, 2018; Olckers 1999, 2000).

Gargaphia decoris is a sap feeding lacebug that reduces the photosynthetic output of *S. mauritianum* trees (Cowie *et al*., 2016a). Nymphs and adults of *G. decoris* cause leaf chlorosis and premature leaf abscission as they continuously feed on *S. mauritianum* (Olckers, 2000). *Gargaphia decoris* was released first in KZN, then later in Limpopo and Mpumalanga provinces in 2001 (Olckers, 2011). The establishment of *G. decoris* has been successful in these provinces, with peak populations observed during the autumn, declining populations in winter and populations recovering during the spring season (Olckers, 2011). Plant quality during winter negatively affects the population of *G. decoris* (Cowie *et al*., 2016a), and generalist predators, such as ants, are believed to be a major cause of low-density populations of *G. decoris* in the established sites (Olckers, 2009). Unfortunately, there has been no assessment of the distribution of *G. decoris* in South Africa for the past two decades (Cowie *et al*., 2018) and *G. decoris* is regarded as an ineffective, trivial biocontrol agent (Klein *et al*., 2011; Henderson, 2020). However, the flowerbud feeding weevil, *Anthonomus santacruzi*, is a more effective biocontrol agent as it reduces seed development and hence the dispersal of *S. mauritianum* (Cowie *et al*., 2018).

The biocontrol by *A. santacruzi* on *S. mauritianum* has been accompanied by numerous challenges, one being the one being the laborious culturing of *A. santacruzi* (Olckers, 1999). Out of many *A. santacruzi* weevils that were imported in 1995, only eighteen weevils survived and were used to sustain a culture until 1997 (Olckers, 1999). Host specificity testing of *A. santacruzi* started in 1998 when two hundred additional weevils were imported from Argentina (Olckers, 1999). Five cultivated plant species, seven exotic plant species, and thirteen native plant species from the Solanaceae were used for host specificity testing (Olckers, 2003). Although non-target feeding and oviposition by *A. santacruzi* was observed on twelve *Solanum* non target test plant species, including eggplant, potato and most South African indigenous *Solanum* plants, more feeding and oviposition was noted on *S. mauritianum* (Olckers, 2003). In 2003, a release application for *A. santacruzi* was submitted to the regulation authorities and while the release application was still in the process, host specificity trials were continued (Olckers, 2009). Concerns were raised by cotton farmers five years after the release application was submitted to the regulation authorities, regarding the potential risks of *A. santacruzi* on cotton production, as *A. santacruzi* is in the same genus as the cotton pest, *Anthonomus grandis* (Boheman) (Olckers, 2008). It was found that *A. santacruzi* poses no risk in cotton production and is host specific to *S. mauritianum* (Olckers, 2008), and *A. santacruzi* was later approved for release in late 2008 (Cowie *et al*., 2018).

The biocontrol potential of *A. santacruzi* is based on its reproduction and feeding behaviour (Olckers, 2003). The adult weevils feed on the flowers and foliage of *S. mauritianum.* The fertilized eggs are oviposited into the unopened flowerbuds of *S. mauritianum,* and the weevil larva feeds on the bud contents as it develops, preventing seed development and bud opening (Olckers, 2003). A maximum of three eggs are laid per bud and the developing larva takes 10-14 days to pupate (Olckers, 1999). From egg to adult, *A. santacruzi* completes its entire developmental cycle within 15-25 days before an adult emerges (Olckers, 2003). The short development time is an added advantage for *A. santacruzi* being a biocontrol agent, as there are overlaps between generations, resulting in high population density of the weevils and high reproductive output (Olckers, 2003). Adult weevils of *A. santacruzi* can live between three to six months, with females ovipositing 16-60 larvae in *S. mauritianum* flowerbuds throughout their entire lifespan (Hakizimana, 2011). Post release surveys have been conducted to assess the

establishment of *A. santacruzi* (Mkhize and Olckers, 2019; Singh and Olckers, 2017), showing restricted distribution.

The establishment of *A. santacruzi* is limited to the lowveld and coastal regions in South Africa due to the unfavourable winter temperatures and humidity conditions elsewhere in the country (Cowie *et al*., 2016b). *Solanum mauritianum* in the highveld remains uncontrolled by *A. santacruzi*, and hence there is a need for additional biocontrol agents (Cowie *et al*., 2018). *Anthonomus morticinus* Clark (Coleoptera: Curculionidae) is another flowerbud feeding weevil, regarded as a potential biocontrol agent of *S. mauritianum* (Cowie *et al.,* 2018; Olckers, 2009; Pedrosa-Macedo *et al*., 2003) and is a subject of this study. *Anthonomus morticinus* weevils were collected in Uruguay within the region of Punta del Este, Rocha and Treina ye Tres, which have been hypothesized to having matching temperatures with the South African highveld regions where *S. mauritianum* occurs (Venter *et al*., 2021). For this to be confirmed, the temperatures of the sites where *A. morticinus* was collected should be compared to the South African highveld, to assess their percentage of similarity using the CLIMEX software.

Rationale

The biocontrol of *S. mauritianum* has so far shown largely trivial results from the two released agents namely *A. santacruzi* and *G. decoris* (Zachariades, 2021). Although there are currently no field surveys done on the impacts of *G. decoris* on *S. mauritianum*, post release evaluations done on *A. santacruzi* reveal that its establishment is limited by climatic unsuitability, particularly low winter temperatures and low relative humidity, especially in the inland regions of South Africa (Cowie *et al.,* 2016b). Additional, cold adapted biocontrol agents are required to control and reduce the spread of *S. mauritianum* further inland in South Africa (Venter *et al*., 2021). *Anthonomus morticinus* was collected from Uruguay and its native range was recorded to include the colder regions of South America (Pedrosa-Macedo *et al*., 2003), making it a suitable candidate for the study of thermal limits (Venter *et al.*,2021). *Anthonomus morticinus* was also reported to have a similar biology to *A. santacruzi* (Pedrosa-Macedo *et al*., 2003; Olckers, 2009). These ecological and life-history traits of *A. morticinus* thus encourage the assessment of the weevil as a potential biocontrol agent of *S. mauritianum* in South Africa. Host specificity tests of *A. morticinus* were conducted using agricultural Solanaceae plants. This study aims to contribute

towards the biocontrol of *S. mauritianum*, the thermo-physiological traits of *A. morticinus* and *A. santacruzi*, and the reproductive capability of *A. morticinus* on *S. mauritianum.*

Aims and objectives

The aim of this study was to contribute to the biocontrol research on *S. mauritianum* in South Africa by investigating the suitability of *A. morticinus* on *S. mauritianum*, particularly for the colder inland regions of the country where the released agent, *A. santacruzi*, has failed to establish. This was achieved by assessing the reproduction, host specificity, and climatic suitability of *A. morticinus* as follows:

Objective 1. Assess the fecundity of *A. morticinus* on *S. mauritianum.* This was carried out by presenting *A. morticinus* with *S. mauritianum* bouquets to allow for oviposition of eggs until the death of the parental generation.

Objective 2. Assess the host range of *A. morticinus* by exposing the weevil to a variety of agricultural Solanaceae plants through a series of no-choice and paired choice trials in quarantine. A risk analysis of *A. morticinus* was completed using the paired choice trial data.

Objective 3. Assess the potential range of *A. morticinus* in South Africa by determining the lower thermal thresholds of *A. morticinus* and relating its thermal limits to the South Africa's climate where *S. mauritianum* has invaded. Then predict the distribution range of *A. morticinus* establishing in South Africa by matching the climates where *A. morticinus* was collected, with South Africa's climate where *S. mauritianum* has invaded.

Dissertation Outline

The first chapter is a general introduction on invasive alien plants and biocontrol as a sustainable long-term mode of managing the spread of IAPs. That chapter proceeds to highlight the biocontrol of *S. mauritianum* and the challenges experienced by the agents currently released against *S. mauritianum*. In chapter two, the fecundity and survivorship of *A. morticinus* on *S. mauritianum* were assessed to observe its biology and longevity on *S. mauritianum* (Objective 1). Chapter three presents the host range testing of *A. morticinus* on *S. mauritianum* and agricultural Solanaceae. The possible non-target feeding, oviposition and development were

assessed, and a risk analysis was completed (Objective 2). Chapter four focuses on the response of *A. morticinus* and *A. santacruzi* to low temperatures by assessing their cold tolerance. CLIMEX modelling was used to match the climate variables of the South African distribution of *S. mauritianum*, to the Uruguayan collection range of *A. morticinus* to predict the potential establishment of *A. morticinus* in South Africa (Objective 3). The final chapter synthesises the three objectives and highlights the potential of *A. morticinus* as a biocontrol agent of *S. mauritianum*.

Chapter 2: Assessing the development and fecundity of *Anthonomus morticinus* Clark (Coleoptera: Curculionidae), on *Solanum mauritianum* Scopoli (Solanaceae).

Abstract

Anthonomus morticinus Clark (Coleoptera: Curculionidae) is a flowerbud feeding weevil native to South America which is currently under assessment for use as a biocontrol agent against *S. mauritianum*. *Anthonomus morticinus* feeds on the leaves, flowers and buds of *S. mauritianum* and relies on flowerbuds for reproductive success. Females produced on average 28 eggs in their lifetime. Eggs take 19-30 days to mature into adult weevils. The first mortality of *A. morticinus* weevils was observed on the 21st day of the experiment, and the parental weevils were all dead on the 105th day. The biology of *A. morticinus* and *A. santacruzi* on *S. mauritianum* is similar based on their feeding, duration of development and fecundity. Furthermore, these two *Anthonomus spp.* coexist in *S. mauritianum's* native distribution in South America. The lifespan, developmental time, and reproductive outputs all suggest that *A. morticinus* may persist on *S. mauritianum* pending release approval.

Keywords: biocontrol agent, mortality rate, oviposition, persistence, progeny.

2.1 Introduction

In weed biocontrol programmes, most insects/agents ideally rely exclusively on their target plants for their feeding, survival, and reproductive success (McFayden, 1998). When environmental and ecological conditions are favourable, agents are observed to have a successful rate of establishment on their target weeds, which leads to the agents managing their target weed populations (McFayden, 1998). Assessing the biology of an agent is important to show its relationship with the target weed in terms of the agent's developmental times, fecundity, longevity, and mortality rate (Olckers, 2000; 2003). The fecundity is the number of offspring a female insect can produce its lifetime (Peters and Barbosa, 1977), and previous studies on the biocontrol of *S. mauritianum* show that the agents of the weed exhibit a relatively high fecundity (Cowie *et al*., 2018).

Gargaphia decoris was observed to produce batches of 34-876 eggs (Olckers, 2000). As the nymphs develop, they feed on *S. mauritianum* leaves, resulting in the scarring and chlorophyll loss of the leaf tissue (Olckers, 2000). The eggs of *G. decoris* take about a month to develop into adults which can live for 20-145 days (Olckers, 2000). Olckers (2003) assessed the biology of *Anthonomus santacruzi*, including the developmental times and the fecundity. *Anthonomus santacruzi* eggs are oviposited inside *S. mauritianum* flowerbuds and take up to 25 days to develop into adults (Olckers, 2003). The adults feed on leaves and flowers of *S. mauritianum* and *A. santacruzi* has a mean fecundity of 16-59 eggs per female (Olckers, 2003). These biocontrol agents of *S. mauritianum* have short developmental times and multiple generational overlaps that contribute to their reproductive success (Cowie *et al*., 2018).

Flowerbud-feeders are regarded as promising agents in controlling *S. mauritianum* since they result in reduced seed output of the weed (Olckers, 2009; 2011). An additional flowerbud feeding weevil, *Anthonomus morticinus* Clark (Coleoptera: Curculionidae), is a potential agent against *S. mauritianum* (Olckers, 2009; Pedrosa-Macedo *et al*., 2003). *Anthonomus morticinus* is distinguishable from *A. santacruzi* by having a more pronounced interfemoral tooth on the forelimbs than *A. santacruzi*, and by having a pair of white bands of scales that are located on the 4th interstria of the elytra (Clarke and Burke, 1996).

There are currently no studies on the biology and the fecundity of *A. morticinus*. In this study, the biological relationship between *A. morticinus* and *S. mauritianum* with regards to the developmental time, fecundity and mortality rate were assessed in quarantine. These factors are important in highlighting the biocontrol potential of *A. morticinus* on *S. mauritianum,* and whether the agent will survive and produce progeny if it is released.

2.2 Materials and methods

Anthonomus morticinus adults were collected from Uruguay (-34,420128; -54,403067) and sustained on *S. mauritianum* bouquets which were placed inside the glass jars filled with water, at the Wits Insectary Quarantine facility. The offspring that were used for this study as parental weevils were reared from *A. morticinus* that was cultured for a year.

Developmental observations of *Anthonomus morticinus*

To assess the development and fecundity of *A. morticinus,* 51 newly emerged adults were confined onto foliage bouquets of *S. mauritianum*. The bouquets were placed inside glass vials filled with water in a 5 liter bucket covered with a gauzed top in room conditions (T= 23.1 ± 0.4 °C; RH= 49%). For the first two weeks of the study, the weevils were sustained on only leaf material of *S. mauritianum* as there was limited floral material available in the field. From the third week until the end of the experiment both foliage material and flowers with flowerbuds were presented to the weevils weekly. Feeding observations, weevil mortality and sex were determined at the end of each change of floral and foliage feeding material. All dead weevils were placed in 70 % ethanol and later dissected under a microscope for sexing. The flowers and buds removed from the culture were placed inside petri dishes having 1 sheet of filter paper (Munktell filter paper; 65 g/m^2) moistened with 2-% bleach solution (sodium hypochlorite). The bud-filled petri dishes were stored in a humidity chamber (i.e., 10-liter gauzed-top containers lined with moist paper towel sprayed with bleach solution) for 5-7 days after which they were dissected to search for larvae. The humidity chamber was placed in a warmer quarantine room for better development of the larvae. The larvae were reared in the humidity chamber with conditions (T= 25.6 ± 0.1 °C; RH= 85 %). Larvae were transferred to fresh flowerbuds and the larval development was observed daily under a dissection microscope. Multiple transfers of larvae to fresh flowerbuds were done whenever necessary (i.e., when the bud contents were

consumed by larvae, and on rare occasions, when there were fungi developing) for the larvae to complete their development. Once the larvae began to pupate, the pupae were transferred into separate petri dishes and the duration of pupation was noted. The F1 adults reared out were added to the *A. morticinus* stock culture.

2.3 Results

Developmental observations of *Anthonomus morticinus*

Anthonomus morticinus adults feed on *S. mauritianum*, particularly on the stem, leaves and flowers. For the first two weeks, the weevils were observed to survive on *S. mauritianum* on only leaves. The weevils used flowerbuds for their reproduction by ovipositing into them. The larvae feed on the stamens and the anthers of the flowers within flowerbuds. As the larvae continuously feed on the bud and floral contents, they produce pellets of frass which they form into a dark brown frass chamber that serves as a protective cocoon, around the larva. Given that there were not enough bud contents for the larva to mature to pupation, the larva was transferred into another flowerbud for pupation. The third instar larvae moults into the pupal phase and feeding stops. The pupae then mature into adult weevils which chew their way out of the buds. The adult weevils mate immediately after they exit the flowers and buds. *Anthonomus morticinus* adults are highly mobile and fly frequently.

Experimental results

Anthonomus morticinus adults survived between 21 and 105 days (Figure 2.1). The larval phase duration from oviposition to pupation took 14 - 25 days (mean ± SE= 18.4 ± 0.3). The pupal phase was between 4 - 7 days (mean ± SE= 5.4 ± 0.1). The overall development from egg to adult took 19-30 days (mean ± SE= 24.3 ± 0.2). Of the 51 P-generation *A. morticinus* weevils, 27 were females and 24 were males and these produced a total of 760 larvae (663 found alive and 97 found dead) recovered from flowers and buds. Only 64 % of the larvae found alive during dissection (423 *A. morticinus*) completed their life cycle, with each female producing an average of 28 eggs.

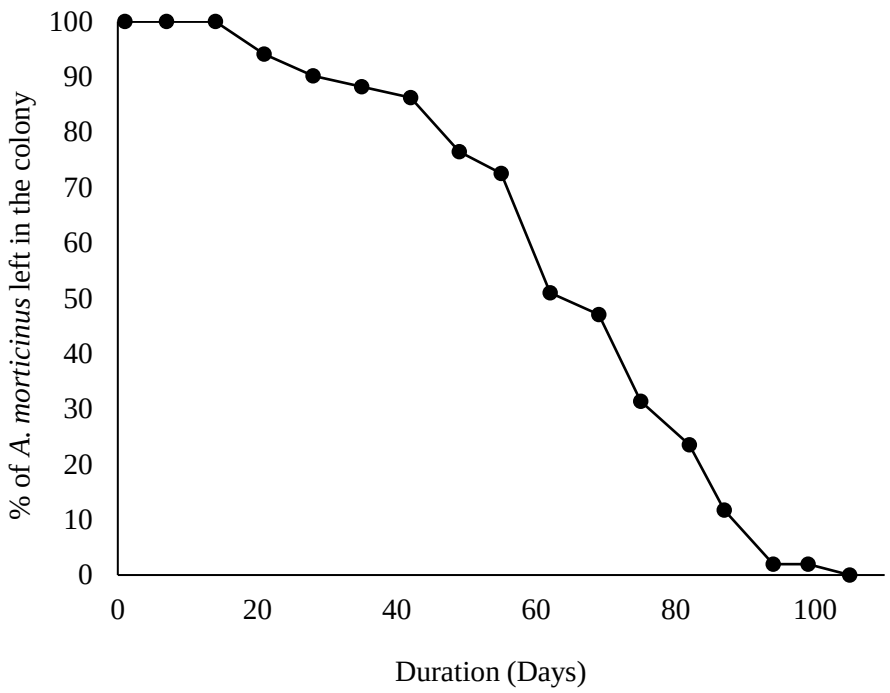


Figure 2.1: *Anthonomus morticinus* weevil survivorship (%) in the colony over a period of 15 weeks. From the third point until the last point of the graph, bouquets of *S. mauritianum* with flowers and leaves were provided. Prior to that leaves only were used. The average mortality rate of *A. morticinus* was 4 weevils per week.

2.4 Discussion

Anthonomus morticinus has a similar biology to that of *A. santacruzi*, which exhibited short developmental times and generational overlaps (Olckers, 2003). The weevil depends on *S. mauritianum* as the host plant for both feeding and reproduction in its native range in South America (Clarke and Burke, 1998).

The field collected *S. mauritianum* used in this study had a reduced quality due to the winter season, as winter temperatures drop below the photosynthetic threshold of *S. mauritianum* in this season (Cowie *et al*., 2016a). Although *S. mauritianum* quality was low, *A. morticinus* sustained a high fecundity which will likely play an important role in managing the spread of *S. mauritianum* if it were to be released in South Africa. The fecundity measured in this trial could have been higher if floral material was available from the onset of this study.

About 13 % of the larvae found were dead during dissection. The possible cause of the dead larvae could have been food shortages in small flowers and buds, which the *A. morticinus* larvae require for complete development. Some larvae also sustained injury during the bud's dissection. *Anthonomus morticinus* presents a low mortality rate, which plays a significant role in maintaining a high fecundity. The weevils being highly mobile and flying on a frequent basis suggest that *A. morticinus* could have potential for dispersal on *S. mauritianum* if it were to be released.

Anthonomus morticinus depends on *S. mauritianum* for its biological life cycle and successful reproduction, as observed in its native region (Olckers, 2011; Pedrosa-Macedo *et al*., 2003). Although the study was conducted mid-winter when the plant quality and floral quantity/quality was reduced, the candidate agent presented a relatively high fecundity and low mortality rate. The suitability for release and the potential of *A. morticinus* establishing in South Africa are both highlighted in chapter 3 and chapter 4, respectively.

Chapter 3: Host specificity testing and risk analysis of *Anthonomus morticinus* Clark (Coleoptera: Curculionidae), a potential biocontrol agent of *Solanum mauritianum* Scopoli (Solanaceae).

Abstract

Solanum mauritianum Scopoli (Solanaceae) is a South American tree invasive in regions outside its native range, most notably in South Africa and New Zealand. The biocontrol agents released to manage the spread of this weed in South Africa are *Gargaphia decoris* Drake (Tingidae) and *Anthonomus santacruzi* Hustache (Coleoptera: Curculionidae), both of which are noted to cause ineffective levels of damage to *S. mauritianum*. There is a need for additional agents to control *S. mauritianum.* Host specificity tests were conducted, using several agricultural Solanaceae in quarantine, on an agent imported from Uruguay, the florivorous weevil *Anthonomus morticinus* Clark (Coleoptera: Curculionidae). Non-target feeding by *A. morticinus* adults was observed on all the *Solanum* test plants including *S. melongena* (eggplant) and *S. tuberosum* (potato) during no-choice trials with only foliage material. The greatest feeding intensity was recorded on *S. mauritianum* and two *S. melongena* varieties (black beauty and little finger), during no-choice trials with foliage material only. There was non-target oviposition and development of *A. morticinus* on some *Solanum* plants, particularly on four of the five *S. melongena* varieties, during no-choice tests with floral material, with the highest oviposition recorded on *S. mauritianum*. During paired choice tests, *A. morticinus* showed a clear preference for *S. mauritianum* when compared to the remaining *Solanum* test plants. Feeding intensity and oviposition was significantly greater on *S. mauritianum*. A risk analysis, using the paired choice data showed that *A. morticinus* had a high non-target feeding risk on four of the five tested eggplant varieties, while the reproductive risk on these eggplant varieties these was low (< 1%). *Anthonomus morticinus* may therefore be a suitable agent of *S. mauritianum* pending further host specificity testing on native *Solanum* species.

Keywords: Bugweed, host specificity, non-target feeding, oviposition, risk analysis.

3.1 Introduction

The South American tree, *Solanum mauritianum* is an invader that remains under limited control in South Africa (Venter *et al*., 2021). This weed has invaded most South African provinces (Henderson, 2020), with its spread over long distances facilitated by frugivores particularly birds (Olckers, 2003). *Solanum mauritianum* has low levels of fruiting in its native range due to the presence of its natural enemies, particularly flower feeding and leaf feeding insects (Olckers, 2011). The lack of natural enemies of *S. mauritianum* in South Africa was the major contributing factor in the high fruiting and hence the success of the weed (Olckers and Hulley, 1989). This led to the onset of biocontrol efforts against *S. mauritianum* in 1984, which saw many candidates being rejected for release because of non-target effects on both native and cultivated Solanaceae plants (Olckers, 1999).

Of the seventeen imported agents, the sap feeding lace bug *Gargaphia decoris* was the first biocontrol agent released against *S. mauritianum* in South Africa, during 1999-2001 (Olckers and Borea, 2009), and later released in New Zealand during 2010 (Olckers, 2011). Although *G. decoris* reduces the photosynthesis of *S. mauritianum* and hence its growth rate and seed output (Cowie *et al*., 2016a), the agent's impacts on the weed are trivial (Cowie *et al.,* 2018; Henderson, 2020). The second biocontrol agent that was released in 2008 against *S. mauritianum* was the flowerbud-feeding weevil, *Anthonomus santacruzi* (Cowie *et al*., 2018).

Anthonomus santacruzi was regarded as a promising agent given its ability to reduce the fruit and seed output of *S. mauritianum* (Olckers, 2003). *Anthonomus santacruzi* adults oviposit eggs into flowers and buds of *S. mauritianum*, which result in their destruction while the larvae develop inside of them (Olckers, 2003). This reduces the quantity of fruits and seeds produced by *S. mauritianum* trees (25% and 66%, respectively), while maintaining the reproductive success of *A. santacruzi* (Cowie *et al.,* 2017). At present *A. santacruzi* has only established along the coastal region of KwaZulu-Natal and in the Sabie region in Mpumalanga and has failed to establish further inland on the South African highveld due to climate constraints, particularly the unfavorable temperature and humidity conditions in the inland regions of South Africa (Cowie *et al*., 2016b; Singh and Olckers, 2017). As a result of this restricted establishment, the overall

control of *S. mauritianum* in South Africa by *A. santacruzi* is currently trivial (Zachariades, 2021), and hence the need for additional biocontrol agents to manage the weed.

Releasing a suite of agents against one target weed is sometimes necessary to improve the efficacy of biocontrol (Stiling and Cornelissen, 2005). This has been the case for several weeds such as *Hypericum perforatum* (Clusiaceae) and *Sesbania punicea* (Fabaceae), both of which had multiple agents released on them to manage their spread and increase the efficacy of biocontrol thereafter (Zachariades, 2018). Once the efficacy of biocontrol is improved over time, the weeds are controlled within manageable levels, and their negative impacts are significantly reduced (McFayden, 1998). It is therefore necessary to introduce another biocontrol agent against *S. mauritianum,* to improve the status of biocontrol of this weed. Before an additional agent can be introduced to control *S. mauritianum* in South Africa, it is mandatory that the potential risks of the agent are assessed in quarantine through host specificity tests (see McConnachie, 2015; Olckers, 2000; 2003, Smith *et al*., 2019) because once an agent is released it cannot be reclaimed (McFayden, 1998).

Assessing the potential risks of agents using the plants in the same taxonomic genus as the target weed by host specificity tests is important to promote safe releases (McConnachie, 2015). The outcomes of host specificity trials in quarantine are often overestimated due to laboratory restrictions, especially during no choice tests where the agent is limited to only one test plant at a time (Fowler *et al*., 2012). Candidate agents often exhibit non-target attacks during no choice tests, whereas they would not naturally exploit those plants in the environment (Groenteman *et al*., 2011). Paired choice tests offer better insights when compared to no-choice tests in this instance, as an agent has the option to feed and oviposit on the target weed in the presence of the test plant (Paynter *et al*., 2015), which may reduce the rejection of host specific agents. Risk analysis is also an essential component in weed biocontrol that helps to reduce the rejection of host specific agents (Wan and Harris, 1997; Paynter *et al*., 2015). It is a valuable tool that was used to promote the release of the two earlier agents for *S. mauritianum* biocontrol (see Olckers, 2003; Olckers and Borea, 2009).

Predicting the likelihood of an agent attacking non-target plants in the field may be done by means of risk analysis (Paynter *et al*., 2015; Weyl *et al*., 2021). This is where the performance of

the agent on non-target plants is compared to the performance of the agent on the target weed (Paynter *et al*., 2015; Wan and Harris, 1997). Olckers (2003), Olckers and Borea, (2009) and Wan and Harris (1997) all considered factors such as the agent's feeding intensity, plant preference, oviposition preference and completed development to adulthood in their risk analysis, all of which were used to conduct the risk analysis for *S. mauritianum* in this study. The feeding intensity and plant preference were used to predict the 'feeding risk', while the oviposition preference and the proportion of larvae developing to adulthood was used to predict the 'reproductive risk' from the host specificity trials (see Olckers, 2003; Olckers and Borea, 2009; Wan and Harris, 1997). Either the 'feeding risk' or the 'reproductive risk' should be less than 5 % on each test plant species to be regarded as 'low risk' (see Olckers, 2003). These factors were assessed on non-target plants relative to the target plant and expressed as ratios to assess the probability of non-target impacts the agent could show in the field. *Anthonomus santacruzi* (Olckers, 2003) and *G. decoris* (Olckers and Borea, 2009) exhibited non-target impacts on some Solanaceae plants (Olckers, 2003), but none of the plants attacked in quarantine were attacked in the field (Hakizimana, 2011; Olckers, 2009; 2011) once the insects had been released.

Flowerbud feeders are important candidates to consider for host specificity testing, as they were observed to result in less fruiting and seed output of *S. mauritianum* in its native range (Pedrosa-Macedo *et al*., 2003; Olckers, 2009). A flowerbud feeding weevil, *Anthonomus morticinus* is a candidate agent of *S. mauritianum* that was imported from Uruguay (Venter *et al.*, 2021), where it is more common in the temperate regions of South America than *A. santacruzi* (Pedrosa-Macedo *et al*., 2003). Olckers (2011) suggested that *A. morticinus* should be considered for assessment as it may be a suitable candidate for *S. mauritianum* if *A. santacruzi* failed to establish in colder regions supporting *S. mauritainum* in South Africa. *Anthonomus morticinus* has potential as a biocontrol agent for *S. mauritianum* (Pedrosa-Macedo *et al*., 2003), as field surveys suggest that *A. morticinus* has a very narrow host range in its native region (Olckers, 2009). In this study, host specificity tests were conducted on the main agricultural Solanaceae plants, which are of commercial importance in South Africa and were of most concern during the host specificity testing of *A. santacruzi*. It was hypothesized that *A. morticinus* should display acceptable levels of host specificity for *S. mauritianum* since it exhibited a similar life cycle and feeding patterns on *S. mauritianum* as *A. santacruzi*, while being in the same genus (Olckers,

2009). A risk analysis was also conducted to assess the possibility of non-target feeding by *A. morticinus* on agricultural Solanaceae if it were to be released.

3.2 Materials and Methods

Culture colony collection

A total of 156 adult *Anthonomus morticinus* weevils were collected from Uruguay (Latitude: -34,420128; Longitude -54,403067) in March 2020 and kept in the Wits Insectary Quarantine Facility at the University of the Witwatersrand, Johannesburg. The quarantine temperature and relative humidity were T= 23.8 ± 0.1 °C, RH= 63%. The imported weevils were assessed using a dissection microscope to ensure that only *A. morticinus* was present. The examination involved counting the number of bands of white scales on the elytra of the weevils. *Anthonomus morticinus* only has one white band on the fourth interstitial segment, while *A. santacruzi* has 4-5 white bands on their elytra (Clark and Burke, 1996; Olckers, 2003). It was found that all the imported weevils were *A. morticinus.* All weevils were maintained on bouquets of *S. mauritianum*.

Culturing of *Anthonomus morticinus*

Fresh apical leaflets, flowers and flowerbuds of *S. mauritianum* were collected. Their stems were cut under water and placed inside the glass jars filled with water. The jars were kept inside 5-liter plastic buckets with gauze tops and lined with a sheet of moist paper towel at the base, to reduce desiccation of the bouquets. The adult weevils were transferred to the *S. mauritianum* bouquets for feeding and oviposition. Every 4-7 days, fresh bouquets were presented to the weevils and a paint brush was used to transfer weevils from old to fresh bouquets. The old flowers and flowerbuds were then collected and placed in petri dishes lined with 1 sheet of filter paper (Munktell filter paper; 65 g/m^2) moistened with 2-% bleach solution (sodium hypochlorite). The bleach solution was used to slow the rate of fungi/mold development on the flowers and flowerbuds. The petri dishes containing old buds were placed inside humidity chambers, using 5 and 10-liter gauzed top containers lined with moist paper towel sprayed with 2-% bleach solution. The buds in the petri dishes were kept inside the humidity chambers for approximately one week to allow the eggs to develop into larvae. All buds were then carefully dissected, and

the developing larvae were individually transferred to a fresh flowerbud of *S. mauritianum* placed in a new petri dish (containing moistened filter paper as above). The petri dishes containing the developing larvae were observed daily under a dissection microscope and larval transfers to fresh flowerbuds were done whenever necessary (i.e., when the bud contents were consumed by larvae, and on rare occasions, when there were fungi developing). Emerging adult weevils of *A. morticinus* were added to the stock culture and maintained for the rest of the study.

On some occasions, the *S. mauritianum* flowers and buds were stored in the humidity chamber for 2-3 weeks before they were dissected, to check if the larvae could survive without being transferred into fresh flowers and buds. Dead larvae, pupae and adult weevils were recovered when the buds were dissected, and some adult weevils were able to emerge without transfer. The larval transfer to fresh flowers and buds was prioritized to increase the numbers of adults reared out for the culture.

***Anthonomus morticinus* host specificity testing.**

The assessment of possible non-target effects (feeding) of *A. morticinus* on cultivated plants was tested using standard host specificity tests (centrifugal testing), followed by a risk analysis as conducted by Olckers (2003). Five horticultural Solanaceae species were tested in the host specificity trials and included: *Capsicum annuum* L. (green pepper), *Lycopersicon esculentum* Mill. (tomato), *Physalis peruviana* L. (Cape gooseberry), five varieties of *Solanum melongena* L. (eggplant) (var. black beauty (BB), black king (BK), black king F1 hybrid (BKF1), little finger (LF), and violet moon (VM)) and two varieties of *Solanum tuberosum* L. (potato) (var. Mondial and Sifra). All these test plants were grown from seed in quarantine.

***Anthonomus morticinus* adult no-choice tests**

No choice tests were divided into both floral and non-floral trials. Non-floral trials were conducted initially to observe whether *A. morticinus* was able to feed and survive on the test plants in the absence of flowers. Test plant species on which feeding was observed in the non-floral trials were then used in floral trials to observe feeding, oviposition and development of *A. morticinus*. For both floral and non-floral trials, *S. mauritianum* bouquets were used as the control test plants, placed either in a separate 5-liter container, or a small cage (L=40 cm, W= 40

cm, and H=40 cm) during the trials. For the Solanaceae species in which feeding by *A. morticinus* was recorded, the trials were replicated six times. Conversely, the trials were repeated three times on Solanaceae plants where feeding was absent.

***Anthonomus morticinus* non-floral, no choice trials**

Ten *A. morticinus* adults were individually presented with bouquets of *S. mauritianum* foliage material, and the potted Solanaceae test plant, respectively. After 4-5 days, feeding intensity was assessed by examining the plants' leaves under a dissection microscope (by counting the number of feeding scars). Weevil mortality was also recorded as the percentage of weevils that died in each trial.

***Anthonomus morticinus* no-choice, floral trials**

Ten *A. morticinus* adults (five males and five females) were released onto each test plant with flowers, for a period of 4-5 days. The sexing of the weevils was achieved by removing mating pairs from the culture, with each pair being assessed under a microscope for assurance that each pair had one male and one female. Alternatively, weevil sexing was done by putting the weevils in a petri dish, which was then placed in a – 40 ° C freezer for 30-45 seconds to make the weevils less mobile. Thereafter, the petri dish was placed on an ice pouch under a dissection microscope to observe the presence or absence of the tergal notch on the ventral side of the weevils' abdomen. The tergal notch is present in male and absent in female weevils (Sasa *et al.*, 2020). On some occasions when there were more males than females or vice versa, ten weevils were randomly selected and sexed after each trial by dissection. The weevils were sexed in order to determine the number of larvae produced per female.

The position of the weevils (whether they were on the test plants or not), feeding intensity, mortality, oviposition, and larval survival on the test plant species were all recorded on the last day of each trial. The trials were replicated four times and ran for a period of 4-5 days for each test plant species. The flowers and buds were collected after each trial and stored inside petri dishes for 4-5 days. Thereafter, the flowers and buds were dissected, and larvae were transferred to fresh buds of the respective test plant species. The developing larvae were observed daily under a dissection microscope for the duration of their development, and emerging adults were added to the stock culture.

Anthonomus morticinus paired choice tests

All test plant species that exhibited feeding by *A. morticinus* during the no choice tests were used in paired choice trials. One *S. mauritianum* bouquet and one potted test plant species with equivalent amounts of floral and foliage material were placed in a cage (L= 60 cm, W= 60 cm and H= 90 cm). A total of four replicates were done per test plant species, having two occasions where *S. mauritianum* and the test plant were in contact, and the other two occasions where the test plants were separated and placed diagonally in the corners of the cage. Twenty newly emerged *A. morticinus* adults were randomly released into the cage for one week. After each trial, the position of the weevils, feeding intensity, oviposition, larval survival, adult weevil sex and mortality were all recorded. Flowers and buds were collected from both plant species and stored inside the humidity chamber for 4-5 days after each trial. The buds were then dissected, and larvae were transferred to fresh flowers and buds of the same species in which oviposition occurred. The development of the larvae was observed daily under a dissection microscope, and emerging adults were recorded, then added to the stock culture.

Risk analysis of *Anthonomus morticinus*

The risk analysis was done to assess the performance of *A. morticinus* adults on each test plant species relative to that of *S. mauritianum* as per Olckers (2003). The feeding and oviposition results of *A. morticinus* that were obtained in paired choice tests were used to complete the risk analysis. The performance criteria that were used are: (i) plant preference (R^1), (ii) food acceptability (R^2), (iii) oviposition preference (R^3), and (iv) larval survival (R^4). Plant preference was determined by the position of the weevils (number of weevils recorded on the test plants). Food acceptability was determined by the feeding intensity of *A. morticinus*, using the number of feeding scars recorded on the test plants. Oviposition preference involved the number of larvae recorded in each test plant species. Larval survival was determined by the number of larvae that completed their development to adults from paired choice tests.

The risk of possible non-target effects by *A. morticinus* on test plants was assessed in two ways:

1. The risk of feeding damage, which was calculated as the product of the plant preference and food acceptability scores (i.e., Feeding risk= $R^1 \times R^2$).

2. The risk of the weevils establishing a reproductively viable population, which was assessed as the product of oviposition preference and larvae survival scores (i.e., Reproductive risk= $R^3 \times R^4$).

The letter (R) represents the performance of *A. morticinus* weevils on a test plant relative to that on *S. mauritianum.* All the zero values were recorded as 0.001 when running calculations (Wan and Harris, 1997).

3.3 Data analysis

A two-by-two Chi-Square test was used to assess the difference in feeding intensity and mortality between *S. mauritianum* and the test plant species during no-choice non-floral trials. For the no-choice floral trials, a two-by-two Chi-Squared tests was conducted to assess the difference in feeding, mortality, oviposition and development between *S. mauritianum* and the test plant species. A paired t-test was conducted to assess the preference, feeding, mortality, oviposition and development between *S. mauritianum* and the respective Solanaceae test plant species during the paired choice trials. The results obtained in paired choice trials were used to complete the risk analysis of *A. morticinus* on non-target plants.

3.4 Results

Anthonomus morticinus no-choice non-floral tests

All *S. melongena* and *S. tuberosum* varieties were fed on by *A. morticinus,* and the adult weevil mortality ranged from 4 – 70 % (Table 3.1). The highest feeding and lowest mortality of *A. morticinus* adults was observed on *S. mauritianum,* both of which were not significantly different from *S. melongena* (var. BB). The test plant species with the highest weevil mortality was *S. melongena* (var. BKF1) and it was also observed to support the least feeding by *A. morticinus.*

Anthonomus morticinus no-choice tests floral trials

By the time the no choice non-floral trials were completed on all the *Solanum* species, the Solanaceae plants outside the *Solanum* genus were already flowering, and hence only floral trials were done on these plants. No feeding, oviposition or development were noted on the test plant species outside of the genus *Solanum*. These plants were *Capsicum annum* (Chilli pepper), *Capsicum annum* group. (Green pepper), *Lycopersicon esculentum* Mill. (tomato), and *Physalis peruviana* (Cape gooseberry) (Table 3.2). Non-target feeding was present in all eggplant varieties, with the highest feeding intensity recorded on *S. mauritianum*. The feeding intensity was significantly different between *S. mauritianum* and all the other test plant species excluding *S. melongena* (var. BB) ($\chi^2 = 0.21271$, df = 1, p = 0.1447) (Table 3.2). The adult weevil mortality was also significantly different between *S. mauritianum* and all the other Solanaceae test plant species excluding *S. melongena* (var. BB) ($\chi^2 = 0.245$, df = 1, p = 0.6206) (Table 3.2). Both the number of larvae, and the number of larvae produced per *A. morticinus* adult females in *S. mauritianum* were significantly higher than in all the eggplant varieties in which oviposition was present. Amongst the eggplant varieties, *S. melongena* (var. BKF1) had the highest adult mortality, lowest feeding intensity and no oviposition. The potato varieties, *S. tuberosum* (Mondial and Sifra) failed to flower and hence were not included in no-choice floral trials and in paired choice tests.

Anthonomus morticinus paired-choice tests

The number of *A. morticinus* adults found on *S. mauritianum* was significantly greater than all the accompanying test plant species in the paired choice tests (Table 3.3; $p < 0.001$). The feeding intensity of *A. morticinus* on *S. mauritianum* was significantly different to that on all the accompanying test plant species (Table 3.3; $p < 0.01$). There was no significant difference in the percentage of buds with larvae between *S. mauritianum* and all the eggplant varieties, except *S. melongena* (var. BKF1) in which oviposition was absent and hence there was no development of *A. morticinus*. The number of larvae recovered from *S. mauritianum* was significantly higher than all the *S. melongena* varieties in all the paired choice trials ($p < 0.01$). However, only 1-4 buds occurred on the non-target plants compared to 60.3 ± 4.7 buds on *S. mauritianum.* The duration of larval development was significantly longer on *S. melongena* (var. BB) than on *S.*

mauritianum (Table 3.3; p = 0.0363), and there was no significant difference in the duration of larval development between *S. mauritianum* and all the other eggplant varieties.

Risk analysis of *Anthonomus morticinus*

Out of the 12 Solanaceae test plants, only seven exhibited non-target feeding of *A. morticinus.* The potato varieties did not flower and hence were not included in the risk analyses. Four of the five *S. melongena* varieties supported *A. morticinus* development. The non-target feeding risks of *A. morticinus* on the eggplant varieties was lowest on *S. melongena* (var. BKF1) (< 5%), higher (>5 %) on *S. melongena* (var. BK), *S. melongena* (var. VM), *S. melongena* (var. LF) and highest on *S. melongena* (var. BB) (Table 3.4). The non-target reproductive risks of *A. morticinus* were low in all eggplant varieties (< 1 %), with *S. melongena* (var. BKF1) having no reproductive risk.

Table 3.1: The host range of *A. morticinus* adults (n=10 weevils per plant species; n=6 trials per plant) as determined by their feeding intensity and mortality on agricultural Solanaceae plants during no choice non-floral trials. The letters in the superscript indicate values that are significantly different from *S. mauritianum* (P< 0.05).

Test plant variety	Mean ± (SE) Feeding scars	Mean ± (SE) % Adult mortality
S. mauritianum	212.2±2.9[a]	3.6±1.1[a]
S. melongena (BB)	208.3±11.1[a]	6.7±3.3[a]
S. melongena (BK)	141.2±6.1[b]	10±3.7[b]
S. melongena (BKF1)	22.3±3.6[b]	70±5.8[b]
S. melongena (LF)	173.7±9.4[a]	8.3±4.8[a]
S. melongena (VM)	171.2±7.6[b]	10±4.5[a]
S. tuberosum (Mondial)	104.3±10.3[b]	15±4.2[b]
S. tuberosum (Sifra)	109.7±5.4[b]	10±2.6[a]

Table 3.2: The host range of *A. morticinus* adults (n=10 weevils per plant species; n=6 trials per plant) as determined by their feeding intensity, adult weevil mortality, oviposition and larval survival on agricultural Solanaceae plants during no choice floral trials. *Solanum tuberosum* varieties did not flower and hence were not included in no-choice floral trials. The letters in the superscript indicate values that are significantly different from *S. mauritianum* ($P< 0.05$).

Test plant variety	Mean ± (SE) Feeding scars	% Adult mortality	Mean ± (SE) Number of larvae per plant	Mean ± (SE) Number of larvae/female	% Larval survival	Mean ± (SE) Number of days to adults
Capsicum annum (Chilli pepper)	0^{a}	66.7^{a}	0^{a}	-	-	-
Capsicum annum group. (Green pepper)	0^{a}	73.3^{a}	0^{a}	-	-	-
Lycopersicon esculentum Mill. (tomato)	0^{a}	70^{a}	0^{a}	-	-	-
Physalis peruviana (Cape gooseberry)	0^{a}	63.3^{a}	0^{a}	-	-	-
S. mauritianum	235.4 ± 5.8^{b}	4.7^{b}	30 ± 1.1^{b}	5.9 ± 0.2^{a}	75.4^{a}	19.9 ± 0.7^{a}
S. melongena (BB)	204.8 ± 11.0^{b}	3.3^{b}	1.8 ± 0.3^{a}	0.3 ± 0.01^{b}	90^{a}	22.3 ± 0.9^{a}
S. melongena (BK)	137 ± 9.2^{a}	20^{a}	1.3 ± 0.3^{a}	0.3 ± 0.1^{b}	37.5^{b}	25.5 ± 2.0^{a}
S. melongena (BKF1)	25.8 ± 2.4^{a}	62.5^{a}	0^{a}	-	-	-
S. melongena (LF)	164.8 ± 8.1^{a}	13.3^{a}	1.5 ± 0.3^{a}	0.3 ± 0.2^{b}	37.5^{b}	25.7 ± 0.7^{a}
S. melongena (VM)	152 ± 0.7^{a}	17.5^{a}	1.8 ± 0.3^{a}	0.5 ± 0.2^{b}	50^{b}	27.7 ± 0.9^{a}

Table 3.3: The host range testing of *A. morticinus* adults (n=20 weevils per pair; n=4 trials per pair) as determined by their position, feeding intensity, oviposition and development on agricultural Solanaceae plants during paired choice tests. *Solanum tuberosum* varieties did not flower and hence were not included in the paired-choice trials. The letters in the superscript indicate values that are significantly different from *S.mauritianum* ($P< 0.05$).

Test plant pair	Mean ± (SE) Number of adults	Mean ± (SE) Number of feeding scars	% Buds with larvae (Mean ± (SE) number of buds)	Mean ± (SE) Number of larvae per plant *spp.*	% Larval survival	Mean ± (SE) Number of days to adults
S. mauritianum	11.8±0.5[a]	238.5±3.8[a]	69.7[a] (n= 66.5±5.2)	46.5±4.5[a]	74.2[a]	18.2±0.4[a]
S. melongena (BB)	6±0.4[b]	153±9.7[b]	77.1[a] (n= 3.25±0.3)	2.5±0.3[b]	75[a]	22.3±1.0[b]
S. mauritianum	14.3±1.3[a]	252±5.4[a]	76.7[a] (n= 48.5±4.5)	36.8±4.4[a]	64.9[a]	19.9±0.4[a]
S. melongena (BK)	2.5±0.3[b]	107.5±5.4[b]	87.5[a] (n= 2.25±0.3)	2±0.4[b]	33.3[a]	29.3±0.3[a]
S. mauritianum	14±1.1[a]	280.8±8.6[a]	69.4[a] (n= 68.5±4.3)	41.5[a]	68.7	19.4±0.4
S. melongena (BKF1*)*	1.8±0.5[b]	28±4.9[b]	0[b] (n= 2.5±0.6)	0[b]	-	-
S. mauritianum	13.5±1[a]	261.5±6.5[a]	64.2[a] (n= 52±4.8)	32.3±1.3[a]	77.9[a]	18.9±0.4[a]
S. melongena (LF)	4.3±0.9[b]	118.8±5.3[b]	43.8[a] (n= 2±0.7)	1.3±0.8[b]	29.2[a]	25.7±0.7[a]
S. mauritianum	14.1±1.2[a]	237.3±5.5[a]	57.8[a] (n= 66±5.7)	38.3±6.7[a]	67.2[a]	21.0±0.4[a]
S. melongena (VM)	3.5±0.6[b]	121.3[b]	25[a] (n=2.5±0.3)	0.8[b]	38[a]	27.5±0.5[a]

Table 3.4: The risk analysis based on *A. morticinus* performance on agricultural Solanaceae plants relative to that on *S. mauritianum* in paired choice tests.

Test plant species	Plant preference ($\mathbf{R^1}$)	Food acceptability ($\mathbf{R^2}$)	Feeding risk ($\mathbf{R^1 \times R^2}$)	Oviposition Preference ($\mathbf{R^3}$)	Larval survival ($\mathbf{R^4}$)	Reproductive risk ($\mathbf{R^3 \times R^4}$)
S. mauritianum	1	1	**1**	1	1	**1**
S. melongena (BB)	0,4444	0.6024	**0.2677**	0.0640	0.0680	$\mathbf{4.35\times10^{-3}}$
S. melongena (BK)	0.1852	0.4232	**0.0784**	0.0512	0.0242	$\mathbf{1.24\times10^{-3}}$
S. melongena (BKF1)	0.1296	0.1102	**0.0143**	0.0010	0.0010	$\mathbf{1\times10^{-6}}$
S. melongena (LF)	0.3148	0.4675	**0.1472**	0.0320	0.0132	$\mathbf{4.23\times10^{-4}}$
S. melongena (VM)	0.2593	0.4774	**0.1238**	0.0192	0.0183	$\mathbf{3.51\times10^{-4}}$

3.5 Discussion

Anthonomus morticinus showed the highest feeding intensity on *S. mauritianum* compared to all the *Solanum* non-target plants during no-choice trials, and the highest oviposition and development was observed on *S. mauritianum* during no-choice floral trials. The non-target feeding, and oviposition observations are more common in no-choice trials, where the agent is restricted to feed on only one host plant which may not be attacked in the field (Baars *et al*., 2003; McConnachie, 2015; Olckers, 2003). The absence of feeding and oviposition in non-*Solanum* agricultural Solanaceae suggests that these plants may have chemical repellants or other defensive compounds that caused *A. morticinus* to avoid them. This is supported by the high mortality observed on non-*Solanum* test plants, which is caused by the absence of a preferred food source required for survival as seen in no choice trials in Olckers (2003). *Solanum melongena* BKF1 was the only exception within the *Solanum* genus which had high mortality and the lowest feeding intensity during the no-choice trials. In the case of *S. melongena* (BKF1), the weevils were likely exploring the only available food source which was not sufficiently palatable for them to continuously feed on for survival. There may be differences in phytochemicals between test plants within the *Solanum* genus vs. the test plants outside the

Solanum genus, which may have resulted in *A. morticinus* avoiding feeding on non-*Solanum* plants as suggested by Olckers (2003) with *A. santacruzi*.

A consistent preference by *A. morticinus* for *S. mauritianum* over all five eggplant varieties was recorded during paired-choice tests. The highest feeding intensity was on *S. mauritianum*, and more larvae were recovered from *S. mauritianum* than all the eggplant varieties during these tests. A similar observation was made by Cagnotti *et al.*, (2007) who found that *Plectonycha correntina* Lacordaire (Coleoptera: Chrysomelidae) preferred *Anredera cordifolia* (Tenore) Steenis (Basellaceae) more than the related plant species during paired choice trials (Snow *et al*., 2012). *Plectonycha correntina* was cleared for release in Australia after four years of host range testing (Snow *et al*., 2012). Compared to the no-choice tests, the non-target feeding intensity declined during paired choice tests in all the non-target *Solanum* plants. Although the duration of *A. morticinus*' development on *S. mauritianum* was not different to that on all the eggplant varieties, excluding *S. melongena* BB, the weevils' development on *S. mauritianum* were relatively shorter than all the eggplant varieties. This suggests that the biology of *A. morticinus* is similar to that of *A. santacruzi*.

The non-target feeding risk by *A. morticinus* was highest on *S. melongena* (BB), followed by *S. melongena* (LF), *S. melongena* (VM), and *S. melongena* (BK). These non-target feeding risks by *A. morticinus* (> 5%) may be due to the small cages that were used during the paired choice trials, which likely exaggerated the non-target feeding and oviposition results in all the eggplant varieties (see Olckers, 2003). The feeding risk by *A. morticinus* was low only on *S. melongena* (BKF1), and this eggplant variety had no reproductive risk given the absence of oviposition. Although the oviposition results may have been exaggerated due to the small cages used, all the other eggplant varieties (*S. melongena* BB, BK, LF and VM) showed low reproductive risks (< 1%). Smith *et al*., (2019) found that *Hydrellia egeriae* Rodrigues (Diptera: Ephydridae) exhibited low feeding risks and reproductive risks (< 2%) on Hydrocharitaceae test plants relative to the target weed, *Egeria densa* Planchon (Hydrocharitaceae) during host specificity tests (Smith *et* al., 2019). This agent was approved for release as a biocontrol agent of *E. densa* (Smith *et* al., 2019), and has established on the Nahoon River, Eastern Cape and in Midmar Dam, KwaZulu Natal (Coetzee *et al*., 2021). McConnachie, (2015) found that the Mexican beetle, *Zygogramma bicolorata* Pallister (Coleoptera: Chrysomelidae), was sufficiently host

specific to *Parthenium hysterophorus* L. (Asteraceae: Heliantheae), with low risks of feeding and development on the plants outside the subfamily tribe of Heliantheae. The beetle was released after showing low, non-target risks on the plants outside the subfamily of the target weed (McConnachie, 2015). The low reproductive risks suggest that the probability of *A. morticinus* being reproductively successful on these non-target plants is very low. Also, the use of pesticides against generalist pests on commercial plants, including *Solanums*, diminishes the risk of any attack by biocontrol agents. This further implies that the populations of *A. morticinus* will likely sustain only on *S. mauritianum* and not utilize eggplant varieties as a reproductive host, should *A. morticinus* be released.

Anthonomus morticinus is relatively fecund (See Chapter 2) and self-sustaining on *S. mauritianum*, with field surveys in South America revealing that *A. morticinus* has a narrow host range (Pedrosa-Macedo *et al*., 2003). This is further supported by the fact that *A. morticinus* adults collected in Uruguay and reared in quarantine for this study were found only on *S. mauritianum* (Venter *et al*., 2021), which suggests that *A. morticinus* is likely to survive on *S. mauritianum* and reduce the quantity of seeds produced, if it were to be released in South Africa. There are no native host records of *A. morticinus* on any of the agricultural crops that exhibited non-target feeding in these trials, particularly the eggplant and potato varieties which are in *Solanum* (Clarke and Burke, 1996). The case is similar to *A. santacruzi*, which had 'exaggerated' feeding, oviposition and development in no-choice and paired-choice trials on potato and eggplant. These appear due to the laboratory restrictions (Olckers, 2003; Hakizimana and Olckers, 2013), and the field surveys showed that none of the native and cultivated *Solanum spp.* were attacked by *A. santacruzi* in South Africa following its release (Olckers, 2011). This suggests that the laboratory findings presented here are also likely exaggerated by quarantine conditions, as seen with *A. santacruzi* (see Olckers, 2003 and Hakizimana, 2011).

Anthonomus morticinus preferred *S. mauritianum* over the other Solanaceae test plants during the paired choice trials, as justified by high feeding intensity and oviposition noted on *S. mauritianum* relative to all the eggplant varieties. There is a need for additional host specificity tests to be conducted in large walk-in cages to reduce the restrictions caused by smaller cages, as also seen in Olckers, (2003) with *A. santacruzi*, and Olckers and Borea (2009) with *G. decoris*. The use of outdoor hardened *Solanum* plants should also be considered for future host specificity

tests, to provide more realistic results. Plants that are grown outdoors could offer more realistic results as they would likely be harder in texture, and this might reduce the number of feeding scars on non-target plants. Another reason is that bugweed was collected in the field, and not grown indoors. Despite these exaggerated paired choice results, *A. morticinus* had a very low (almost zero percent) reproductive risk for all the eggplant varieties, excluding *S. melongena* (BKF1) which appeared to have no reproductive risk. Although there are still more *Solanum* test plants needed for host range testing with *A. morticinus* (see Olckers, 2003), the findings in this study are similar to those found by Olckers, (2003) where non-target feeding, and oviposition was found only on *Solanum* plants. *Anthonomus morticinus* may thus be a suitable agent for *S. mauritianum,* and additional host specificity tests on the native and exotic *Solanum* plants are currently underway.

Chapter 4: Assessing the thermal tolerance of *Anthonomus morticinus* Clark (Coleoptera: Curculionidae).

Abstract

The establishment of the flowerbud-feeding weevil *Anthonomus santacruzi* is apparently limited by climate constraints in South Africa, and hence the control of *Solanum mauritianum* is currently incomplete. There is a need for additional biocontrol agents, preferably cold adapted, to manage the spread of *S. mauritianum* in regions where *A. santacruzi* has failed to establish. Another flowerbud feeding weevil, *Anthonomus morticinus* was imported from Uruguay. CLIMEX software was used to predict the potential of *A. morticinus* establishing in South Africa where *S. mauritianum* has invaded. The east coast of South Africa matches best with the Uruguayan collection range of *A. morticinus*, followed by the Western Cape region and the midlands region. The highveld and the lowveld regions had a low percentage match with the *A. morticinus* collection range. The lower thermal thresholds, particularly the critical thermal minima (CT_{min}) and lower lethal temperatures (LT_{50}) of *A. morticinus* and *A. santacruzi* were assessed. *Anthonomus morticinus* (CT_{min} = 1.71 ± 0.1 °C; LT_{50} = - 9.5 °C) was more cold tolerant than *A. santacruzi* (CT_{min} = 4.93 ± 0.18 °C; LT_{50} = -5.8 °C). The CT_{min} of *A. morticinus* was lower than the mean minimum winter temperatures in regions where *S. mauritianum* is found in South Africa, and the mean minimum winter temperatures in the highveld and the KZN midlands. *Anthonomus santacruzi* experiences cold stress in the highveld and the midlands, whereas the establishment of *A. morticinus* being hindered by cold temperatures anywhere in South Africa is less likely. *Anthonomus morticinus* has the potential of being a climatically suitable biocontrol agent of *S. mauritianum.*

Keywords: biocontrol agent, critical thermal minima, cold stress, lower lethal temperatures, establishment, thermal threshold

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4.1 Introduction

Solanum mauritianum Scopoli (Solanaceae) is a South American tree that was first observed in South Africa in the KwaZulu-Natal (KZN) province during the 1860s (Olckers and Zimmerman, 1991; Olckers, 2003). The weed has since invaded several other South African provinces, occupying an area of over 1.76 million hectares (Versfeld *et al*., 1998), where it is more prominent in the eastern high rainfall areas of the country (Witkowski and Garner, 2008). Frugivorous birds are the major vectors for dispersing the *S. mauritianum* seeds over long distances as they feed on the yellowish, ripe fruits, with each fruit containing about 150 seeds (Olckers, 2011). This weed is self-compatible, with high seedbank densities (Witkowski and Garner, 2008), and there are reported cases of its invasion in some agricultural lands, conservation areas, forestry plantations and riparian zones in South Africa (Cowie *et al.*, 2018; Olckers, 2011).

The negative environmental impacts caused by the *S. mauritianum* invasion in South Africa led to the implementation of mechanical and chemical control efforts (Cowie *et al*., 2018; Olckers, 2011). However, these efforts have been ineffective, as regrowth of *S. mauritianum* was observed in cleared areas (Mtetwa, 2019), and are expensive when implemented on a large scale (Olckers, 2009). The biological control (biocontrol) of *S. mauritianum* is an alternative option that has been used to manage the spread of this invasive weed (Olckers, 2003). Since the commencement of a *S. mauritianum* biocontrol programme in 1984, several candidates have been imported for host range testing, but only two were permitted for release namely *Gargaphia decoris* Drake (Hemiptera: Tingidae) and *Anthonomus santacruzi* Hustache (Coleoptera: Curculionidae) (Olckers, 2009, 2011).

Flowerbud feeding weevils in the *Anthonomus* genus Germar (Coleoptera: Curculionidae) are regarded as promising agents that are responsible for low fruiting/ seeding of the plants in their native range (Olckers, 2009). This is because the adult weevils feed on the flowers, oviposit inside the flowers and buds, and cause the abscission and abortion of flowers and buds while the larvae develop inside them (Olckers, 2009). Of the two agents released against *S. mauritianum*, the flowerbud-feeding weevil, *A. santacruzi* was thought to be promising due to its ability to interfere with the development of flowers, and hence, seed production (Olckers, 2011). The weevil was released from 2008-2014 (Cowie *et al*., 2018). The establishment of *A. santacruzi*

has largely been restricted to the coastal region of KZN, and within the Sabie region of Mpumalanga (Cowie *et al*., 2016b, 2018; Olckers, 2011). Cold stress and moisture stress, experienced during winter, temperatures < 4 °C and humidity < 50 % restricted the establishment of *A. santacruzi* elsewhere in South Africa (Cowie *et al*., 2016b). This conclusion was supported by the field surveys conducted by Singh and Olckers, (2017) who found that the abundance of *A. santacruzi* decreased from the coast towards the inland regions of South Africa due to climate constraints, particularly low temperature and humidity. *Solanum mauritianum* is not the only invasive weed with restricted control due to climatic conditions. Other weeds such as *Solanum sisymbrifolium* (Solanaceae) (Byrne *et al.*, 2002) and *Pontederia crassipes* (Pontederiaceae) (Coetzee *et al*., 2007; Rogers *et al.*, 2021) have agents released on them which were restricted by climatic conditions, especially during winter. It is therefore important for biocontrol agents to have their thermo-physiological traits assessed in order to determine where they could possibly establish and perform to their optimum, or fail (Cowie *et al.*,2016b; Rogers *et al.*, 2021). It is also convenient to have the current and future biocontrol agents assessed using climate prediction models in order to predict the regions where they could potentially be restricted or established (Denslow and D'Antiono, 2005).

Another flowerbud feeding weevil, *Anthonomus morticinus* Clark (Coleoptera: Curculionidae) is being considered as a potential biocontrol agent for *S. mauritianum* because it is found at higher elevations and potentially colder, or more temperate regions of Argentina, Brazil (Pedrosa-Macedo *et al*., 2003) and Uruguay (Venter *et al*., 2021). The host specificity of *A. morticinus* is still under investigation and the agent has not yet been released. The climatic tolerance of *A. morticinus* ideally should be more suited to lower temperatures in the highveld regions of South Africa.

Winter temperatures often restrict the establishment of biocontrol agents as they accumulate cold stress over time (Coetzee *et al*., 2007). As a result, the released agents are unable to persist and overwinter on their target plants due to unfavorable conditions such as temperature (Coetzee *et al*., 2009). Although *A. santacruzi* and *S. mauritianum* both originate from the same native range, *S. mauritianum* invaded South Africa over a century ago and might have adapted to South Africa's climate conditions, while *A. santacruzi* establishment remains restricted to KZN and Sabie in Mpumalanga province (Cowie *et al*., 2016b). *Anthonomus morticinus* is likely to be

more adapted to cold temperatures than *A. santacruzi* and therefore, the lower thermal limits of *A. morticinus* were assessed. It was hypothesized that *A. morticinus* would display better cold tolerance than *A. santacruzi,* and therefore would be a more suitable biocontrol agent to establish further inland in South Africa, if it is sufficiently host specific to *S. mauritianum.*

4.2 Materials and Methods

Culture maintenance of the weevils.

Anthonomus morticinus weevils were field collected and imported from Uruguay (Latitude: -34,420128; Longitude -54,403067) in March 2020, and *A. santacruzi* weevils were collected from an established site in KZN, Pietermaritzburg (S29°33'20" E30°21'21") during January 2021. *Anthonomus santacruzi* was reared in quarantine prior to thermal experiments and only the weevils from the F_2 generation were used in the thermal trials. *Anthonomus morticinus* and *A. santacruzi* were maintained under the same conditions with room temperatures of T= 25.5 ± 0.4 °C and the average relative humidity of RH= 57 % and sustained on *S. mauritianum* floral and foliage material at the Wits University Insectary quarantine facility.

Climate parameters in localities invaded by *S. mauritianum*

The climate prediction software, CLIMEX DYMEX, version 4 (Maywald *et al.*, 2015) was used to extract climate data, and compare the overall percentage match between the known Uruguayan range of *A. morticinus* and the South African localities of *S. mauritianum* in the coastal, highveld, lowveld, midlands and the Western Cape (WC) regions. The mean minimum temperatures, mean annual rainfall, mean annual relative humidity, rainfall pattern and soil moisture are the climatic parameters that were compared between the Uruguayan *A. morticinus* range and the *S. mauritianum* localities in South Africa.

The mean minimum temperatures of the South African eastern coastal towns (Bashee, Cape Hermes, Cape St Lucia, Cape St Francis, Durban, East London, Great Fish Point, Mt Edgecombe and Port Shepstone), and localities in the highveld (Johannesburg and Pretoria), lowveld (Barberton, Lydenburg, Nelspruit, Pilgrims Rest and Pusella), midlands (Cedara, Dundee, Grootspruit, Ladysmith, Mistley, Newcastle, Paulpietersburg and Pietermaritzburg) and the WC (Cape Columbine, Danger Pt, Dasseneiland, Jonkershoek and Langebaanweg), were compared to

the Uruguayan collection range (Punta del Este, Rocha and Treina y Tres) by averaging the monthly data as done by Cowie *et al.*, (2016b).

Critical thermal minimum temperatures

For the measurement of thermal tolerances, the critical minimum (CT_{min}) temperatures of *A. morticinus* and *A. santacruzi* adults were assessed using a programmable water bath (Julabo F32-ME, Seelbach, Germany) as per Cowie *et al*., (2016b). Individual weevils were weighed using a mass balance before they were inserted into clear Eppendorf tubes which were submerged in a water bath set at 25 °C. Water was cooled at the rate of 0.25 °C per minute and the temperatures at which *A. morticinus* and *A. santacruzi* weevils failed to self-right and respond to a stimulus of a fine brush were recorded. Thereafter, the weevils were allowed to recover in petri-dishes for 72 hours. No data were recorded for individuals that immediately responded to the stimulus, as this indicated that they were not at their CT_{min}.

Low lethal temperatures

The same procedure was used in assessing the lower lethal temperature (LLT_{50} of *A. morticinus* and *A. santacruzi*). The results obtained by Cowie *et al*., (2016b) were used to select the starting target temperature for *A. morticinus* and *A. santacruzi*. According to Cowie *et al*., (2016b), 100 % of *A. santacruzi* weevils were unable to survive at –7 °C, therefore this temperature was selected as the starting target temperature for both *A. morticinus* and *A. santacruzi* respectively. Thereafter, the target temperatures were increased by 1 °C until 100 % survival of the weevils of both species was observed and decreased by 1 °C until 0 % survival was observed for both *A. morticinus* and *A. santacruzi*. The rate of cooling was 0.25 °C per minute from 25 °C for all target temperatures, and the weevils were kept for two hours at each target temperature. For *A. santacruzi*, 10 weevils were tested at each target temperature of -2, -3, -4, -5, -6, -7, -8 and -9 °C. For *A. morticinus*, 10 adult weevils were tested at each target temperature of -3, -4, -5, -6, -7, -8, -9, -10, -11, -12, -13, and -14 °C. After exposure, the weevils were removed from the Eppendorf tubes and individually placed inside a petri dish with fresh floral and leaf material of *S. mauritianum* at 25 °C for 72 hours to allow for recovery. The last two target temperatures at which 0 % and 100 % survival of *A. morticinus* and *A. santacruzi* were noted, and the experiments were concluded.

4.3 Data analysis

CLIMEX was used to extract the annual climatic meteorological data for the South African invaded range of *S. mauritianum* and the Uruguayan collection range of *A. morticinus*. The composite match indices were generated using the *Match Climates (Regional)* feature in CLIMEX. The mean minimum temperatures for each region of the *S. mauritianum* invaded range in South Africa were compared to the Uruguayan collection range (Punta del Este, Rocha and Treina y Tres) using one-way analysis of variance (ANOVA) with repeated measures, and a Tukey HSD post hoc was used to assess the statistical significance using R-studio software (Team R, 2020). A t-test was conducted to assess the difference in mass of the weevils used in the CT_{min} and LT_{50} trials, between *A. morticinus* and *A. santacruzi*. A probit analysis was conducted respectively for *A. morticinus* and *A. santacruzi* in order to estimate the lethal temperatures at which 50 % of the weevil population died. The lethal temperatures (LT_{50}) of *A. santacruzi* and *A. morticinus* were compared by means of a T-test, to assess which of the two *Anthonomus spp*. was more cold tolerant.

4.4 Results

Climate matching

Overall, the climatic parameters of the South African regions occupied by *S. mauritianum* (Table 4.1) had a mean match of 60% to the Uruguayan collection range of *A. morticinus* (Table 4.2). The east coast displayed the highest match of 65%, followed by a 63% match in the Western Cape region and 61% in the midland regions of *S. mauritianum*'s South African distribution (Table 4.2). The lowveld and the highveld had the lowest matches of 56% and 54% respectively. The South African eastern coastal region is significantly warmer than all other South African regions invaded by *S. mauritianum*, and significantly warmer than the Uruguayan collection sites of *A. morticinus* ($F_{5,6} = 37.43$; $P < 0.01$) (Figure 4.1). The mean minimum temperature of the Uruguayan collection sites was not significantly different to the mean minimum temperature of the highveld, lowveld, midlands and the Western Cape regions of *S. mauritianum* ($P > 0.05$).

Table 4.1: Comparison of climate parameters of the South African distribution of *Solanum mauritianum* with the collection sites of *Anthonomus morticinus* in Uruguay, South America.

Location	**Mean annual rainfall (mm)**	**Mean annual minimum temperatures (°C) (minimum/maximum)**	**Mean annual minimum relative humidity (%) (minimum/maximum)**	**Mean annual maximum relative humidity (%) (minimum/maximum)**
Uruguay collection range	1038	11.6 (7.1/16.4)	65.9 (59.7/70.9)	78.7 (71.9/84)
South Africa: Highveld	749	10.1 (3.5/14.9)	39.4 (29.1/50.6)	69.4 (59.1/76.6)
Midlands	846	10.2 (3.6/15.2)	52.0 (44.1/59.9)	61.1 (55.3/67.6)
Lowveld	835	11.9 (5.8/16.5)	49.9 (42.7/58.8)	77.2 (70.7/83.6)
East coast	960	15.9 (11.1/20)	70.9 (64.1/ 74.9)	78.9 (73.9/84.2)
Western Cape region	489	11.7 (9.0/14.3)	69.3 (65/72.8)	86.9 (81.3/90)

Table 4.2: The CLIMEX % match indices of the South African distribution of *Solanum mauritianum* with the Uruguayan collection range of *Anthonomus morticinus*. Superscript numbers represent the number of selected localities in each region.

South African sites of *Solanum mauritianum*	**Overall match**	**Soil moisture**	**Mean annual relative humidity**	**Mean annual rainfall**	**Rainfall pattern**	**Minimum temperature**	**Potential of establishment**
Highveld[2]	**54**	47	9	69	60	73	Less likely
Midlands[8]	**61**	43	29	79	61	75	Moderate (localized)
Lowveld[5]	**56**	44	22	77	57	72	Less likely
East coast[9]	**65**	62	40	82	76	55	Likely (abundant)
Western Cape region[5]	**63**	57	40	51	80	75	Moderate (localized)

Critical thermal minimum and lethal temperatures

The critical thermal minimal temperatures ranged from 1.2 °C to 3.0 °C and from 3.8 °C to 6.7 °C for *A. morticinus* and *A. santacruzi* respectively. The CT_{min} for *A. morticinus* was 1.71 ± 0.1 °C (*n*= 20) was significantly lower ($p< 0.001$) than that of *A. santacruzi* at 4.93 ± 0.18 °C (*n*= 20). The lethal temperature (LT_{50}) for *A. morticinus* was -9.5 °C (*n*= 10 per temperature class) (Figure 4.2), and for *A. santacruzi* the LT_{50} was -5.8 °C (*n*= 10 per temperature class) (Figure 4.3). The overall mass of *A. morticinus* and *A. santacruzi* weevils did not differ significantly ($p> 0.05$) at 1.54 ± 0.008 mg and 1.38 ± 0.005 mg respectively.

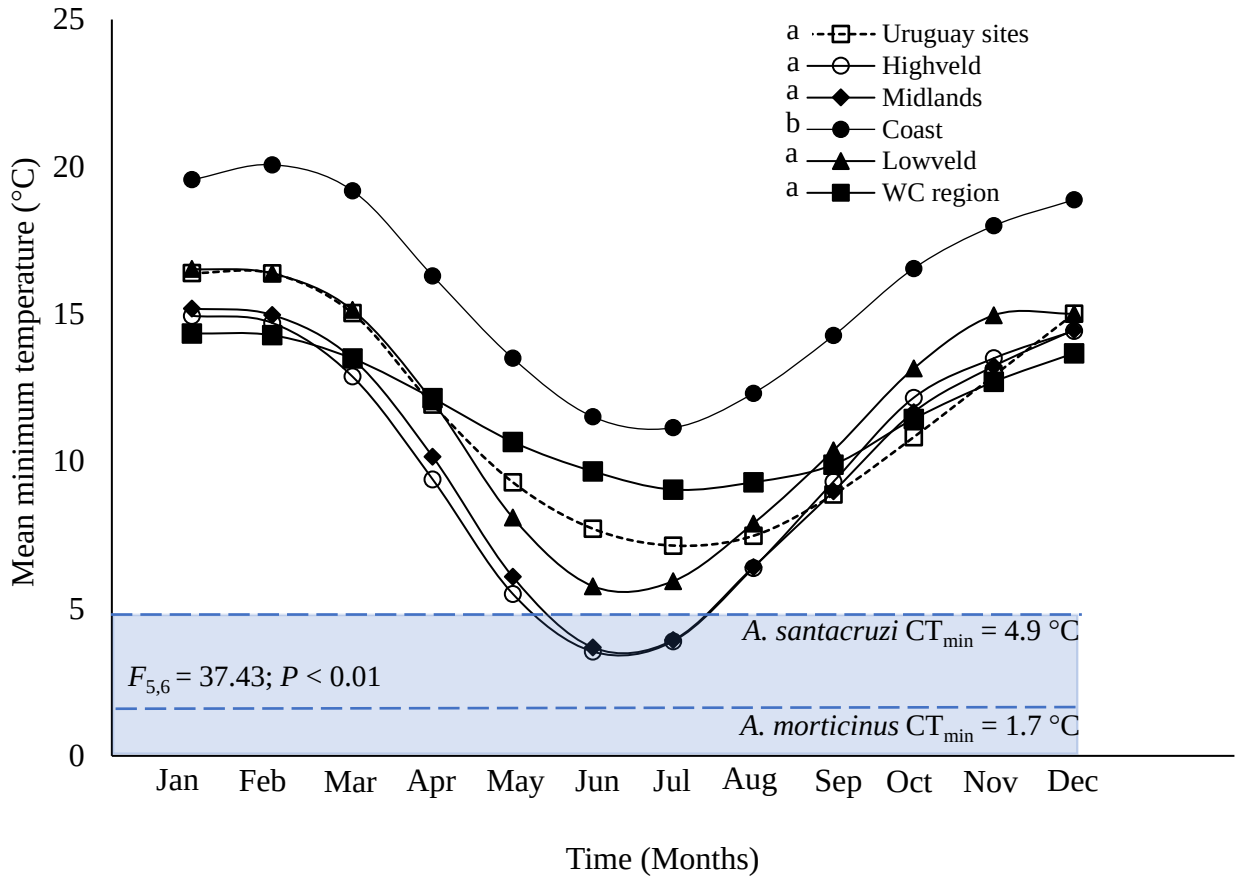


Figure 4.1. Mean minimum temperature for the Uruguayan collection range of *Anthonomus morticinus*, compared to selected South African localities of *Solanum mauritianum* on the coastal, highveld, lowveld, midlands and WC regions. The climatic data were generated from the CLIMEX software. Significant statistical differences in the mean minimum temperatures ($p < 0.05$) between localities is indicated by the lower-case letters before each legend on the graph. The overall difference of the mean minimum temperatures between localities is indicated by the *Fstat* and the *P-value* on the graph using repeated measures ANOVA. Temperatures below 4.9 °C and 1.7 °C as highlighted in the graph indicate the onset of cold stress for *A. santacruzi* and *A. morticinus* respectively.

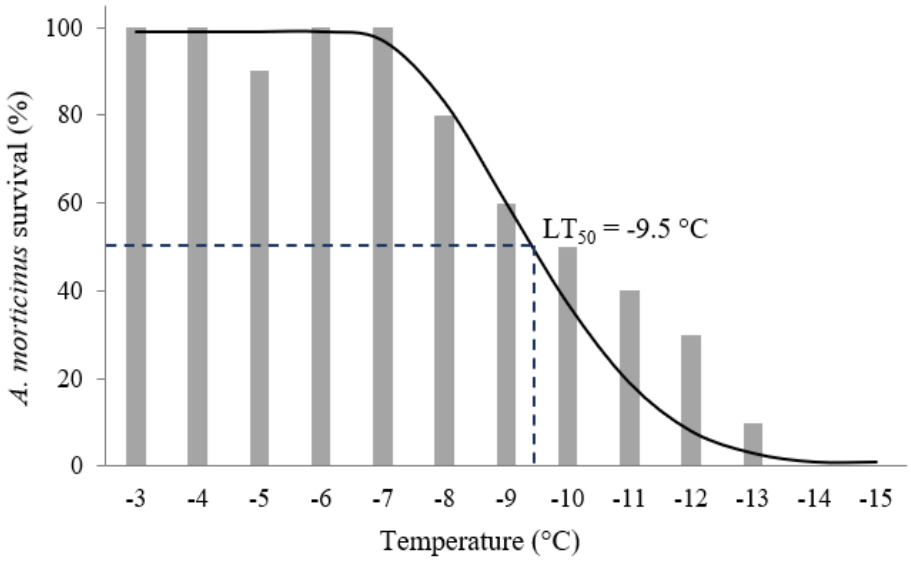


Figure 4.2. The survival of *Anthonomus morticinus* adults after being exposed for 2 hours to the following temperatures: -3, -4, -5, -6, -7, -8, -9, -10, -11, -12, -13, -14 and -15 °C. Ten adult weevils were used in each temperature class and allowed to recover for 72 hours. The survival data of *A. morticinus* were used in the Probit function to determine the (LT50). A total of n= 130 *A. morticinus* adults were used in this experiment.

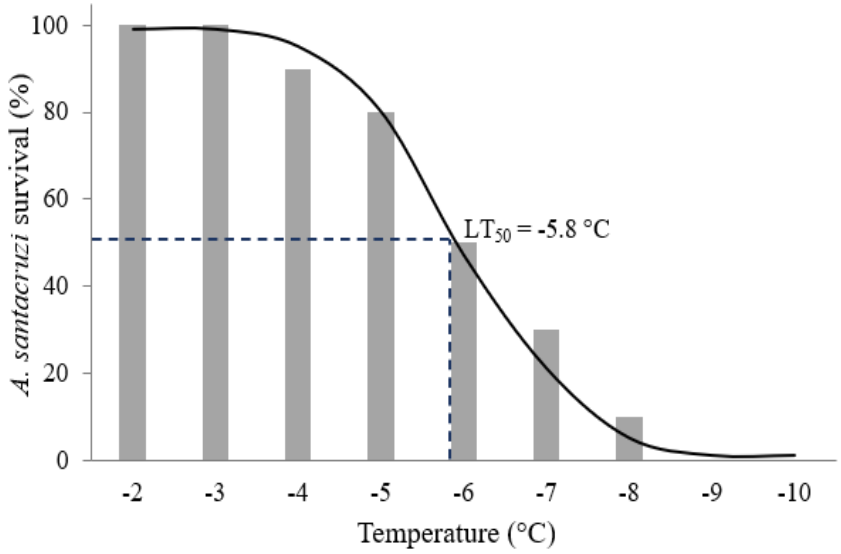


Figure 4.3. The survival of *Anthonomus santacruzi* adults after being exposed for 2 hours to the following temperatures: -2, -3, -4, -5, -6, -7, -8, -9 and -10. Ten adult weevils were used in each temperature class and allowed to recover for 72 hours. The survival data of *A. santacruzi* were used in the Probit function to determine the (LT50). A total of n= 90 *A. santacruzi* adults were used in this experiment.

4.5 Discussion

Environmental conditions play a significant role in the establishment and distribution of biocontrol agents (Byrne *et al*., 2002; Coetzee *et al*., 2007; Harms *et al*., 2021). *Anthonomus santacruzi* is currently abundant along the coastal region of KZN (Singh and Olckers, 2017), which has the best climate match of 72 % with its native range (Cowie *et al*., 2016b). *Anthonomus morticinus*' Uruguayan native range has the best overall match with *S. mauritianum's* distribution along the East coast of South Africa, followed by the WC region, the Midlands, Lowveld and highveld (Table 2). The potential of *A. morticinus* to establish in South Africa may vary from the coast to the highveld, due to colder temperatures and lower humidity with increasing altitude as observed with *A. santacruzi* (Cowie *et al*., 2016b).

In this study, laboratory reared *A. santacruzi* exhibited cold tolerance with a CT_{min} of 4.9 °C, which is similar to the figure obtained by Cowie *et al*., (2016b) of 4.1 °C for field caught specimens. The slight difference could be caused by using *A. santacruzi* weevils that were reared in the laboratory. *Anthonomus morticinus* with a CT_{min} of 1.7 °C displays a significantly better cold tolerance than *A. santacruzi*, which suggests that *A. morticinus* has a greater potential of establishing across a wider range of *S. mauritianum*, particularly within the inland regions of South Africa (Venter *et al*., 2021). Chill coma or cold stress accumulates in insects exposed to environmental temperatures that are equal to, or below their CT_{min} (Byrne *et al*.,2002; Cowie *et al*., 2016b; Sinclair *et al*., 2015). Therefore, temperature is less likely to restrict the establishment of *A. morticinus* in South Africa as mean minimum winter temperatures are warmer than 1.7 °C where *S. mauritianum* is found in South Africa, while *A. santacruzi* is likely to be restricted by winter mean minimum temperatures that are colder than 4.9 °C on the highveld and midlands. In addition, the lower lethal temperature of *A. morticinus* is 3.7 °C lower than that of *A. santacruzi*, suggesting that *A. morticinus* populations are less likely to die from cold stress when compared to *A. santacruzi* anywhere in South Africa.

Anthonomus santacruzi is likely to establish the WC region, with the mean minimum winter temperatures in this region being warmer than 4.9 °C (Cowie *et al*., 2018; Venter *et al*., 2021). *Anthonomus santacruzi* has been released in the WC region, but its establishment status remains unconfirmed (Fig. 3 of Cowie *et al*., 2018). Cold temperatures during winter not only

detrimentally affect the physiology of these two *Anthonomus spp.*, but also the physiology and flowering of *S. mauritianum* (Cowie *et al*., 2016a).

The photosynthesis of *S. mauritianum* is reduced when exposed to temperatures below 20 °C (Cowie *et al*., 2016a), which further reduces the plant quality and floral quality/quantity which both *A. morticinus* and *A. santacruzi* need for reproduction. Julien *et al*., (1996) noted that the temperature conditions to which a target weed, and its natural enemy/enemies are exposed to may affect their respective growth rates, reproductive rate, nutrient intake, and hence the rate at which the agent manages the weed population. This suggests that *A. morticinus* and *A. santacruzi* are likely to have reduced population size, lower oviposition, and longer developmental duration due to reduced *S. mauritianum* quality during winter. This is supported by English and Olckers, (2018) who found that adult *A. santacruzi* and larval abundance are both at peak when the floral production is at its highest in late summer, then decline during winter and steadily increase during the spring season in KwaZulu-Natal. The East coast and WC regions are relatively warmer than other regions in winter and are likely to have a relatively better quality of *S. mauritianum*, suggesting better establishment, survival and persistence of *A. morticinus* during winter in the WC region and the coast (Cowie *et al*., 2018; Venter *et al*., 2021).

Anthonomus morticinus is more cold tolerant compared to *A. santacruzi* and is less likely to accumulate cold stress during the winter season in South Africa. It is less likely for *A. morticinus* to be exposed to lethal thermal limits and experience cold stress in South Africa, and hence *A. morticinus* has an improved potential to control *S. mauritianum* further inland should the agent be cleared for release. Although it is less likely for *A. morticinus* to accumulate cold stress during winter in South Africa, a study that assesses the humidity impacts on the potential establishment of *A. morticinus* in South Africa is currently underway, because moisture stress also restricted the establishment of *A. santacruzi* as shown by Cowie *et al* (2016b). Future studies should consider assessing the fecundity of *A. morticinus* and *A. santacruzi* after being exposed to cooler temperatures, in order to determine the temperatures at which these *Anthonomus spp*. stop producing eggs and compare these temperatures to the minimum winter temperatures in all South African regions supporting *S. mauritianum*. This will provide accurate information about the South African distribution range of *S. mauritianum* where the agent(s) could potentially control or not control the weed.

Chapter 5: Synthesis and conclusion

5.1 Overview

Solanum mauritianum is a South American perennial tree that has devastating impacts in regions outside its native range (Henderson, 2020). The weed's seed dispersal is rapid because it is facilitated by frugivorous birds over long distances (Olckers, 2009). As a result, the distribution of *S. mauritianum* has expanded in regions outside its native range such as South Africa, New Zealand, and other tropical and sub-tropical regions in the world (Cowie *et al*., 2018). The use of herbicides to manage the weed has been ineffective and expensive (Mtetwa, 2019), with mechanical control posing a health hazard to the workers (Cowie *et al*., 2018). The biocontrol of this weed was launched in South Africa over 35 years ago (Olckers, 2009). Of all the tested candidates so far, only two agents namely *Anthonomus santacruzi* and *Gargaphia decoris*, have been released to manage the spread of *S. mauritianum* (Olckers, 2009; Hakizimana, 2011; Olckers and Borea, 2009).

Anthonomus santacruzi is a florivorous weevil that reduces the quantity of seeds produced by *S. mauritianum* (Olckers, 2003). The weevil was regarded as the most promising agent relative to all the candidates that were tested since the biocontrol efforts of *S. mauritianum* commenced, because of its life cycle on *S. mauritianum* (Olckers, 2009; Hakizimana, 2011). Due to the climate constraints experienced by *A. santacruzi* in South Africa (Cowie *et al*., 2016b), *A. santacruzi* has established in the coastal region of KZN where it reduces the seed recruitment of the weed (Mkhize and Olckers, 2019), and is localized to the Sabie region in Mpumalanga (Cowie *et al*., 2018). As a result, *A. santacruzi* causes limited damage to the weed in South Africa (Henderson, 2020) and this advocates for testing of a new, cold adapted agent that may control *S. mauritianum* in regions where *A. santacruzi* failed to establish. *Anthonomus morticinus* was imported from the cold regions in Uruguay and reared in quarantine for host specificity testing. The biological relationship between *A. morticinus* and *S. mauritianum*, particularly the fecundity of *A. morticinus* was assessed. Host specificity testing of *A. morticinus* on agricultural Solanaceae was done. Lastly, the thermal tolerance of *A. morticinus* was assessed and the climate model (CLIMEX) was used to predict the potential of the weevil establishing in South Africa if it were to be released. This study adds to the biocontrol efforts of *S. mauritianum*

in South Africa. This final chapter will highlight the overall findings, and the potential of *A. morticinus* establishing in South Africa if it were to be released.

5.2 Biology and fecundity of *A. morticinus*

Anthonomus morticinus naturally occurs on *S. mauritianum* in South America (Pedrosa-Macedo *et al.,* 2003; Venter *et al*., 2021), and is not found on any other Solanaceae plants (Clark and Burke, 1996). For both survival and reproductive success, *A. morticinus* relies on *S. mauritianum* as its host (Chapter 2), as observed with *A. santacruzi* in quarantine (Olckers, 2003), and after it was released in the field (Cowie *et al*., 2018; Hakizimana, 2011; Olckers, 2009). These *Anthonomus spp.* are morphologically similar, except for the number and position of scales that are found on their elytra (Clarke and Burke, 1996). The two *Anthonomus spp.* exhibit similar reproductive cycles and duration of development on *S. mauritianum. Anthonomus morticinus* and *A. santacruzi* coexist on *S. mauritianum* in South America, and result in low fruiting of the weed in its native range (Olckers, 2009). *Anthonomus morticinus* has a high fecundity on *S. mauritianum* and can persist on shoot tips and foliage even when floral material is absent during the winter season. This suggests that *A. morticinus* could improve the control of *S. mauritianum* along with *A. santacruzi* in South Africa if it is sufficiently host specific to the weed.

5.3 Host specificity and risk analysis of *Anthonomus morticinus*

In weed biocontrol, host specificity tests on candidate agents are prioritized for the safety of indigenous and cultivated plants related to the target weed (McFayden, 1998). These tests are conducted to prevent unwanted non-target attacks, and their results are used to formulate the risk analysis that predicts the probability of non-target impacts in the environment after biocontrol agents are released (see Baars *et al*., 2003; Hakizimana and Olckers, 2013; McConnachie, 2015; Olckers, 2003). The agents of *S. mauritianum* were released because they showed acceptable levels of host specificity, which were accompanied with low risks of non-target attacks (Venter *et al*., 2021). The agents established in localized invaded sites of *S. mauritianum* in South Africa, with *A. santacruzi* having established a wider range in the coastal region of KZN, a few sites in Sabie and some parts of the Eastern Cape (Nic Venter pers. comm.). So far, the two agents released for the past 14 to 22 years have not controlled *S. mauritianum* sufficiently due to the ecological and environmental challenges the agents experienced in the field. *Anthonomus*

*santacruzi'*s establishment is limited by climate constraints (Cowie *et al.,* 2016b; Singh and Olckers, 2017), and *Gargaphia decoris* is preyed on by ants (Cowie *et al.,* 2018). *Anthonomus morticinus* is a candidate agent collected from Uruguay, South America, was tested for host specificity and a risk analysis was conducted thereafter to assess if the candidate was a suitable for release (Chapter 3).

No-choice tests (floral and non-floral) and paired choice tests (floral) on *A. morticinus* were conducted using agricultural Solanaceae. The results showed that *A. morticinus* fed and developed more on *S. mauritianum* than all tested plants, particularly the *Solanum* plants during no-choice trials. Feeding and oviposition by *A. morticinus* was absent in Solanaceae outside the *Solanum* genus, as observed with *A. santacruzi* during the no-choice tests (see Olckers, 2003; Hakizimana, 2011). *Anthonomus morticinus* showed a consistent preference for *S. mauritianum* during paired choice tests, with more adults developing on *S. mauritianum* and higher feeding intensity observed on the weed than any other test plants during the trials. The risks of non-target feeding were high (> 5%) on four of the five *S. melongena* varieties (BB, BK, LF and VM) during paired choice tests, while *S. melongena* (BKF1) exhibited low risks (< 2%). The reproductive risks of *A. morticinus* were low in the above four mentioned *S. melongena* varieties, and *S. melongena* (BKF1) showed no reproductive risk. The agricultural Solanaceae are of economic significance and a risk analysis showed that the probability of *A. morticinus* developing in these Solanaceae plants in the natural environment are low. The released agent, *A. santacruzi,* also exhibited non-target impacts in quarantine and after the agent was released it has only persisted on *S. mauritianum*, with no reported non-target attacks in the field (Cowie *et al*., 2018). More tests are currently being conducted on the indigenous Solanaceae plants and so far, the results are promising. *Anthonomus morticinus* may be a suitable agent of *S. mauritianum* and should be considered for release pending outstanding host specificity trials, to manage the spread of the weed in South Africa.

5.4 Thermal tolerance of *Anthonomus morticinus*

Climate matching of the native regions of invasive weeds with their biocontrol agents, and the regions where these weeds have invaded is important to predict the range of establishment of potential biocontrol agents (Harms *et al.,* 2021). Temperature is one of the important climatic parameters that impact an agent's distribution (Byrne *et al*., 2002; Cowie *et al*., 2016b; Harms *et*

al., 2021). Biocontrol agents are observed to be abundant, survive and reproduce optimally on their target weeds when temperature conditions are favorable, hence the physiological performance of agents in introduced regions is affected by their thermal tolerance (Porter *et al*., 2019). The range of establishment of candidate agents in invaded regions could be improved when the temperature conditions of the invaded range matches best with the native range of candidate agents (Harms *et al*., 2021). Temperature mismatches, and lack of thermal tolerance have resulted in the agent of *S. mauritianum, Anthonomus santacruzi*, failing to widely establish and hence control the weed in the inland regions of South Africa (Cowie *et al*., 2016b). *Anthonomus morticinus* was collected at more temperate sites in Uruguay and its thermal tolerance was assessed and compared to *A. santacruzi* collected from the established sites in KZN (Chapter 4).

Anthonomus morticinus is more cold tolerant than *A. santacruzi* and is less likely to experience cold stress anywhere in South Africa. A study by Porter *et al*., (2019) compared the thermal limits between *Eccritotarsus catarinensis* (Hemiptera: Miridae) reared in a laboratory, and from one of the colder weed sites in Kubusi River 23 years after release. Populations of *Eccritotarsus catarinensis* from Kubusi River were more cold tolerant than laboratory populations. Porter *et al*., (2019) proposed that the agent be released in the inland South African regions where other agents failed to establish on water hyacinth. Therefore, *A. morticinus* should be considered for release to manage *S. mauritianum* in the inland regions of South Africa. Furthermore, the lethal limits of *A. morticinus* being lower than the lethal limits of *A. santacruzi* suggest that the candidate agent may overwinter in the inland regions of *S. mauritianum* where *A. santacruzi* has failed to establish. Both *A. morticinus* and *A. santacruzi* could improve the control of *S. mauritianum* more effectively in the field because these *Anthonomus spp.* should coexist on *S. mauritianum* in South Africa if *A. morticinus* is released.

The climate of the native range of *A. morticinus* in Uruguay has the best match with the east coast of South Africa, and the lowest match with the Highveld. This suggests that *A. morticinus* will be most abundant on the east coast, followed by the Western Cape region and Midlands. The Lowveld and the Highveld regions will likely have the least abundance of *A. morticinus*. Other climate parameters such as the relative humidity are poorly matched in the midlands, lowveld and the highveld, which reduced the overall match in these regions. *Anthonomus morticinus* does

not experience death at low humidity, but instead goes into reproductive diapause (Yaron Keizan's pers. comm.). The weevils seek refuge in the curled leaves which contain a higher relative humidity than the surrounding environment (Yaron Keizan's pers. comm.). This suggests that although the relative humidity is poorly matched in the midlands, lowveld and the highveld, *A. morticinus* may have seasonal outbreaks during the rainy seasons where relative humidity in the environment is favorable for its reproductive demands in these regions.

5.5 Conclusion and recommendations

The release of *A. morticinus* as a biocontrol agent of *S. mauritianum* should be considered, as the weevil appears sufficiently host specific on to the weed. All the potentially vulnerable *Solanum* test plants should undergo multichoice large cage trials with *A. morticinus,* because the results will likely reduce the overestimation of non-target effects as seen in the results with small cages in this study. The phytochemistry of Solanaceae plants should be assessed and compared with the phytochemistry of *S. mauritianum.* This will give an indication of the preferred biological compounds in *S. mauritianum* relative to the non-target Solanaceae plants and may highlight the possible reason as to why the non-*Solanum* plants were not attacked by *A. morticinus* as compared to the *Solanum* plants. Although temperature is less likely to inhibit the establishment of *A. morticinus* because the CT_{min} of *A. morticinus* is lower than the lowest winter temperatures in South Africa where *S. mauritianum* is found, humidity is also an important factor to consider since it plays an important role in the development of insects (Cowie *et al*., 2016b). The cold tolerance of *A. morticinus* suggests that the candidate agent could potentially establish further inland, and hence control *S. mauritianum* where *A. santacruzi* could not establish. The humidity studies are currently ongoing on *A. morticinus*, and future studies should consider assessing the reproductive output of *A. morticinus* and *A. santacruzi* on *S. mauritianum* at low temperatures. This will give more accurate results on temperatures at which *A. morticinus* and *A. santacruzi* stop producing larvae, and hence stop controlling *S. mauritianum*. The biology assessment, cold tolerance and host specificity results all suggest that *A. morticinus* may overwinter further inland in South Africa and improve the biocontrol of *S. mauritianum*.

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