Assessing the climatic suitability and environmental responses of *Anthonomus morticinus* Clark (Coleoptera: Curculionidae), a potential biological control agent of *Solanum mauritianum* Scopoli (Solanaceae) in South Africa

by

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Declaration

I, Yaron Yakov Keizan, 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: 1 October 2024

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Chapter 1: Problem statement, general introduction, and rationale of the study

1.1 Problem statement

Arthropod biological control agents have long been recognized to offer a sustainable alternative way to control alien invasive plant species (Smith, 1919). Critical to the success of these initiatives is the relationship between the physiological attributes of the agent and the environments of the regions into which they are introduced (Cowie *et al*., 2016b; Huffaker and Messenger, 1976; Van Driesche *et al*., 2009). So crucial is this relationship that close to half of all such initiatives are limited as a result of the climatic unsuitability of the agent to the locations in which they are released (Harms *et al*., 2020; McEvoy and Coombs, 2001; Stiling, 1993). Despite this, climatic unsuitability is largely overlooked in biocontrol programs due to the complexities it presents. Illustrative of this is the performance of the weevil *Anthonomus santacruzi* Hustache (Coleoptera: Curculionidae), a flower-bud feeder native to South America introduced as a biological control agent in South Africa to manage *Solanum mauritianum* Scopoli (Solanaceae) (Olckers, 2003; Klein, 2011). Since its release in 2008, the weevil has shown a restricted distribution largely limited to the east coast of South Africa with continual failure of establishment throughout the inland regions, despite repeated releases. Post-release surveys have confirmed that the colder climate of the South African highveld and inland regions prevents the successful establishment of *A. santacruzi* (Cowie *et al*., 2016a; Singh and Olckers, 2017). The closely related *Anthonomus morticinus* Clark (Coleoptera: Curculionidae) is therefore currently being investigated as a putative biological control agent for release against *S. mauritianum* (Cowie *et al*., 2018). *Anthonomus morticinus* has been observed at cooler and drier sites in South America relative to that of *A. santacruzi*, generating optimism for its climatic suitability to the regions of South Africa where *A. santacruzi* has failed to establish (Pedrosa-Macedo *et al*., 2003; Venter *et al*., 2021). However, the climatic limits of *A. morticinus* remain unknown and therefore it cannot be concluded that the weevil will achieve much greater coverage than its predecessor.

Therefore, based upon the colder native range distribution of *A. morticinus* relative to *A. santacruzi*, this study aims to answer the hypothesis that *A. morticinus* should occupy a broader geographic range in South Africa than that of the observed range of *A. santacruzi* in South

Africa. Based upon previous research (Cowie *et al*., 2016b), relative humidity (RH) was shown as a contributing factor to the climatic unsuitability of *A. santacruzi* in South Africa. These tests did not, however, include the examination of how *A. santacruzi* would interact with the microclimate of the plant which is the main realized niche in terms of humidity. Therefore, the relationships that exist between the physiology and reproductive behaviours of *A. morticinus* at varying environmental humidities were explored to determine its realized relative humidity niche and whether low RH will negatively impact its distribution in South Africa. The research presented here adds useful information to the understanding of the biocontrol of *S. mauritianum* in South Africa, with potential application to control of the weed worldwide. Moreover, the methods used in this dissertation present a Species Distribution Modelling (SDM) framework novel in biological control, utilizing the Ensemble of Small Models (ESM) technique to predict the potential range of putative biocontrol agents with limited occurrence datasets.

1.2 Introduction

'Biological invasion' describes the phenomenon where a particular organism gains a competitive edge after the removal of a limitation to its distribution, leading to rapid growth of the species, often in a new and non-native ecosystem (Simberloff *et al*., 2013). Often, the alien invasive organism's population becomes widespread in the non-native regions where it establishes (Valéry *et al*., 2008). These events drive ecological changes in the introduced environments, typically in a negative manner. The introduced species are often able to outcompete native fauna and flora for resources leading to the loss of biodiversity and animal and plant communities becoming homogeneous (Olden *et al*., 2011). Moreover, the spread of the alien species comes with costs to natural resources such as water and fertile land (Carneiro *et al*., 2024). Additionally, the economy can be negatively affected by invasive species. The cost resulting from invasive alien species has exceeded $423 billion since 2019, with costs having quadrupled each decade since 1970. These expenses primarily stem from the damage invasive species impose on the environment and human activities, with 92% of costs attributed to such damages and only 8% to management efforts (IPBES 2023).

1.2.1 Alien invasive plants

Alien invasive plant species present a serious danger to the biodiversity of the ecosystems that they invade (van Wilgen *et al*., 2020). Furthermore, these weeds may impact the economy through damage to agricultural land, infrastructure or by driving water loss (De Lange and van Wilgen, 2010; Wilson *et al*., 2009). Global invasions of many of these plant species has been the result of the human trade and movement, transporting alien plant species either intentionally, such as for ornamental use, or accidentally such as contaminants in animal feed (Hill *et al*., 2020; Wilson *et al*., 2009). Once introduced, some plants can rapidly establish and proliferate in the novel ecosystem where their natural enemies are absent (Hill *et al*., 2020). In South Africa alone, it is estimated that over 1400 alien plant species occur, with about 180 of these species covering more than ten million hectares in the country (Versfeld *et al*.,1998). The management of these plants costs the country approximately two billion ZAR each year, a figure which is expected to grow with the adverse effects of global climate change and increased globalisation (van Wilgen *et al*., 2020).

1.2.2 Management of alien invasive plant species

The control of invasive alien plants may take various forms that can be used in conjunction for a more integrated type of management. One form of management is through the physical removal of weeds either by hand or using machinery, known as mechanical control (Culliney, 2005). While this technique may prove effective in the short-term, such operations are often expensive and labour intensive. Moreover, the plant is often able to reinvade the cleared space making this technique ineffective in the long-term (Witkowski and Garner, 2008). Another form of control is through the use of chemicals such as herbicides (Hill *et al*., 2020a). The application of such chemicals can be done effectively and is a suitable technique for blanketing larger areas in a shorter period of time. However, this method is often associated with high costs, requiring repeated applications which demand growing investment of logistical and monetary resources. Moreover, the chemical application may also affect non-target species in the. Furthermore, once the site has been controlled, reinvasion of the target plant is again possible.

Another form of control is biological control, otherwise known as biocontrol. The classical definition of biocontrol is using of one or more organisms to control the population of a target undesirable species. This typically involves the use of ‘natural enemies’ of the target species which are usually found in its original native ecosystem and are released into the invaded regions

to manage the populations of the target organism (Baars, 2022; Smith, 1919). Many classical biological control programs utilize arthropod species as biological control agents to control alien invasive plant populations (Hajek *et al*., 2016). Although not necessary for the region of concern to resemble the native habitat of the natural enemy being introduced, it is important that climatic conditions meet the agent's requirements to allow it to establish and subsequently control its host (Boivin *et al*., 2006; De Cal *et al*., 2020; Harms *et al*., 2021). In addition, it is typical that in the native range of the invasive organism, there is a multitude of natural enemy species of the target species. Therefore, to identify which of these potential agents to introduce to achieve the highest levels of success, the following criteria should be met: (1) the agent should be suitably host-specific as to not pose a risk to non-target species in the area of introduction, (2) its lifecycle is synchronous with the target species and it can increase its population densities when the host does, (3) it only requires a minimal presence of the host to complete its life cycle, (4) it is able to search for the host species and (5) the agent is able to establish and persist within the area where it is introduced (Coetzee *et al*., 2009; Murdoch *et al*., 1985). The necessity that the agent is host specific is of utmost importance as generalist species are typically not synchronous with the host species (Murdoch *et al*., 1985) and, more importantly, an introduced agent should not damage or limit native or agricultural flora (Howarth, 1991). Furthermore, the agent must be capable to persist and reproduce at the environmental conditions of the ecosystems where it is introduced and be able to inflict significant damage to the target species (Coetzee *et al*., 2009; Cowie *et al*., 2016b).

1.2.3 Factors influencing the success of biological control agents

As biocontrol removes the necessity for environmentally damaging activities such as large-scale clearances and harmful chemical applications, this technique offers a sustainable method for managing alien plant populations within invaded ranges. However, it is subject to a group of limitations which may impact the success of biocontrol projects (Hoelmer and Kirk, 2005). For instance, a lack of taxonomic knowledge of plant or agent species may lead to the failure of the agent to manage target weed populations (Rosen, 1986). This may result from confusion with cryptic species, incorrect species identification or unforeseen genetic differences within and between populations of target host or agent species (Fernando and Walter, 1997). Furthermore, biotic factors affecting agent populations such as biotype selection or pressure from predation

may drastically influence the effectiveness of species to manage target plant populations (Lotter, 2004; Olckers, 2009). However, the main factor influencing the viability of biological control programs is the climatic suitability of the released agent to the environment in which it is released (Stiling, 1993). Hoelmer and Kirk (2005) discussed biological control efforts related to *Aphis glycines* Matsumura (Hemiptera: Aphididae), the soybean aphid, in North America using a number of insect parasitoids, emphasizing that inadequate climate matching may lead to the failure of these agents to establish. The paper cautions against sourcing biocontrol agents from a region with a significantly different climate to that of the area intended for release which may result in the inability of the agent to effectively establish and reproduce. In fact, climatic unsuitability is responsible for the failure of close to half of all arthropod related biocontrol initiatives (Barrat *et al*., 2018; Hoelmer and Kirk, 2005). With high costs in the pre-release processing of candidate agents, failed initiatives can represent a substantial waste of these precious assets (Byrne *et al*., 2002). Therefore, it is crucial that the climatic suitability of an agent be predicted before any large-scale investment in the species. This remains true for any agent, even where the introduced and native ranges share similar latitudes because the two regions differ in climate (Cowie *et al*., 2018).

1.2.4 Solanum mauritianum

The genus *Solanum* contains many alien invasive species of plant recognized around the world. *Solanum mauritianum*, colloquially known as bugweed in South Africa, or woolly nightshade in Australasia, is native to regions of southern Brazil, northern Argentina, Uruguay and Paraguay and has become established in many countries either intentionally as a decorative plant, or accidently by colonists (Olckers, 2009; Roe, 1972). The plant has become established and reached invasive status in more than 15 countries worldwide including several Asian and African countries, The United States, Australia, New Zealand and islands in Oceania (Olckers, 2011; ISSG, 2006). The most severe invasion of bugweed has taken place in South Africa, where the weed now ranges over 80 500 hectares (ha), primarily in the eastern higher rainfall areas (Marais *et al*., 2004) (Refer to figure 3.3). The plant has caused significant damage to agricultural and forestry, freshwater ecosystems, and conservation areas of the country (Olckers, 2009).

Solanum mauritianum is a small to intermediate broadleaf perennial tree capable of reaching about four meters in height but can start flowering at about 1.5 meters (Witkowski and Garner,

2008). Its leaves and stems are shielded in dull white trichomes that give it a hair-like feel. Small, purple flowers eventually turn to bunches of small, green berries that ripen to become yellowish in colour (Olckers, 2009). The biology and reproductive ability of the plant predisposes it to invasion as it is able to produce large quantities of fruits and seeds year-round (Olckers and Zimmermann, 1991). Moreover, pollinators of the plant are typically generalist species such as bees and the fruits are eaten by many frugivorous birds which exploit the large number of fruits on each tree (Jordaan *et al*., 2011). Furthermore, *S. mauritianum* has a rapid growth rate, able to reach several meters in height within two or three years and a short generation time with individual plants living for around 15 years (Olckers, 2011). Germination of seeds can be triggered by many disturbances like fire, floods or from clearing operations. Many attempts to clear the species, whether mechanical or chemical, have typically only aggravated the invasion of the plant (Witkowski and Garner, 2008). Bugweed will aggressively invade disturbed areas such as agricultural land and forestry plantations as well as urban and semi-urban regions. *Solanum mauritianum* is considered a 'transformer species' in South African weed legislation (Henderson, 2001) as it is able to dominate sub-canopy and canopy levels in natural and semi-natural areas where it shades out other plants and seedlings in plantations, causing harm to the functioning and stability of the ecosystem (Henderson, 2001).

1.2.5 Current status of biological control of *S. mauritianum* in South Africa

Currently, biocontrol is being reconsidered as a sustainable solution to control *S. mauritianum* in South Africa (Mtetwa, 2019). Attempts to find effective biocontrol agents for bugweed in South Africa began in 1984 and despite the project being suspended in the early 1990s, efforts resumed in 1994 (Olckers *et al*., 2002). Surveys of the natural enemies of *S. mauritianum* occurred within its native range of southern Brazil, northern Argentina, and Paraguay (Roe, 1972). Although over 80 insect species have been identified from these surveys only five species were considered suitably host-specific for further consideration (Pedrosa-Macedo *et al*., 2003). Of the suitable species, two have since been released against bugweed, with another undergoing trials (Olckers, 2011; Venter *et al*., 2021).

The primary agent introduced in South Africa against bugweed was the lace-bug *Gargaphia decoris* Drake (Hemiptera: Tingidae). Females of this species lay large egg-batches underneath the leaves. Nymphs hatch and feed together with the adults on the leaf sap leading to chlorosis

and early leaf abscission (Lotter, 2004; Olckers, 2000). Through no choice trials, *G. decoris* was observed consuming a few native *Solanum* species as well as eggplant, *Solanum melongena* L. (Solanaceae). However, it was shown that this was due to an expansion of the insect's host range and would not be significant in the field as *G. decoris* showed a strong affiliation to *S. mauritianum* over the other species presented during choice tests (Olckers and Lotter, 2004). Post release studies show that despite releases of over a million individuals, the influence of *G. decoris* has been ineffective on populations of *S. mauritianum* (Klein, 2011). It is suspected that predation from generalist predators as well as the preference of *G. decoris* for more shaded areas could be among the reasons for these results (Patrick and Olckers, 2014). Despite *G. decoris* not being the most effective agent against the plant in South Africa, its release is a step in the right direction for building a complex of species for biocontrol of *S. mauritianum*. Due to the successful introduction of the lace bug in South Africa, New Zealand began its own biological control attempts against *S. mauritianum* with the eventual release of, *G. decoris* in the country at the end of 2010 (Withers *et al*., 2002; Winks, 2014). While the species is expected to have a wide distribution in New Zealand (Falla *et al*., 2021), the insects' preference for shaded and low UV conditions may drastically limit its effectiveness (Falla *et al*., 2023).

The second biocontrol agent which was released in 2008 to manage *S. mauritianum* in South Africa is the flower bud-eating weevil *Anthonomus santacruzi* (Olckers, 2011). The adults mainly eat the shoots and the floral material, while the females oviposit into new buds. After the larvae have hatched, they eat the inside of the bud thereby destroying it as they mature (Olckers, 2003). The feeding and reproduction of *A. santacruzi* reduces the ability of bugweed to flower and produce seeds (Cowie *et al*., 2017). The initial host specificity trials revealed that the weevils accepted native *Solanum* species throughout the no choice experiments which gave rise for concern, however during choice tests it showed a continuous affinity for *S. mauritianum* (Hakizimana, 2011). Further studies were conducted to test if the weevil would be suitable for release in New Zealand, which determined that while *A. santacruzi* continually shows preference for *S. mauritianum* it did also feed and oviposit on the New Zealand native *S. aviculare* (Hakizimana and Olckers, 2013). Due to this, *A. sanatcruzi* was determined not to be fit for bugweed control in New Zealand (Cowie *et al*., 2018). However, the effectiveness of *A. santacruzi* in South Africa is still unknown (Cowie *et al*., 2018). It appears that a restricting factor for the species success is the climate, primarily colder temperatures (< 4°C) and dry

relative humidity levels (<50%) (Cowie *et al*., 2016b). These apparent constraints were further supported by Singh and Olckers (2017) who observed that *A. santacruzi* were found in greater numbers at lower altitudes in South Africa and decreased in abundance further inland despite the availability of the host.

Anthonomus morticinus Clarke is also a species of flower bud-feeding weevil found overlapping in distribution with *A. santacruzi* within its native range (Olckers, 2003). The biology and reproductive strategies of the weevil resemble those of *A. santacruzi* as well as being similar in appearance, but *A. morticinus* is slightly larger. Although the two species occur in similar regions and habitats, *A. morticinus* has been recorded at inland and at higher altitude sites in Brazil (Pedrosa-Macedo *et al*., 2003) suggesting this species may survive and establish populations in areas with lower temperatures and humidity than *A. santacruzi* (Refer to figure 3.1 for native range occurrences). Therefore, *A. morticinus* may be a better agent against *S. mauritianum* in the more inland and cooler regions of South (Cowie *et al*., 2017).

Climatic suitability of biocontrol agents to the conditions present at release sites is influential in determining the agent's efficacy at controlling the populations of the target plant. Agents sourced from regions climatically similar to those intended for release tend to offer greater chances for effective management (Hoelmer and Kirk, 2005). Climate matching approaches offer valuable insight, revealing areas within the weed's native range where climatically suitable agents are to be expected (Robertson *et al*., 2008). For instance, Byrne *et al* (2002) investigated the effect of the South African climate on the establishment of the tortoise beetle *Gratiana spadicea*, a biocontrol agent against *Solanum sisymbriifolium*. While the beetle has successfully established throughout much of the country, it appears unable to persist within the cooler and drier Highveld of the Mpumalanga province. *Gratiana spadicea* individuals released in South Africa had been sourced from Argentinian populations at lower altitudes with warmer and more humid conditions. Climate matching analyses revealed that the Highveld is significantly less humid and subject to colder winters than the conditions where the insect was collected. Thus, *G. spadicea* populations may not be adapted to the conditions of the Highveld. The study further suggested that had climate matching been performed prior to the acquiring the agent, surveys could have focused on sourcing agents from regions more climatically similar to the Highveld.

To determine whether *A. morticinus* is expected to have a greater range in South Africa than *A. santacruzi*, the climatic factors affecting *A. morticinus* need to be determined. A niche is classified as the parameters within which a species can persist and breed successfully (Begon *et al*., 1986). The principle that each species has its own niche is useful for understanding the capacity of a species to establish and invade novel ecosystems and define factors that would limit its ability to spread (Chase and Leibold, 2003). This concept can further be broken down into two categories, the fundamental niche and the realized niche. The fundamental niche consists of the measurable abiotic factors and resources that determine the survival and growth of the species, while a realized niche also includes biotic factors such as predation, parasitism, interspecific competition and the response of the insect to the microclimates present on the host plant (Hutchinson, 1957). Thus, if the fundamental niche of *A. morticinus* were to show a greater tolerance to colder temperatures and lower humidity levels than *A. santacruzi* it could be a suitable biocontrol agent of bugweed in cooler environments in South Africa.

Although *A. morticinus* is anticipated to exhibit a wider possible range in South Africa compared to *A. santacruzi*, the effect of climatic conditions in the field on its population growth remains unknown. The restricted range of *A. santacruzi* in South Africa, particularly its failure on the Gauteng highveld and other cooler regions, emphasise the influence of climatic suitability on the efficacy of biocontrol initiatives (Cowie *et al*., 2018; Singh and Olckers, 2017). Therefore, estimating the potential range of *A. morticinus* before further testing is conducted is of importance.

1.2.6 Species Distribution Modelling

Species Distribution Modelling (SDM) is a valuable means for understanding the role of environmental conditions in insect distributions (Guisan and Zimmerman, 2000). These statistical models are generally employed in the fields of ecology and conservation biology to predict the probability of a species' occurrence within a defined geographic area based on climatic similarities to that of the species' native range. The models integrate species-related data with environmental variables to produce maps indicating where a species is likely to exist and the habitats suitable for supporting its populations (Buckley *et al*., 2010; Elith and Leathwick, 2009). In biological control, SDMs prove useful for estimating the potential distribution of the introduced natural enemies aimed at controlling the target species as done for *A. santacruzi* by

Cowie *et al*. (2016b) and for *G. decoris* by Falla *et al*. (2021). There are multiple techniques available to conduct an SDM, but the method chosen is typically based on the kind of data available. Mechanistic SDMs for example, use known physiological and ecological parameters, typically collected from laboratory or field experiments, to characterize the species' fundamental niche (Evans *et al*., 2015). For biological control agents which have undergone formal testing to determine their ecophysiological parameters, mechanistic SDMs offer valuable information detailing the species predicted distribution (Kearney and Porter, 2009). The CLIMEX ecoclimatic modelling software (Hearne, Scientific Software Pty Ltd, Australia) has successfully utilized this modelling approach to estimate the ranges of introduced species or to predict how a species future range may shift with climate change (Sutherst *et al*., 1995; Sutherst and Maywald, 1985). Alternatively, correlative SDMs use statistical frameworks for estimating a species range by identifying climatic patterns common across the species occurrence sites (Guisan and Thuiller, 2005). Unlike mechanistic SDMs that count on explicit understanding of the physiological or environmental traits of a species, correlative models only require the species occurrence records (Rougier *et al*., 2015). Therefore, these models become particularly useful for estimating species range when physiological data is lacking as is the case for many potential and untested biological control agents.

While SDMs yield valuable insights into the possible geographic range of species based on broader climatic conditions, they do not explain the underlying biological and behavioural mechanisms utilized by insects to reduce stresses from unfavourable conditions. For an animal to persist through periods of adverse climatic conditions, species must be able to navigate the different microenvironments which make up their surrounding ecosystem (Kaiser *et al*., 2017). This is particularly valid for insects and other arthropods owing to their ectothermic physiology (Byrne *et al*., 2002). Microclimates often have differing environmental conditions to its surroundings often in the form of varying temperature, humidity or light (Caillon *et al*., 2013). Arthropods actively seek out microhabitats with conditions more suited to their needs such as the underside of leaves, crevices in bark, or burrows in the soil (Pike *et al*., 2012). This behaviour allows these taxa to regulate their internal physiology by avoiding extreme weather events and protecting themselves from predators. Displays of microclimate preferences are evident in the behaviour of *G. decoris* which actively avoid *S. mauritianum* plants in bright light conditions, opting to mainly occur on host plants in shade (Patrick and Olckers, 2014). The flowers of many

plants also offer refugia for insects, particularly against low environmental relative humidity (RH) which is particularly relevant for insects prone to desiccation. Floral humidity is typically higher than that of the surrounding ecosystem with floral structures often having increased humidity resulting from evaporating nectar (Willmer, 1980; Willmer, 1982). Inflorescence-inhabiting species such as *A. morticinus* and *A. santacruzi* may exploit these floral microenvironments to regulate their internal biology and avoid the risks of dry periods.

1.3 Rationale and hypothesis

Host-specificity trials for *A. morticinus* are ongoing and *A. morticinus* appears to have a strong affinity for *S. mauritianum*. Despite the similar biology and behaviours of *A. morticinus* and *A. santacruzi*, because *A. morticinus* has been observed at colder and dryer locations in South America, the species is expected to be more climatically suited to South Africa than *A. santacruzi*. Therefore, it is hypothesized that *A. morticinus* has the potential to match more of the *S. mauritianum* invaded range in South Africa than *A. santacruzi*.

This study uses Conviron (Conviron Ltd., Winnipeg, Canada) chambers to investigate the impacts of RH on the survival and reproduction of *A. morticinus*. Furthermore, niche mapping techniques novel to biological control are employed, which can predict the geographic distributions of species with minimal native range occurrence records. These techniques are applied to investigate the differences in predicted ranges of both *Anthonomus* species, examining if *A. morticinus* may exhibit greater distribution than *A. santacruzi* in South Africa.

1.4 Aims and objectives

The aim of the study is to assess if *A. morticinus* will show greater climatic suitability to the *S. mauritianum* invaded regions of South Africa relative to *A. santacruzi*.

To address the aim of this study, influences in physiological and reproductive behaviours of *A. morticinus* were investigated. Firstly, the responses of *A. morticinus* to low relative humidities where *S. mauritianum* microclimates are present were investigated. This objective explains the reliance of the weevil on its host for more than just food and reproduction, but for shelter as well. Secondly, the effect of climatic factors on the geographic distribution of both *A. morticinus* and *A. santacruzi* was investigated with particular focus on South Africa. Furthermore, this objective

aims to predict the ranges of both weevil species based solely on occurrence data, exploring new approaches for mapping potential biological control agents with no known physiological data. The projections generated were compared to those created using physiological species data in a mechanistic model.

The objectives of this MSc are threefold: (i) Firstly, to assess the physiological and behavioural responses of *A. morticinus* to low environmental relative humidity levels; (ii) to predict the estimated distributions of *A. morticinus* and *A. santacruzi* throughout South Africa; and (iii) to use novel approaches for estimating the potential distributions of biological controls agents when known physiological data is lacking. This study investigates the potential establishment of *A. morticinus* against *S. mauritianum* in the cooler areas of South Africa where *A. santacruzi* has failed.

Chapter 2: The effect of relative humidity on the survival and oviposition of *Anthonomus morticinus* Clark (Coleoptera: Curculionidae), a candidate biological control agent for *Solanum mauritianum* Scopoli (Solanaceae)

2.1 Abstract

Insect biology is greatly influenced by the surrounding climate. Desiccation, owing to low relative humidity, poses a significant threat to insect populations, especially those with small body sizes. To avoid exposure to low relative humidity (RH) levels, insects actively seek out nearby microenvironments with raised RH. The flowerbud feeding weevil *Anthonomus morticinus* is an optimistic agent to limit the spread of the highly invasive *Solanum mauritianum* throughout South Africa. However, the restricted geographic range shown by *A. santacruzi* in South Africa due to climatic unsuitability questions how *A. morticinus* will respond to climatic variables, particularly humidity. The effect of low temperatures has shown to be a limiting factor by Mkhomazi (2022) on the survival of *A. morticinus*, but the effects of RH remains untested. This study examined the influence of RH on the survival and oviposition of *A. morticinus* inhabiting *S. mauritianum* inflorescences. Furthermore, this study also intended to reveal the influence of lethal humidities on *A. santacruzi* cultures compared to that of *A. morticinus* when sheltered by host inflorescences. *Anthonomus morticinus* colonies inhabiting *S. mauritianum* bouquets were kept at seven relative humidities, from ~80% to ~20% for a period of 14 days. Survival and oviposition, observed by dissection of buds, were recorded. For comparison, colonies of *A. morticinus* and *A. santacruzi* were maintained at 20% and 46% relative humidity for seven days to determine if the species had differing responses at the lower RH levels. *A. morticinus* survival and oviposition showed no significant differences at the decreasing humidity levels. Moreover, no significant differences were observed in the survival between *A. morticinus* and *A. santacruzi* at 46% and 20% RH. Weevils residing within the *S. mauritianum* flowers and shoots were shielded from the unfavourable environmental humidities owing to higher humidity levels within these microclimates. These results highlight the dependence of biological control agents on their hosts for more than just food and reproduction but also for physiological functionality.

Keywords: climatic unsuitability, floral humidity, microclimate, temperature

2.2 Introduction

Climatic unsuitability of biological control agents is a leading factor contributing to failed biocontrol initiatives (Heimpel and Cock, 2018; Hoelmer and Kirk, 2005). Insect physiology is largely tied to climatic conditions which directly impact the survival, development, and geographic distributions of insect populations (Angilletta *et al*., 2002). Temperature and relative humidity (RH) are both highly significant drivers of insect abundance and distribution (Byrne *et al*., 2002). However, the effect of humidity on biocontrol agents is a regularly excluded topic in biological control programs as such experimentation is often highly complex and unfeasible and thus often remains untested (Cowie *et al*., 2016b). Relative humidity (RH) is key in shaping the biology, behaviours, and movements of insect populations and therefore should not be ignored (Norhisham *et al*., 2013). Lower humidities greatly challenge the ability of many insect species to successfully overwinter (Danks, 1991) and contribute to desiccation, particularly in species with smaller body sizes (Chown and Nicolson, 2004).

With the challenges posed by environmental conditions, insects need to be able to successfully navigate their environment to avoid exposure to unfavorable conditions (Kaiser *et al*., 2017). While an ecosystem may be enveloped by a larger macroclimate, environmental variables are not always homogenous across an ecosystem especially at finer scales (Scheffers *et al*., 2013). This heterogeneity exists on several temporal and spatial scales and creates a range of microclimates within a given region (Caillon *et al*., 2013). It is critical for arthropod species to navigate between these spaces in order to seek out microhabitats characterized by conditions ideal for their survival and development (Pike *et al*., 2012).

Plant material itself can often present an assortment of microclimates based on the plants' physical and biochemical features (Kaiser *et al*., 2017). Molina-Montenegro *et al*., (2006) detailed this behaviour in *Eriopis connexa* and *Hippodamia variegate*, two coleopterans native to the Andes Mountain range of central Chile. During extreme periods of cold, both species sought out thermal refuges under the canopies of the nearby cushion plant species *Azorella monantha* and *Laretia acauliscertain*. The microclimates below the leaves of these plants were shown to offer greater water availability and more stable temperatures than the surrounding ecosystem (Molina-Montenegro *et al.*, 2006).

For many insects, the microclimate in flowers is key to their ecology. For instance, *Cyclocephala colasi* beetles in the French Guiana spend much of their time feeding, mating, and resting in the thermogenic floral chambers of species such as *Philodendron solimoesense*. These floral refuges show temperatures above that of the surrounding ambient air, offering these coleopteran pollinators heat rewards for visiting the plant (Seymour *et al*., 2003). Another climatic trait of many floral microhabitats is the increased levels of relative humidity within the flower itself when compared with the surrounding ecosystem. Floral humidity is generally the result of evaporation of nectar but may also occur through transpiration (Corbet *et al*., 1979). Insect pollinators such as hawkmoths and bumblebees have been shown to be highly sensitive to these different relative humidities, using floral humidity as an indication of nectar availability (Harrap *et al*., 2020; von Arx *et al*., 2012).

The South American tree *Solanum mauritianum* has become a prevalent invader in many regions around the world (Roe, 1972; Witkowski and Garner, 2008). The weed is particularly problematic and widespread in South Africa especially within the higher rainfall reaches of the country impacting forestry, agriculture, and conservation initiatives (Henderson, 2001; Olckers, 2011). Research efforts began in 1984 to identify suitable natural enemies on *S. mauritianum* for biological control of the plant in South Africa (Olckers, 1999). The flowerbud feeding weevil *Anthonomus santacruzi* offered a potential solution to combat the substantial seedbank of the plant by feeding and ovipositing on flowers and developing buds (Olckers, 2011). However, the agent has shown a distribution restricted to South Africa's east coastline and central areas of the Limpopo and Mpumalanga provinces (Cowie *et al*., 2018). Climatic constraints in the cooler drier regions of the country have been identified as the main reason for the weevils' restricted range (Singh and Olckers, 2017). Cowie *et al*., (2016b) investigated the fundamental niche of the weevil by directly exposing individuals to low temperatures and relative humidities, providing severed floral or non-floral *S. mauritianum* material as a food source only thus being too small to provide a microhabitat for *A. santacruzi*. The lethal humidity (LH_{50}) at which the cultures tested experienced 50% mortality was ~46%, with 100% mortality being displayed at and below relative humidities of 30%. Therefore, Cowie *et al*., (2016b) concluded that low RH is constraining the range of *A. santacruzi* in South Africa.

Another potential biocontrol agent for *S. mauritianum* is the flowerbud feeding weevil *Anthonomus morticinus* which is currently undergoing research for its possible release. This species has garnered much optimism owing to populations being found in colder and drier regions of South America than those of *A. santacruzi* (Pedrosa-Macedo *et al*., 2003; Venter *et al*., 2021). This observation gives rise to the hypothesis that the species is more cold and dry tolerant than the previously released weevil and may therefore show greater establishment in South Africa (Cowie *et al*., 2018). Recent laboratory studies conducted on the lower thermal thresholds for both *Anthonomus* species found that *A. morticinus* has a thermal tolerance of roughly 3 °C below that of *A. santacruzi* (Mkhomazi, 2022). It is hypothesized that *A. morticinus* will show a greater tolerance to dry conditions than *A. santacruzi*. Nevertheless, with the apparent constraints imposed by low RH on the range of *A. santacruzi* (Cowie *et al*., 2016b) and the characteristic dry conditions throughout South Africa (Singh and Olckers, 2017), there is concern that low humidity will have a similar limiting effect on the establishment of *A. morticinus* in South Africa. However, the Cowie *et al*. (2016b) study recorded the mortality of *A. santacruzi* kept in plastic containers with severed *S. mauritianum* leaves or flowers. This method directly exposed the weevils to the eight decreasing RH levels (80, 70, 60, 50, 40, 30, 20 and 10%) used, achieved through the Winston and Bates RH methodology, and thus only exploring the humidity of the *A. santacruzi* fundamental niche. This study aims to detail the survival and reproductive responses of *A. morticinus* to differing RH levels when *S. mauritianum* microclimates are present, thus exploring the realized niche of the species. Furthermore, this study aims to assess the responses of *A. morticinus* and *A. santacruzi* to low humidity while providing floral microhabitats for the weevils.

2.3 Materials and methods

2.3.1 Effects of relative humidity on *Anthonomus morticinus* survival and oviposition

Seven different relative humidities were used to test the survival and oviposition of *A. morticinus* which included 20%, 30%, 40%, 50%, 60%, 70% and 80% while temperature was maintained at a constant 25 °C (Cowie *et al*., 2016b). *Solanum mauritianum* bouquets, containing floral and non-floral material, were collected from a park in Johannesburg (-26.135854, 28.101454) or the University of the Witwatersrand campus (-26.188820, 28.032513) and placed into 100 ml glass

vials filled with tap water. Cages were constructed using 4-liter clear plastic buckets each containing a single *S. mauritianum* floral bouquet and were tightly fitted with a gauze covering as the lid. Each cage received eight weevils at a 1:1 sex ratio. Sexing was achieved by exposing the weevils to -40 °C for 45 – 60 seconds to immobilize them allowing for sex determination under a dissecting microscope as conducted by Mkhomazi (2022). Males can be identified by the presence of the tergal notch which is missing in the females (Sasa *et al*., 2020). Each cage was then placed into a walk-in Conviron growth room (Conviron PGV40, Winnipeg, Canada) at the respective humidity for a period of 14 days. Survival was recorded after seven days for each cage and the old bouquet was replaced with a new one. The buds from the old bouquets were deposited into a petri dish lined with damp filter paper for approximately 5 days to allow for larval development. Subsequently, the buds were dissected to determine the number of larvae found from each humidity. Cages were removed from each growth room after 14 days and the final levels of survival were recorded. The buds from the second bouquets followed the same procedure as the first. Four replicates were conducted at each RH level.

2.3.2 Comparison of the effect of humidity on the survival of *A. santacruzi* and *A. morticinus*

A cage of ten randomly selected *A. morticinus* adults were placed into a Conviron at 46% relative humidity next to a cage of ten randomly selected *A. santacruzi* adults. Each cage was supplied with *S. mauritianum* bouquets containing both floral and non-floral material. After seven days, the survival of beetles in each cage was recorded. This experiment was repeated using an RH of 20% representing an extreme low humidity. Four replicates per weevil species were conducted simultaneously for both RH levels.

2.3.3 Data analysis

The survival scores for *A. morticinus* at 20, 30, 40, 50, 60, 70 and 80% RH were compared using a binomial generalized linear model (GLM) calculated using the mortality rates. This GLM was chosen as the data does not follow a normal distribution.

Oviposition of *A. morticinus* at 20, 30, 40, 50, 60, 70 and 80% RH were compared using one-way ANOVAs. The survival of the two species at 46% and 20% RH was compared using a t-test for each relative humidity.

The survival of the two species at 46% and 20% RH was compared using a t-test for each relative humidity.

All calculations were conducted in R version 4.2.1 (R Core Team, 2024) and data was visualized using the 'ggplot2' R package (Wickham, 2016).

2.4 Results

2.4.1 Effects of relative humidity on *Anthonomus morticinus* survival and oviposition

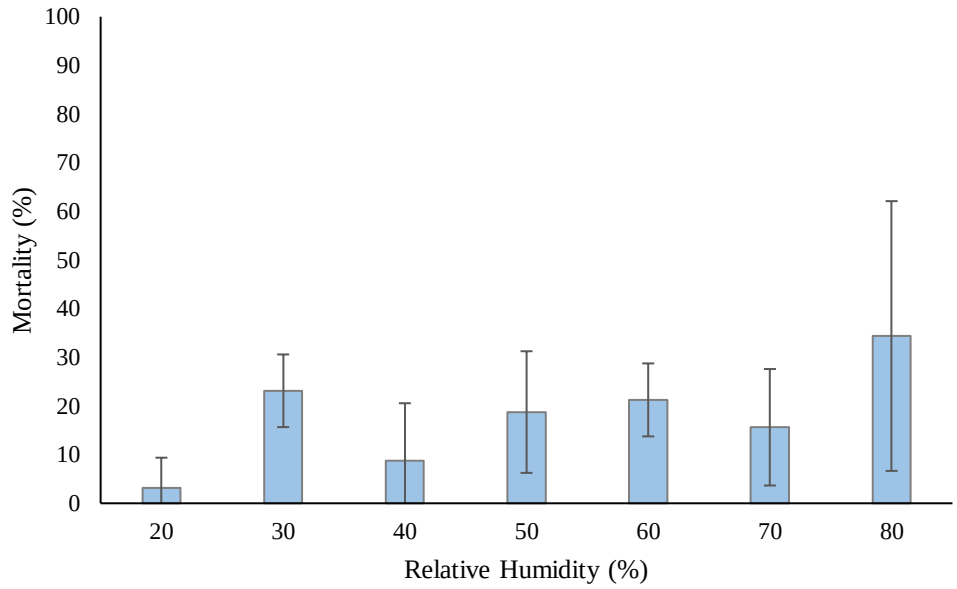


Figure 2. 1: The mortality levels of *Anthonomus morticinus* adults after 14 days of exposure to differing relative humidities while *S. mauritianum* flowers and leaves were present and temperature remained constant at 25 ℃. Eight adult weevils were used for each RH with four repetitions per humidity class. A total of n= 224 *A. morticinus* adults were used in this experiment.

The mean mortalities from the seven humidities showed the highest mortality (34.4%) at 80% and lowest (3.1%) at 20% with no observable pattern between them (Figure 2.1). The results from the binomial GLM found no significant differences between the RH levels ($P_{20\%}$ = 0.232; $P_{30\%}$ = 0.473; $P_{40\%}$ = 0.747; $P_{50\%}$ = 0.532; $P_{60\%}$ = 0.496; $P_{70\%}$ = 0.583; $P_{80\%}$ = 0.362; > 0.05).

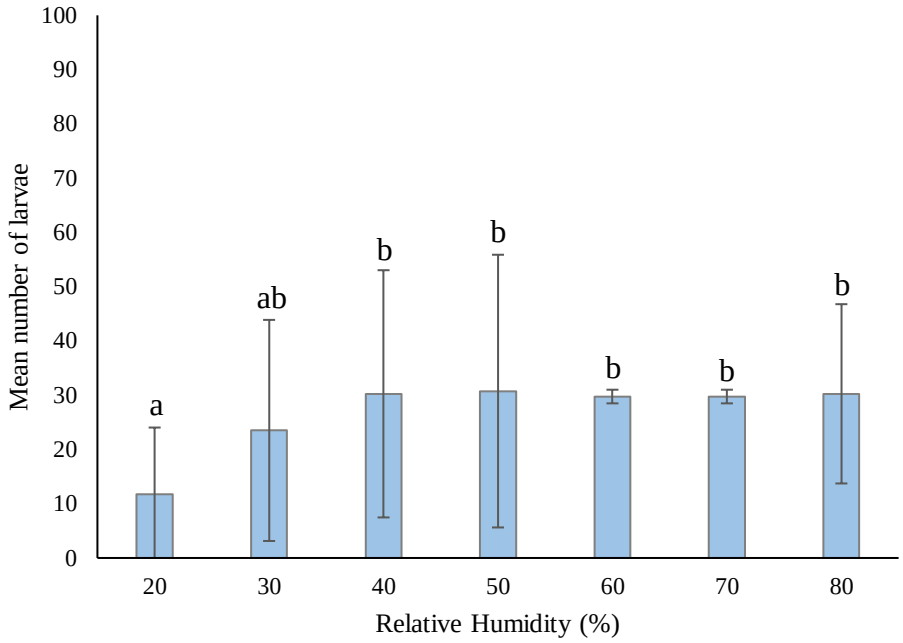


Figure 2. 2: The larval output of *Anthonomus morticinus* adults after 14 days of exposure to differing relative humidities while *S. mauritianum* flowers and leaves were present and temperature remained constant at 25 °C. Eight adult weevils at a sex ration of 1:1 were used for each RH with four repetitions per humidity class. A total of n= 224 *A. morticinus* adults were used in this experiment. The 20% RH was shown to be significantly different to all other RH levels except for the 30% RH which showed no significant differences in oviposition to the other RH levels as indicated by the Tukey HSD post-hoc analysis.

Consistent levels of oviposition were found between 80 and 40% and then declining at 30 and 20% RH (Figure 2.2). The negative binomial GLM shows that all the RH levels besides the 30% show significant differences ($P_{20\%} = 0.000000000000184 < 0.05$; $P_{30\%} = 0.1336 > 0.05$; $P_{40\%} = 0.0396$; $P_{50\%} = 0.0362$; $P_{60\%} = 0.0433$; $P_{70\%} = 0.0433$; $P_{80\%} = 0.0396$; < 0.05) in oviposition levels between the seven humidities. Using a Tukey HSD post-hoc test it was found that 20% RH was significantly different to all the other RH levels except for the 30%, which showed no significance to any other RH level.

2.4.2 Comparison of the effect of humidity on the survival of *A. santacruzi* and *A. morticinus*

All weevils of both species subjected to 46 and 20% RH for one week showed no mortality, concluding that there was no significant difference between the RH tolerances of *A. morticinus* and *A. santacruzi*. Oviposition could not be determined due to technical difficulties at the facility. The results from both of the binomial GLMs returned no significant differences.

2.5 Discussion

As ectothermic animals, insects are dependent on their immediate environment for physiological function (Chown *et al*., 2010). Relative humidity of that environment also plays a significant role in insect physiology, influencing species' behaviour, ecology, and geographic range (Han *et al*., 2008). To avoid prolonged exposure to unfavourable conditions, many insect species actively search for, and exploit microclimates present on plants (Danks, 2002; Willmer, 1982). The results indicate that relative humidity is not an important factor in *A. morticinus* survival and reproduction in the presence of host plant inflorescences and non-floral material. Furthermore, at low and high humidities, *A. morticinus* and *A. santacruzi* showed no difference in survival when given *S. mauritianum* bouquets. This has consequences for the probable distributions of these weevils in South Africa and suggests that physiological testing for distribution modelling should be done by taking microclimates into account.

Insects, especially those of smaller body size, are vulnerable to desiccation when exposed to low relative humidities (Chown and Nicolson, 2004). Therefore, insects are forced to find solutions to limit their exposure to dry conditions (Kaiser *et al*., 2017). The findings of this study imply that *A. morticinus* is able to endure and continue to reproduce through periods of low environmental humidities if *S. mauritianum* inflorescences are available. The lack of significant differences in both the survival and oviposition trials implies that *A. morticinus* persistence is linked to the microclimates found on *S. mauritianum* inflorescences. This finding is further fortified in the RH trials where both *A. morticinus* and *A. santacruzi* experienced no mortality at low humidity levels. These conclusions contradict that of Cowie *et al*. (2016b) who found an LH_{50} for *A. santacruzi* at 46% RH and complete mortality at 30%. However, Cowie *et al*. (2016b) recorded the mortality of *A. santacruzi* kept in plastic containers with pieces of *S. mauritianum* leaves or flowers, therefore directly exposing the weevils to eight RH levels (80, 70, 60, 50, 40, 30, 20 and 10%) done using the Winston and Bates RH methodology. This technique involves the use of saturated salt solutions to achieve desired humidity levels in small enclosed spaces for biological research. The RH of the enclosed spaces can be determined by recording the internal temperatures and nature of the salt solutions used (Winston and Bates, 1960). The current study presented weevils with full bouquets in open mesh cages within Convirons, thus investigating the realized niche of the insects.

Despite the lack of significant differences with regard to survival and oviposition, the findings of this study offers insight as to how *A. morticinus* is able to cope with low relative humidities. While the mortality of *A. morticinus* is not significantly different at 20% RH, oviposition did show significantly lower results to the higher RH levels.

The biology and reproductive behaviours of both *A. morticinus* and *A. santacruzi* are inextricably linked to the *S. mauritianum* inflorescences which they inhabit (Cowie *et al*., 2018; Olckers, 1999). Adults of both species feed predominantly on the floral material and shoot tips of the plant and females oviposit their eggs into premature buds. The soft bodied larvae hatch and begin to feed on the developing floral material enclosed within the bud and the pupal stage occurs entirely within the bud's chamber (Olckers, 2003). These weevils may live out their entire lives on the inflorescences of the host plant, rarely venturing out of its confines. Therefore, as long as the weevils remain within the microenvironment of the *S. mauritianum* inflorescences, they are unlikely to suffer much from low external relative humidities. This observation infers the importance of the quality and the quantity of *S. mauritianum* inflorescences on the survival and growth of both *Anthonomus* species populations. English and Olckers (2018) discovered that *A. santacruzi* establishment in the KwaZulu Natal Province was highly influenced by fluctuating floral quantity of the host plant. Declines in weevil population sizes were observed during the winter months, aligning with declining production of *S. mauritianum* floral material. While *S. mauritianum* flower production often varies from year to year (Henderson, 2001), low temperatures negatively influence flowering of the plant (Lubke *et al*., 2021). At temperatures below 20 °C, the photosynthetic capabilities of *S. mauritianum* are greatly reduced which in turn leads to reduced quantity and quality of the plant's inflorescences (Cowie *et al*., 2018). Thus, it appears that temperature is a significant factor determining the success of *Anthonomus* populations both directly, through the effects on the insect's thermal physiology (Chown *et al*., 2010), and indirectly, as a driver of the weevils required microhabitats and food sources (Olckers, 2011; Singh and Olckers, 2017).

The thermal physiology of insects is usually reflective of the environments in which they have evolved (Robertson *et al*., 2008). However, estimating the relationships between climatic conditions and insect tolerances does not always yield reasonable conclusions as it generally ignores the influence of the microclimates in which the insects reside. Coetzee *et al* (2007)

studied the effect of low winter temperatures of the South African highveld on the distribution of the mirid *Eccritotarsus catarinensis* (Order: Hemiptera), a biological control agent of water hyacinth *Pontederia crassipes* (Order: Pontederiacea). Much of the highveld is subject to winter frost, a characteristic which contributes to the death of the above water biomass of *P. crassipes*. The authors discuss the impact that the cold temperatures have on *E. catarinensis* which is further compounded as the agent loses the microhabitats it requires to avoid exposure to the dangerous environmental conditions. For these reasons, the mirid has been unable to successfully establish in much of the highveld particularly around Johannesburg. A later study by Griffith *et al*. (2019) identified that populations of *E. catarinensis* established at different localities in South Africa exhibit a degree thermal plasticity and evidence of microevolution in their thermal tolerances. The study discovered that individuals from Kubusi river (32°35033.25″S, 27°25019.38″E), representing the coldest site where the insect has established, had a CT_{min} of 3.7 ℃ which was found to be significantly lower than that of individuals collected at the hottest site at Enseleni River (28°41026.60″S, 32°00048.69″E) with a CT_{min} of 4.8 ℃. Short-term acclimation of laboratory reared *E. catarinensis* by Porter *et al*. (2019) further represents the characteristics of insect thermal plasticity, showing that individuals exposed to colder conditions for a period of only five days had significantly lower thermal limits than those kept at ideal conditions. The findings of these studies identify the restrictions of colder climates on a biological control agent but also suggest that the insect is capable of adapting to cooler climatic conditions.

Similar to *E. catarinensis*, temperatures below the *A. santacruzi* CT_{min} restrict the distribution of the weevil in South Africa (Cowie *et al*., 2016b; Mkhomazi, 2022). Despite multiple release attempts, *A. santacruzi* has consistently failed to establish within the cooler Highveld region of South Africa (Cowie *et al*., 2018; Singh and Olckers, 2017). The field observations of this study show failure of *A. santacruzi* to establish along the south coast of South Africa. Furthermore, low temperatures serve to limit the ability of *S. mauritianum* to produce the high-quality inflorescences required for successful *A. santacruzi* establishment (Cowie *et al*., 2018; English and Olckers, 2008). Mkhomazi (2022) showed that *A. morticinus* has a CT_{min} of 1.71 ℃ which is significantly lower than the CT_{min} of *A. santacruzi* at 4.93 ℃. Moreover, the lower thermal limits of *A. morticinus* are similar to the winter temperatures found along the southern coast of South Africa (CLIMEX Match Climates) indicating that the insect is expected to show greater

establishment along the south coast than *A. santacruzi*. However, similar to *A. santacruzi*, the conditions present on the Highveld are expected to prevent successful establishment of *A. morticinus*.

2.6 Conclusion

The present study investigated the effects of relative humidity on the survival and reproduction of *A. morticinus* when *Solanum mauritianum* inflorescences are present. The survival of *A. morticinus* and *A. santacruzi* were compared when the RH was at lethal levels based upon *A. santacruzi*'s fundamental niche (Cowie *et al*., 2016b). The results of this study showed that humidity is of little significance for either the survival or reproduction of *A. morticinus* when inflorescences are available. Survival of *A. morticinus* and *A. santacruzi* showed no difference at 46% or at extreme RH levels of 20%. It is thus hypothesized that both species adjust their behaviour, remaining within the confines of the flowers to avoid exposure to unfavourable external conditions. Thus, the distributions of these insects is largely tied to temperature and the availability of *S. mauritianum* inflorescences. With decreased flowering of the weed in winter (English and Olckers, 2018), the microhabitats of the weevil are reduced during the winter months particularly in the colder regions of its invaded range, thereby reducing microhabitat availability and prolonged exposure to cold temperatures. This observation provides insight into the contributing factors of why *A. santacruzi* has been limited to the warmer regions of the South African east coast with continued failure in the colder highveld. Moreover, with its similar biology, *A. morticinus* is likely to experience similar limitations in South Africa should it be released. However, with a greater cold tolerance (Mkhomazi, 2022) and collection from cooler localities in South America than *A. santacruzi* (Olckers, 2003; Pedrosa-Macedo *et al.,* 2003; Venter *et al*., 2021), *A. morticinus* is expected to stand a greater chance to establish along the South African south coast where *A. santacruzi* had failed. The findings of this study highlight the role of microclimates in the success of insect populations and the dependence of insect biological control agents on the microenvironments on their plant hosts for their physiological function. When researching possible agents, the influence of microhabitats on agent establishment and reproduction should be investigated through field surveys, ecophysiological experiments and analysis of the conditions within the microclimates selected by the agent. This study would benefit from investigating the conditions, both temperature and humidity, within and amongst the

floral material present on the *S. mauritianum* host. Such an investigation would be valuable in future biological control analyses of *S. mauritianum* as no literature currently exists describing the humidity levels within the plants' flowers.

Chapter 3: The Ensemble of Small Models: a novel framework to screen potential biological control agent for climate matching

3.1 Abstract

Close to half of all insect biological control agents fail or are limited in their establishment due to climatic unsuitability of the species to the areas into which they are introduced. This is detrimental to biological control as agents that fail to establish represent a significant waste of the time and funding invested in pre-release efforts. Species Distribution Modelling (SDM) is a useful technique for predicting the geographic distribution a species is expected to have in a given region. However, most SDM approaches require either known species physiological data or large occurrence datasets to produce accurate models, both of which are unavailable for many potential biological control agents. Therefore, SDMs are normally not performed in biological control initiatives, particularly before release. The Ensemble of Small Models (ESM) is a technique first developed for the modelling of rare species with small occurrence datasets, similar to that of many biological control agents. This study used the ESM approach to model the distributions of *Anthonomus santacruzi* and *Anthonomus morticinus*, potential biocontrol agents of the invasive South American weed *Solanum mauritianum* in South Africa. The projections of these models were then compared to an ESM of *S. mauritianum* to eliminate areas overpredicted by the model which fall outside of the hosts actual range. Finally, CLIMEX mechanistic models were generated for both weevil species using ecophysiological data from previous studies then compared to the ESMs. The ESM for *A. morticinus* using only 17 native range occurrence data depicted an expected range wider than that of *A. santacruzi*, particularly along the South African south coast. The CLIMEX models showed very similar projections to that of the ESMs, suggesting that both models produce accurate results and therefore the modelling technique should be chosen according to the type of data available for the organism. This study advocates for the use of the ESM in modelling the expected distributions of potential biological control agents without known ecophysiological tolerances and thus narrowing the search for climatically suitable candidates for future biological control initiatives.

Keywords: agent selection, biological control, climatic unsuitability, ensemble of small models, establishment, species distribution model, temperature

3.2 Introduction

Species distribution modelling (SDM) is a statistical modelling technique utilized in conservation biology and ecology to estimate the probability for occurrence of a given species over a specified geographic range (Guisan and Zimmerman, 2000). This approach integrates species data and environmental variables to map where the species is likely to occur based on habitats suitable for supporting its population (Buckley *et al*., 2010; Elith and Leathwick, 2009). The modelling technique used is generally dependent on the data available. For instance, mechanistic SDMs utilize known physiological or ecological parameters of a species often collected from experiments, such as the tolerance to differing temperatures, moisture, or light levels (Evans *et al*., 2015). This data is then used to estimate the fundamental niche of the species, mapping the estimated distribution the species will exhibit (Kearney and Porter, 2009; Monahan, 2009). Mechanistic SDMs are commonly used to estimate the climatic suitability of biocontrol agents and is often produced using the CLIMEX ecoclimatic modelling software (Hearne, Scientific Software Pty Ltd, Australia) (Hong, 2004). For instance, Falla *et al* (2021) used existing ecophysiological data to estimate the distribution of the lace bug *Gargaphia decoris*, a biocontrol agent on *Solanum mauritianum*, within its native South American and introduced South African and New Zealand ranges to estimate its potential distribution in areas where the species has yet to be introduced. This approach yields valuable information for biocontrol programs; however, modelling is limited to agents that have undergone formal testing to determine their ecophysiological parameters (Kearney and Porter, 2009; Tourinho and Vale, 2023). For agents where this is not an option, a more universal approach is required.

An alternative approach is correlative species distribution modelling using statistical frameworks to estimate the distribution the species is expected to have based on environmental conditions and the occurrence data of that species (Guisan and Zimmermann, 2000; Low *et al*., 2021). This approach produces a model able to accurately predict the potential range of the species while also offering insight into the environmental conditions influencing its distribution (Wu and Smeins, 2000). To avoid overfitting which occurs when a model is too closely aligned to its training data and to increase the predictive performance of these models, large occurrence datasets are often required (Collart and Guissan, 2023). This is especially true for models using a

large number of environmental predictors which may prove inaccurate when integrated with few occurrence records (Peduzzi *et al*., 1996). However, this requirement often means that correlative approaches cannot be used to forecast the distributions of biocontrol agents as their occurrence data is often very scarce (Mukherjee *et al*., 2021).

Rare species have characteristically small occurrence datasets similar to that of biological control agents, and therefore cannot be modelled using classical correlative techniques (Lomba *et al*., 2010). Ensemble modelling has become a popular machine learning technique which uses a combination of multiple models or data sources thus improving the prediction accuracy and robustness of the model (Wu and Levinson, 2021). These techniques often outperform single models by leveraging diverse information and reducing individual model weaknesses (Ogutu *et al*., 2022; Dietterich, 2007). There are two distinct forms of ensemble modelling namely the multiple algorithm and multiple predictors approaches. The first method emphasizes the use of multiple models and algorithms to make predictions and inferences, rather than relying solely on a single best model, thereby accounting for model selection uncertainty. This method enhances the robustness of predictions and provides a richer set of quantitative evidence, improving the overall reliability of scientific conclusions (Anderson and Burnham, 2002; Burnham and Anderson 2002). The second approach makes use of a singular modelling approach over multiple predictor variables. One such example of this approach is the Ensemble of Small Models (ESM) approach as used by Lomba *et al* (2010) has been shown to produce accurate and descriptive models for rare species with scarce occurrence data. This method fits multiple bivariate models with predictors from a series of selected environmental variables which are then subsequently integrated using weighted averages to produce a final ensemble (Lomba *et al*., 2010). With the similar characteristics of few records in occurrence datasets, the ESM approach may prove useful in successfully predicting the distribution of potential biological control agents. However, this has not yet been used for predicting the potential geographic distributions of putative agents.

The flowerbud feeding weevil *Anthonomus santacruzi* was introduced as a biological control agent on *S. mauritianum* in South Africa with the hope that the insect would drastically reduce the reproductive ability of the plant in the country as a result of its feeding and reproductive behaviours (Olckers, 1999). *A. santacruzi* populations have successfully established along the

east coast of KwaZulu Natal and a few sites in central Limpopo and Mpumalanga Provinces (Cowie *et al*., 2016b; Olckers 2011). However, weevils released within the Gauteng highveld failed to persist suggesting climatic unsuitability to the region (Singh and Olckers, 2017). Other releases of *A. santacruzi* in the Western and Eastern Cape have yet to be confirmed for successful establishment (Cowie *et al*., 2018). Using ecophysiological data from *A. santacruzi* laboratory trials, Cowie *et al*. (2018) produced a mechanistic CLIMEX model to map the predicted distribution of *A. santacruzi* in South Africa. The model predicted that *A. santacruzi's* establishment would be restricted mainly to the South African east coast, from East London in the south until just below the Mozambican border in the north and inland to include Pietermaritzburg. Furthermore, isolated pockets for potential establishment were shown to exist in the Sabie region of Mpumalanga province, Tzaneen in the Limpopo province and along the southern coast from Knysna to Cape St Francis. The limitations of South Africa's climate on the weevil's distribution thus greatly limit the cover of control offered by the agent.

With the constraints of the potential distribution of *A. santacruzi*, another agent is currently being investigated for biological control of *S. mauritianum* in South Africa. *Anthonomus morticinus* is a flowerbud feeding weevil within the same genus as *A. santacruzi* and with similar feeding and reproductive behaviours which target the same host plant (Olckers *et al*., 2002). Despite its resemblance to *A. santacruzi*, *A. morticinus* has been collected from higher altitudes and therefore cooler temperatures than that of *A. santacruzi's* native range (Pedrosa-Macedo *et al*., 2003). *Anthonomus santacruzi* was not found to occur in the cooler regions of Uruguay (Venter *et al*., 2021). Moreover, studies on the thermal tolerances of *A. santacruzi* have shown the critical thermal minimum (CT_{min}) of the species to be between 3.8 and 6.7 °C, while that of *A. morticinus* has been found to be between 1.2 and 3.0 °C (Cowie *et al*., 2016b; Mkhomazi, 2022). The combination of the higher altitude occurrences and the lower CT_{min} scores for *A. morticinus* gives rise to the hypothesis that *A. morticinus* is likely to show a wider distribution in South Africa than *A. santacruzi*.

The goal of this study was to consider the performance of the Ensemble of Small Models technique in answering key questions regarding the potential distribution of biological control agents. This was illustrated by employing the ESM to answer the hypothesis of *Anthonomus*

morticinus having a wider estimated geographic distribution than *Anthonomus santacruzi* in South Africa. Consequently, the accuracy of the *Anthonomus* models were investigated, observing their predicted ranges within the confines of the *Solanum mauritianum* invaded regions of South Africa. Lastly, using known physiological species data, mechanistic SDMs for both *Anthonomus* species were produced in CLIMEX to compare to the projections of the ensemble of small models.

3.3 Materials and methods

3.3.1 *Anthonomus santacruzi* establishment in South Africa

The occurrence of *A. santacruzi* was surveyed along the South African south coast, starting in Cape Town, and ending in East London (Figure 3.1a). Sampling was conducted along this route between the 19th to the 22nd of February 2023 starting at 08:00 and ceasing after 18:00. The floral material and new leaves of *S. mauritianum* plants at and around *A. santacruzi* release sites (Cowie *et al*., 2018) were thoroughly examined for presence or absence of the species. Sampling was also conducted on plants found between these release sites to assess the weevil's range expansion (if any) in the region. At each sampling site, available floral material was collected in sealed zip lock bags which were examined for *A. santacruzi* individuals at the completion of each day.

3.3.2 *Anthonomus morticinus* and *Anthonomus santacruzi* Ensemble of Small Models

3.3.2.1 The modelling framework

Correlative species distribution modelling uses statistical frameworks to predict the realized niche of a given species by recognizing patterns of the environmental variables present at species occurrence sites (Guisan and Thuiller, 2005; Guisan and Zimmermann, 2000). This approach aims to accurately predict the potential distribution of a species while also providing insight into the environmental conditions that influence the species' distribution (Wu and Smeins, 2000). However, the statistical algorithms typically used in classical SDM techniques require large amounts of species occurrence data to yield accurate results and avoid overfitting (Jeliazkov *et al*., 2022). The Ensemble of Small Models approach utilized in this paper fits multiple bivariate

models with predictors from a series of selected environmental variables which are then subsequently integrated using weighted averages to produce a final ensemble (Lomba *et al*., 2010).

3.3.2.2 Species occurrence data

Both *A. morticinus* and *A. santacruzi* are native to the subtropical reaches of eastern South America with collections in southern Brazil, northern Argentina, and Paraguay. *Anthonomus morticinus* has however also been recorded in Uruguay while *A. santacruzi* has not (Figure 3.1b). Since its release, *A. santacruzi* has been distributed widely in eastern South Africa and has successfully established along the KwaZulu Natal east coast and central Mpumalanga and Limpopo Provinces (Figure 3.1a) (Cowie *et al*., 2018, Singh and Olckers, 2017). A total of only seventeen collection locations are known for *A. morticinus* in South America (Olckers *et al*., 2002; Pedrosa-Macedo *et al*., 2003; Venter *et al*., 2021) while *A. santacruzi* has only nine known collection localities in South America (Olckers, 2002, Pedrosa-Macedo *et al*., 2003). However, forty-five known occurrences exist for *A. santacruzi* in its introduced range in South Africa (Cowie *et al*, 2018, Inaturalist.org, Singh and Olckers, 2017). Therefore, the 17 native range data points for *A. morticinus* were used to calculate the ecological niche of the weevil while the forty-five non-native South African records were used for that of *A. santacruzi* as nine occurrences proved too few even for the ESM model.

3.3.2.3 Environmental predictors

The environmental predictors used in the ESMs for *A. morticinus* and *A. santacruzi* were selected according to their level of influence on the species distributions within the study range (South America for *A. morticinus* and South Africa for *A. santacruzi*). Climatic predictors used were the 19 WorldClim bioclimatic variables obtained from http://worldclim.org/data/worldclim21.html at a spatial resolution of 30 arc-seconds which has cell sizes of ~ 1 km^2. These include a range of precipitation and temperature related variables (Hijmans *et al*., 2005). The global rasters were resized to fit the extents examined which included the southern Brazil, northern Argentina, Paraguay, and Uruguay range for *A. morticinus* (Figure 3.1b) and South Africa for *A. santacruzi* (Figure 3.1a) to fit the species climatic niches. All 19 bioclimatic rasters were clipped in QGIS (version 3.34.3) to obtain the specific rasters needed.

Each of these databases were imported into R (version 4.2.1) for analysis and a raster stack of the 19 bioclimatic variables was produced by employing the 'raster' (Hijmans *et al*., 2024) and 'terra' (Hijmans *et al*., 2023a) packages. As the ESM uses the bivariate approach, there is no need to assess the collinearity of the predictors. Species presences, 1000 background points and the environmental data were then integrated into a single dataset which was used by the model to recognize relationships between occurrence sites and the environmental conditions present.

3.3.2.4 Model fitting and evaluation

Classical SDM techniques often require many occurrence records (Elith *et al*., 2006; Guisan and Thuiller, 2005) often lacking for many potential biocontrol agents. Furthermore, biological control agents usually lack absence data, limiting the number of modelling approaches to produce SDMs for potential agents with limited occurrence data (Wisz and Guisan, 2009). A number of studies (Chefaoui and Lobo, 2008; Elith and Leathwick, 2007; Graham *et al*., 2004) suggest the use of pseudo-absence data to help overcome the restrictions characteristic in presence-only models, thus adding to the amount of eligible modelling techniques offered to species with small datasets (Elith *et al*., 2006). Pseudo-absences help build SDMs when only presence data is available. These data are generated by selecting random points from within the study area where the species has not been recorded but climatic conditions are presumed to be suitable for species presence (Barbet-Massin *et al*., 2012). The data was then split into two groups, one containing 80% and the other with 20% of the data, using the 'zealot' (Teetor and Teetor, 2022) package, with the larger set being used to train the model and the other to be used to assess the models accuracy. The models were trained using Maxent algorithms made possible by employing the 'dismo' (Hijmans *et al*., 2023b) and 'SDMtune' (Barras *et al*., 2023) packages, to identify the most apparent environmental variables at the occurrence sites. The trained models were then able to highlight the climatic variables of greatest significance influencing the distributions of both weevil species. This study follows the recommendations of Ferrier *et al* (2002) to generate an amount of randomly sampled pseudo-absences corresponding to the number of presences within the appropriate rasters (Guisan *et al*., 2017). This involved the generation of seventeen pseudo-absences in South America for *A. morticinus* and forty-five in South Africa for *A. santacruzi*.

Another limitation presented by limited occurrence datasets is that predictive models are often unable to use multiple environmental predictors for an SDM and therefore cannot profile the entire climatic niche of the species (Guisan and Zimmermann, 2000). Harrell Jr. *et al*. (1996) advised that for models with binary outcomes (presence / absence), the number of predictors should be less than one-tenth of the occurrences in the less frequent outcome. This drastically limits the informative ability for modelling species with scarce occurrences such as that of many biocontrol agents. The Ensemble of Small Models approach presents an alternative method which has been used to overcome such limitations through generating many bivariate models which are subsequently averaged into a final weighted ensemble (Lomba *et al*., 2010).

This study employed the 'flexsdm' R package (Velazco *et al*., 2021) to predict the species ranges for *A. morticinus* and *A. santacruzi* in South Africa using the ESM approach. The Generalized Linear Model (GLM) regression algorithm was selected for fitting the models owing to its capability in handling many data types and its ability to capture complex statistical relationships (Hastie and Tibshirani, 1990; Li and Wang, 2013). In SDMs using a GLM, the probability of species occurrence can be calculated against the linear predictor, comprised of a combination of multiple environmental variables, by using a link function (Guisan and Zimmerman, 2000). For GLMs using binary data, a logit function is typically used. The logit function is the logarithm of the ratio of the probability of a species being present to the probability of the species being absent. The model returns a probability of species occurrence at a given location based on the environmental predictors (Guisan *et al*., 2002).

The accuracy of the models produced were assessed by using the area under the curve (AUC) scores obtained as a 'flexsdm' output. This method makes use of a split-sample process through setting aside 20% of the initial data to validate the models predictions (Thuiller *et al*., 2009). This study employed the AUC score only as it does not depend on a probability threshold to distinguish between sites of predicted establishment and sites where establishment is unlikely (Elith *et al*., 2006; Walther *et al*., 2007). The models were deemed accurate if the AUC score was above 0.7 (Brotons *et al*., 2004).

3.3.3 Adjusting *Anthonomus* species predicted distributions to host range

The ranges of both weevil species are dependent on the range of their host. This allows for further adjustment of the ESM predictions for the agents by restricting their estimated ranges to that of *S. mauritianum*.

The range of *S. mauritianum* in South Africa was mapped by applying the same methods for environmental predictor selection and Ensemble of Small Models approach as that of both *Anthonomus* species. A total of 2158 recorded observations for the plant in South Africa were obtained from GBIF.org for use in the model. The ESM for *S. mauritianum* was set to return a range within South Africa where weevil establishment probabilities were 65% and above to represent only areas where establishment is highly probable.

The ranges of *A. morticinus* and *A. santacruzi* were fitted to that of the plant. The ESMs for both weevils species were filtered to return distributions of probabilities 65% and higher. The *Anthonomus* and *S. mauritianum* distributions were then mapped onto South Africa in QGIS. The weevil ranges were clipped to that of *S. mauritianum*, eliminating regions of weevil potential establishment where *S. mauritianum* is expected to be absent.

3.3.4 Mechanistic species distribution models

3.3.4.1 CLIMEX model parameters and input data

Temperature index: The temperature index comprises four thermal values which includes a lower and upper temperature threshold (DV0 and DV3) and a lower and upper optimal temperature (DV1 and DV2). The threshold values represent the lowest and highest temperature extremes outside of which species survival is greatly impaired. The optimal temperatures represent a thermal range ideal for species survival and development between the lower and upper optimal temperatures. The lower temperature thresholds for both *Anthonomus* species were set to correspond with the CT_{min} from Cowie *et al* (2016b) who estimated a CT_{min} for *A. santacruzi* at 4.1 °C while Mkhomazi (2022) observed a CT_{min} for the species at 4.9 °C. Thus, the

DV0 for *A. santacruzi* was set as the average of these two findings at 4.5 °C. The DV0 for *A. morticinus* was set to 1.7 °C corresponding with the CT_{min} determined by Mkhomazi (2022). The optimal temperatures were set to the average minimum and maximum temperatures of known species occurrences in their native range. While both species were found in Corrientes province in Argentina, only *A. morticinus* was collected in Uruguay. Thus, the lower optimal temperature (DV1) for *A. santacruzi* was set to the minimum average temperature of Corrientes (16 °C) while that of *A. morticinus* was set to the minimum average temperature of Montevideo (11 °C). The upper optimal temperature (DV2) for both species was set to the maximum average temperature of Corrientes (29 °C). Lastly, the upper thermal thresholds (DV3) for both species were set to the maximum temperature recorded at the Corrientes collection site (34 °C) as neither species has not been observed at locations with a greater temperature.

Moisture index: The moisture index relates to soil moisture which is a determining factor for the moisture available to vegetation at a given site (Sutherst *et al*., 2007; Kriticos *et al*., 2015). Like the temperature threshold, soil moisture has lower and upper moisture thresholds (SM0 and SM3) and lower and upper optimal moistures (SM1 and SM2). Soil moisture values are dimensionless and were fitted using the Köppen climate classification and the localities from the native ranges for both *Anthonomus* species (Alvares *et al*., 2014). As the soil moisture corresponds more with *S. mauritianum* requirements instead of the insect, the moisture index fitted for both species was the same. The lower soil moisture threshold (SM0) was given a value of 0.1, representing a value near to 0 at which plants face permanent wilting or death (Sutherst *et al*., 2007). The lower and upper optimal soil moistures (SM1 and SM2) were set to 0.7 and 1.3 representing the subtropical conditions of the two weevil's native range. The upper soil moisture threshold (SM3) was set at 2, representing the more tropical and wet areas where both weevil species are not currently reported.

Cold stress: The cold stress threshold (TTCS) of each species was adjusted to their respective CT_{min} values (4.5 °C for *A. santacruzi* and 1.7 °C for *A. morticinus*). The cold stress accumulation rates (THCS) were fitted through iterative adjustment to match the known establishment records for both species (Falla *et al*., 2021).

Heat stress: The heat stress thresholds (TTHS) were 34 °C for both species which corresponds with the highest temperature at which the weevils have been observed being the

Argentinian Province of Corrientes. The heat stress accumulation rates (THHS) were iterated to represent locations with the highest temperature in which the weevils are found.

Dry stress: The dry stress thresholds (SMDS) were set to 0.1 for both *Anthonomus* species. The dry stress accumulation rate (HDS) was adjusted to yield a range corresponding with the *S. mauritianum* ESM (Figure 3.3) as both species are host specific on the plant.

Wet stress: The wet stress thresholds (SMWS) were set at 2 for both *A. santacruzi* and *A. morticinus* with the wet stress accumulation rates (HWS) iteratively adjusted to fit *the S. mauritianum* ESM.

CLIMEX estimates species growth using an Annual Growth Index (GI_A) calculated as the product of the Temperature Index (TI) and the Moisture Index (MI). The Stress Index (SI) is the species response to climatically unsuitable environments comprised of the stress indices (CS, HS, DS, WS). The Ecoclimatic Index (EI) for a species is the product of the GI_A and the SI (Sutherst and Maywald, 2005). EI ranges from 0 (unsuitable) to 100 (perfect), with higher values indicating areas ideal to sustain permanent populations (Sutherst, 2003; Kriticos *et al*., 2015).

Table 3. 1: Parameter values used to estimate the species distributions of *Anthonomus morticinus* and *Anthonomus santacruzi* in South Africa using the CLIMEX mechanistic modeling approach. The highlighted cells indicate where major differences between the species were found.

Parameter	**Description**	***A. santacruzi***	***A. morticinus***	**Units**
Temperature	DV0 = lower temperature threshold	4.5	1.7	°C
	DV1 = lower optimal temperature	16	11	°C
	DV2 = upper optimal temperature	29	29	°C
	DV3 = upper temperature threshold	34	34	°C
Soil moisture	SM0 = lower soil moisture threshold	0.1	0.1	
	SM1 = lower optimal soil moisture	0.7	0.7	
	SM2 = upper optimal soil moisture	1.7	1.7	
	SM3 = upper soil moisture threshold	2	2	
Cold stress	TTCS = cold temperature threshold	4.5	1.7	°C
	THCS = cold stress accumulation rate	-0.006	-0.006	Week $^{-1}$
Heat stress	TTHS = hot temperature threshold	34	34	°C
	THHS = hot stress accumulation rate	0.002	0.002	Week $^{-1}$
Dry stress	SMDS = dry stress threshold	0.1	0.1	
	HDS = dry stress accumulation rate	-0.005	-0.005	Week $^{-1}$
Wet stress	SMWS = wet stress threshold	2	2	
	HWS = wet stress accumulation rate	0.0015	0.0015	Week $^{-1}$

3.3.5 Ensemble of Small Models vs CLIMEX models

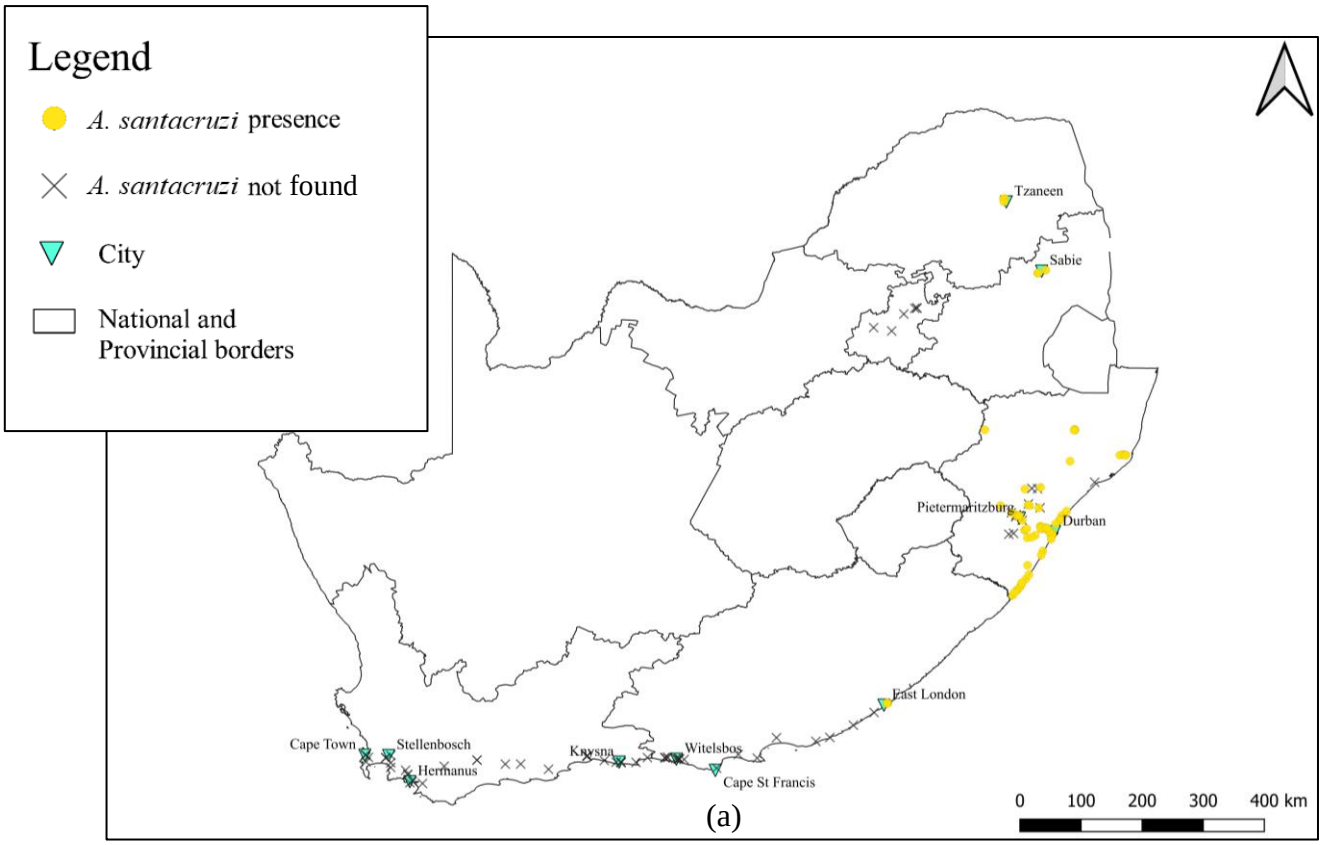
For visual comparison of the outputs of the two modelling techniques the ESM and CLIMEX predictions for both weevil species were mapped for all rasters with a 65% probability of establishment or with EI scores of 65 and above.

3.4 Results

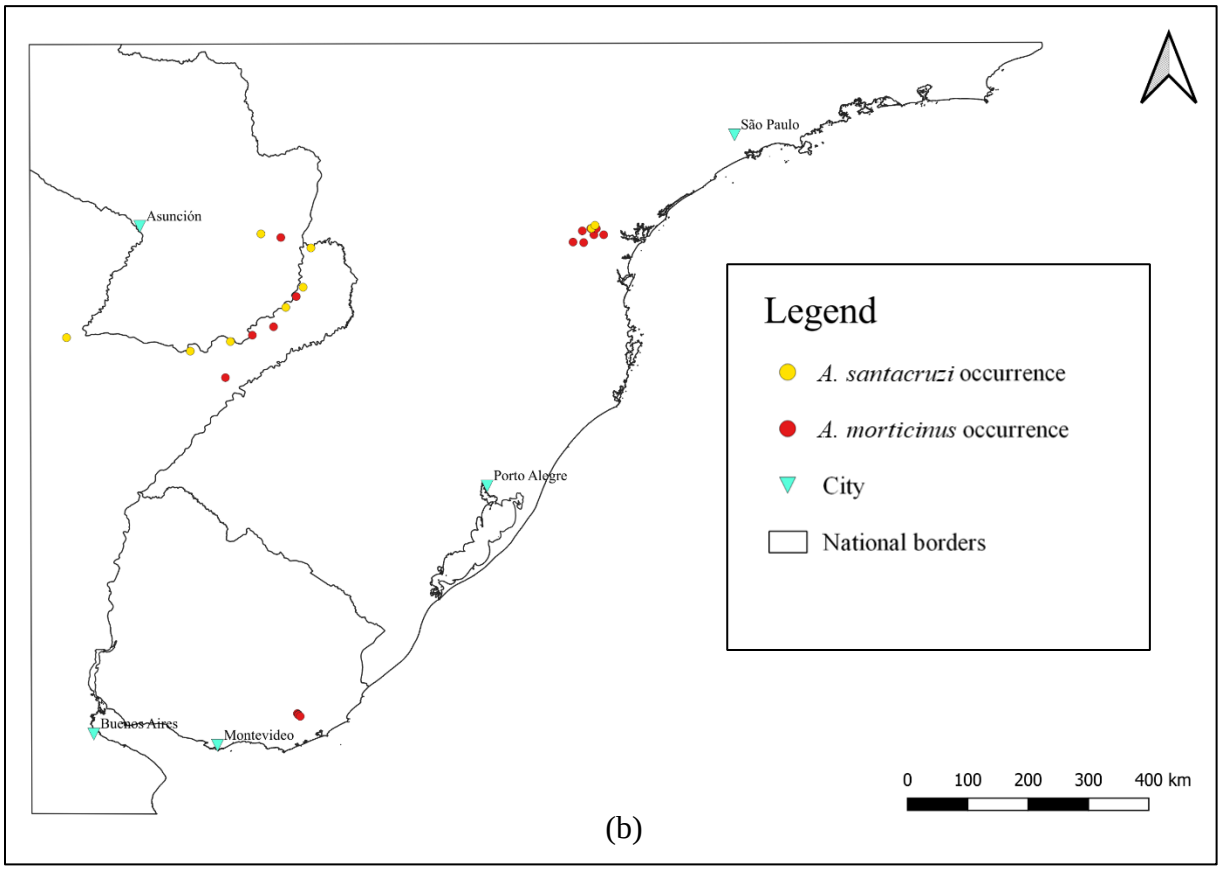
3.4.1 *Anthonomus santacruzi* establishment in South Africa

Table 3. 2: Localities throughout the Western and Eastern Cape Provinces where *Solanum mauritianum* plants were sampled to identify the establishment of *Anthonomus santacruzi* along the South African south coast. Sites where *A. santacruzi* individuals were not observed were recorded as 'Absent' and locations where the weevil was found were recorded as 'Present'.

Locality name	Longitude	Latitude	Date	Status
Signal Hill	18.409925	-33.924686	19/02	Absent
Rondebosch	18.466841	-33.967735	19/02	Absent
Spier wine farm site 1	18.782757	-33.974548	19/02	Absent
Spier wine farm site 2	18.785237	-33.973159	19/02	Absent
Spier wine farm site 3	18.782757	-33.973602	19/02	Absent
Karwyderskraal road site 1	19.182684	-34.277275	19/02	Absent
Karwyderskraal road site 2	19.183356	-34.280444	19/02	Absent
Hermanus	19.270782	-34.404919	19/02	Absent
Swellendam site 1	20.449339	-34.016846	20/02	Absent
Swellendam site 2	20.449286	-34.016496	20/02	Absent
Heidelberg	20.966537	-34.090263	20/02	Absent
George site 1	22.467184	-33.941277	20/02	Absent
George site 2	22.456551	-33.944534	20/02	Absent
George site 3	22.457375	-33.940044	20/02	Absent
George site 4	22.457401	-33.943882	20/02	Absent
Knysna site 1	23.084381	-34.058151	21/02	Absent
Knysna site 2	23.081867	-34.057865	21/02	Absent
Knysna site 3	23.080973	-34.057545	21/02	Absent
Knysna site 4	23.071432	-34.053574	21/02	Absent
Plettenberg bay	23.354080	-34.058125	21/02	Absent
Storms river village site 1	23.888363	-33.972687	21/02	Absent
Storms river village site 2	23.885033	-33.974636	21/02	Absent
Storms river village site 3	23.881336	-33.971447	21/02	Absent
Storms river petroport site 1	23.930071	-33.967251	21/02	Absent
Storms river petroport site 2	23.932972	-33.968536	21/02	Absent
Witelsbos site 1	24.081371	-33.989883	21/02	Absent
Witelsbos site 2	24.119047	-33.994629	21/02	Absent
Witelsbos site 3	24.131996	-33.995773	21/02	Absent
Witelsbos site 4	24.134089	-33.996044	21/02	Absent
Witelsbos site 5	24.138729	-33.996429	21/02	Absent
Witelsbos site 6	24.150738	-33.996830	21/02	Absent
Oudebosch farmstall	24.233013	-34.004868	21/02	Absent
Nahoon estuary nature reserve	27.935535	-32.981365	22/02	Present
Nahoon	27.941423	-32.989037	22/02	Absent
Nahoon	27.942322	-32.984715	22/02	Present
Bonza Bay	27.957743	-32.971737	22/02	Present
Bonnie Doon	27.919905	-32.981033	22/02	Present



(a)



(b)

Figure 3. 1: (a) All *Anthonomus santacruzi* occurrence sites in South Africa from Cowie *et al* (2018), Singh and Olckers (2017) and occurrences recorded during field work. Absences of *A. santacruzi* observed along the south coast are also shown. (b) Occurrence data for both *Anthonomus morticinus* and *Anthonomus santacruzi* in their native South American range is displayed based on data recorded by Olckers (1999), Pedrosa-Macedo *et al* (2003) and Venter *et al* (2021).

No evidence of *A. santacruzi* establishment was found near any of its release sites in the Western Cape province near Cape Town (18.409925, -33.924686), Stellenbosch (18.782757, -33.974548), Hermanus (19.270782, -34.404919) or Witelsbos (24.131996, -33.995773). Furthermore, individuals were not found between these sites or beyond the Witelsbos release site. However, *A. santacruzi* was found to have established at 4 of the 5 locations sampled in East London (27.957743, -32.971737) (Table 3.2) (Figure 3.1a).

3.4.2 Anthonomus morticinus and *Anthonomus santacruzi* Ensemble of Small Models

Using the Maxent variable importance function from the rJava package (Urbanek, 2023), five variables were recognized as important for *A. morticinus* distribution in its native range consisting of the (1) precipitation of the driest quarter (BIO 19), (2) maximum temperature of the warmest month (BIO 5), (3) precipitation seasonality (BIO 15), (4) mean diurnal range (BIO 2) and (5) mean temperature of driest quarter (BIO 9) (Table 3.3).

The ESM estimated establishment for *A. morticinus* along the South African south coast, from the coast of St Helena Bay (-32.818608, 17.898041) until Richards Bay (-28.727074, 32.034347). Establishment was also predicted in the Cederburg region northeast of Cape Town, the Lesotho highlands and central Mpumalanga and Limpopo provinces (Figure 3.2a). This model had an AUC of 0.76 indicating a high probability.

Table 3. 3: Bioclimatic variables associated with the distribution of *Anthonomus morticinus* in its native South American range calculated using Maxent. The percentage contribution refers to the weight of the variable in determining the model while the permutation importance refers to the weight of the predictor on species distribution.

Variable code	Variable name	Percent contribution	Permutation importance
BIO19	Precipitation of Coldest Quarter	69.60	67.64
BIO05	Max Temperature of Warmest Month	12.76	11.27
BIO15	Precipitation Seasonality	11.21	16.02
BIO02	Mean Diurnal Range	4.68	3.17
BIO09	Mean Temperature of Driest Quarter	0.98	1.24

The bioclimatic variables for *A. santacruzi* found five climatic predictors of significance driving the establishment of the weevil in South Africa. They comprise of the (1) annual precipitation (BIO12) with the highest permutation importance at 81% followed by the (2) minimum temperature of the coldest month (BIO 6), (3) precipitation seasonality (BIO 15), (4) isothermality (BIO 3) and (4) temperature seasonality (BIO 4) (Table 3.4).

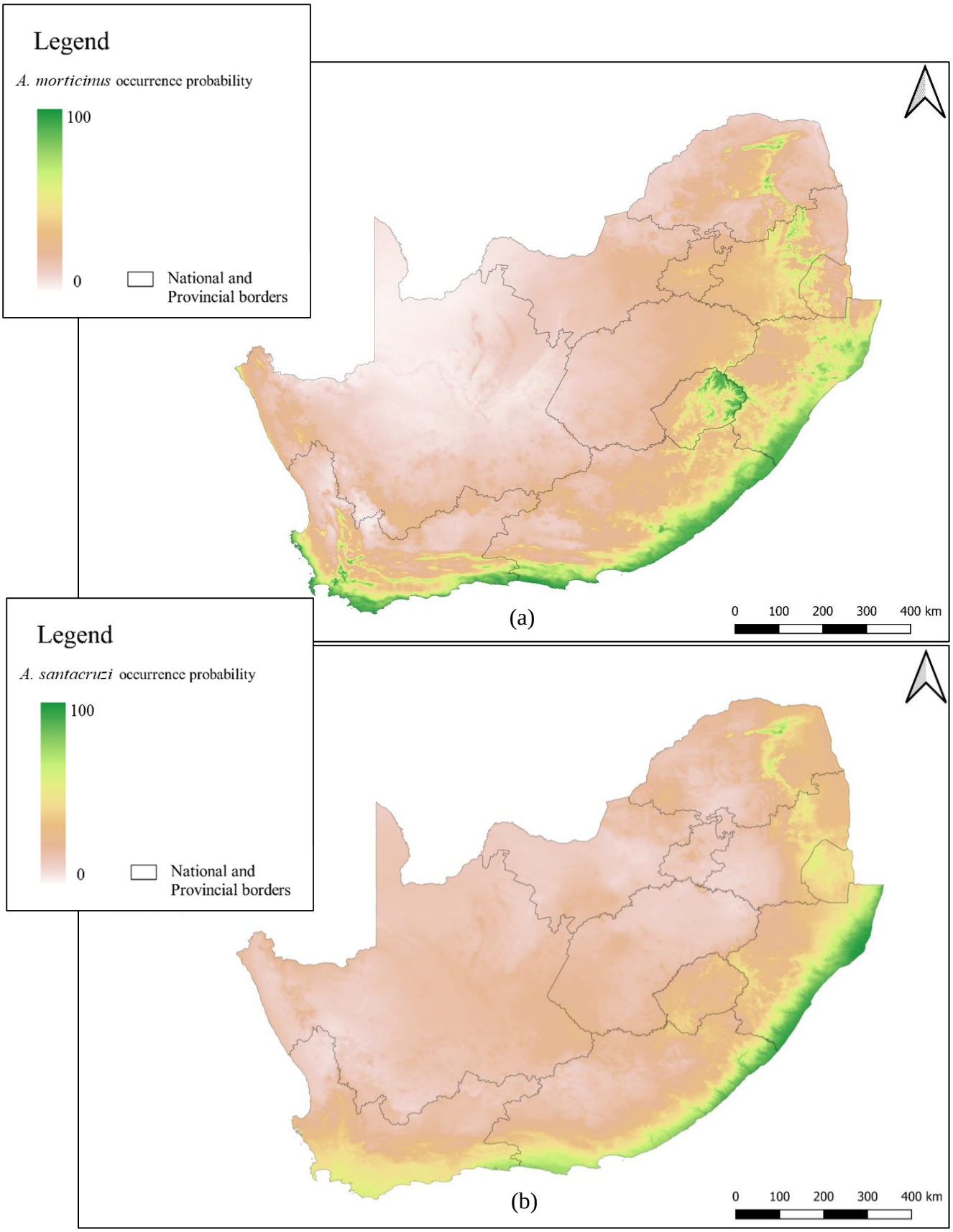


Figure 3. 2: Distributions predicted using the Ensemble of Small Models for *Solanum mauritianum* biocontrol agents (a) *Anthonomus morticinus*, mapped using 17 native South American occurrence data points from Olckers (1999), Pedrosa-Macedo *et al* (2003) and Venter *et al* (2021), and (b) *Anthonomus santacruzi* using non-native South African occurrence data points from Cowie *et al* (2018), Singh and Olckers (2017) and occurrences recorded during ground truthing.

Table 3. 4: Bioclimatic variables associated with the distribution of *Anthonomus santacruzi* within its introduced South African range calculated using Maxent. The percentage contribution refers to the weight of the variable in determining the model while the permutation importance refers to the weight of the predictor on species distribution.

Variable code	Variable name	Percent contribution	Permutation importance
BIO12	Annual Precipitation	44.96	81.49
BIO06	Min Temperature of Coldest Month	19.89	5.58
BIO15	Precipitation Seasonality	16.95	5.74
BIO03	Isothermality	12.21	3.80
BIO04	Temperature Seasonality	5.17	2.52

The *A. santacruzi* ESM projected a range similar to the Cowie *et al* (2018) mechanistic model showing establishment along the east coast and the Cape St Francis to Knysna coast. The central regions of Mpumalanga and Limpopo Provinces are also predicted to show *A. santacruzi* establishment with a lesser distribution than that of *A. morticinus*. The AUC value for the *A. santacruzi* ESM showed very high levels of accuracy at 0.92.

3.4.3 Adjusting *Anthonomus* species predicted distributions to host range

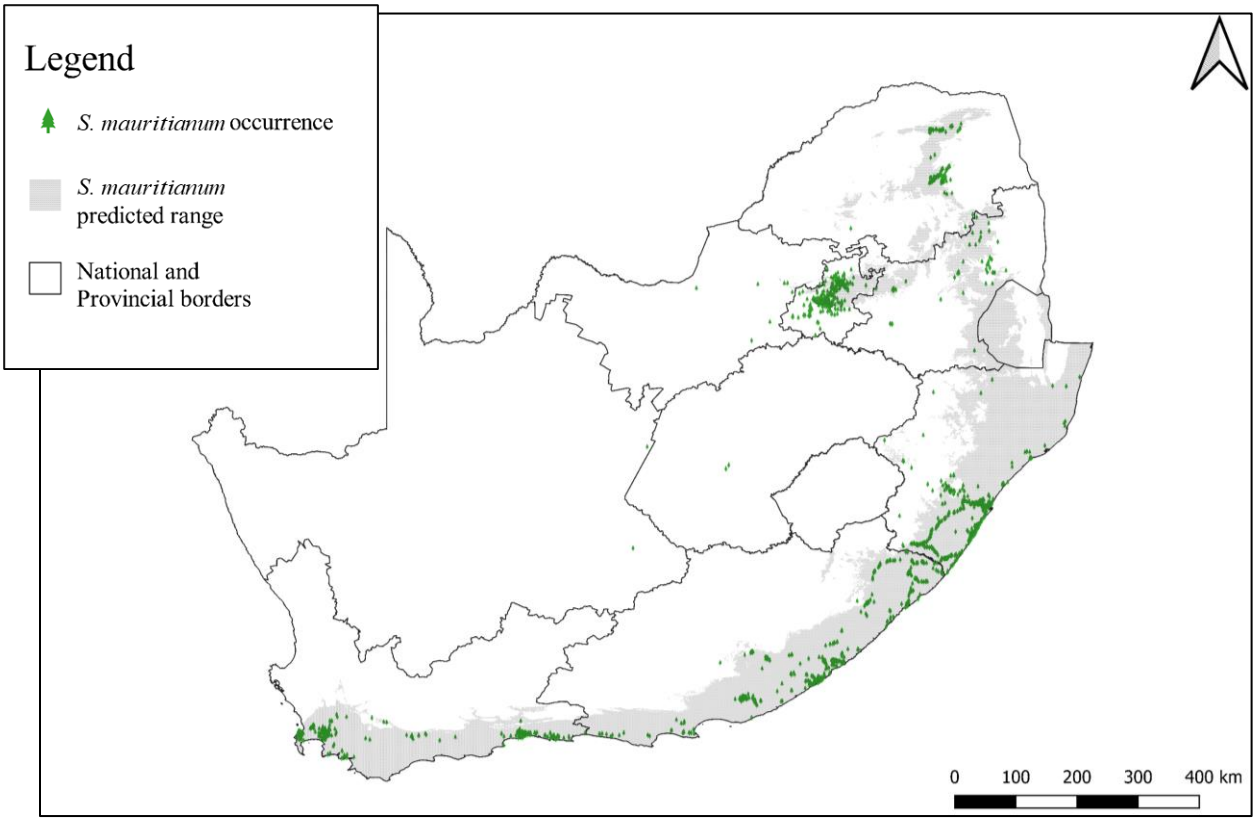


Figure 3. 3: Ensemble of Small Models projection predicting the distribution of *Solanum mauritianum* in South Africa with an establishment probability of above 65%. The model was generated using *S. mauritianum* occurrence records in South Africa obtained from GBIF.org.

Seven bioclimatic variables were identified in the distribution of *S. mauritianum* in South Africa based on South African occurrence records. These include (1) temperature annual range (BIO 7), (2) maximum temperature of warmest month (BIO 5), (3) isothermality (BIO 3), (4) precipitation of driest month (BIO14), (5) precipitation of wettest month (BIO13), (6) minimum temperature of coldest month (BIO 6) and (7) mean temperature of wettest quarter (BIO 8) (Table 3.5).

Table 3. 5: Bioclimatic variables associated with the distribution of *Solanum mauritianum* within its non-native South African range calculated using Maxent. The percentage contribution refers to the weight of the variable in determining the model while the permutation importance refers to the weight of the predictor on species distribution.

Variable code	Variable name	Percent contribution	Permutation importance
BIO07	Temperature Annual Range	35.51	17.19
BIO05	Max Temperature of Warmest Month	23.99	36.37
BIO03	Isothermality	10.72	7.42
BIO14	Precipitation of Driest Month	10.63	8.76
BIO13	Precipitation of Wettest Month	9.11	10.44
BIO06	Min Temperature of Coldest Month	6.62	5.33
BIO08	Mean Temperature of Wettest Quarter	3.42	14.49

The ESM model for *S. mauritianum* included areas where establishment of the plant was 65% and above. The range shows establishment along the south coast from Cape Town to the Mozambican border on the eastern coast. Its range includes the northern and central regions of the Gauteng Province, eastern Eswatini and central Mpumalanga and Limpopo Provinces. The occurrences used in this model were plotted with 85% of the observations falling within the range produced (Figure 3.3). The AUC for this model had high accuracy with a value of 0.95.

Anthonomus morticinus' and *A. santacruzi*' predicted ranges were plotted at 65% probability against that of their hosts range. While *A. morticinus* showed some areas outside of the plant's range including north of Cape Town and Lesotho (Figure 3.4a), *A. santacruzi* showed a range completely within that of *S. mauritianum* (Figure 3.4b). As the weevil cannot survive without its host, the *A. morticinus* range was clipped to fit within the *S. mauritianum* predicted range.

3.4.4 Mechanistic species distribution models

The *A. morticinus* CLIMEX model using known ecophysiological data predicted the highest probability of establishment along the east coast of South Africa and the Knysna to Cape St Francis coast. Occurrence was also predicted in Cape Town and Hermanus and more inland including western Eswatini and central Mpumalanga and Limpopo Province areas (Figure 3.5a).

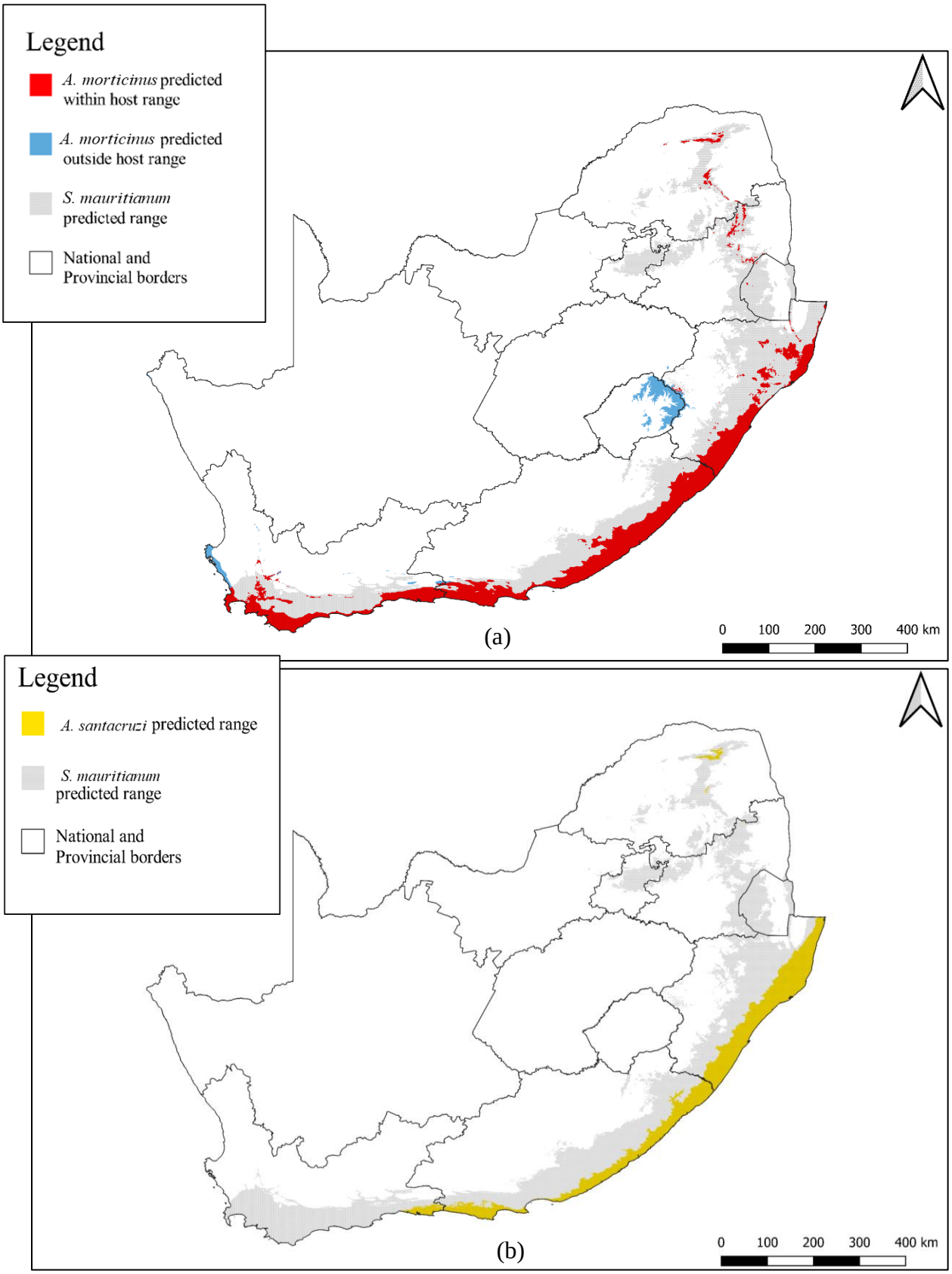


Figure 3. 4: The Ensemble of Small Models projections for the geographic distributions of (a) *Anthonomus morticinus* and (b) *Anthonomus santacruzi* showing estimated ranges at probabilities of 65% and higher in South Africa. Each projection depicts the range of the respective species within the predicted range of the *Solanum mauritianum* host in South Africa.

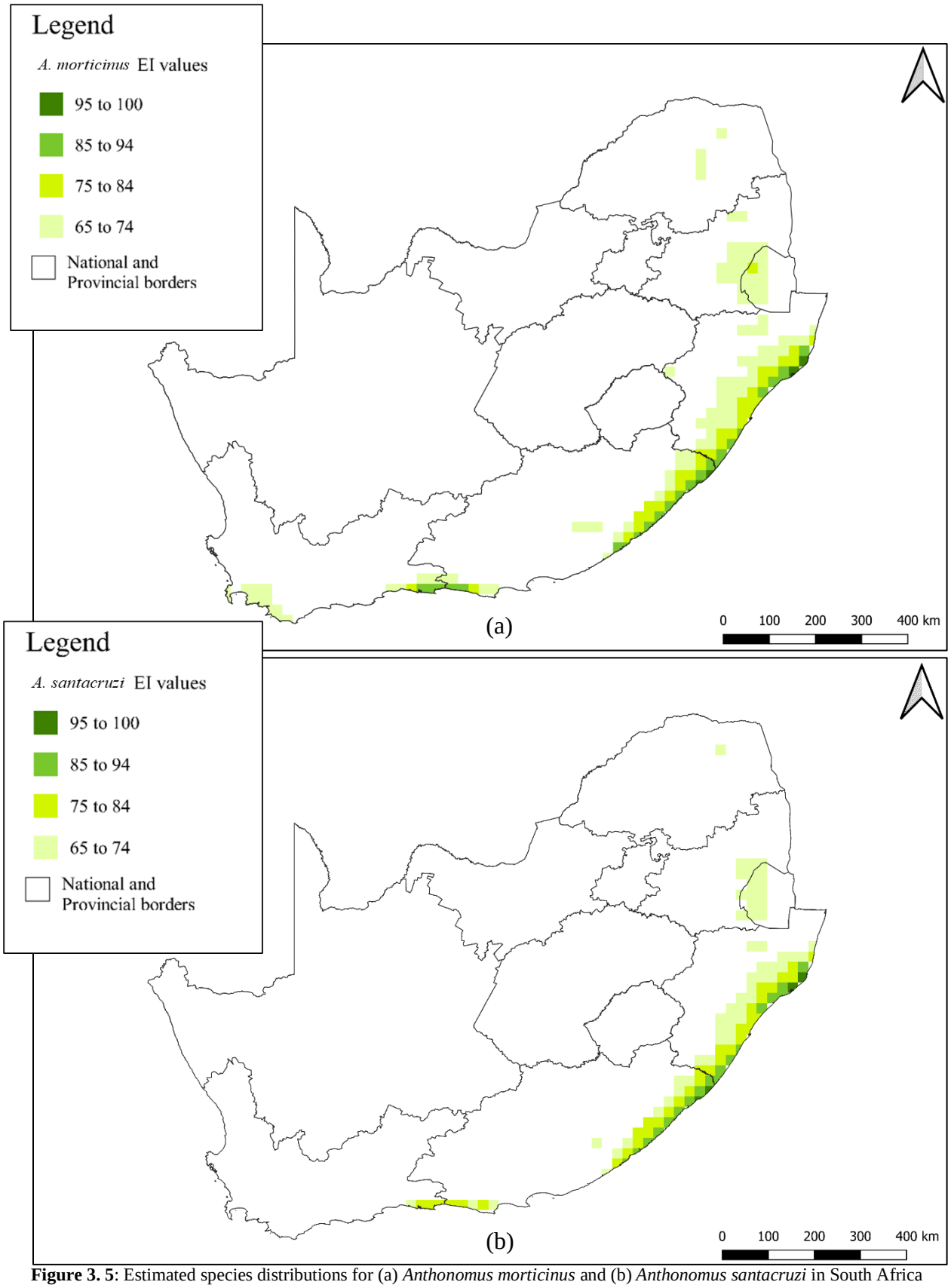


Figure 3. 5: Estimated species distributions for (a) *Anthonomus morticinus* and (b) *Anthonomus santacruzi* in South Africa based on projections generated using the mechanistic CLIMEX modelling approach utilizing known species ecophysiological data

The *A. santacruzi* model is more restricted than *A. morticinus* with establishment predicted along the eastern coast of KwaZulu Natal and Eastern Cape Provinces. Establishment is also predicted from Knysna to Cape St Francis and western Eswatini. Single rasters also show possible species occurrence north of the Eswatini border and in northern Limpopo Province (Figure 3.5b).

3.4.5 Ensemble of Small Models vs CLIMEX models

The predicted range for *A. morticinus* distribution in South Africa is similar in both the ESM and CLIMEX models (Figure 3.6). Both approaches show the species range along the east coast, the Knysna to Cape St Francis stretch and occurrence in Cape Town and Hermanus. Furthermore, both maps estimate species occurrence in central Mpumalanga and Limpopo Provinces. However, the maps differ in Eswatini with the ESM predicting no species occurrence in the country while CLIMEX predicts *A. morticinus* occurrence in the western regions of Eswatini (Figure 3.6). The ESM is able to detail the species range to a greater extent as the model uses ~ 1 km^2 bioclim rasters while Climond uses rasters at ~ 340 km^2. This is evident along the southern coastline where the ESM predicts small regions for species occurrence while CLIMEX cannot.

The ESM approach and CLIMEX mechanistic approach returned visually similar distributions for *A. santacruzi* in South Africa (Figure 3.7). Both maps predict species range along the eastern coast of South Africa and the Knysna to Cape St Francis coastline. The maps also estimate species occurrence in the Tzaneen area in northern Limpopo Province. The maps do however differ in their predictions in Eswatini, with the ESM showing no species occurrence in the country while CLIMEX estimates species occurrence in the county's western regions (Figure 3.7).

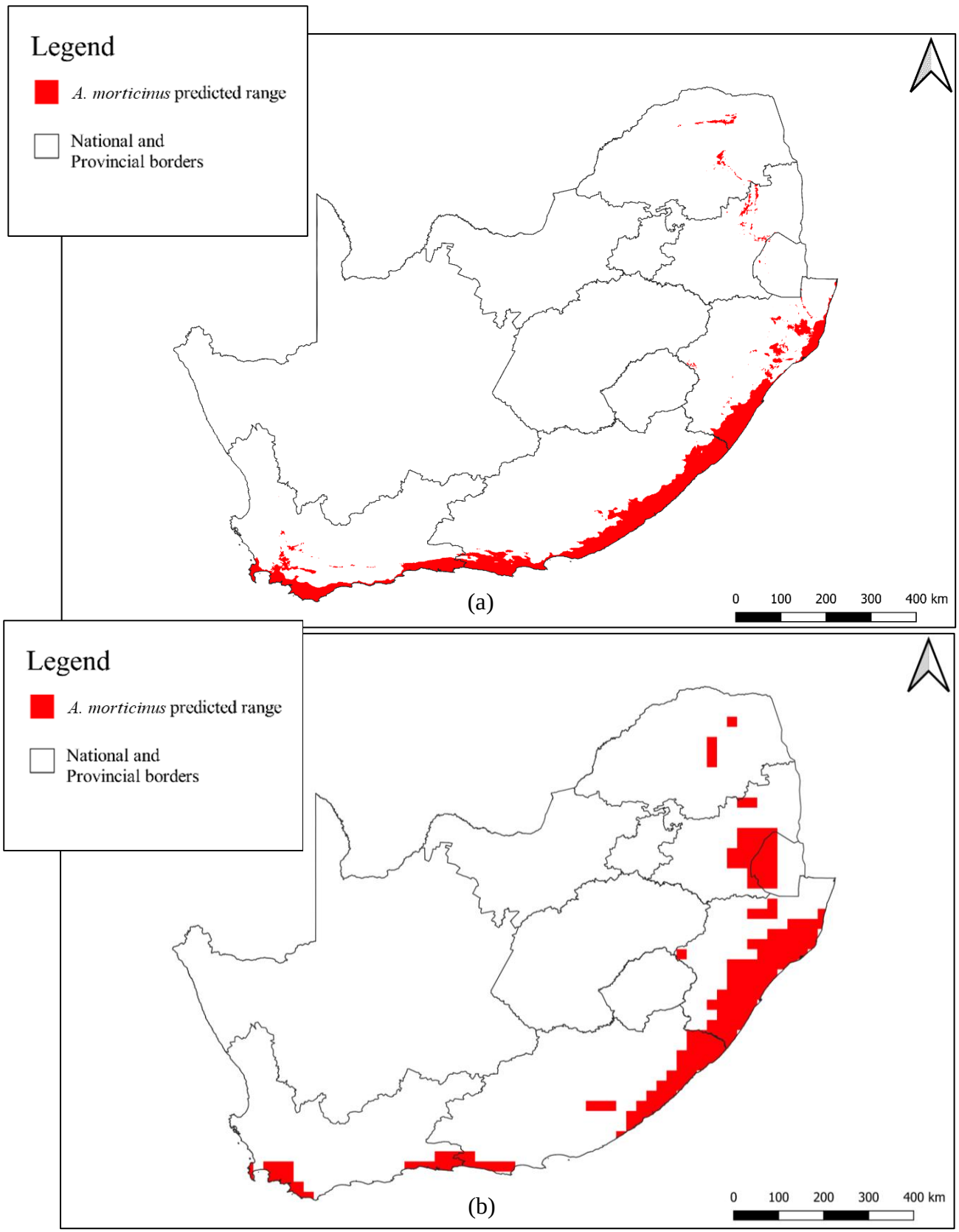
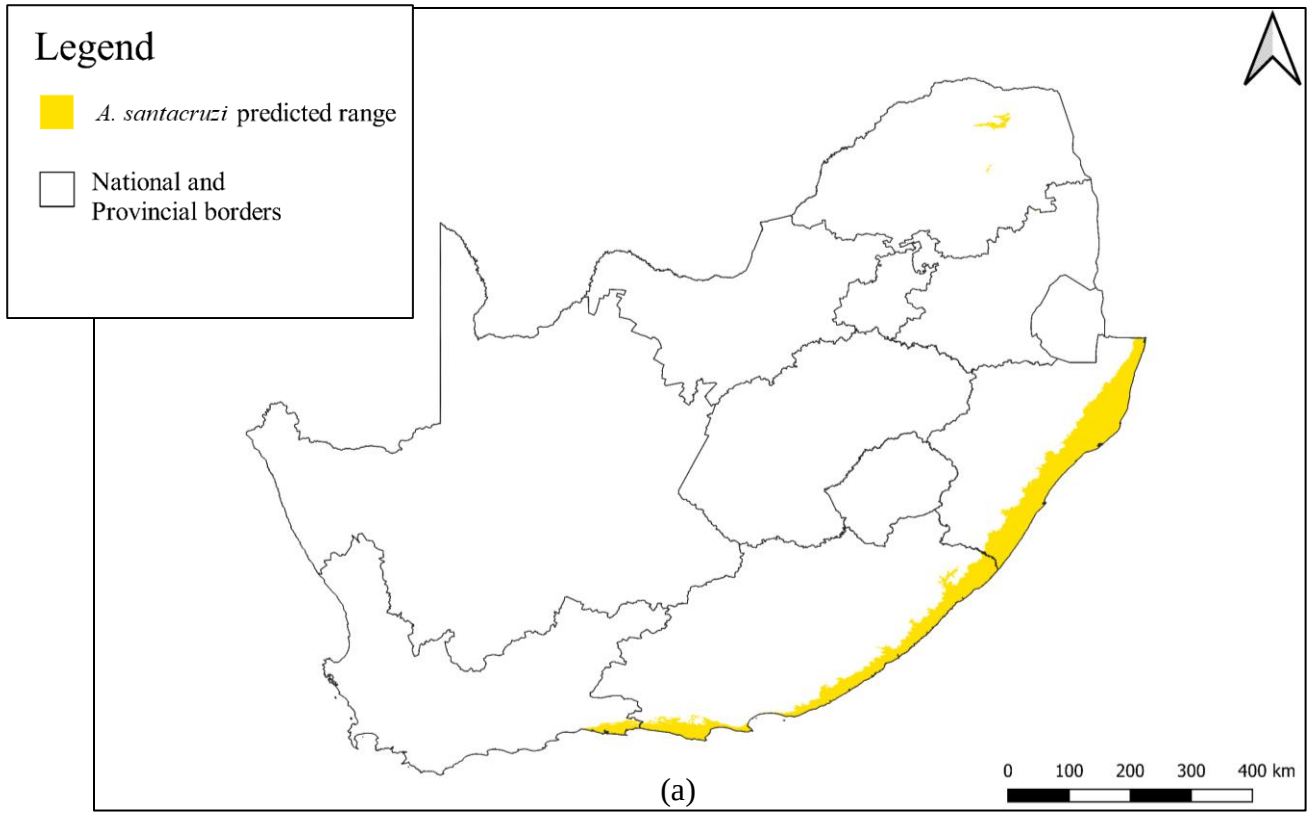
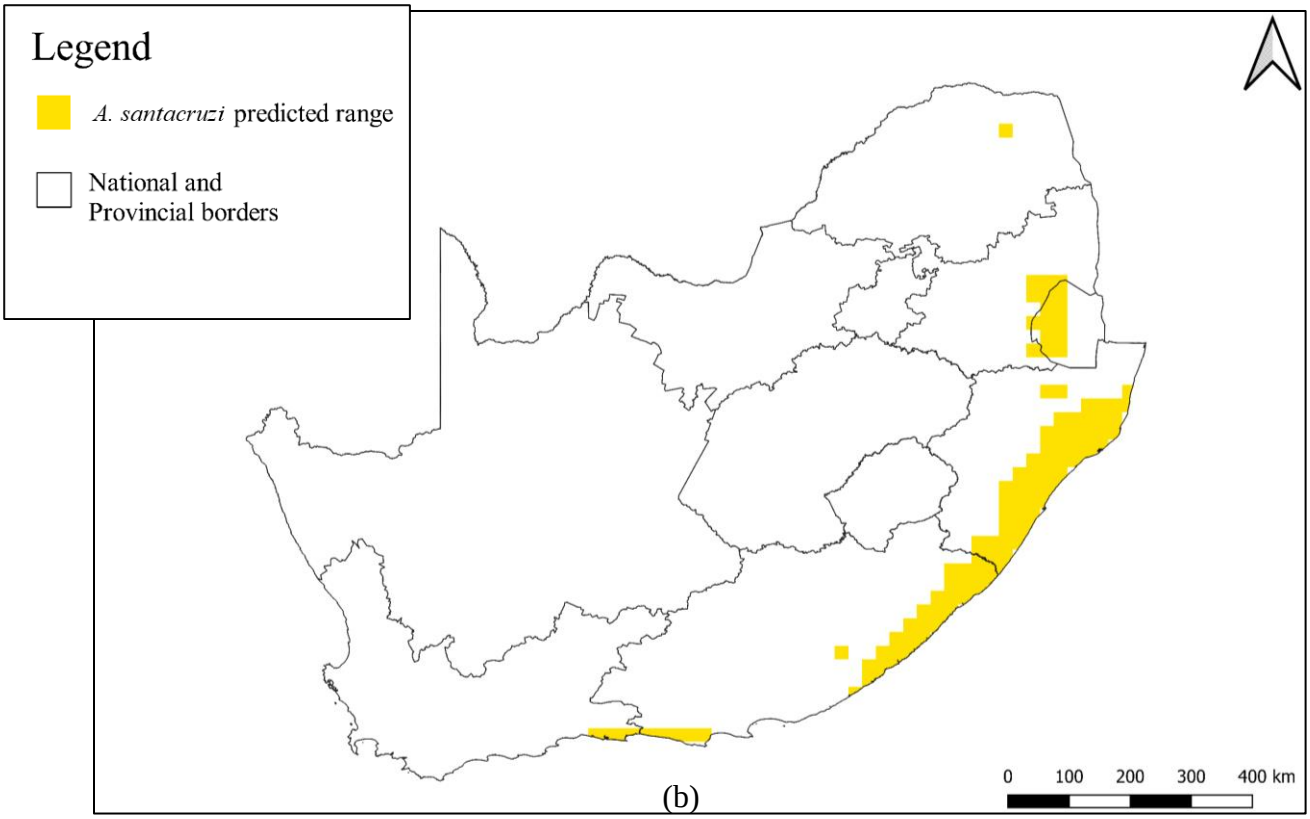


Figure 3. 6: Predicted distribution for *Anthonomus morticinus* in South Africa based on projections using (a) the Ensemble of Small Models approach with >65% probability of occurrence and (b) CLIMEX mechanistic approach at >65 Ecoclimatic Index score for species establishment.



(a)



(b)

Figure 3. 7: Predicted distribution for *Anthonomus santacruzi* in South Africa based on projections using (a) the Ensemble of Small Models approach with >65% probability of occurrence and (b) CLIMEX mechanistic approach at >65 Ecoclimatic Index score for species establishment.

3.5 Discussion

The climatic suitability of biological control agents remains the most significant point in determining the extent of their post-release success (Byrne *et al*., 2004; Muskett *et al*., 2020; Stiling, 1993). However, most of the classical species distribution modelling techniques available are unable to predict agent distribution owing to the small occurrence datasets characteristic of such species (Guisan and Thuiller, 2005). The aim of this study was to reveal if the Ensemble of Small Models (ESM) approach, often used in modelling the distribution of rare species, would be able to estimate the distributions of biological control agents with limited occurrence data. The predictive performance of the model was illustrated by identifying climatically suitable regions in South Africa for supporting populations of *Anthonomus santacruzi* and *Anthonomus morticinus*. The ensemble models proved useful in comparing the estimated ranges of the weevils in South Africa, with *A. morticinus* showing a wider distribution along the south coast and inland regions than that of *A. santacruzi.* The *S. mauritianum* ESM indicated some areas of overprediction with the *A. morticinus* model particularly in the Lesotho highlands and west coast regions, which were subsequently adjusted for, while *A. santacruzi* showed no predictions outside of the host range. Results from the ensemble models and the CLIMEX models for both *Anthonomus* species yielded visually similar projections, estimating a broader geographic range for *A. morticinus* than *A. santacruzi* particularly along the south coast of South Africa. Furthermore, the models produced for *A. santacruzi* show significant resemblance the models produced by Cowie *et al*. (2016b).

Interestingly, despite the predictions of both the ESM and CLIMEX models for *A. santacruzi* establishment along the south coast between Knysna and Cape St Francis, no individuals were found during the field assessments (Table 3.2). This seemingly contradictory observation questions the validity of both modelling techniques used and establishes that such models may not always be accurate. One possible way to explain this apparent contradiction could point to the influence of biotic factors found along the south coast. Species distribution modelling predicts the range of a species based solely on known occurrence data and may thus exclude a number of biotic factors which may influence the distribution actually experienced by the species (Sutherst, 2003). For example, Cowie *et al* (2018) produced a CLIMEX mechanistic SDM for *Gargaphia decoris* in South Africa which indicated that the lace bug would occur in most of

Gauteng and KwaZulu Natal, along the south and east coasts of the country and the central expanses of the Mpumalanga and Limpopo provinces. However, the range of *G. decoris* has remained limited in the country (Klein, 2011). Predation by generalist predators such as ants on the vulnerable egg and nymph life stages of *G. decoris* has shown to decrease densities of the agent's population (Lotter, 2004). Furthermore, the lace bug has shown an affinity for *S. mauritianum* plants in shade and semi-shade conditions therefore reducing the habitats available to the agent (Cowie *et al.*, 2016a, Patrick and Olckers, 2014). Similarly, it may be possible that a biotic factor such as a predator species or another unforeseen element is present in these pockets along the south coast, preventing successful *A. santacruzi* establishment. However, previous studies have indicated that predation is not expected to present a significant risk to *A. santacruzi* survival and population growth (Hakizimana and Olckers, 2013).

An alternative explanation for the limited establishment of *A. santacruzi* in South Africa is the interaction of environmental temperatures and the lower thermal limits of the weevil. For instance, the mirid *Eccritotarsus catarinensis* was released at multiple sites in South Africa as a natural enemy against water hyacinth, *Pontederia crassipes* in 1996 (Hill *et al*., 1999). The insect managed to successfully establish at many release sites but failed when released in Johannesburg. Coetzee *et al* (2007) discovered that the mirid has a CT_{min} of around 1.2 °C, which is similar to temperatures often experienced during winter at the Johannesburg release site. The study estimated that *E. catarinensis* was unable to develop at a fast enough rate to successfully overwinter at the Johannesburg release site due to the low temperatures. To further challenge the persistence of the mirid over winter at this site, *P. crassipes* experiences greater levels of mortality in winter due to exposure to frost which is characteristic of the region (Hill and Cilliers, 1999). As the mirid resides and feeds on the weed, food and habitat availability for *E. catarinensis* is drastically reduced over these months (Coetzee *et al*., 2007). While these factors are assumed to be the underlying reasons for the insects failure in Johannesburg, the authors do not rule out the possibility of the effect of other aspects such as plant nutrients or ineffective agent release also contributes to this result. Mkhomazi (2022) calculated the CT_{min} for *A. santacruzi* to range from 3.8 to 6.7 °C. Average minimum temperatures of the *A. santacruzi* release sites along the south coast range from 6.7 to 8.5 °C with the exception of the East London site which has a minimum of 10.1 °C (BIO6 of www.worldclim.org). Thus, temperatures may border or even drop into the CT_{min} of the weevil during the coldest winter periods at these

sites therefore limiting species development. Furthermore, *S. mauritianum* is predicted to experience reduced photosynthesis when exposed to temperatures below 20 °C leading to a reduction in plant nutrients and floral material available (Cowie *et al*., 2016a). The abundance of *A. santacruzi* adults and larvae is both directly tied to the availability and quality of *S. mauritianum* floral material (English and Olckers, 2018), therefore further hindering the ability of the weevil to overwinter along the south coast.

Additionally, this explanation also describes the reasons why *A. santacruzi* was observed at release sites in East London while not being found at the sites further south. East London experiences average and minimum temperatures that are consistently about 2 °C above the temperature range of the other south coast release sites (CLIMEX 'match locations'). Thus, development of *A. santacruzi* is less likely to be unaffected in East London as a drop in temperature would most likely still not fall below the CT_{min} of the weevil. Despite being the most likely explanation for the absences observed along the south coast, it is possible that other factors such as the nature or number of the agents released, or an environmental event may also be responsible (McEvoy and Coombs, 2001). It should be noted that the *A. santacruzi* individuals released along the south coast were sourced from sites along the east coast such as Mt Edgecomb and Umkomaas (B. Cowie pers. comm). These locations have average minimums above 11 °C (CLIMEX 'match locations') and are thus much more similar in climate to East London than the other south coast release sites. Robertson *et al* (2008) suggested that biological control agents sourced from locations with similar climatic conditions to that of the release sites are expected to have already adapted to environmental conditions akin to those of the target area. Furthermore, Griffith *et al* (2019) compared the CT_{min} values of two *Eccritotarsus catarinensis* populations, one collected from the hottest site where the agent has established in South Africa, and the other from the coldest site. The study discovered that the thermal physiology of the agent was significantly influenced by season and site conditions, with insects from the cold site displaying significantly lower CT_{min} levels than those at the hottest site. This observation suggested that a degree of post-release local adaptation to the climatic conditions had occurred through microevolutionary processes (Griffith *et al*., 2019). Similarly, *A. santacruzi* has managed to establish at many warm sites such as Mt Edgecomb and Umkomaas but has also established in Pietermaritzburg which experiences temperatures more similar to that of the south coast release sites. This suggests that it may be possible for *A. santacruzi* to successfully establish at these

sites should they be sourced from field sites in or around Pietermaritzburg. Therefore, the predictions made by the ESM for *A. santacruzi* in the Western and Eastern Capes cannot be ruled out unless reevaluated and alternate methods for the agent's release continue to prove ineffective.

Similarly, this explanation provides insight into the reasons why *A. morticinus* is expected to have greater success than *A. santacruzi* along the south coast. While the CT_{min} values for *A. santacruzi* range from 3.8 to 6.7 °C, Mkhomazi (2022) also found significantly lower CT_{min} values for *A. morticinus* ranging between 1.2 and 3.0 °C. With average minimum temperatures for the Cape Town, Stellenbosch, Hermanus and Witelsbos release sites being between 6.7 and 8.5 °C (BIO6 of www.worldclim.org), the difference in temperatures is far larger for the *A. morticinus* CT_{min} than that of *A. santacruzi*. Furthermore, many other regions along the south coast have average minimums only slightly below that of the *A. santacruzi* release sites thus explaining why *A. morticinus* is likely to occur throughout much of the region. This observation is supported by both the CLIMEX mechanistic model, and the correlative ESM model produced.

This study, based upon keyword literature searches, is the first of its kind to utilize the ESM approach to model the estimated species range of putative biological control agents. The spatial projections generated for both *A. santacruzi* and *A. morticinus* using the ESM approach advocates for the use of this modelling technique for potential agents with similarly limited occurrence data. Furthermore, when compared to the CLIMEX models, the ESMs for both *Anthonomus* species returned highly comparable results. This comparison is significant as the ESMs used limited occurrence datasets while the mechanistic models were produced using a range of known physiological responses for both *Anthonomus* species. These findings further imply that both approaches are valid for testing the climatic suitability of an agent, and the method used should be chosen based on the type of data available.

The incorporation of ensemble modelling in biocontrol initiatives could yield major implications for the direction of future studies. For example, Olckers (1999) reviewed a range of potential candidates for biological control of *S. mauritianum* in South Africa. This review included agents now released in South Africa such as *G. decoris* and *A. santacruzi*. Many other agents assessed were rejected owing to their broad host ranges while others, including *A. morticinus*, were unsuccessfully cultured. Another candidate unsuccessfully cultured was the stem-boring beetle *Adesmus hemispilus* (Cerambycidae). The larvae of this beetle spend between 7 – 12 months

boring inside the pith of *S. mauritianum* stems while adults feed on the midribs and veins of the leaves (Olckers, 1999). The potential damage that the insect could inflict on *S. mauritianum* plants may thus be of value to its biological control in South Africa. Thirty-six occurrences currently exist for *A. hemispilus* in South America, originating around Rio de Janeiro to Porto Alegre in Brazil, and as far inland as the Misiones province of Argentina (www.inaturalist.org). Therefore, should interest in the species be renewed, as no physiological data currently exists for mechanistic models and with the limited amount of occurrence data available for standard correlative models, the ESM approach offers a useful technique to estimate the climatic suitability of the insect to the South African climate. Thus, the ESM could determine if the beetle would be suitable for the task before even embarking on an expedition into its native range.

Predicting distributions for biological control agents has many other potential applications of interest to biocontrol initiatives. One application may show areas where inter-specific competition can be predicted between multiple species of biocontrol agents with similar feeding habits. For example, *A. santacruzi* and *A. morticinus* both feed on *S. mauritianum* flowers and the larvae of both species mature and grow in the immature buds of the host plant (Olckers *et al*., 2002). The SDMs generated in this study indicate regions where competition between the two species may occur based on climatic overlap. Another possible application is determining what indigenous plant species are required for host range testing. While it is ideal to test as many potential non-target species as possible, it is not always feasible to do so, and it may not even be relevant. For example, *Solanum burchellii* is a species of Solanaceae native to the western parts of South Africa. The plant has been used to test for host specificity in both *A. santacruzi* and *A. morticinus* (Olckers, 2003), but the climate analyses challenge why such a species should tested when the predicted ranges of the weevils do not overlap with the plant (www.GBIF.org). Thus, it may be possible to narrow down non-target trials by testing only species found in the predicted ranges of the insects. The predicted ranges can also be added to risk analyses for non-target species, possibly lowering the risk the agent poses to species outside its predicted range. However, while focusing non-target species trials based on the predicted geographic ranges of the agent can improve efficiency, it does not fully mitigate risk. For example, *Rhinocyllus conicus* Froelich (Coleoptera: Curculionidae), a biological control agent released against *Carduus nutans* (Asteraceae) in the United States of America showed an expansion of its host range to include native thistles in the United States, including species previously considered

outside its predicted distribution (Buntin and Murphy, 2018; Sauer and Bradley, 2008). This highlights the potential for biological control agents to adapt or extend their geographic and ecological ranges, posing unanticipated risks to non-target species and ecosystems.

Despite the various applications of climatic suitability modelling for biocontrol agents, it is not without its limitations. These models are based on the climatic variables present at occurrence sites affecting the establishment and distribution of insect species thus limiting such models. Biotic factors such as predation, food availability and host plant quality are important determining features of the realized niche of a species (Hamby *et al*., 2016; Klein, 2011). As seen with the lace bug *Gargaphia decoris*, predation and its specific habitat requirements have proven to be the main contributing factors to their limited distribution and population densities (Cowie *et al*., 2018). Another factor which climate analysis cannot account for is the quality of the host plant and the effect of that quality on the agent (Awmack and Leather, 2002; Salgado and Saastamoinen, 2019). An example of this can be seen in the floral material of *S. mauritianum* plants which drops in quality and quantity under 20 °C (Cowie *et al*., 2016a). Populations of agents such as *A. santacruzi* are linked to the abundance and quality of the hosts floral material and therefore populations are expected to decrease with decreasing quality and availability of the food source (English and Olckers, 2018). In spite of these limitations, species distribution modelling remains a useful first filter for finding suitable biocontrol agents.

3.6 Conclusion

In conclusion, the climatic suitability of biological agents to the areas intended for their release should be a major consideration in the search for suitable agents. With the limitations of classical correlative and mechanistic species distribution modeling for biological control initiatives due to limited occurrence and ecophysiological data, the Ensemble of Small Models approach provides a novel method for evaluating the likely range a biological control agent will occupy. To demonstrate the performance of the model, the correlative ESM and mechanistic CLIMEX approaches were used to investigate the hypothesis the *Solanum mauritianum* biological control agent *Anthonomus morticinus* would exhibit a wider predicted range than that of the already released *Anthonomus santacruzi* in South Africa. The models produced revealed that *A. morticinus* is, in fact, expected to have a wider distribution than *A. santacruzi* particularly along the south coast and more inland regions of South Africa. The projections were then compared to

an ESM of *S. mauritianum* within the country, which ensured that the *Anthonomus* models were aligned with the predicted habitat of the host. These results were then compared to mechanistic models produced using CLIMEX to allow for the comparison of the two techniques. The ESMs and CLIMEX models showed very similar expected distributions for each weevil in South Africa, thus indicating the validity of the ESM technique for biological control programs as a viable replacement for mechanistic models when ecophysiological data is unavailable. These findings demonstrate that the distribution of introduced insect populations is greatly influenced by their climatic suitability to the new region. Furthermore, this study offers a framework in upcoming biological control research, allowing for the identification of agent climatic suitability prior to the large-scale investment of time and resources required of pre-release trials. Research into modeling techniques similar to that of the ESM may further streamline agent climatic suitability assessments.

Chapter 4: Synthesis and Conclusion

4.1 Background and rationale

Anthonomus morticinus is anticipated to have a wider range in South Africa than *Anthonomus santacruzi* but its climatic suitability remains unknown. The limited distribution of *A. santacruzi* highlights the importance of understanding the climatic suitability of biocontrol agents before their release. Species Distribution Modelling (SDM) offers valuable insight and estimations into the potential geographic ranges agents may experience (Elith and Leathwick, 2009; Guisan and Zimmerman, 2000). Furthermore, agent performance can also be better understood through analysis of their biological and behavioural responses to unfavourable conditions (Kaiser *et al*., 2017). This study investigated the responses of *A. morticinus* to some abiotic environmental pressures. Moreover, this study uses SDM techniques novel to the field of biocontrol, estimating the predicted range of *A. morticinus* in South Africa using the ESM approach for evaluating the potential distributions of future biological control agents. Therefore, this research not only adds to *S. mauritianum* biocontrol efforts but further contributes to the field of biological control.

4.2 Effect of environmental relative humidity on *Anthonomus morticinus*

This research considers the impact of relative humidity (RH) on the survival and reproduction of *A. morticinus* when inhabiting *S. mauritianum* inflorescences. The study also compares the responses of *A. morticinus* to that of *A. santacruzi* to low RH. The findings suggested that environmental humidity does not significantly affect the survival or reproduction of *A. morticinus* or *A. santacruzi*, with both weevil species altering their behaviour to avoid exposure to unfavourable environmental conditions. Owing to their high humidity, *S. mauritianum* inflorescences serve as suitable microhabitats for both *Anthonomus* species to take refuge from unsuitable environmental conditions. The findings of this study imply the potential challenges *A. morticinus* may face, particularly in the colder regions during the winter months when *S. mauritianum* inflorescences are reduced in availability and quality. The dependence of these agents on the floral microhabitat emphasizes the importance of considering such variables when attempting to estimate the distribution of a potential insect biocontrol agent. Furthermore, this study provides valuable insights applicable to future research into insect-plant interactions and

the need to consider microclimates when aiming to develop effective biological control strategies.

4.3 Estimating *Anthonomus morticinus'* distribution in South Africa

Species Distribution Modelling (SDM) has proven valuable as a tool for predicting a species' range based on its climatic tolerances. Therefore, this approach is ideal for predicting the climatic suitability of a biological control agent before it has been introduced to a target area. However, most classical SDM techniques, whether mechanistic or correlative, require data of a quality or quantity often unavailable for potential agents. Thus, this research aimed to utilize novel SDM techniques to predict the range that *Anthonomus morticinus* is expected to experience in South Africa. Furthermore, this study created an SDM for *A. santacruzi* to compare which agent is expected to have a wider distribution in the country. The Ensemble of Small Models (ESM), typically used for rare species, produced an SDM for each *Anthonomus* species based on available occurrence datasets (17 for *A. morticinus* native occurrences and 45 non-native occurrences for *A. santacruzi*). Mechanistic SDMs for the two species were also created using existing physiological data collected from previous studies. The ESMs for both species returned spatial projections and confirmed *A. morticinus* is likely to have a wider geographic range in South Africa than *A. santacruzi*. Moreover, the mechanistic models produced using CLIMEX returned distribution estimations for both species similar to that of the ESM models. Thus, not only is it implied that *A. morticinus* is expected to have a greater distribution than *A. santacruzi* in South Africa, but the study also presents the ESM approach, novel to the discipline of biocontrol, as a useful new tool for estimating the climatic suitability of a species.

4.4 Conclusion and suggestions for future research

Ultimately, this study has revealed a number of valuable findings. Firstly, it was discovered that both *A. morticinus* and *A. santacruzi* are able to greatly reduce their exposure to low environmental relative humidity levels by seeking refuge within the microclimates present within *S. mauritianum* inflorescences. When analyzed in conjunction with Cowie *et al* (2016b), these conclusions emphasize the value of differentiating between a species' fundamental and realized niches. The fundamental niche is more empirical in nature, grounded in experimental observations of a species' response to abiotic environmental conditions. In contrast, the realized

niche represents the actual response of the species, taking biotic factors into account, such as competition, habitat selection, and other interactions which influence species distribution and resource use (Severtsov, 2013; Soberón and Arroyo-Peña, 2017). While Cowie *et al* (2016b) identified that direct exposure to low RH significantly reduced the survival of *A. santacruzi*, this study discovered that both *A. morticinus* and *A. santacruzi* avoid the impacts of low RH by seeking refuge within the microclimates of the *S. mauritianum* inflorescences, where humidity levels are heightened. Therefore, this study questions the significance of low environmental humidity on the mortality and distribution of these species, postulating that their ability to regulate their internal physiology is more closely linked to the availability and quality of host plant inflorescences as opposed to ambient relative humidity levels. Furthermore, these results suggest that environmental conditions alone may not be adequate in predicting the actual species survival and establishment as this method excludes the influence of biotic variables such as habitat selection. These factors should be considered when performing species distribution modelling using only climatic variables.

The second finding of this study concluded that *A. morticinus* is expected to have a broader geographic distribution owing to its greater climatic suitability to South Africa's climate than the already present *A. santacruzi*. Owing to the collection of *A. morticinus* from higher altitudes and cooler climates than that of *A. santacruzi* (Pedrosa-Macedo *et al*. 2003, Venter *et al*., 2021), previous research hypothesized that *A. morticinus* would show a different geographic distribution in South Africa than *A. santacruzi* (Cowie *et al*., 2018; Singh and Olckers, 2017). This finding affirms that *A. morticinus* is expected to have a wider range in South Africa, offering a more complete level of control against the weed than that of *A. santacruzi*. Therefore, this study advocates for the completion of the host-specificity testing on *A. morticinus* and its subsequent release in South Africa.

Lastly, this study used the Ensemble of Small Models (ESM) approach to identify potential distributions and climatic suitability of biological control agents using limited occurrence data only. First developed by Lomba *et al*. (2010) for modelling of rare species with limited occurrence data, this technique is novel in biological control. Many previous studies on potential agents with limited occurrence data have only investigated climatic suitability once ecophysiological data was available to construct mechanistic models, risking investing resources

into potentially unsuitable agents (Cowie *et al*., 2016b; Falla *et al*., 2021). For Cowie *et al*. (2016b) to estimate the distribution of *A. santacruzi* in South Africa using the CLIMEX mechanistic approach, the RH and thermal limits of the weevil had to be determined through ecophysiological experimentation. The *A. santacruzi* distribution displayed by Cowie *et al*. (2018) was generated using CLIMEX which required significant physiological data from previous studies (Cowie *et al*., 2016b). The ESMs generated in this study showed a high level of visual similarity to the CLIMEX models produced, representing the validity of this approach in biological control. Furthermore, both the ESM and CLIMEX *A. santacruzi* models yielded similar distributions found by Cowie *et al*. (2018). Should the ESM method have been chosen to predict the distribution of *A. santacruzi* in South Africa as opposed to the CLIMEX approach, the need for ecophysiological experimentation on the weevil would have been eliminated. This concept can help predict the potential distributions and climatic suitability for many biocontrol agents before investing resources in experiments to determine their ecophysiological limitations. Additionally, the ESM approach provides a useful tool for identifying suitable candidates for biocontrol initiatives before committing to extensive funding and labour for expeditions, should occurrence data already be known, and host-specificity testing.

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