# Aspects influencing the release and establishment of the flowerbud weevil, *Anthonomus santacruzi* Hustache (Coleoptera: Curculionidae), a biological control agent for *Solanum mauritianum* Scopoli (Solanaceae) in South Africa

By

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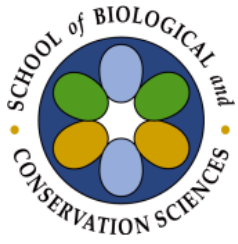

**PREFACE**

The research described in this dissertation was carried out at the facilities of the School of Biological and Conservation Sciences (Pietermaritzburg campus) from February 2009 to June 2011 under the supervision of Dr T. Olckers.

The work presented in this dissertation represents the original work of the author and has not been otherwise submitted in any other form for any degree or diploma to any other University. Where use has been made of the work of others, it has been duly acknowledged in the text.

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**ABSTRACT**

*Solanum mauritianum* (bugweed, woolly nightshade) is a perennial tree native to South America that has invaded many countries including South Africa and New Zealand. In South Africa, after 143 years of naturalization, the plant is ranked as the country's sixth worst weed and has invaded 1.76 million ha. Invaded areas include agricultural lands, forest plantations, water courses and conservation areas, especially in the eastern higher rainfall regions. The success of the spread of this weed is due to its production of very high numbers of bird-dispersed seeds. Since conventional control methods are unsustainable in the long term, the weed has been targeted for classical biological control since 1984.

Following exploration work in its native range, biological control experts recommended that agents that are able to limit the weed's reproductive potential would help to manage the spread and invasiveness of this weed. *Anthonomous santacruzi*, a flower-feeding weevil found throughout the native range of the weed, was imported and tested between 1998 and 2002. Following approval for its release in South Africa in 2007, a new colony was imported and propagated at the University of KwaZulu-Natal Pietermaritzburg. This study was initiated to investigate aspects that could influence the release and establishment of this agent. Three aspects were investigated namely: (1) reassessing the weevil's host range to confirm that the new colony is not different from the colony tested originally and to assess the risks associated with the release of the weevil in New Zealand; (2) surveying the arthropods associated with *S. mauritianum* in the field to identify groups of predators that could interfere with the establishment of the weevils as well as to investigate, through laboratory-based trials using spiders as surrogate, the impact of these predators on the survival and proliferation of the weevils; and (3) propagation and release of the weevil and monitoring of its establishment.

Host-specificity tests revealed that the host range of new colony is not different from that of the originally tested culture. In no-choice trials, the weevils fed and reproduced on some non-target Solanaceae species but reverted back to *S. mauritianum* in the choice tests. Although the risks for releasing the weevils in New Zealand were calculated to be very low, additional evidence is needed to demonstrate this conclusively. Future research to provide this evidence includes open-field trials complemented with a chemical ecology study, to resolve the case of two species, a New Zealand native and South African native, which have shown higher risks in comparison to the other tested species.

For arthropods associated with *S. mauritianum* in the field, Araneae (especially Thomisidae), Thysanoptera, Hemiptera (especially Miridae) and Hymenoptera (especially Formicidae) were identified as generalist predators that could interfere with the establishment of *A. santacruzi*. However, their numbers in the field appear to be too low to provide a major threat. Also, laboratory trials using spiders as a surrogate suggested that *A. santacruzi* populations can survive and reproduce in the presence of such predators.

The weevils were released at four sites in KwaZulu-Natal and monitoring of three of these has confirmed establishment at the warmest site along the South Coast but not at the coldest site in the Midlands. Further releases in the province are intended to complement these promising results, while additional studies are intended to facilitate the weevil's release in New Zealand.

Key words: Agent establishment, biological weed control, bugweed, insect host range, invasive alien plants.

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# CHAPTER 1: GENERAL INTRODUCTION AND BACKGROUND

## 1.1. Invasive plants

Ecosystems provide services that meet basic human needs as well as services that enhance human wellbeing (Van Wilgen *et al.* 2008). As a result, a well managed biodiversity is necessary for full realization of these services. Alien plants contribute to the loss of biodiversity and degrade ecosystems, hence reducing ecosystem services (Pysek & Richardson 2010). Invasions by alien species are currently recognized as the second most important threat to biodiversity after habitat destruction (Olckers *et al*. 1998; Walker & Steffen 1999; Mooney & Hobbs 2000; Van Wilgen *et al.* 2008). Invasive alien plants involve species that have been intentionally or accidentally introduced into new habitats (Van Wilgen *et al.* 2008), with negative consequences (Mack 1995). Due to the absence of their natural enemies (Elton 1958), amongst other factors, these plants grow rapidly and vigorously outcompete native plants, causing the loss of many species and hence threatening biodiversity (Harley & Forno 1992; Mack 1995; Mack *et al.* 2000; Pearson & Callaway 2003; Van Wilgen *et al.* 2008; De Lange & Van Wilgen 2010). Lack of natural enemies (Elton 1958), complemented by their high reproductive output, especially the production of numerous viable seeds, have been among the main reasons for the rapid spread and persistence of invasive alien plants worldwide (Witkowski & Wilson 2001). Seeds are often, but not always, the main means by which invasive alien plants spread and invade new and disturbed areas. Also, generalist avian frugivores are among the most important seed dispersal agents and often play an important role in the processes of naturalization and invasion of invasive alien plants (Renne *et al.* 2002; Gosper *et al.* 2005). As a result, bird-dispersed weeds, such as the subject of this study, represent major challenges for conservation and weed management.

In this introductory chapter, I will discuss the problem of invasive alien plants in South Africa and how biological control has been used as a tool for their management. In this section, the theory and philosophy behind the use of biological control and some successful examples from South Africa will be discussed. After that, this chapter will focus on *Solanum mauritianum* Scopoli (Solanaceae), the targeted weed in this study and a promising natural enemy, *Anthonomus santacruzi* Hustache (Curculionidae), as well as the general use of flower-feeders as biological control agents. Thereafter, I will provide the rationale behind this study and introduce the main aims and objectives. Finally, I will outline the structure of the dissertation with a brief description of each chapter.

### 1.2. Invasive alien plants in South Africa

Since the arrival of European colonists in 1652, exotic plant species have been continuously introduced into South Africa (Moran *et al.* 2005). Though some plants were introduced by accident, about 75% were deliberately introduced as ornamentals and food plants, for timber and firewood and for stabilizing sand dunes (Olckers *et al*. 1998; Van Wilgen *et al.* 2008). In South Africa, the spread of these problematic plants began in the 1700s in the Fynbos biome and 150 years later, these spread to the other major plant biomes with the potential to reach their optimum densities in 2150 (Richardson *et al.* 1997; De Lange & Van Wilgen 2010). These invasive alien plants have already invaded more than 10.1 million ha with severe impacts on land devoted to agriculture, forestry, grazing or recreational activities, as well as in watercourses, lakes and dams, and in national parks, conservation areas and gardens (Richardson *et al*. 2004). Most of these impacts are caused by about 198 plant species which are considered to be the most important and most damaging and have thus been declared under the Conservation of

Agricultural Resources Act (CARA) in South Africa (Henderson 2001, 2007). The Fynbos biome, which has a long history of invasive alien plants, has been invaded by about 160 species and four of them, which are the most problematic, have covered about 50% of the total area of this biome (Van Wilgen *et al.* 2008).

Apart from threatening many native plant species due to competition (Gould & Gorchov 2000), alien plants are reported to transform habitats, degrade landscapes, drive soil erosion (Vitousek & Walker 1989), and greatly contribute to wildfires (Brooks *et al.* 2004) which cause losses to vegetation, animals and property. In addition, alien plants have been associated with degrading grazing land, causing loss of pastures and the poisoning of livestock (ISSG 2010). In an attempt to estimate the impact of invasive alien plants in South Africa, Van Wilgen *et al*. (2008) used the current infestation and future potential infestation of 56 invasive species to calculate losses in relation to the provision of water, provision of grazing land for livestock and biodiversity. These authors found a current loss of 7% of the total national water supply (Le Maitre *et al.* 2000) which could increase to 56% if these invasive alien plants managed to reach their optimum densities in the future (De Lange & Van Wilgen 2010; Richardson 2011). Invasive alien plants also affect the production of livestock. Current infestations cause a loss of 1% of livestock production due to the loss of grazing land, and this could increase to 71% if optimum densities are reached (Van Wilgen *et al.* 2008; De Lange & Van Wilgen 2010; Richardson 2011). Currently, biodiversity in many South African biomes is affected, with some more than others (e.g. the Fynbos biome). However, the loss of biodiversity would be increased from the current level of 30% to 71-89% for the major biomes in South Africa if the potential spread by invasive alien plants is realized (Van Wilgen *et al*. 2008; De Lange & Van Wilgen 2010; Richardson 2011).

De Lange & Van Wilgen (2010) quantified in monetary terms the values of ecosystem services and found that in the absence of invasive alien plants, the services of ecosystems would be valued at R152 billion of which 63% would be contributed by water, 22% by grazing land and 15% by biodiversity (Van Wilgen *et al.* 2008; De Lange & Van Wilgen 2010; Richardson 2011). The economic losses due to invasive alien plants were calculated to reach R6.5 billion (De Lange & Van Wilgen 2010) which was 0.3% of the gross domestic product of about R2000 billion in 2009 in South Africa (Richardson 2011). Such loss of ecosystem services is projected to increase to 5.2% of the country's gross domestic product if these plants spread to their maximum densities (Richardson 2011).

These considerations created a great need to control these problematic plants and provided the rationale behind the Working for Water Programme which has played a major role in the management of invasive plants in South Africa (Zimmermann *et al*. 2004; Moran *et al.* 2005). Biological control which provides a long term, self-sustaining solution to many problematic plants (Moran *et al*. 2005) started in 1913 in South Africa, and since then a significant investment has been made (Zimmermann *et al.* 2004). Economic savings resulting from different alien plant control measures was estimated at R41.7 billion, of which biological control alone accounted for between R2.1 to R31.3 billion (De Lange & Van Wilgen 2010; Richardson 2011).

### 1.3. Biological control as a management tool for invasive alien plants

Traditionally, invasive alien plants are targeted using either pesticides or by removing them mechanically. In South Africa, it was calculated that about R6.97 billion would be needed to

chemically and mechanically clear the 10.1 million ha of land invaded by alien plants (Versfeld *et al*. 1998). In addition, more resources would be required to follow up and keep the cleared land clean of reinvasion. Furthermore, in some areas, the cost of clearing the land exceeded the actual value of the land (Olckers *et al*. 1998). These considerations made such operations economically unviable, thus necessitating additional strategies which would be economically viable and effective in reducing the impact of invasive plants. Biological control provides such a strategy.

Biological control utilizes natural enemies (parasites, predators and pathogens) for the regulation of weed population densities (Harley & Forno 1992; McFadyen 1998; Zimmermann *et al*. 2004). This concept is based on the assumption that, in the absence of their natural enemies, exotic species become invasive because they escape their regulating influence (Keane & Crawley 2002). Also implicit in this concept is that the natural enemies are host specific and are confined to the target. Biological control thus attempts to introduce the alien plants' natural enemies (agents) into their new habitats (McFadyen 1998; Mitchell & Powers 2003) in the hope that these will reduce the plants' competitive advantages (Davis *et al*. 2006) to the level of that of the natural invaded vegetation (Harley & Forno 1992).

There are different approaches towards biological control, notably classical biological control, augmentation and conservation, with classical biological control the favoured option in targeting invasive weeds (Van Driesche *et al.* 2008). Classical biological control entails the importation and release of introduced natural enemies, from the weed's country of origin (McFadyen 1998; Mitchell & Powers 2003), with the expectations that these will become established, increase in number and then reduce the weed populations to the extent that no further releases are needed (Hunt-Joshi et *al.* 2005). This differentiates it from augmentation,

which involves regular releases of the natural enemies, whether native or introduced (Van Driesche *et al.* 2008), and conservation, which involves creating environmental conditions that favour such natural enemies (Van Driesche *et al.* 2008). In theory, the introduced agents reduce the mean equilibrium density of the weed to below some economic and ecological threshold (Smith & Van den Bosch 1967 in Pearson & Callaway 2003). This is achieved through a direct negative effect in which the biological control agents feed on and negatively affect the weed, leading to positive indirect effects on native species. At the same time, because of their host specificity, the reductions in host plant density leads to a negative feedback that reduces and regulates the biological control agents' own populations.

The earliest successful biological control of an invasive weed was recorded in 1836, when the cochineal bug *Dactylopius ceylonicus* (Hemiptera: Dactylopidae), native to Brazil, was released in India against the invasive cactus *Opuntia vulgaris* (Olckers & Hoffmann 1995; Klein 2011). Following the control of the cactus, *Dactylopius ceylonicus* was introduced into many countries, including South Africa in 1913 (Olckers *et al*. 1998) where it facilitated the first successful biological control programme against a weed (Olckers & Hoffmann 1995; Zimmermann *et al*. 2004; Klein 2011). Since 1913, some 271 natural enemies were introduced into quarantine in South Africa as part of biocontrol attempts against 67 invasive alien plant species in 23 plant families (Klein 2011). Of these, 109 were released as biocontrol agents and 83 became established on 47 invasive alien plant species in 14 plant families. These established agents have resulted in either the complete control, or a substantial degree of control in 28 plant species. Seven years ago, Zimmermann *et al*. (2004) estimated that the use of biological control in weed management programmes in South Africa has reduced the overall control costs by 19.8% which was equivalent to $US276 million dollars. In addition, a recent study by De Lange

& Van Wilgen (2010) demonstrated biological control to have saved R 2.1 to R 31.3 billion that would have been lost due to the degradation of ecosystem services by invasive alien plants (Richardson 2011). Although there have been several successes, one of the more difficult of these programmes has been that against *Solanum mauritianum* Scopoli (Solanaceae).

### 1.4. *Solanum mauritianum*

*Solanum mauritianum* (bugweed, woolly nightshade) is a perennial tree, 2-4 m tall, which is native to several countries in South America (Olckers 2009) and has invaded many countries, possibly via the Portuguese trade routes in the 16$^{th}$ Century (Roe 1972 in Olckers 1999). The plant has since become naturalized in Africa, Australia, India and Islands in the Atlantic, Indian and Pacific oceans (Olckers 1999). The weed has been in South Africa for at least 143 years and has invaded agricultural lands, forestry plantations, watercourses, and conservation areas, especially in the eastern higher rainfall regions (Fig. 1.1) (Olckers 1996, 1999, 2000a, 2011; Olckers & Zimmermann 1991; Henderson 2001, 2007). Versfeld *et al*. (1998) estimated that *S. mauritianum* has invaded some 1.76 million ha in South Africa.

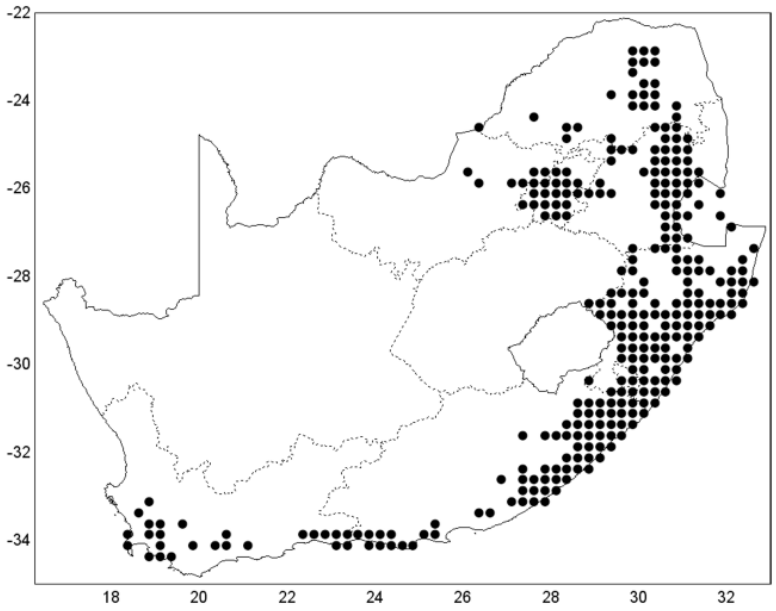


Fig. 1.1: Distribution of *Solanum mauritianum* in South Africa (from Olckers 2011).

*Solanum mauritianum* has also become recognized as an important invasive species in New Zealand (Olckers & Borea 2009; ISSG 2010). First recorded in the Auckland region in 1883 (Olckers & Borea 2009; ISSG 2010), the weed has spread to at least seven more regions including the Bay of Plenty, Hawke's Bay, Manawatu-Wanganui, Northland, Waikato, Wellington and Marlborough (ISSG 2010). In these areas, the weed invades pastoral land, native forest margins, plantations, roadsides, watercourses and urban open space.

In addition to changing plant succession patterns, the weed is also poisonous to livestock and humans, especially children (ISSG 2010). The weed's success as an invader is due to extremely high fruit set and long-range seed dispersal by frugivorous birds which prefer *S. mauritianum* over indigenous plants (Olckers 1999, 2009). Witkowski & Garner (2008) found that seed production occurs on plants of $\geq 1.5$ m tall and that the number of seeds increases linearly with increasing plant height, reaching between 100 000 to 200 000 seeds/plant/year for

plants taller than 3 m. In addition, *S. mauritianum* spreads vegetatively and can regrow rapidly from severed roots and stems. Witkowski & Garner (2008) also investigated the long term response of this plant to clearing operations and found that densities often increase after clearing due to re-sprouting and the recruitment of bird-dispersed seedlings. This renders conventional control efforts unsustainable as clearing operations involve considerable efforts and resources and require regular follow-up (Olckers 1999).

Even though *S. mauritianum* is easily killed by herbicides, the current extent of invasion and rapid recruitment of seedlings in cleared areas has also made the use of chemicals unsustainable (Olckers 1999). Also, *S. mauritianum* typically invades watercourses and agricultural lands where the use of chemicals presents a threat to crops and water purity. Biological control, which is self-sustaining and environmentally friendly, was thus deemed necessary to augment conventional control methods and render integrated control sustainable (Olckers 1999).

### 1.5. Biological control of *S. mauritianum*

*Solanum mauritianum* was first targeted for biological control in 1984 and South Africa was the first country to have imported, tested and released biological control agents against this weed (Olckers 1999, 2009, 2011; Olckers & Borea 2009). Following these initiatives, New Zealand has also launched a biological control programme against the weed (Hayes 2009, Olckers 2011). In the search for potential agents, some 80 phytophagous insects were recorded on *S. mauritianum* and related plants in its country of origin (Olckers 1999). Potential agents were prioritized based on their relative abundance, and desired impacts on the weed population

(Olckers 1999). It was intended that agents that reduce fruiting would be released to limit long range seed dispersal and hence post-clearing reinvasion. Also, agents that could reduce the weed's high growth rates and the density of existing infestations were required (Olckers 1999).

Two agents have been released on *S. mauritianum* in South Africa, to date, the sap-sucking lace bug *Gargaphia decoris* Drake (Tingidae) and the flowerbud-feeding weevil *Anthonomus santacruzi* Hustache (Curculionidae). *Gargaphia decoris*, which was released in 1999 (Olckers 2000a) has become established at several sites in South Africa but its population dynamics and impact on the weed has not yet been evaluated. A starter colony of *G. decoris* was shipped to New Zealand in 2010 as part of a collaborative biocontrol programme and releases have since been made (Olckers 2011). *Anthonomus santacruzi*, the subject of this study, is a more recent initiative and will be discussed in further detail below.

### 1.5.1 Flower feeders as biological control agents

Flower feeders have been used as biological control agents against alien invasive weeds in several countries. In the USA, for example, spotted knapweed, *Centaurea stoebe* Lamarck, an Eurasian perennial plant which has invaded more than 3 million ha of land, was targeted with flower feeders among other biological control agents (Story *et al.* 2008, 2010). Also, purple loosestrife, *Lythrum salicaria* (Lythraceae), a wetland perennial weed from Europe and Africa that, since the 1800s has invaded all states of the USA and nine provinces of Canada, degrading agricultural lands, pastures and affecting wetlands, was targeted with the flower weevil *Nanophyes marmoratus* Goeze to complement other biological control agents (Blossey 1995; Blossey & Schroeder 1995; Blossey *et al.* 2001). In South Africa, eight biological control agents

that attack flowers have been released and established on six weed species, of which 67% are either under substantial control or complete control (Klein 2011). These include *Sesbania punicea* (Cav.) Benth, an invasive tree that was problematic in wetlands and rivers and was targeted for biological control in the 1980s. The bud-destroying weevil *Trichapion lativentre* (Bequin-Billecocq) is one of the three biological control agents that have successfully controlled this weed (Hoffmann & Moran 1991, 1998).

Many researchers believe that florivory, which is common in natural systems, may surpass leaf herbivory in both magnitude and impact. Florivory includes any damage to developing floral buds or mature flowers before the development of the seed coat (Louda 1982; Louda & Potvin1995; McCall & Irwin 2006). Different views have been expressed about the effect of florivory on the reproductive biology of the plant, seed formation, seedling recruitment, plant population structure and the fitness of individual plants. One view is that, although the reproductive success of individual plants may be affected (Andersen 1989) plants produce flowers in excess (Burd 1998) so that even excessive levels of florivory may not necessarily affect the size of plant populations (Stephenson 1981). The same applies to agents that attack the fruits/seeds of plants. For example, although a seed-feeding beetle destroyed 98% of the seeds produced by populations of the invasive *Ulex europaeus* L. in New Zealand, the remaining 2% of seeds sustained the average plant population so that spreading was not reduced (Harper 1977; Lewis & Gripenberg 2008).

Investigations on the impact of flower feeders on plant population dynamics have suggested otherwise (Louda 1982; Hoffmann & Moran 1991, 1998; Hoffmann 1995; Louda & Potvin 1995). Several studies (Louda 1982; Louda & Potvin 1995) have concluded that, under natural conditions, flower feeders can potentially limit seed production, seedling recruitment,

plant density and the maternal fitness of plants. In Montana (USA), for example, where spotted knapweed, *Centaurea stoebe*, invaded about 1.6 million ha of land, 12 biological control agents were released of which five were seed-head insects (Story *et al.* 2008, 2010). A study investigating the impact of these flower-feeding agents found a combination of the seed-head fly, *Urophora affinis* Frauenfeld and two seed-head weevils, *Larinus obtusus* Gyllenhal and *Larinus minutes* Gyllenhal, to have reduced seed production of this weed by 96% in Western Montana where these agents are well established (Story *et al.* 2008, 2010). This was also revealed in a study on the invasive tree *Sesbania punicea* in South Africa, where the flowerbud weevil *Trichapion lativentre* destroyed 98% of the seeds and in combination with the seed weevil *Rhyssomatus marginatus* Fåhraeus reduced seed production by 99% (Hoffmann & Moran 1991, 1998). This indicated that even though flower feeders alone may not control the weed, they hinder fecundity hence curbing invasiveness and complementing other agents or other control methods. Recommended as a 'first line of attack' in biological control of weeds (Hoffmann & Moran 1998), flower feeders clearly have the potential to limit plant reproduction and contribute to the control of invasive alien plants. The *Sesbania* case history provided the rationale for the introduction of *A. santacruzi* against *S. mauritianum* in South Africa.

### 1.5.2 *Anthonomus santacruzi*

The adult weevils are black and about 2 to 3 mm in length. They have striated elytra and conspicuous patches of white scales that appear as thin vertical bands on the second to sixth interstrial regions (Clark & Burke 1996; Fig. 1.2). The weevil has a wide distribution in South America which follows the range of *S. mauritianum*, including northern Argentina, southern Brazil and Bolivia (Clark & Burke 1996; Olckers *et al*. 2002; Pedrosa-Macedo *et al*. 2003).

*Anthonomus santacruzi* was introduced into quarantine in 1998 and after extensive biology studies and host range tests (Olckers 2003, 2009) was cleared for release in South Africa in 2007. Adults feed on the flower buds, open flowers and shoot tips of newly formed leaves (Olckers 2003, Barboza *et al*. 2009). The females oviposit in the mature and immature flower buds which are consumed by the larvae during their development. Usually one larva is recovered from a single bud, but 2 to 3 larvae may be recovered when flower buds are limited. The short pre-oviposition period lasts about four days, while the longer oviposition period ranges from 43 to 114 days (Olckers 2003). Larval development in the flower buds is fairly rapid, with the time from oviposition to pupation ranging from 10 to 18 days and the time from oviposition to adult emergence ranging from 15 to 25 days (Olckers 2003).

Fig. 1.2: Larva and adult of *Anthonomus santacruzi* (images by K.J. Hope).

Several factors suggested that *A. santacruzi* was a very promising biological control agent for *S. mauritianum*. These included their: (i) high fecundity (16 - 59 larvae per female); (ii) adult longevity (99 - 122 days on floral material and 131 - 182 days on non-floral material); (iii)

good dispersal ability; (iv) short generation times (15 - 25 days) resulting in overlapping generations and rapid population increases and; (v) the destructive nature of adult and larval feeding. Adults feed on the stamens of open flowers and unopened buds, causing their abortion and abscission, but can sustain themselves on shoot tips in the absence of floral material (Olckers 2003, Barboza *et al*. 2009). The developing larvae consume the entire contents of the flower buds and prevent flowering (Olckers 2003). The destructive nature of both adult and larval feeding prevents fruit set (Olckers 2003) and thus has the potential to reduce the weed's extensive seed dispersal and seedling recruitment in the field.

### 1.6. Rationale for the study

The production of excessive numbers of bird-dispersed seeds is presumably the main reason for the rapid spread and persistence of *S. mauritianum* in South Africa and elsewhere in the world. Targeting this aspect of the weed's biology is thus important if weed populations are to be managed effectively. In contrast to the high fruit set of *S. mauritianum* populations in South Africa, which is caused in part by very low numbers of fruit-reducing insects (Olckers & Hulley 1995), populations in South America suffer high levels of damage by flower-feeding insects and thus display considerably lower levels of fruit set (Olckers 2003). These differences suggested that biological control using fruit-reducing agents was an attractive prospect. *Anthonomus santacruzi*, which occurs throughout the range of *S. mauritianum* in South America and which appeared to be the most destructive of the florivorous agents, was thus considered to be the most promising agent that could contribute to the long-term management of this weed.

Following the completion of host-specificity testing (Olckers 2003), considerable delays in obtaining approval for the weevil's release resulted in the loss of the quarantine laboratory culture in 2004. However, following approval for the release of *A. santacruzi* in 2007, this study was initiated as part of the programme to propagate reintroduced cultures of the weevil for releases at selected sites in KwaZulu-Natal (KZN). Following the extension of the *S. mauritianum* programme to New Zealand, additional host-range tests were carried out to determine its suitability for release in this country. Finally, aspects that could influence the establishment and success of the weevil in South Africa, notably predation by generalist predators, were also investigated.

### 1.7. Objectives of the study

The main objectives of this study included:

1. Reassessment of the host range of *A. santacruzi* to confirm that it is suitable for release in New Zealand and that the host range of the reintroduced population does not differ from that of the population that was tested originally and is thus safe for release in South Africa.
2. Investigation of the role of generalist predators, notably spiders, that are associated with *S. mauritianum* inflorescences in the field in limiting the establishment and proliferation of *A. santacruzi* populations.
3. Propagation of *A. santacruzi* to facilitate releases at selected sites in KwaZulu-Natal and monitoring of the release sites to determine whether the founder populations have established and are proliferating.

## 1.8. Outline and previews of the chapters

### 1.8.1. Chapter 2: Host range determination

This chapter will discuss the need for the reassessment of the weevil's host range and will outline the procedures that were used to determine the host specificity of *A. santacruzi*. The results of the no-choice, choice and walk-in cage trials will be discussed in the context of a risk assessment. Conclusions will be drawn on the weevil's suitability for release in New Zealand and whether the results conform to those from the population that was tested originally (Olckers 2003).

### 1.8. 2. Chapter 3: Predator surveys and predation trials

This chapter will introduce the importance of investigating the impact of predation on weed biological control agents. It will outline the protocol that was adopted in assessing the diversity and abundance of native predators that are associated with the inflorescences of *S. mauritianum* in the field and which of these may be likely to negatively affect *A. santacruzi*. An experimental procedure aimed at quantifying the potential impact of key predators on the survival and proliferation of weevil populations will also be presented. The results will be discussed in the context of whether the potential impacts of *A. santacruzi* are likely to be hindered by generalist native predators in South Africa.

### 1.8.3. Chapter 4: Propagation, releases and monitoring of establishment

This chapter will outline the procedures that were adopted in releasing founder populations of *A. santacruzi* at selected sites in KZN and in monitoring the outcomes of these. The results will be discussed in relation to the weevil populations' persistence and proliferation.

### 1.8.4. Chapter 5: General discussion and conclusions

This chapter will provide a final discussion on the results of the preceding chapters and draw some conclusions on the potential of *A. santacruzi* for the biological control of *S. mauritianum* in South Africa and New Zealand**.**

# CHAPTER 2: REASSESSING THE RISKS ASSOCIATED WITH THE RELEASE OF NEW STOCKS OF THE FLOWERBUD WEEVIL *ANTHONOMUS SANTACRUZI* AGAINST *SOLANUM MAURITIANUM*

## 2.1. Introduction

*Solanum mauritianum* Scop. (Solanaceae), a 2-10 m tall perennial tree that is native to South America (Olckers 2009, 2011), is a major environmental weed in South Africa, New Zealand and several other tropical and subtropical regions worldwide. The plant has been in South Africa for about 143 years, where it is ranked as the sixth worst invader (Henderson 2007, Olckers 2011). In total, some 1.76 million ha have been invaded (Versfeld *et al.* 1998) and comprise mostly agricultural lands, forestry plantations, watercourses, and conservation areas, especially in the country's higher rainfall regions (Henderson 2001, 2007).

The weed has been targeted for biological control in South Africa for several years (Olckers & Zimmermann 1991; Olckers 1999, 2011) and has recently been targeted in New Zealand (Olckers & Borea 2009; Olckers 2009, 2011).The plant's invasiveness is mostly due to excessive fruit production that facilitates long-distance seed dispersal by fruit-eating birds. Olckers & Hulley (1989, 1991a, 1991b) surveyed *S. mauritianum* in South Africa and found very few flower-feeding and other herbivorous insects and hence negligible feeding damage. On the contrary, surveys in South America, the region of its origin, revealed that *S. mauritianum* supports considerably more floral and foliar herbivores and thus displays low levels of fruiting (Olckers 2003, 2011). Under the assumption that flower-feeding insects could reduce the high levels of fruiting in invaded habitats, biological control efforts in South Africa have focused on the establishment of agents that are able to reduce fruiting (Olckers 1999, 2003, 2011).

*Anthonomus santacruzi* Hustache (Curculionidae), a flower-feeding weevil, was considered to be the most important florivorous natural enemy of *S. mauritianum* in its countries of origin. This is because the weevil occurs throughout the range of *S. mauritianum* in South America and was thought to contribute largely to the plant's low fruit set in these regions (Olckers 2003, 2011). Following the importation of *A. santacruzi* into quarantine in South Africa in 1998 and the completion of host range testing in 2002, the agent was finally approved for release in South Africa in 2007 (Olckers 2008, 2009, 2011). Since the original quarantine cultures had died out in the interim, during 2008-2009 new stocks of the weevil were imported from the same region in Argentina where the original stocks were collected (Olckers 2011). These were cultured at the University of KwaZulu-Natal, Pietermaritziburg, in South Africa to facilitate additional studies and releases.

This study was initiated to firstly assess the host range of the new stocks of *A. santacruzi* to confirm that this does not differ substantially from that of the stocks that were tested originally. In addition, the host-range testing was expanded to include native and cultivated New Zealand *Solanum* species and thereby to assess the risks associated with the release of the weevil in New Zealand.

## 2.2. Material and methods

### 2.2.1. Insect cultures

New stocks of *A. santacruzi* were collected in Argentina (Misiones Province) on two occasions in 2008 and in 2009 to establish and sustain a new laboratory culture in South Africa. The weevils were cultured at 24 ± 2 $^{o}$C and a 12 h photophase in the insectary at the University of

KwaZulu-Natal (Pietermaritzburg) using the technique of Olckers (2003). Weevil colonies were maintained in 10-litre plastic containers with gauze-covered tops. Field-collected bouquets of *S. mauritianum* containing flowers, flowerbuds and apical leaflets were placed in water in small plastic jars that were sealed with sponge stoppers, which were added to the rearing containers. The bouquets were presented to the weevils for feeding and oviposition. Fresh bouquets were provided every 4 to 5 days and the old buds were placed in glass Petri dishes to allow the immature stages to complete their development. Moist pieces of sponge were placed in the Petri dishes to increase humidity and reduce desiccation of the buds and developing larvae. After 4 - 5 days, all floral material was dissected to remove any developing larvae which were then transferred to fresh buds in clean Petri dishes. Emerging adults were added to the culture or used in the host-specificity tests.

### 2.2.2. Test plants

Following a more intensive earlier study (Olckers 2003) in which a range of plants in the family Solanaceae were tested, the current host-specificity testing focused on selected species in the genus *Solanum* (Table 2.1). Besides *S. mauritianum*, nine non-target species were tested. These included four varieties of *Solanum melongena* L. which are cultivated in South Africa and New Zealand as well as two species which are cultivated in New Zealand. The remaining test plants comprised three South African native species and three New Zealand native species (Table 2.1).

Table 2.1: Species of *Solanum* used in the host-specificity tests and their status in South Africa and New Zealand.

| **Test plant species (variety)** | **Status** | **Previously tested** |
|---|---|---|
| *Solanum mauritianum* Scop. | Target plant (control) | Yes (Olckers 2003) |
| *S. aculeastrum* Dunal | South African native | No |
| *S. americanum* Mill. | New Zealand native | No |
| *S. aviculare* G. Forst | New Zealand native | No |
| *S. laciniatum* L. | New Zealand native | No |
| *S. giganteum* Jacq. | South African native | No |
| *S.* cf. *linnaeanum* Hepper and Jaeger | South African native | Yes (Olckers 2003) |
| *S. melongena* L. (Black Beauty) | Cultivated (SA & NZ) | Yes (Olckers 2003) |
| *S. melongena* L. (Japanese Long) | Cultivated (SA & NZ) | No |
| *S. melongena* L. (Louisiana Long Green) | Cultivated (SA & NZ) | No |
| *S. melongena* L. (Violet Prince) | Cultivated (SA & NZ) | No |
| *S. muricatum* Ait. | Cultivated (NZ) | No |
| *S. quitoense* Lam. | Cultivated (NZ) | No |

The test plants were grown from seeds or cuttings and were propagated in pots that were maintained in a shade house in the University of KwaZulu-Natal's Botanical Garden. The New Zealand native species were monitored for fruits which were removed and destroyed to prevent any seed dispersal.

### 2.2.3. Host-specificity tests

Host-range tests were carried out in both no-choice and multi-choice arenas and included: (i) two sets of no-choice tests involving bouquets with either floral material (flowers and flower buds) or non-floral material (shoot tips with apical leaves); (ii) two sets of multi-choice tests in small cages involving floral and non-floral bouquets and; (iii) two sets of multi-choice tests in large walk-in cages involving potted plants with floral and non-floral material. Non-floral material was included since the weevils easily sustain themselves on this for extended periods by feeding on the shoot tips and young leaves (Olckers 2003, Barboza *et al*. 2009).

#### *2.2.3.1. Adult no-choice tests*

Adult no-choice trials were designed to determine physiological host range, these tests were used for the initial screening of *A. santacruzi* in order to determine whether the weevil can survive on both the non-floral and floral material of the test plant species. In the trials with non-floral material, bouquets containing apical leaflets were inserted into wet Oasis to provide moisture and placed in small 500 ml plastic containers. Five newly-emerged adults were confined on each test plant species for 4 days, after which feeding and mortality was recorded. Adults were also confined on *S. mauritianum* as the control. To assess feeding damage, the number of feeding scars per weevil was recorded. Each test plant species was tested on 3 to 4 separate occasions.

In the trials with floral material, bouquets containing flowers and flower buds of each test plant species were placed in small water-filled plastic containers that were sealed with a sponge and placed in a 10-litre plastic bucket. Ten newly-emerged adults were confined on each test

species for 4 days and oviposition and mortality were recorded. Adults were also confined on *S. mauritianum* as the control.

After 4 days, the numbers of open flowers, mature buds, immature buds and dead adults were recorded. The floral material was then placed in glass Petri dishes to allow the immature stages to complete their development. After another 4 days, the floral material was dissected to record the numbers of larvae and pupae. Developing larvae were then transferred to fresh flower buds of the same test plant species in clean Petri dishes for completion of larval development. The number of adults that emerged and the time taken to complete their development were recorded. Because sexing of adults is very difficult, the adults used were dissected and sexed according to their male or female genitalia (Clark & Burke 1996) in order to determine the numbers of larvae and adults produced per female.

*2.2.3.2 Adult multi-choice tests in small cages*

The adult multi-choice trials were designed to determine ecological host range, these tests included trials with non-floral and floral material and involved non-target species that supported feeding and oviposition during the no-choice tests. In trials with non-floral material, bouquets with shoot tips and apical leaflets were used as before. During each trial, four bouquets of different test plant species (that included *S. mauritianum*) that were placed in water-filled jars (see above) were randomly arranged in a bell-shaped cage of 60 cm × 60 cm × 75 cm. Ten newly-emerged adults were released in the cage and exposed to the plants for 4 days. After 4 days, the position of the weevils, adult mortality and the number of feeding scars were recorded on each test plant species. Each test plant species was tested on 3-4 separate occasions.

Trials with floral material were similarly carried out using the same design, except that the bouquets contained flowers and flower buds and 20 reproductively-active adults were released in the cages. After 4 days, the positions of the weevils, adult mortality and the numbers of open flowers and mature/immature flower buds were recorded. The flower buds were then transferred to glass Petri dishes to allow development of the immature stages. The flower buds were dissected after 4 to 5 days to record the number of larvae/pupae and larvae were transferred to fresh buds of the same test species as before. The numbers of adults that emerged from each test plant species were recorded. Each test plant species was tested on 3 to 4 separate occasions as before.

*2.2.3.3. Adult multi-choice tests in walk-in cages*

Although open-field trials are preferable, the limited stocks of adult weevils coupled with the low recovery rates of insects that are typical during such trials led to the use of large walk-in cages (250 cm × 150 cm × 200 cm) to determine the ecological host range of *A. santacruzi*. These tests, which included potted plants with either floral or non-floral material, were less restrictive and were designed to reflect true insect behaviour in the field. The walk-in cage was erected inside a glasshouse in the UKZN Botanical Garden. Only test plant species that were exploited for feeding and oviposition during the comparative choice tests in small cages were used in these trials. During trials with non-floral material, 20 adults were exposed to the test plants that were randomly arranged in the walk-in cage. The position of the adults and number of feeding scars were recorded on each test plant species after 4 days. Each test plant species was tested at least three times on separate occasions.

Trials with floral material were similarly carried out using the same design, except that the potted plants contained flowers and flower buds. Twenty sexually-mature adults were released into the cage and after 4 days the position of the adults on each test plant species was recorded. All flower buds were placed in Petri dishes and dissected after 4 to 5 days to record the presence of larvae/pupae. Developing larvae were transferred to fresh buds as required and adult emergence was recorded. Each test plant species was tested at least three times on separate occasions.

*2.2.3.4 Risk assessment*

Given the differing results obtained by the different host-range tests, the overall risks to non-target *Solanum* species were quantified (see Wan & Harris 1997) by measuring the weevil's performance on each test species as a proportion of that on *S. mauritianum*. Four criteria, namely plant preference ($R^1$), food acceptability ($R^2$), oviposition preference ($R^3$) and larval survival ($R^4$), were used to represent different stages in the host selection process and involved data from both choice and no-choice tests. Choice data from the walk-in cages were given preference over those from the small cages, except where the former were not recorded. Plant preference involved the number of weevils recorded on each test plant (position) during the choice trials with non-floral material. Food acceptability involved the levels of feeding damage (number of scars per plant) recorded during the no-choice trials with non-floral material. Oviposition preference considered the number of larvae recovered during the choice trials with floral material. Finally, larval survival considered the number of larvae that survived to adulthood during the no-choice trials with floral material.

The potential impact of *A. santacruzi* on the non-target plants was assessed in two ways. Firstly, the risks of 'spillover' feeding damage were determined as the product of the plant preference and food acceptability scores ($R^1 \times R^2$). Secondly, the risks of the weevil establishing viable reproductive adult populations were determined by the product of the oviposition preference and larval survival scores ($R^3 \times R^4$). For each criterion, R represents the weevil's performance on the test species relative to that on *S. mauritianum*. To facilitate calculations, zero values were assigned scores of 0.001 as proposed by Wan & Harris (1997) and used by Olckers (2003).

### 2.2.4. Data analysis

For the data in which the assumptions for normality and homogeneity of variances were met, One-way ANOVA, followed by Least Significant Difference (LSD) multiple range tests, were used to test for statistically significant differences between the test plant species in relation to the recorded variables. Where percentage data were recorded, these were first arcsine-square root transformed and then subjected to the tests as above. Data which did not meet the assumptions of normality and homogeneity of variances were transformed using square root transformation. Data that still did not meet these assumptions following transformations were subjected to non-parametric tests, namely Kruskal-Wallis tests followed by Mann-Whitney U-tests to compare each test species with the control.

## 2.3. Results

### 2.3.1. No-choice tests

The no-choice tests were designed to determine physiological host range during the initial screening of *A. santacruzi* in order to determine whether the weevil can survive on both the non-floral and floral material of the test plant species.

Table 2.2: Physiological host range of *Anthonomus santacruzi* as determined by adult mortality and feeding intensity in the no-choice trials with non-floral material.

| Test species (variety) | Mean (± SE) % of adult mortality | Mean (± SE) number of feeding scars /weevil |
|---|---|---|
| *Solanum mauritianum* | 10.0 ± 0.1 | 29.4 ± 6.6 |
| *S. aculeastrum* | 10.0 ± 0.1 | 19.0 ± 4.6 |
| *S. americanum* | 15.0 ± 0.1 | 4.5 ± 1.9* |
| *S. aviculare* | 5.0 ± 0.1 | 10.6 ± 1.4* |
| *S. laciniatum* | 15.0 ± 0.1 | 6.5 ± 1.0* |
| *S. giganteum* | 5.0 ± 0.1 | 5.3 ± 1.9* |
| *S.* cf. *linnaeanum* | 0 | 17.4 ± 2.9 |
| *S. melongena* (Black Beauty) | 10.0 ± 0.1 | 19.9 ± 9.8 |
| *S. melongena* (Japanese Long) | 25.0 ± 0.1 | 5.5 ± 0.9* |
| *S. melongena* (Louisiana Long Green) | 10.0 ± 0.1 | 3.9 ± 1.4* |
| *S. melongena* (Violet Prince) | 15.0 ± 0.1 | 15.3 ± 3.7 |
| *S. muricatum* | 5.0 ± 0.1 | 17.7 ± 4.3 |
| *S. quitoense* | 25.0 ± 0.1 | 4.0 ± 0.7* |

* Values were significantly different from *S. mauritianum*.

In the no-choice trials with non-floral material, adult mortality amounted to 10 % on *S. mauritianum* and varied from 0 - 25 % on the non-target species (Table 2.2). The % insect

mortality data were arcsine-square root transformed but did not meet the assumptions for normality. Kruskal-Wallis tests found no significant differences (H = 11.646, P = 0.475) in % adult mortality between the test plant species.

During these trials, all 13 test plant species/varieties supported feeding to varying degrees (Table 2.2). The mean number of feeding scars per weevil were normally distributed (Z = 1.053, P = 0.217) and had equal variances (F = 0.00, P = 1.00). One-way ANOVA followed by LSD multiple comparison tests found significant differences (F = 3.9, df = 12, P = 0.001) in the number of scars between the test species. The highest number of feeding scars per weevil was recorded on *S. mauritianum* and the mean number of feeding scars was significantly lower on seven test species, including the native New Zealand species, *S. americanum*, *S. aviculare* and *S. laciniatum* and the cultivated *S. quitoense* and *S. melongena* (varieties Louisiana Long Green and Japanese Long), than on *S. mauritianum* (Table 2.2). Feeding on the remaining five test species, including the South African native *S.* cf. *linnaeanum* and *S. aculeastrum*, and the cultivated *S. muricatum* and *S. melongena* (varieties Black Beauty and Violet Prince), was not significantly lower than on *S. mauritianum* (Table 2.2).

During no-choice trials with floral material, adult mortality was 7.5 % on *S. mauritianum* and varied from 0 – 25 % on the non-target species (Table 2.3). These data were arcsine square-root transformed but still did not meet the assumptions for parametric tests. The data were compared using a non-parametric Kruskal-Wallis test which revealed no significant differences in mean % adult mortality between test species (H = 17.093, df = 12, P = 0.146).

Table 2.3: Physiological host range of *Anthonomus santacruzi* as determined by the mean (± SE) percentage adult mortality, number of larvae, number of larvae per female, number of adults emerged and time to adult emergence during no-choice tests with floral material.

| Test species (variety) | % Adult mortality | Number of larvae | Number of larvae/female | Number of adults | Time to adult emergence (days) |
|---|---|---|---|---|---|
| *S. mauritianum* | 7.5 ± 0.1 | 3.3 ± 0.8 | 0.8 ± 0.3 | 2.8 ± 1.7 | 8.1 ± 2.4 |
| *S. melongena* (Black Beauty) | 7.5 ± 0.1 | 0 * | 0* | 0* | - |
| *S. aviculare* | 3.3 ± 0.0 | 3.7 ± 0.7 | 0.6 ± 0.2 | 2.3 ± 0.3 | 11.7 ± 0.6 |
| *S. laciniatum* | 16.7 ± 0.1 | 2.3 ± 1.9 | 0.4 ± 0.3 | 0* | - |
| *S. melongena* (Japanese Long) | 17.5 ± 0.1 | 0* | 0* | 0* | - |
| *S. melongena* (Louisiana Long) | 0 | 0* | 0* | 0* | - |
| *S. muricatum* | 25.0 ± 0.1 | 0* | 0* | 0* | - |
| *S.* cf. *linnaeanum* | 0 | 6.5 ± 1.2* | 1.2 ± 0.2 | 3.8 ± 0.6 | 9.5 ± 1.9 |
| *S. aculeastrum* | 10.0 ± 0.0 | 1.8 ± 1.0 | 0.6 ± 0.4 | 0.3 ± 0.3* | 18.0 ± 5.7* |
| *S. giganteum* | 7.5 ± 0.1 | 1.5 ± 0.9 | 0.4 ± 0.2 | 0* | - |
| *S. americanum* | 15.0 ± 0.1 | 0* | 0* | 0* | - |
| *S. quitoense* | 13.3 ± 0.1 | 0* | 0* | 0* | - |
| *S. melongena* (Violet Prince) | 12.5 ± 0.1 | 0.3 ± 0.3* | 0.1 ± 0.1* | 0* | - |

* Values were significantly different from *S. mauritianum*.

Besides *S. mauritianum*, six test species/varieties supported oviposition, while six did not (Table 2.3). Kruskal-Wallis tests revealed significant differences in larval recovery (overall and per female) between the test plants ($H = 32.478$, $P = 0.001$). Mann-Whitney U-tests were then used to compare each test species with the control and in five tests species, including two native New Zealand and three native South African *Solanum* species, the number of larvae per female

was not significantly different from *S. mauritianum* (Table 2.3). No or very few larvae were recovered from any of the cultivated species/varieties.

Larval development to adulthood occurred in three of the six non-target species/varieties that supported oviposition (Table 2.3). A Kruskal-Wallis test revealed a significant difference in the mean number of adults reared from the different test species ($H = 44.801$, $P = 0.005$). Mann-Whitney U-tests were used to compare each test species with the control and although the highest number of adults emerged from *S. mauritianum*, the differences were not significant in the case of *S. aviculare* and *S.* cf. *linnaeanum.* Only *S. aculeastrum* displayed a significantly lower adult emergence (Table 2.3). The larvae recovered from *S. melongena* (Violet Prince) and *S. giganteum* did not survive to adulthood.

Of the larvae recovered on *S. mauritianum*, 87 % developed into adults in 8 days, compared with 62.9 % and 59.2 % on *S. aviculare* and *S.* cf. *linnaeanum* in 11.7 and 9.5 days respectively (Table 2.3). Only 14.6 % of the larvae recovered on *S. aculeastrum* developed into adults, taking 18 days. None of the larvae recovered on *S. giganteum* and *S. laciniatum* survived to adulthood (Table 2.3). Overall, there was a significant difference in larval developmental times between the test species that supported development to adulthood ($F = 6.962$, $P = 0.010$). Although larval developmental time was shorter in *S. mauritianum*, there were no significant differences between the control and *S. aviculare* and *S.* cf. *linnaeanum*. However, larval developmental time was significantly higher in *S. aculeastrum* (Table 2.3).

### 2.3.2. Multichoice tests in small cages

The multi-choice trials were designed to determine ecological host range, these tests included trials with non-floral and floral material and involved only non-target species that supported feeding and oviposition during the no-choice tests.

During choice trials with non-floral material in small cages, adults of *A. santacruzi* were recovered on all except one test species (*S. quitoense*) and displayed variable levels of feeding on these (Table 2.4). The data for % number of insects recorded on each test species were arcsine square root transformed and were both normal and had equal variances ($P > 0.05$; One-sample Kolmogorov-Smirnov and Levene's tests). One way ANOVA revealed significant differences in the proportions of adults recovered between the test species ($F = 3.633$, $df = 11$, $P = 0.001$). Significantly more adults were recorded on *S. mauritianum* than on any of the test species (LSD multiple range comparisons) (Table 2.4).

All test plant species supported feeding to some degree, except *S. quitoense* which had no feeding scars. After the numbers of feeding scars were square root transformed, the assumptions for normality and equality of variances were met ($P > 0.05$ for both One-sample Kolmogorov-Smirnov and Levene's tests). There were significant differences in feeding intensity between the test plant species (One way ANOVA; $F= 6.894$, $P = 0.001$) and all non-target test plant species displayed significantly lower numbers of feeding scars relative to *S. mauritianum* (LSD multiple range comparisons) (Table 2.4).

Table 2.4: Host selection of *Anthonomus santacruzi* adults as determined by their position and feeding intensity on each test species during multichoice tests with non-floral material in small cages.

| Test species (variety) | Mean (± SE) % of adults | Mean (± SE) number of feeding scars |
|---|---|---|
| *S. mauritianum* | 53.3 ± 0.1 | 79.7 ± 25.0 |
| *S. aviculare* | 3.3 ± 0.0* | 2.3 ± 2.3* |
| *S. laciniatum* | 16.7 ± 0.1* | 6.7 ± 6.7* |
| *S. melongena* (Japanese Long) | 10.0 ± 0.1* | 2.8 ± 2.1* |
| *S. melongena* (Louisiana Long) | 13.3 ± 0.1* | 1.7 ± 1.7* |
| *S. muricatum* | 22.5 ± 0.1* | 4.3 ± 3.6* |
| *S.* cf. *linnaeanum* | 12.0 ± 0.1* | 2.8 ± 2.3* |
| *S. aculeastrum* | 26.3 ± 0.1* | 10.9 ± 6.0* |
| *S. giganteum* | 21.4 ± 0.1* | 1.6 ± 1.0* |
| *S. americanum* | 20.0 ± 0.1* | 7.5 ± 7.5* |
| *S. quitoense* | 0* | 0* |
| *S. melongena* (Violet Prince) | 13.3 ± 0.1* | 3.2 ± 1.6* |

* Values were significantly different from *S. mauritianum*.

During multichoice trials with floral material, adults of *A. santacruzi* were recovered on all of the *Solanum* species tested (Table 2.5). Data on adult recoveries (%) were arcsine square-root transformed and fulfilled the requirements for normality (One sample Kolmogorov-Smirnov test; $P > 0.05$) and equality of variances (Levene's test; $P > 0.05$). One way ANOVA revealed significant differences in the proportions of adults recorded on the different test plants ($F = 2.433$, $df = 7$, $P = 0.039$) (Table 2.5) and LSD multiple range tests found that *S. mauritianum* attracted significantly higher numbers of adults than *S. melongena* (Japanese Long), *S.* cf. *linnaeanum* and *S. laciniatum*, but not in the case of *S. aculeastrum* and *S. giganteum*.

Table 2.5: Host selection of *Anthonomus santacruzi* adults as determined by their position, oviposition and development to adulthood on each test species during multichoice trials with floral material in small cages.

| Test species (variety) | Mean (± SE) % of adults | Mean (± SE) number of larvae | Mean (± SE) number of adults emerging |
|---|---|---|---|
| *S. mauritianum* | 47.7 ± 0.1 | 4.6 ± 1.3 | 3.4 ± 1.0 |
| *S. melongena* (Japanese Long) | 10.0 ± 0.1* | 0* | 0* |
| *S.* cf. *linnaeanum* | 25.7 ± 0.1* | 1.3 ± 0.6* | 0.9 ± 0.3 |
| *S. aculeastrum* | 36.7 ± 0.1 | 0* | 0* |
| *S. giganteum* | 55.0 ± 0.2 | 4.5 ± 2.2 | 3.0 ± 2.1 |
| *S. melongena* (Violet Prince) | 40.0 ± 0.2 | 0.3 ± 0.3* | 0* |
| *S. aviculare* | 16.7 ± 0.1* | 0* | 0* |
| *S. laciniatum* | 13.3 ± 0.1* | 0* | 0* |

* Values were significantly different from *S. mauritianum*.

Besides *S. mauritianum*, oviposition and larval development was recorded on three test plant species, namely *S. giganteum*, *S.* cf. *linnaeanum* and *S. melongena* (Violet Prince), with no larvae recovered on the remaining three species (Table 2.5). The larval recovery data were both normal and had equal variances ($P > 0.05$ for both One-sample Kolmogorov-Smirnov and Levene's tests). One Way ANOVA revealed significant differences in the numbers of larvae recorded on the different test plants ($F= 2.63$, $df= 7$, $P = 0.028$) (Table 2.5). The LSD multiple range tests indicated that, with one exception (*S. giganteum*), significantly more larvae were recovered on *S. mauritianum* than on the other test species.

Larvae were reared to adulthood on two of the three non-target species that supported oviposition (Table 2.5). While *S.* cf. *linnaeanum* supported a lower survival of larvae to adults,

the numbers of larvae that developed to adults were similar in *S. giganteum* and *S. mauritianum* (Table 2.5). However, these data did not meet the assumptions of normality, even after square root transformation and Kruskal-Wallis tests revealed no significant differences ($H = 13.6$, $P = 0.059$) in the number of adults that emerged between the test plants that supported development of adults. Adults were reared on *S. giganteum* despite no adults being reared on this plant during the no-choice tests (Table 2.3).

### 2.3.3 Multichoice tests in walk-in cages

These tests, which included potted plants with either floral or non-floral material, were less restrictive and were designed to reflect true insect behaviour in the field.

During less restrictive choice tests conducted in a walk- in cage, that involved potted plants with non-floral material, adult weevils were recovered on six of the nine non-target plants tested (Table 2.6). Data on adult recoveries (%) were arcsine square-root transformed and fulfilled the requirements for normality (One-sample Kolmogorov-Smirnov test; $P > 0.05$) and equality of variances (Levene's test; $P > 0.05$). One way ANOVA revealed significant differences in the proportions of adults recovered on the different test plant species ($F = 5.053$, $df = 9$, $P = 0.001$). Significantly higher numbers of adults were recovered on *S. mauritianum* than on any of the test plants (LSD multiple range comparisons) (Table 2.6).

Table 2.6: Host selection of *Anthonomus santacruzi* adults as determined by their position and feeding intensity on each test species during multichoice trials with non-floral material in walk-in cages.

| Test species (variety) | Mean (± SE) % of adults | Mean (± SE) number of feeding scars |
|---|---|---|
| *S. mauritianum* | 54.3 ± 13.2 | 41.3 ± 6.3 |
| *S. melongena* (Black Beauty) | 0* | 0* |
| *S. melongena* (Japanese Long) | 3.0 ± 3.0* | 3.0 ± 3.0* |
| *S. melongena* (Louisiana Long Green) | 0* | 0* |
| *S. melongena* (Violet Prince) | 3.0 ± 3.0* | 1.7 ± 1.7* |
| *S. aviculare* | 0* | 0* |
| *S. laciniatum* | 3.0 ± 3.0* | 4.3 ± 4.3* |
| *S.* cf. *linnaeanum* | 17.0 ± 9.5* | 18.3 ± 11.7* |
| *S. giganteum* | 10.3 ± 5.4* | 12.7 ± 7.2* |
| *S. quitoense* | 9.0 ± 5.9* | 0* |

* Significantly different from *S. mauritianum*.

Feeding occurred on all but four of the nine non-target plants tested (Table 2.6). After a square root transformation, the feeding damage data met the assumptions for normality and equality of variances ($P > 0.05$ for both One-sample Kolmogorov-Smirnov test and Levene's tests). There were significant differences in the mean number of feeding scars between test plants (One way ANOVA; $F = 4.916$, $df = 9$, $P = 0.001$), with feeding intensity always significantly higher on *S. mauritianum* (LSD multiple range comparisons).

Trials in walk-in cages involving potted plants bearing floral material were carried out on the two non-target species that were accepted for oviposition during the multichoice trials in small cages. One way ANOVA (% adult recovery data arcsine square root transformed)

indicated significant differences in the position of the adults between the test species (F = 10.684, df = 2, P = 0.006) (Table 2.7). The proportion of adults recovered was significantly higher on *S. mauritianum* than on *S.* cf. *linnaeanum* and *S. giganteum*. Similarly, there were significant differences in oviposition between the test plants (F = 24.737, P = 0.00), with the highest number of larvae recovered on *S. mauritianum*, significantly fewer recovered on *S.* cf. *linnaeanum* and none recovered on *S. giganteum* (Table 2.7). The same pattern was observed with the number of larvae that developed to adulthood (F = 17.657, P = 0.001).

Table 2.7: Host selection of *Anthonomus santacruzi* adults as determined by their position, oviposition and development to adulthood on different test species during multichoice trials with floral material in walk-in cages.

| Test plant | Mean (± SE) % of adults | Mean (± SE) number of larvae recovered | Mean (± SE) number of adults emerging |
|---|---|---|---|
| *S. mauritianum* | 64.5 ± 1.4 | 8.8 ± 0.5 | 7.5 ± 0.5 |
| *S. giganteum* | 15.3 ± 6.8* | 0* | 0* |
| *S.* cf. *linnaeanum* | 27.0 ± 5.6* | 2.0 ± 2.0* | 2.0 ± 2.0* |

* Significantly different from *S. mauritianum*.

### 2.3.4. Risk assessment

Given the variable data generated by the different host-range tests (Tables 2.2-2.7), the overall risks to non-target *Solanum* species were quantified by measuring the weevil's performance on each test species as a proportion of that on *S. mauritianum*.

In most non-target test species, the risk of 'spillover' feeding was < 6 % and these included all New Zealand native and most cultivated species of *Solanum* (Table 2.8). However, there were three notable exceptions where high feeding-risk scores were calculated and these included the South African native *S. aculeastrum* (32 %) and *S.* cf. *linnaeanum* (18 %) and the cultivated *S. muricatum* (25 %) (Table 2.8).

Table 2.8: Risk analysis on the performance of *Anthonomus santacruzi* on non-target *Solanum* species relative to that on *S. mauritianum.**

| Test plant (variety) | Plant preferences ($R^1$) | Food acceptability ($R^2$) | Feeding risks ($R^1 \times R^2$) | Oviposition preferences ($R^3$) | Larval survival ($R^4$) | Reproductive risks ($R^3 \times R^4$) |
|---|---|---|---|---|---|---|
| *S. mauritianum* | 1 | 1 | 1 | 1 | 1 | 1 |
| *S. melongena* (Black Beauty) | 0.001 | 0.66 | $6.6 \times 10^{-4}$ | 0.001 | 0.001 | $1 \times 10^{-6}$ |
| *S. aviculare* | 0.001 | 0.36 | $3.6 \times 10^{-4}$ | 0.001 | 0.52 | $5.2 \times 10^{-4}$ |
| *S. laciniatum* | 0.06 | 0.22 | 0.013 | 0.001 | 0.001 | $1 \times 10^{-6}$ |
| *S. melongena* (Japanese Long) | 0.06 | 0.19 | 0.011 | 0.001 | 0.001 | $1 \times 10^{-6}$ |
| *S. melongena* (Louisiana Long Green) | 0.001 | 0.13 | $1.3 \times 10^{-4}$ | 0.001 | 0.001 | $1 \times 10^{-6}$ |
| *S. muricatum* | 0.42 | 0.60 | 0.25 | 0.001 | 0.001 | $1 \times 10^{-6}$ |
| *S.* cf. *linnaeanum* | 0.31 | 0.59 | 0.18 | 0.23 | 0.83 | 0.19 |
| *S. aculeastrum* | 0.49 | 0.65 | 0.32 | 0.54 | 0.06 | 0.032 |
| *S. giganteum* | 0.19 | 0.18 | 0.034 | 0.97 | 0.001 | $9.7 \times 10^{-4}$ |
| *S. americanum* | 0.38 | 0.15 | 0.057 | 0.001 | 0.001 | $1 \times 10^{-6}$ |
| *S. quitoense* | 0.17 | 0.14 | 0.024 | 0.001 | 0.001 | $1 \times 10^{-6}$ |
| *S. melongena* (Violet Prince) | 0.06 | 0.52 | 0.031 | 0.07 | 0.001 | $8 \times 10^{-5}$ |

*Zero values were designated a score of 0.001 to facilitate calculations.

In contrast, the calculated risks for host range extension were very low, with all but two non-target species displaying reproductive risk scores of < 1% (Table 2.8). The two exceptions were the South African native *S.* cf. *linnaeanum* (19 %) and *S. aculeastrum* (3 %).

## 2.4. Discussion

In the no-choice tests involving non-floral material, all 12 non-target test species/varieties supported feeding to different degrees, although the highest levels were always recorded on *S. mauritianum*. In no-choice tests with floral material, oviposition occurred on six non-target species with three species (*S.* cf. *linnaeanum, S. aculeastrum, S. aviculare*) supporting survival to adulthood. Such results are typical of no-choice trials where insects routinely exploit plants that would not be attacked in the field and are well known from previous studies on the host specificity of agents for *S. mauritianum* (Olckers 1998, 1999, 2000 a, b, 2003).

A different trend was observed in the choice tests that were carried out using bouquets of either non-floral or floral material in small cages. The results of the trials with non-floral material were similar to those of the no-choice tests in that feeding occurred on 11 of the 12 test plant species, with most feeding recorded on *S. mauritianum*. However, oviposition was recorded on fewer non-target plants/varieties (three versus six in the no-choice tests) with adults emerging from two species (*S.* cf. *linnaeanum* and *S. giganteum*). In these trials, no larvae were recovered on *S. aviculare* and *S. aculeastrum* which were exploited in the no-choice trials.

The tests with potted plants in the walk-in cage, which best approximated field conditions, provided the most realistic results. Indeed, the use of excised plant material in host-range testing

(as in the previous two sets of trials) may not always provide results that are consistent with those from intact plants. In trials with non-floral material, feeding occurred on six of the nine non-target test plant species/varieties, with the highest levels again recorded on *S. mauritianum*. In the trials with floral material, larval recovery and survival to adulthood was recorded on *S. mauritianum* and *S.* cf. *linnaeanum* only, with the highest larval recovery and adult survival recorded on *S. mauritianum.*

The overall risk assessment predicted low probabilities of either 'spillover' feeding or host-range extension, with most non-target plants reflecting probabilities of < 6 % for 'spillover' and < 1 % for host range extension. None of the New Zealand native and only one cultivated species (*S. muricatum*) reflected probabilities of >6 %. Although *S. muricatum* and two native South African *Solanum* species displayed relatively high feeding-risk scores, only one species, the native South African *S.* cf. *linnaeanum*, displayed a reproductive risk of >4 %. The high feeding and reproductive risks calculated for *S.* cf. *linnaeanum* in this study (18 % and 19 % respectively) were similar to those calculated during the previous study (13 % and 23 % respectively; Olckers 2003).

These results indicate that *A. santacruzi* can feed, oviposit and develop to adulthood on a limited number of non-target *Solanum* species during restricted laboratory situations. These trends were not consistent between the different testing procedures (i.e. no-choice versus choice tests) and were also not different from those observed during the initial host-range testing where similar ambiguous results were reported (Olckers 2003). Even though the insect's physiological host range did not extend beyond the genus *Solanum*, under laboratory conditions it was able to exploit a few non-target plants including cultivated and native South African species. These trends are further examples of expanded host ranges under artificial laboratory conditions, which

have confounded the host-range testing of virtually all candidate agents for *S. mauritianum* (Olckers 1998, 1999, 2000 a, b, 2003). As in the previous trials, the results of the walk-in cage trials are considered to be the most accurate measure of the weevil's true host range. Initially, Olckers (2003) had predicted that the South African native *S.* cf. *linnaeanum* was the non-target species that was most at risk and these results support this contention.

There have been many arguments put forward to justify why *A. santacruzi* would be safe for release in South Africa and elsewhere in the world. These include host records which were supported by surveys in Argentina (Olckers *et al.* 2002) and southern Brazil (Pedrosa-Mecedo *et al* 1999, Olckers 2003) that confirmed that *A. santacruzi* has a narrow host range. In addition, the failure of *S. mauritianum* to be exploited by native South African insects (Olckers & Hulley 1989, 1991a, b) suggested unique phytochemistry (e.g. the presence of deterrents or lack of required attractants) that would similarly preclude introduced agents from exploiting native plants (Olckers 2003).

In conclusion, these data do not deviate substantially from the results of the original quarantine tests (Olckers 2003). As was previously experienced, the weevils are capable of surviving and producing progeny on a few non-target *Solanum* species during no-choice tests, but revert to *S. mauritianum* during the choice tests, as observed in the small cages but particularly in the walk-in cages. Overall, one may conclude that, at worst, the plants that would be most at risk are the two South African natives, *S.* cf. *linnaeanum* and *S. aculeastrum*. Consequently, none of the native New Zealand or cultivated *Solanum* species are deemed to be at risk and the weevil can be considered to be safe for release in New Zealand.

# CHAPTER 3: POTENTIAL IMPACT OF NATIVE GENERALIST PREDATORS ON THE ESTABLISHMENT AND PERSISTENCE OF *ANTHONOMUS SANTACRUZI*

## 3.1. Introduction

The success of biological control relies on the release and proliferation of specialist insect herbivores to restore top-down control of the target plants in their introduced range (McFadyen 1998, Mitchell & Powers 2003). Besides being climatically compatible, these herbivores need to be freed from the numerous specialist predators, competitors and parasites that would otherwise suppress them and reduce their impact (Jeffries & Lawton 1984, Hunt-Joshi et *al.* 2005, Davis *et al*. 2006). However, even though introduced agents escape their coevolved specialist predators and parasites, they may still be vulnerable to generalist predators and parasitoids in their new range, with similar disruptive effects (Hunt-Joshi *et al.* 2005).

Relatively few weed biological control projects have succeeded worldwide (Carson *et al*. 2008). Several have failed (Crawley 1989, McFadyen 1998,) with only 30 % of the programmes producing successful plant suppression (Crawley 1989) and others delivering variable results (Fowler *et al*. 2000). As Chacon *et al.* (2008) have pointed out, the success of a classical biological control agent depends on many factors including interactions with resident species in the introduced range. If resident species cause biotic interference, which can occur via a range of trophic interactions such as predation, parasitism and competition (Pratt *et al.* 2003, Sebolt & Landis 2004), the ability of the agent to establish and provide effective control can be inhibited (Goeden & Louda 1976, Briese 1986, Reimer 1988, Snyder & Ives 2001, Sebolt & Landis 2004).

Predation is the most common form of biotic interference against arthropod biological control agents (Goeden & Louda 1976, Reimer 1988, Muller & Goeden 1990, Muller *et al.* 1990,

Ehler 1998, Snyder & Ives 2001, Pratt *et al.* 2003, Sebolt & Landis 2004). Despite much evidence that such opportunistic natural enemies can limit the survival and establishment of introduced herbivores (Goeden & Louda 1976, Crawley 1989), generalist predation is not often studied in weed biological control (Hunt-Joshi *et al.* 2005) and determining whether biotic interference is likely to occur with an introduced agent should increase the predictability of biological control (Chacon *et al*. 2008). Plants often provide suitable habitats for predators as they use them for foraging, mating and shelter (Romero & Vasconcellos-Neto 2004, 2005a, b, c, Morais-Filho & Romero 2010). In particular, spiders, especially Thomisidae (crab spiders), are often associated with the inflorescences of *S. mauritianum* in the field in South Africa, presumably feeding on the plant's pollinators. Having observed these spiders capturing the weevils during the propagation of cultures in the laboratory, they were suspected to be the greatest potential threat to the establishment of *A. santacruzi*.

This study was thus initiated to identify, through field sampling of inflorescences and foliage, the groups of potential predators and competitors associated with *S. mauritianum* and to test whether these differ across sites and seasons. Also, the study investigated, through laboratory trials using thomisid spiders as a surrogate, whether generalist predators could inhibit the establishment and proliferation of *A. santacruzi* populations.

## 3.2. Materials and methods

### 3.2.1. Surveys of *S. mauritianum* populations to assess potential predation and competition

Four field sites in KwaZulu-Natal (KZN) were selected to assess the groups of potential native predators and competitors that are associated with the inflorescences and foliage of *S.*

*mauritianum*. These sites were also earmarked for the release of *A. santacruzi* (Table 3.1) and varied slightly in relation to climatic conditions (warmer areas along the coast and colder areas within the midlands of KZN). Sampling was carried out once in each of the four seasons in 2010, namely summer (February), autumn (May), winter (July) and spring (October) to determine any influence of season. On each sampling occasion, 50 inflorescences and 50 shoot tips were removed from at least 25-30 randomly selected trees at each site and placed in Ziploc bags for preservation (freezing) and later inspection in the laboratory.

Table 3.1: Sites in KwaZulu-Natal where seasonal sampling was carried out to assess the arthropods associated with *Solanum mauritianum* populations.

| Site | GPS position | Zone | Description | Mean annual temperature |
|---|---|---|---|---|
| Hilton | 29.32.36.87S<br>30.17.59.70E | Inland | Municipal wasteland | 16.4 $^{o}$C |
| Pietermaritzburg | 29.38.30.05S<br>30.24.34.03E | Inland | Municipal land along watercourse | 18.4 $^{o}$C |
| Hillcrest | 29.47.50.25S<br>30.46.31.20E | Coastal | Municipal land along roadside | 18.7 $^{o}$C |
| Umkomaas | 30.12.07.71S<br>30.47.06.62E | Coastal | Nature reserve | 19.8 $^{o}$C |

During the processing of the frozen inflorescences, open flowers, mature buds, immature buds and fruits were inspected under the microscope and the numbers of all associated arthropods were recorded. The frozen foliage samples were processed in a similar manner. Specimens were identified to family level and groups of potential predators and competitors were compared across sites and seasons (Figs 3.1 & 3.2).

### 3.2.2. Impact of spiders on laboratory populations of *A. santacruzi*

Laboratory predation trials involved four densities of weevils (5, 10, 15 and 20 per inflorescence) and two densities of spiders (0, 1). The spiders used in the trials were collected from *S. mauritianum* inflorescences in the field and were mostly Thomisidae. During testing, a trial consisted of each of the four densities of weevils being exposed to each of the two levels of spiders (i.e. present or absent) on one excised inflorescence that was placed in a small water-filled plastic container that was sealed with a sponge and placed in a 10-litre plastic bucket. Each trial was continued until all the weevils were dead and was repeated three times.

Inflorescences were changed after 4 to 5 days and the numbers of surviving weevils were recorded. The changed buds were put in Petri dishes to allow the immature stages to complete their development and the flower buds were later dissected to record the presence of larvae. The recovered larvae represented the reproductive success of the populations and the original weevils used were dissected to determine the number of females and hence population fecundity per female. Recorded data included the number of surviving weevils, the number of days to 100 % population mortality, the number of larvae (progeny) produced and the sex ratios of weevils to determine the number of larvae per female.

### 3.2.3. Data analysis

For the analysis of the groups of potential predators and competitors, the incidence of each group (% of samples in which the group was represented in all sites and seasons combined) was determined for the inflorescence and foliage samples. The mean numbers of predators and competitors per inflorescence/foliage sample between sites and seasons were compared using

Log linear modeling (Generalized Linear Model) followed by Sequential Sidak multiple comparisons to test if there were any significant differences across treatments and any interactions.

For the predation trials, survivorship and fecundity curves were produced and the numbers of larvae/female (reproduction) and time taken to reach 100 % population mortality were compared between the different weevil densities and spider densities. Log linear modeling (Generalized Linear Model) followed by Sequential Sidak multiple comparisons were similarly used to test if there were any significant differences across treatments and any interactions.

## 3.4. Results

### 3.4.1. Potential predators and competitors

Arthropods recorded in flowers and foliage of *S. mauritianum* at four sites and during four different sampling seasons comprised six orders namely Acari, Hymenoptera, Hemiptera, Thysanoptera, Araneae and Coleoptera (Tables 3.2 & 3.3). The families of Acari, Thysanoptera and Araneae were common in both inflorescences and foliage samples. Thirteen families of Hemiptera were recorded, of which 7 families were common in both inflorescences and foliage samples. Two families (Cixiidae and Membracidae) were uniquely sampled in inflorescences while four families (Pentatomidae, Cicadellidae, Coreidae and Scutelleridae) were uniquely recorded in the foliage samples. For Hymenoptera, four families were recorded of which three (Formicidae, Eurytomidae and Eucharitidae) were common in both inflorescences and foliage while Braconidae was only recorded in the foliage samples. In the order Coleoptera, five families

were recorded of which four were common in both inflorescence and foliage samples and one family (Chrysomelidae) was only recorded in the inflorescence samples (Tables 3.2 & 3.3).

The most common group of arthropods in the inflorescence samples were Thysanoptera with 30.9 % incidence and an abundance of 1.21 thrips per inflorescence. Thrips were followed by the Acari with 21.1 % incidence and an abundance of 1.56 mites per inflorescence. The third most common group was the Miridae (Hemiptera) with 18.2 % incidence and 0.32 mirids per inflorescence. Mirids were followed by Araneae, Formicidae and Aphididae with incidences of 14.7 %, 9.7 % and 9.2 % respectively and abundances of 0.17 spiders, 0.22 ants and 0.23 aphids per inflorescence. The remaining groups were considerably less common (Table 3.2).

In the foliage samples, the most common groups were Formicidae followed by Thysanoptera with incidences of 18 % and 10 % respectively and abundances of 0.50 ants and 0.12 thrips per foliage sample. These were followed by Aphididae, Araneae and Miridae with incidences of 9.1 %, 7.5 % and 6.5 % respectively and abundances of 0.34 aphids and 0.08 for each of spiders and mirids per foliage sample. Similar to the inflorescence samples, the remaining groups were less common (Table 3.3).

Table 3.2: Arthropods associated with inflorescences of *Solanum mauritianum* and their potential interference with the life stages of *Anthonomus santacruzi*.

| Order/Family | Incidence* | Abundance** | Interference (stage affected) |
|---|---|---|---|
| **Hemiptera** | | | |
| Miridae | 18.2 | 0.32 ± 0.03 | Predator (eggs and larvae) |
| Aphididae | 9.2 | 0.23 ± 0.04 | Competitor (adults and larvae) |
| Reduviidae | 1.1 | 0.01 ± 0.01 | Predator (eggs and larvae) |
| Pseudococcidae | 0.5 | 0.01 ± 0.01 | Competitor (adults and larvae) |
| Cercopidae | 0.5 | 0.06 ± 0.05 | None |
| Derbidae | 0.4 | <0.01 | None |
| Cixiidae | 0.2 | <0.01 | None |
| Membracidae | 0.4 | 0.01 ± 0.00 | None |
| Tingidae | 0.4 | 0.01 ± 0.00 | None |
| **Hymenoptera** | | | |
| Formicidae | 9.7 | 0.22 ± 0.04 | Predator (adults) |
| Eurytomidae | 0.2 | <0.01 | Parasitoid (larvae) |
| Eucharitidae | 1.2 | 0.02 ± 0.01 | Parasitoid (larvae) |
| **Coleoptera** | | | |
| Meloidae | 5.5 | 0.09 ± 0.05 | Competitor (adults and larvae) |
| Scarabaeidae | 2.9 | 0.03 ± 0.01 | Competitor (adults and larvae) |
| Coccinellidae | 0.7 | 0.01 ± 0.00 | None |
| Chrysomelidae | 1.1 | 0.02 ± 0.01 | Competitor (adults and larvae) |
| Curculionidae | 6.5 | 0.28 ± 0.05 | Competitor (adults and larvae) |
| **Araneae** | 14.7 | 0.17 ± 0.02 | Predator (adults) |
| Acari | 21.1 | 1.56 ± 0.19 | Predator (eggs and larvae) or Competitor (adults and larvae) |
| **Thysanoptera** | 30.9 | 1.21 ± 0.11 | Predator (eggs and larvae) or Competitor (adults and larvae) |

* Percentage of samples (n = 800) in which the specimens were found.

**Mean number of specimens per inflorescence over the entire sampling period.

Table 3.3: Arthropods associated with foliage of *Solanum mauritianum* and their potential interference with the life stages of *Anthonomus santacruzi*.

| Order/Family | Incidence* | Abundance** | Interference (stage affected) |
|---|---|---|---|
| **Hemiptera** | | | |
| Miridae | 6.5 | 0.08 ± 0.01 | Predator (eggs and larvae) |
| Aphididae | 9.1 | 0.34 ± 0.08 | Competitor (adults) |
| Reduviidae | 0.4 | <0.01 | Predator (eggs and larvae) |
| Derbidae | 0.4 | <0.01 | None |
| Tingidae | 4.7 | 0.08 ± 0.02 | Competitor (adults) |
| Pentatomidae | 0.4 | 0.01 ± 0.00 | None |
| Cicadellididae | 0.9 | 0.01 ± 0.00 | None |
| Pseudococcidae | 1.6 | 0.03 ± 0.02 | None |
| Cercopidae | 0.6 | 0.01 ± 0.00 | None |
| Coreidae | 0.1 | 0.01 ± 0.01 | None |
| Scutelleridae | 0.4 | <0.01 | None |
| **Hymenoptera** | | | |
| Formicidae | 18.0 | 0.50 ± 0.07 | Predator (adults) |
| Eurytomidae | 0.6 | 0.01 ± 0.00 | Parasitoid (larvae) |
| Braconidae | 0.1 | <0.01 | Parasitoid (larvae) |
| Eucharitidae | 1.2 | 0.01 ± 0.00 | Parasitoid (larvae) |
| **Coleoptera** | | | |
| Scarabaeidae | 1.7 | 0.02 ± 0.01 | Competitor (adults)/None |
| Coccinellidae | 0.6 | 0.01 ± 0.00 | None |
| Meloidae | 2.0 | 0.02 ± 0.01 | Competitor (adults)/None |
| Curculionidae | 0.6 | <0.01 | None |
| **Acari** | 3.9 | 0.11 ± 0.03 | Predator (eggs and larvae) or Competitor (adults) |
| Araneae | 7.5 | 0.08 ± 0.01 | Predator (adults) |
| Thysanoptera | 10.0 | 0.12 ± 0.01 | Predator (eggs and larvae) or Competitor (adults) |

* Percentage of samples (n = 800) in which the specimens were found.

**Mean number of specimens per foliage sample over the entire sampling period.

The most common groups of potential competitors and predators comprised spiders, mirids, thrips, ants and mites (Tables 3.2 & 3.3). Spiders and mirids can potentially prey on the adults and eggs and larvae, respectively of *A. santacruzi*. In the inflorescence samples, spiders were consistently present in all seasons and at all sites with the highest abundance recorded at one of the coastal sites (Umkomaas). All parameters (site, season and their interactions) were

significant ($P < 0.05$; Figs 3.1 & 3.2). Similar trends were observed in the foliage samples and more spiders were recorded at the Umkomaas site and the differences due to sites were statistically significant ($P < 0.05$; Fig. 3.2). For the sampling seasons, even though a relatively higher abundance of spiders was recorded in summer, the difference was not significant ($P > 0.05$ Fig. 3.2).

Miridae were rarely sampled at Umkomaas but were most abundant at Pietermaritzburg and were mostly sampled in summer (Fig. 3.1). The difference was statistically significant and the sites, seasons and their interactions were significant for inflorescence samples ($P < 0.05$; Fig. 3.1). Similar trends were observed for foliage samples with more mirids recorded at inland sites and relatively low abundance recorded at Umkomaas (Fig. 3.2). However, there was no significant difference due to site observed in the foliage samples ($P > 0.05$). More mirids were sampled in summer and autumn and the differences were significant ($P < 0.05$; Fig. 3.2).

Thrips, mites and ants are also potential threats to *A. santacruzi* since all three groups can include predators while thrips and mites can also include phytophages that may compete with *A. santacruzi.* Thrips were common in all seasons and at most of the sites, except one of the coastal sites (Umkomaas) where the number was relatively low for both inflorescence and foliage samples, and the differences were significant ($P < 0.05$; Fig. 3.1). Sites, sampling seasons and their interactions showed significant differences ($P < 0.05$) in the inflorescence samples, but only sampling season showed a significant difference in the foliage samples ($P < 0.05$; Fig. 3.2). Ants were the only arthropods that were more frequently encountered in the foliage samples than in the inflorescences (Figs 3.1 & 3.2). Although sites and seasons indicated significant differences in abundance ($P < 0.05$; Figs 3.1 & 3.2), ants were common in all seasons and at all sites with the lowest incidence of 9.7 % in inflorescences and the highest incidence of 18 % in foliage samples

(Figs 3.1 & 3.2). Finally, mites were common at inland sites (Hilton and Pietermaritzburg) and in the first two sampling seasons (summer & autumn) and there were no significant differences ($P > 0.05$) for sampling seasons and for the interaction between seasons and sites for both inflorescence and foliage samples (Figs 3.1 & 3.2). However, the number of mites recorded across the sites for the inflorescence samples were significantly different ($P < 0.05$).

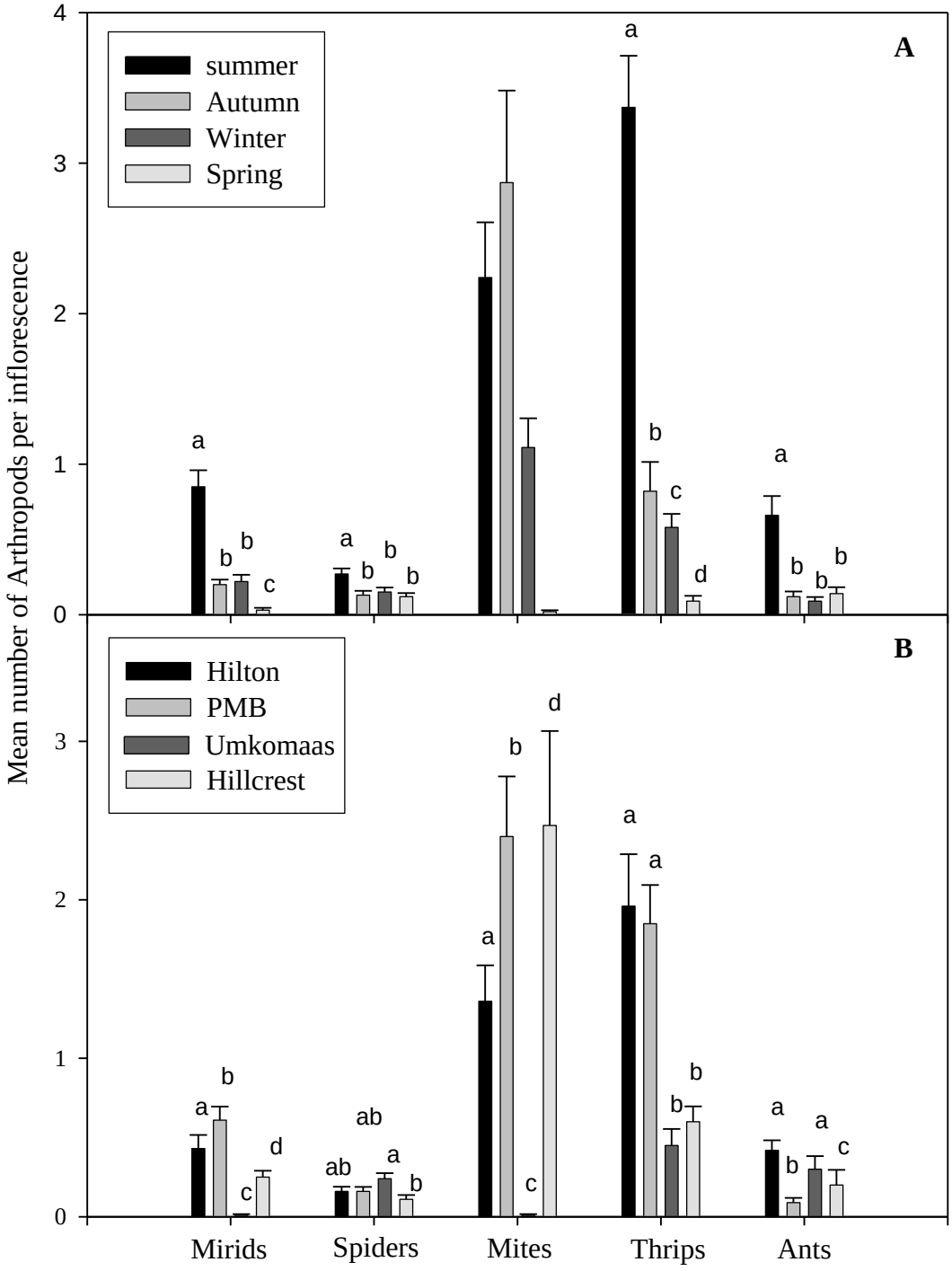


Fig. 3.1: Mean (± SE) number of arthropods per inflorescence of *Solanum mauritianum* recorded across (A) seasons and (B) study sites. Different letters above the bars indicate statistically different means ($P < 0.05$).

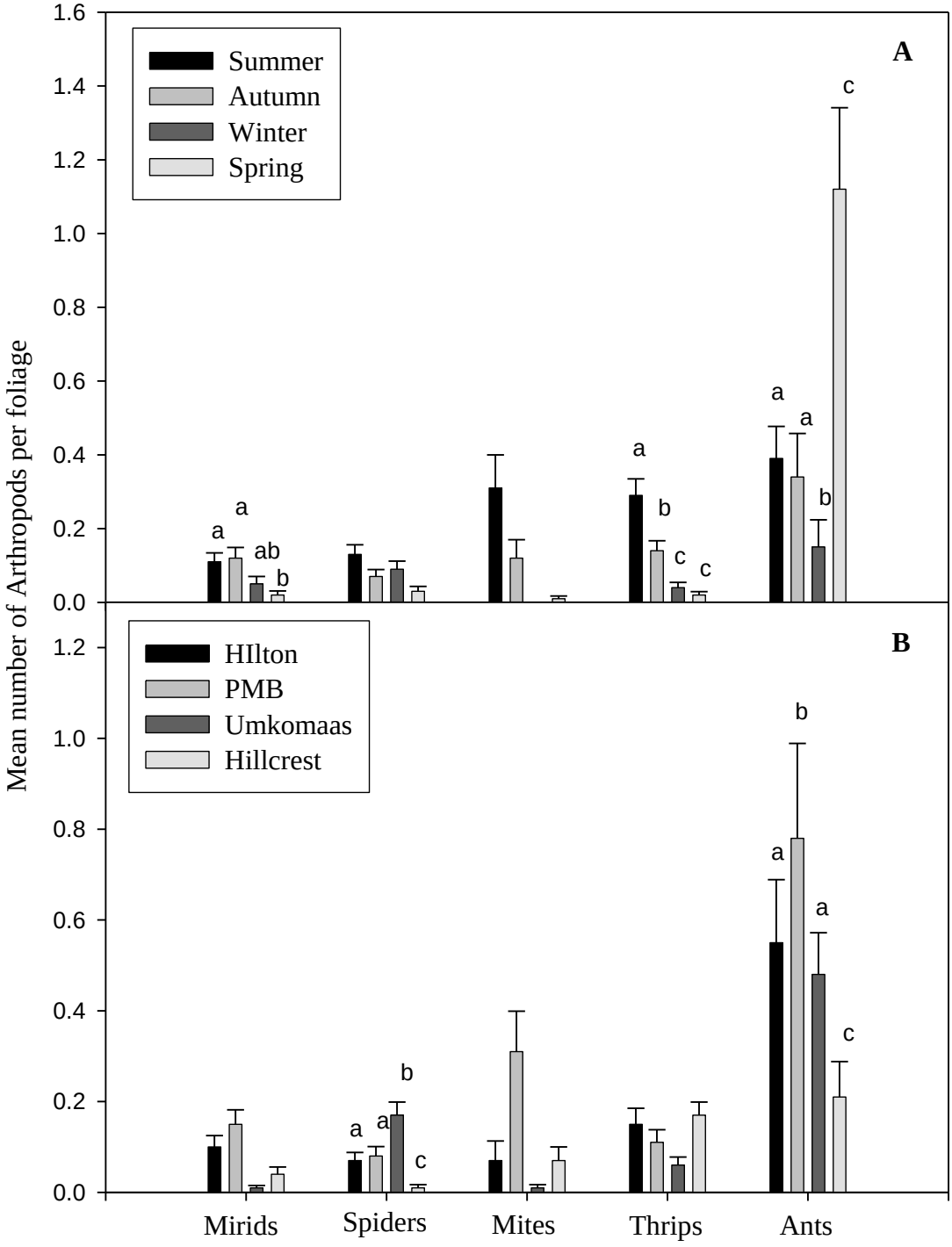


Fig. 3.2: Mean (± SE) number of arthropods per foliage sample of *Solanum mauritianum* recorded across (A) seasons and (B) study sites. Different letters above the bars indicate statistically different means ($P < 0.05$).

### 3.4.2. Predation trials using spiders

With the exception of populations of five weevils that survived for the longest period (20 weeks), there was a general trend of decreased survival times in the presence of spiders (Fig. 3.3A). For example, populations of 15 weevils were the shortest lived and only survived for eight weeks, followed by populations of 10 and 20 weevils which survived for 11 weeks. On other hand, populations that were not exposed to spiders were longer lived. For example, populations of 10 weevils survived for 16 weeks followed by populations of five and 15 weevils that survived for 15 weeks each. Lastly, populations of 20 weevils survived for 12 weeks in the absence of spiders (Fig. 3.3A).

In relation to the cumulative mean number of larvae produced by different population densities of *A. santacruzi*, populations of 10 and 15 weevils produced higher numbers of larvae in the treatments where spiders were absent (Fig. 3.3B). On the contrary, populations of five and 20 weevils produced more larvae in the treatments where spiders were present (Fig. 3.3B). Populations of 15 weevils produced the highest numbers of larvae (about 50) in the absence of spiders, but produced the lowest numbers of larvae (about five) in the presence of spiders (Fig. 3.3B). Populations of 10 weevils followed a similar trend although the difference was slight as both treatments produced less than 20 larvae (Fig. 3.3B). Unexpectedly, populations of five and 20 weevils produced fewer larvae in the treatments where spiders were absent. For example, populations of five weevils produced around 25 larvae in the presence of spiders, but only around seven larvae where spiders were absent (Fig. 3.3B). Similarly, populations of 20 weevils produced slightly more larvae (about 23) in the presence of spiders than in their absence (about 17 larvae) (Fig. 3.3B).

There were general trends of longer population persistence in the absence of spiders, except for populations of five weevils where the converse was true (Fig. 3.4A). In treatments where spiders were absent, populations of 20 weevils survived the longest (almost 80 days) and the remaining densities survived for between 50 and 60 days on average (Fig. 3.4A). In the treatments where spiders were present, populations of five weevils survived the longest (about 75 days) followed by populations of 20 weevils (about 50 days) and the remaining populations of 10 and 15 weevils survived for about 35 days (Fig. 3.4A). Despite these biological differences, high variability in the data sets ensured that there were no significant differences overall (i.e. neither the presence/absence of spiders, nor the densities of weevils, nor their interactions were significant ($P > 0.05$)).

Similar trends were observed with the mean number of larvae produced per female; with higher numbers of larvae produced in treatments were spiders absent (Fig 3.4B). However, populations of five weevils produced almost the same numbers of larvae in the presence (6.5 larvae per female) and absence (6 larvae per female) of spiders (Fig. 3.4B). In treatments where spiders were absent, the highest numbers of larvae (8 larvae per female) were recorded for populations of 15 weevils with the lowest numbers (5 larvae per female) recorded for populations of 20 weevils (Fig 3.4B). In those where spiders were present, the highest numbers were produced by populations of five weevils (6.5 larvae per female) and the lowest numbers by populations of 15 weevils (1 larva per female) (Fig. 3.4B). The differences in larvae produced in the presence and absence of spiders were significant ($P < 0.05$), but there were no significant differences in relation to weevil population size or any significant interactions between population size and spider presence ($P > 0.05$).

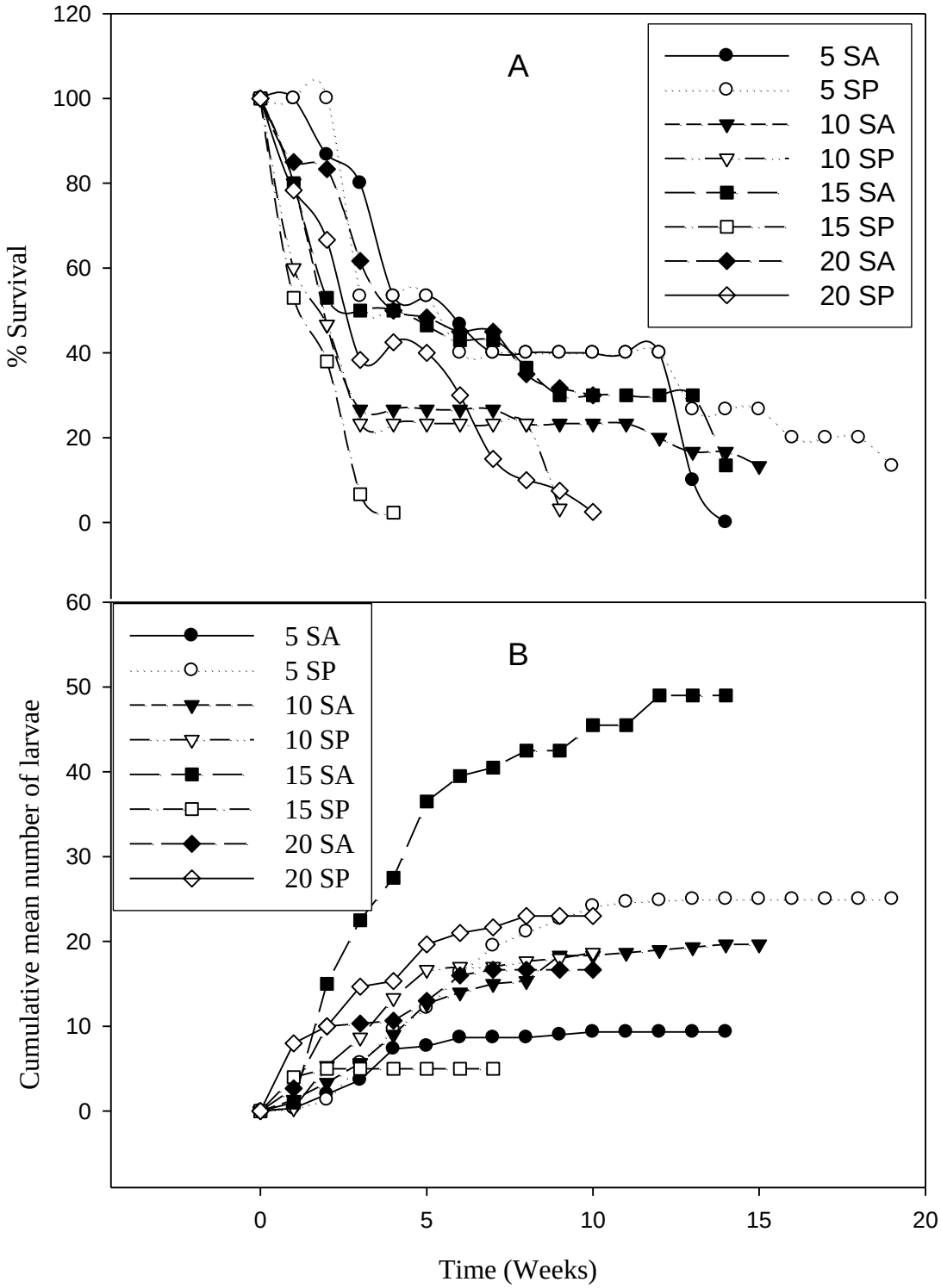


Fig. 3.3: Percentage survival (A) and cumulative mean number of larvae (B) in *Anthonomus santacruzi* populations of varying sizes (5-20 individuals) over time, in the presence (SP) and absence (SA) of spiders.

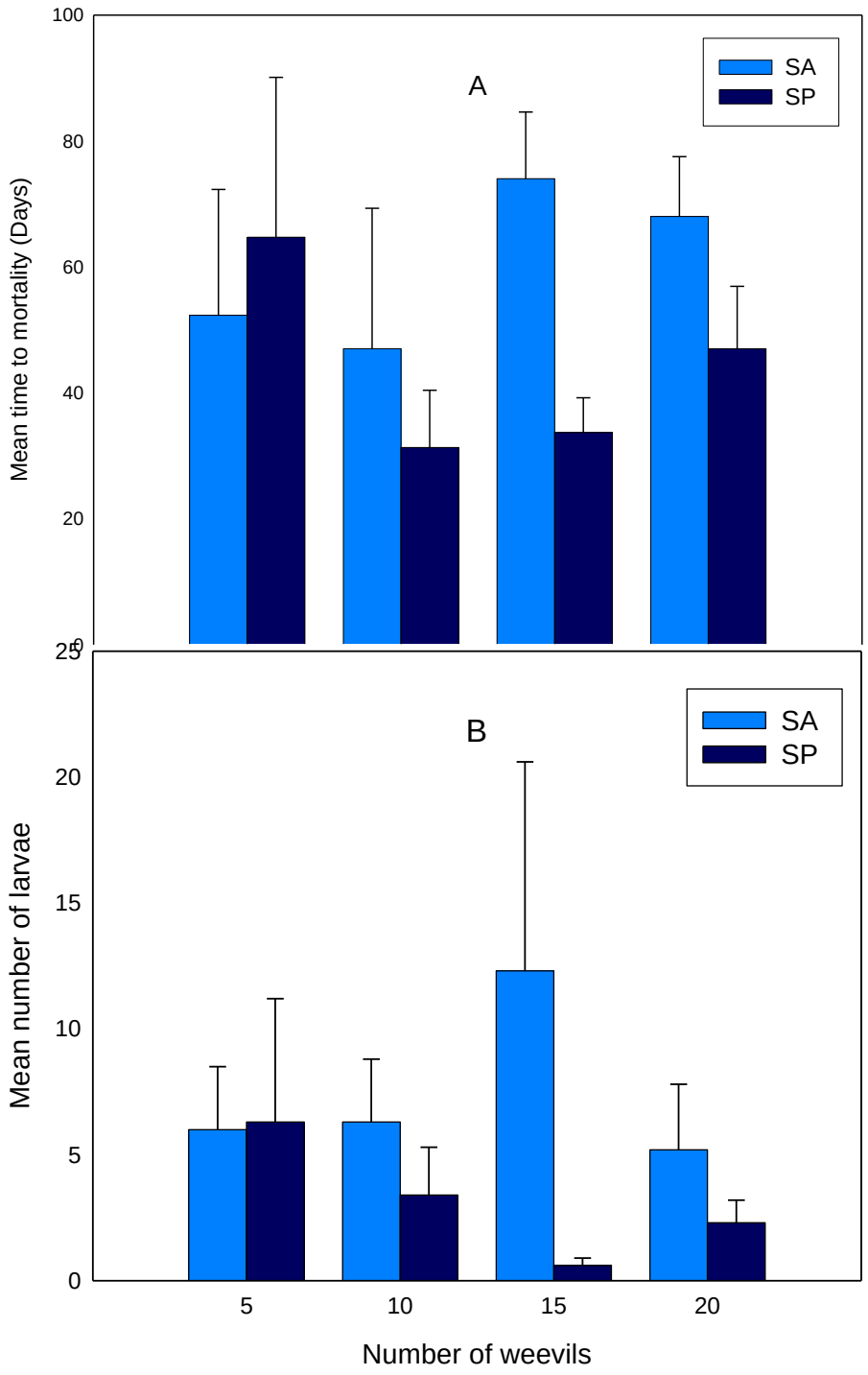


Fig. 3.4: Mean (± SE) number of days to 100 % mortality (A) and mean (± SE) number of larvae per female (B) in *Anthonomus santacruzi* populations of varying sizes **(**5-20 individuals), in the presence (SP) and absence (SA) of spiders.

### 3.5. Discussion

Predators comprise species with a life stage that kills and eats living animals for development, sustenance and reproduction (Van Driesche *et al.* 2008). These predators select their habitats on the basis of the abundance of their prey and the suitability of other parameters such as shelter and mating opportunities (Abrams 2007). On the other hand, prey individuals choose their habitats based on the availability of resources and the risk of predation (Abrams 2007). As a result, predation has been considered the most common form of biotic interference against arthropod biological control agents (Goeden & Louda 1976, Reimer 1988, Muller & Goeden 1990, Muller *et al.* 1990, Ehler 1998, Snyder & Ives 2001, Pratt *et al.* 2003, Sebolt & Landis 2004). The reason for this is that predation risk creates a "landscape of fear" to foraging arthropods resulting in a trade-off between acquiring appropriate food and avoiding predation (Romero *et al.* 2011).

The current survey of arthropods utilizing the inflorescences and foliage of *S. mauritianum* identified Araneae (spiders), Hymenoptera (mostly Formicidae) and Hemiptera (notably Miridae) as groups of potential predators and Acari (mites) and Thysanoptera as potential predators/competitors. Thomisidae (crab spiders) which prey on Coleoptera and several other insect taxa (Jennings 1974) is the largest spider family, with some 2000 species in 160 genera and are the most common spiders in South Africa (Dippenaar-Schoeman & Jocque 1997). Several crab spider species are ambush predators that hunt on flowers and capture flower-dwelling species (Thery 2007, Defrize *et al.* 2011). Thomisidae show some degree of crypsis allowing them to match the colour backgrounds of their foliage or flower substrates (Romero *et al.* 2011). Colour-matching predators are assumed to have an advantage over those without this ability in terms of an increased prey encounter rate or a higher prey capture rate (Brechbuhl *et al.* 2010). Though the current survey indicated low levels of abundance of the spiders (0.17 and 0.08

spiders per inflorescence and foliage sample respectively), which are unlikely to pose serious threats to the establishment of *A. santacruzi*, the literature indicates flower-dwelling arthropods to be constantly at risk of predation when foraging. In particular, a meta-analysis by Romero *et al.* (2011) showed that predation risk increased flower avoidance by flower-dwelling arthropods which significantly decreased visitation rates by 36 % and time spent on flowers by 51 %. Also, it is possible that spider densities may increase as populations of *A. santacruzi* become established and proliferate. This necessitated the need for further investigation through laboratory-based trials to quantify any threats posed by spiders.

Hemiptera represent another group of potential predators and species in the family Miridae were the most abundant. Miridae represent the largest hemipteran group and predatory species occur in all subfamilies (Wheeler 2001). Predatory bugs, especially mirids, feed on the immature stages of a wide range of arthropod herbivores including whiteflies, aphids, spider mites and leaf miners (Moayeri *et al.* 2006) and have been used to control many arthropod pest species (Blaeser *et al.* 2004) including Lepidoptera larvae (Athanassiou *et al.* 2003). Since both egg and larval stages of *A. santacruzi* occur inside the flower buds, generalist predatory mirids might have limited access to them and hence not pose a major threat to them.

Hymenoptera, mostly predatory ants, may pose a potential threat to the establishment of *A. santacruzi*. Ants are considered to be the most abundant hymenopterans, geographically, numerically and ecologically (Breed *et al.* 1982). In addition, some species of ants prey on several organisms including insects (Breed *et al.* 1982). Though there is no recorded evidence of this with *S. mauritianum*, ants often serve as anti-herbivory agents that protect plants for rewards such as food, shelter and nectar provided by the plants (Koptur 1979, Keeler 1981, Fernandes *et al.* 2005). In these mutualistic interactions, they act as a biotic defence (Janzen 1966, Rosumek *et*

*al.* 2009) by fending off herbivores that would attack the plants' vegetative and reproductive organs (Keeler 1981, Oliveira 1997). A comparison of biotic, physical and chemical plant defence strategies found that mutualistic interactions with ants were the most effective anti-herbivore defence strategy for plants (Massad *et al.* 2011). Consequently, ants have the potential to hinder the efficacy of specialist herbivores used in biological control programmes (Mathews *et al.* 2011).

Thysanoptera (thrips) were the most common arthropods and were encountered at all the sites surveyed and potentially comprise both competitors and predators. Thrips are very numerous and some 5500 to 7400 species have been described worldwide (Jaramillo *et al.* 2010, Reitz *et al.* 2011). There are two groupings of thrips, both of which could interfere with the establishment of *A. santacruzi*. Firstly, plant-feeding thrips (suborder Terebrantia, with 90% in the family Thripidae) comprise the most invasive species and include the world's leading crop pests (Mound 2005, Mound *et al.* 2010, Reitz *et al.* 2011). These thrips attack several non-woody plant materials including foliage, flowers, flower buds and fruits (Kirk 1984, Reitz *et al.* 2011). Adult and immature thrips feed in localized areas, using their specialized mouth parts to extract fluid from foliage, floral tissues, pollen grains and fruits (Kirk 1984, Reitz *et al.* 2011). In addition to causing deformation of leaves and flowers, feeding damage also results in necrotic patches on foliage, flowers and fruits (Reitz *et al.* 2011). Thrips have high reproductive rates and short generation times (Pianka 1970). Plant-feeding thrips lay eggs throughout their adulthood and feed mostly on pollen but also prey on the eggs of some arthropods (Trichilo & Leigh 1988, Wilson *et al.* 1991, 1996). This nitrogen-rich diet stimulates oviposition, shortens larval developmental time, increases female fecundity and leads to multiple overlapping generations (Reitz 2008, Reitz *et al.* 2011). Plant-feeding thrips were commonly encountered on both the

inflorescences and foliage of *S. mauritianum*. Besides egg predation, thrips may compete with *A. santacruzi* for oviposition sites in the flower buds and if extensive feeding damage by thrips causes abscission of flower buds, weevil larvae may fail to complete their development.

Predatory thrips (suborder Tubulifera, mostly in the family Phlaeothripidae) comprise both specialized and generalist predators (Cox *et al.* 2006, Grimaldi & Engel 2006, Reitz *et al.* 2011). This group comprises about 60% of all described thrips and at least 50 species are generalist predators which feed on the immature stages of many arthropods including scale insects, mites, whiteflies, other thrips and beetles (Cox *et al.* 2006, Grimaldi & Engel 2006, Jaramillo *et al.* 2009, 2010, Reitz *et al.* 2011). *Karnythrips flavipes* Jones (Phlaeothripidae) is generalist predator that attacks the eggs of the coffee berry borer *Hypothenemus hampei* (Ferreri) (Curculionidae), a worldwide pest among coffee growers (Jaramillo *et al.* 2009, 2010). *Hypothenemus hampei* oviposits in coffee berries in a manner similar to *A. santacruzi* ovipositing in the flower buds of *S. mauritianum* and larval feeding and development is similar to that of *A. santacruzi* (Jaramillo *et al.* 2009). *Karnythrips flavipes* insert their eggs inside the berries, through the small oviposition holes created by the beetles, where the larvae complete their development and the adults prey on the immature stages of the beetles (Jaramillo *et al.* 2009, 2010). Should a similar situation arise with *A. santacruzi*, then such predatory thrips could hinder its establishment.

Mites are similarly capable of competing with and preying on the immature stages of *A. santacruzi*. While about 27 families of mites are known to prey on or parasitize invertebrates (Van Driesche *et al*. 2008), only 8 families, which include phytoseiids and non-phytoseiids, prey on the eggs and smaller instar larvae of many insects (Sabelis & Van Rijn 1997) and these may well pose a threat to *A. santacruzi*. These predatory mites are mostly plant inhabitants and due to

their smaller size have the potential to reach the immature stages of *A. santacruzi* inside the flower buds.

Despite the above mentioned potential threats posed to *A. santacruzi* by predators and competitors associated with *S. mauritianum*, there are several reasons why these may be insufficient to hinder the establishment of *A. santacruzi*. Adult beetles in general are less vulnerable to predation due to their hard protective exoskeletons (Romero *et al.* 2011) and since insect predators are typically larger than their prey (Van Driesche *et al*. 2008), smaller-sized predators like mites and thrips are unlikely to impact on the adult weevils, leaving only spiders and ants likely to pose any risk. The immature stages of *A. santacruzi* are endophagous and, with the exception of very small predators like mites and thrips that may be able to enter the flower buds, should escape predation by several of the larger-sized predators (e.g. mirids, spiders, ants). In any event, the incidence and abundance of potential competitors and predators on the inflorescences and shoot tips of *S. mauritianum* were low and therefore unlikely to pose severe threats during the initial stages of establishment. These results are consistent with those of Olckers & Hulley (1989, 1991a, b) who found very low numbers of native insect herbivores associated with *S. mauritianum* populations in South Africa and suggested that several vacant niches were available for the establishment of biocontrol agents. Flower-dwelling insects are also known to be able to detect past predation events on flowers (Abbott & Dukas 2009) and can learn and memorize information on hazardous flowers (Ings & Chittka 2008, 2009), thereby developing mechanisms to avoid predators (Romero *et al.* 2011). Some flower-dwelling insects rely on olfactory cues to avoid predators (Romero *et al.* 2011, Weiss 2011), enabling them to avoid even cryptic ambush predators such as crab spiders.

Despite their relatively low incidence and abundance during the field surveys, spiders (especially Thomisidae) were observed capturing the weevils in the laboratory and were suspected as the greatest potential threat to the establishment of *A. santacruzi*. However, the laboratory trials revealed that at different weevil densities spiders did not consistently cause substantial reductions in population survival and progeny production. In particular, the lack of any effects of predation at the lowest weevil density (5 per inflorescence) suggests that establishment is unlikely to be prevented by spiders. Although spiders play a major role in suppressing insect populations in the field (Nyffeler 1999), information on feeding preferences are lacking (Jennings 1974). Spiders are unlikely to survive and reproduce on a diet of a single prey species and generally feed on several species (Greenstone 1999). During the laboratory trials, the weevils were the only available prey and it is possible that in the field they will be ignored in favour of larger-sized and less protected (i.e. softer) prey like bees and other pollinators.

In conclusion, this study has suggested that the levels of potential generalist predators or competitors that are associated with the inflorescences and foliage of *S. mauritianum* in the field are insufficient to inhibit the establishment of *A. santacruzi*. Furthermore, the perceived risk of inflorescence-inhabiting spiders is unlikely to be realized as the weevils were able to survive and increase their population densities in their presence.

# CHAPTER 4: RELEASES AND MONITORING OF ESTABLISHMENT OF *ANTHONOMUS SANTACRUZI* IN KWAZULU-NATAL

## 4.1. Introduction

Field establishment of introduced natural enemies, notably their ability to form self-sustaining wild populations (Lodge 1993, Williamson 1996, Williamson & Fitter 1996) is a critical step in classical biological control (Shea & Possingham 2000). Once established, populations need to proliferate, increase their densities and distribution and thereby have the desired impact on the target. Several studies have identified various factors that affect the establishment of biological control agents. These include: the number of insects released and the number of releases made (Memmott *et al.* 1998, Grevstad 1999, Kolar & Lodge 2001); complexity of the release sites; predation and interspecific competition; life history of the released agents (Kolar & Lodge 2001) and; climate (Ulrichs & Hopper 2008).

The aim of this study was to propagate, release and monitor the performance of founder populations of *A. santacruzi* in order to establish self-sustaining populations in the field. This chapter will outline the procedures that were adopted in propagating and releasing founder populations of *A. santacruzi* at selected sites in KwaZulu-Natal (KZN) and in monitoring the outcomes.

## 4.2. Material and methods

### 4.2.1 Agent propagation

Propagation of *A. santacruzi* in the laboratory is difficult and labour intensive because of the need to transfer developing larvae to fresh floral material (Olckers 2003, 2011). The present

cultures were initiated following the reintroduction of new stocks of *A. santacruzi* from northeastern Argentina in 2008 and 2009. The first introduction was to establish new cultures following the loss of the original cultures, while the latter introduction was for boosting the genetic variability of these cultures.

The weevils were propagated in 10-litre plastic containers in the UKZN insectary, under conditions described previously (see Chapter 2). As before, field-collected bouquets of *S. mauritianum* containing flowers, flower buds and apical leaflets, were presented to the weevils for feeding and oviposition. Non-floral material was included since the weevils easily sustain themselves on this for extended periods by feeding on the shoot tips and young leaves (Olckers 2003, Barboza *et al*. 2009). Fresh bouquets were provided every 4 to 5 days and the old buds were placed in glass Petri dishes to allow the immature stages to complete their development. The Petri dishes were supplied with moist pieces of sponge to increase humidity in the Petri dishes and reduce desiccation of the buds. Developing larvae were dissected from buds which had desiccated or rotted and were transferred to fresh buds collected in the field. Emerging adults were placed in holding cages until sufficient numbers had been obtained for releases.

### 4.2.2 Releases of *A. santacruzi* at selected sites

In the absence of pre-release studies to determine suitable sites, adult weevils were released at four selected sites (Table 4.1) with different climatic conditions (warmer areas along the coast and colder areas within the midlands of KZN). The release sites ranged from municipal land to a private nature reserve. The initial aim was to release founder populations of 500 adults but numbers were later adjusted according to weevil availability and the success of preceding

releases. Generally, more than one release was carried out at each site and releases were continued until the desired numbers were achieved.

Table 4.1: Details of releases of *Anthonomus santacruzi* carried out at sites in KwaZulu-Natal.

| Release site | Status of site (*) | Coordinates | Dates of releases | Size of release | Total released |
|---|---|---|---|---|---|
| Hilton: Municipal land near Hilton Hotel | Midlands: Cooler (16.4 $^{o}$C) | 29.32.36.87S 30.17.59.70E | 12/10/2008<br>02/27/2009<br>04/24/2009<br>05/20/2009<br>06/06/2009<br>06/13/2009 | 70<br>72<br>73<br>103<br>102<br>80 | 500 (6 releases) |
| Pietermaritzburg: Municipal land along stream | Midlands: Warmer (18.4 $^{o}$C) | 29.38.30.5S 30.24.34.3E | 05/11/2011<br>06/01/2011<br>08/01/2011<br>08/11/2011 | 50<br>100<br>150<br>200 | 500 (4 releases) |
| Umkomaas: Empisini Nature Reserve | Coastal: Warmer (19.8 $^{o}$C) | 30.12.07.71S 30.47.06.62E | 06/24/2009<br>08/13/2009<br>09/21/2009 | 120<br>82<br>100 | 302 (3 releases) |
| Hillcrest: St Hellier Road along freeway | Coastal: Cooler (18.7 $^{o}$C) | 29.47.50.25S 30.46.31.20E | 06/30/2010<br>07/14/2011 | 100<br>100 | 200 (2 releases) |

* = Mean annual temperature.

### 4.2.3 Monitoring for confirmation of establishment

Monitoring of the founder populations of *A. santacruzi* involved collections of both inflorescences and shoot tips at the release sites. Monitoring started six months after the final release at each site and was carried out at only three sites, namely Hillcrest, Hilton and Umkomaas. The fourth site at Pietermaritzburg was not sampled because the releases were too recent (i.e. 6 months since the final release had not elapsed). On each sampling occasion, 50 inflorescences and 50 shoot tips were removed from randomly selected trees at each site and

placed in Ziploc bags for later inspection in the laboratory. Sampling was carried out once in each of the four seasons in 2010 and 2011, namely summer (February), autumn (May), winter (July) and spring (October).

The numbers of adult *A. santacruzi* on each inflorescence/shoot tip were recorded. The shoot tip samples were stored in a freezer for later processing. The flowers and flower buds from one site where weevil persistence was noticed were placed in glass Petri dishes to allow development of the immature stages, while floral samples from the other sites were frozen and dissected later. Emerging adults were continuously removed from the glass Petri dishes and recorded. About one week after the removal of the last adults emerging in the Petri dishes, the floral material was dissected under a microscope to record the numbers of any additional larvae and pupae. Also, any adults of *A. santacruzi* that might have been missed in the frozen samples were recorded.

## 4.3. Results

### 4.3.1 Propagation and releases of *A. santacruzi*

Good numbers of insects were propagated during this study. Although the majority of these were used for laboratory tests, some 1500 adults were released at four sites (Table 4.1). The first release site (Hilton), in a cooler higher altitude area in the KZN midlands, received the largest founder population of 500 adults, resulting from six smaller releases ranging from 70 to 103 insects. These releases were carried out over 6 months, during December 2008 to June 2009. The second site (Umkomaas), in a warmer coastal area, received a smaller founder population of 302 adults, resulting from three releases,

ranging from 82 to 120 insects. These releases were carried out over three months, from June to September 2009. The third site (Hillcrest), in a cooler coastal area, received only 200 adults in two releases of 100 weevils each during June 2010 and July 2011. The last release site (Pietermaritzburg), in a warmer higher altitude area in the KZN midlands, also received 500 weevils from four releases, each release ranging from 50 to 200 weevils over four months from May to August 2011.

### 4.3.2 Establishment of *A. santacruzi*

Despite the higher numbers released, establishment was not confirmed at the cooler higher altitude site (Hilton), where there were no recoveries of either adults or larvae on any of the sampling occasions (Table 4.2). In contrast, establishment was confirmed at one warmer coastal site (Umkomaas), where adults and larvae were recovered on all sampling occasions. At this site, weevil densities increased with each subsequent sampling occasion (Table 4.2, Fig. 4.1), ranging from less than one individual per inflorescence in March 2010 to around six individuals per inflorescence in July 2010. However, this site was cleared prior to the spring (October 2010) samples, with most of the plants cut down. Although a few weevils were observed on the few remaining trees, samples were not taken at the site (to protect the last few survivors) but from trees in close proximity and these revealed no signs of the weevil. However, further sampling, which commenced in March 2011 and was conducted on trees surrounding the study site, confirmed that the population had survived and was proliferating. Weevil densities soon recovered from this setback with average numbers of 0.98, 1.43 and 3.6 weevils per inflorescence in the ensuing summer, autumn and winter samples, respectively (Table 4.2, Fig. 4.1).

Because releases were more recent, the Hillcrest site was not sampled in 2010. However, sampling in 2011 revealed no signs of establishment from the initial release of 100 adults, as neither adults nor larvae were recovered from sampled inflorescences (Table 4.2). A follow-up release of 100 adults was carried out in July 2011. The most recent releases were carried out at the Pietermaritzburg site (May to August 2011) which has not yet been monitored.

Table 4.2: Numbers of individuals of *Anthonomus santacruzi* recorded during seasonal monitoring of three release sites.

| Release site | Status of site | Season (year) | Dates | Mean (± SE) number per inflorescence |
|---|---|---|---|---|
| Hilton: Near Hilton Hotel | Midlands: Cooler | Summer (2010) | 02/18/2010 | 0 |
| | | Autumn (2010) | 05/30/2010 | 0 |
| | | Winter (2010) | 07/31/2010 | 0 |
| | | Spring (2010) | 10/29/2010 | 0 |
| | | Summer (2011) | 03/27/2011 | 0 |
| | | Autumn (2011) | 05/30/2011 | 0 |
| | | Winter (2011) | 07/14/2011 | 0 |
| | | Spring (2011) | Not yet | - |
| Umkomaas: Empisini Nature Reserve | Coastal: Warmer | Summer (2010) | 03/17/2010 | 0.12 ± 0.12 |
| | | Autumn (2010) | 05/18/2010 | 5.28 ± 0.75 |
| | | Winter (2010) | 07/30/2010 | 5.72 ± 0.91 |
| | | Spring (2010) | 10/25/2010 | Site cleared * |
| | | Summer (2011) | 03/26/2011 | 0.98 ± 0.38 |
| | | Autumn (2011) | 05/14/2011 | 1.43 ± 0.62 |
| | | Winter (2011) | 07/14/2011 | 3.60 ± 1.59 |
| | | Spring (2011) | Not yet | - |
| Hillcrest: St Hellier Road along freeway | Coastal: Cooler | Summer (2010) | Not sampled | - |
| | | Autumn (2010) | Not sampled | - |
| | | Winter (2010) | Not sampled | - |
| | | Spring (2010) | Not sampled | - |
| | | Summer (2011) | 03/26/2011 | 0 |
| | | Autumn (2011) | 05/14/2011 | 0 |
| | | Winter (2011) | 07/14/2011 | 0** |
| | | Spring (2011) | Not yet | - |

* No weevils were recorded on plants sampled in close proximity to the cleared site.
** A second release of 100 weevils was carried out after samples were taken.

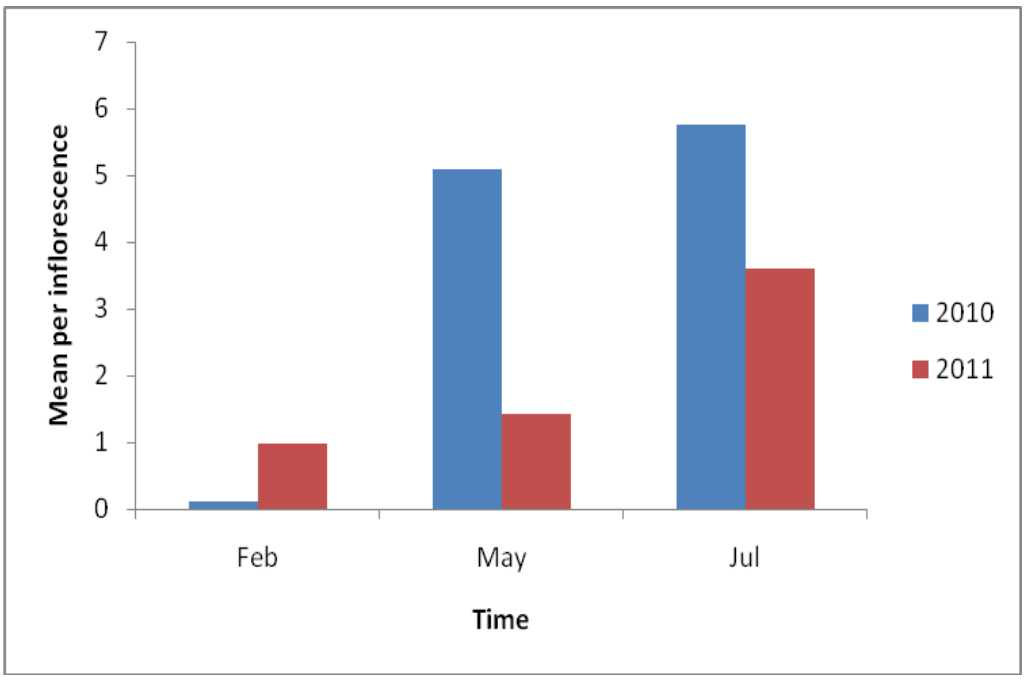


Fig.4.1: Seasonal abundance of *Anthonomus santacruzi* at the Empisini Nature Reserve (Umkomaas) where the population has persisted. Numbers are expressed as means per inflorescence sampled. Note that the site was cleared prior to the October 2010 samples, so no data are shown for this month.

### 4.4. Discussion

Several factors influence the establishment of weed biological control agents and include: total numbers of individuals released and the number of releases made (Memmott *et al.* 1998, Grevstad 1999, Kolar & Lodge 2001); life history of the released agents (Kolar & Lodge 2001) and; climate combined (Ulrichs & Hopper 2008). The potential influences of some of these factors on the persistence *A. santacruzi* are discussed below.

Establishment success is expected to increase with greater release efforts, as shown by several studies (Pimm 1991, Williamson 1996). Smaller founder populations face several challenges including stochastic demographic, environmental and genetic fluctuations which may increase the risks of extinction (Pimm 1991). When introduced species are released in greater

numbers, they are more likely to escape such threats than smaller founder populations. However, in the case of *A. santacruzi*, increased release efforts did not necessarily influence establishment. The insect failed to establish at one site where the highest number of individuals was released, while a release that was 40% smaller resulted in establishment (Table 4.2).

Higher numbers of release attempts, including releases over several years, may also influence establishment success of introduced biological control agents, and is independent of the total numbers released (Crawley 1986). However, higher numbers and higher numbers of release attempts did not overcome the disadvantage of releasing the weevil at the Hilton site. The weevil failed to establish at this site despite the highest number of releases (six) being carried out here, but established at the Umkomaas site with fewer (three) releases.

Life history traits of the biological control agent may also play an important role in establishment success. Agents with higher rates of fecundity, development and population growth escape the risks associated with remaining at a small population size (Moulton & Pimm 1986, Pimm *et al*. 1988, Pimm 1991). The biology of *A. santacruzi* includes several attributes, notably high fecundity, adult longevity, and short generation times (Olckers 2003), that suggested good potential for establishment.

Predation and competition have also been cited as major factors hindering agent establishment in classical biocontrol with establishment more likely in species-poor than in species-rich communities (Elton 1958). In particular, attacks on founder populations of biocontrol agents by generalist predators and parasitoids can prevent establishment, while limited predation/parasitism favours success (Elton 1958, Maron & Villa 2001, Keane & Crawley 2002, Parker & Hay 2005). Following the release of *A. santacruzi*, the impact of predators (notably

spiders inhabiting inflorescences) and potential competitors was investigated in both field and laboratory trials (Chapter 3). Although the field trials revealed considerable variation in both predators and potential competitors between the study sites, with low intensities of these recorded overall, the laboratory trials indicated that *A. santacruzi* can coexist with spiders. Therefore, neither predation nor competition can explain the variation in establishment success recorded in this study.

Climate has a major influence on agent establishment, with higher chances of success when the environmental conditions at the sites of introduction are similar to those at the sites of origin of the agent (Mack 1996, Williamson 1996, Williamson & Fitter 1996). Because environmental and climatic factors often determine the distribution of species (Messenger 1959, 1976, Cammell & Knight 1992, Mack 1996, Williamson 1996), many researchers have emphasized the role of climate in the establishment of introduced species (Worner 1988, Panetta & Mitchell 1991). Insects are particularly prone to environmental constraints, with their distribution and abundance directly influenced by climatic factors (Sutherst & Maywald 1985, Cammell & Knight 1992, Hughes & Evans 1996). Temperature has a direct effect on the physiology of insects and hence has the greatest influence on insect ecology (Messenger 1976). Humidity also plays a major role because insects are often prone to desiccation due to evaporative water loss (Messenger 1976). In addition, climate has a major influence on the quality of food resources. Unsuitable climatic conditions may alter the physiology and morphology of the plant resulting in reduced food quality (Cammell & Knight 1992) which could influence the insect's distribution (Worner 1988). Because climatic factors influence the survival, reproduction and development of insects (Messenger 1959) which are crucial

components of field establishment, the use of climate matching in biological control has been emphasized (Worner *et al.* 1989).

*Anthonomus santacruzi* occurs throughout the range of *S. mauritianum* in South America and was thus considered to be suitably adapted to South African habitats (Olckers 2003). However, in the absence of pre-release thermal tolerance assessments and climate matching studies to determine suitable release sites, the weevils were released at sites with different climatic conditions (warmer coastal versus cooler inland) to gain some insight into the possible influence of climate. Although these data are preliminary, the differing outcomes at the coastal versus inland sites suggest that climate may play a role in the establishment of *A. santacruzi*.

### 4.5. Conclusion

Given the difficulties involved with the mass-rearing of *A. santacruzi*, signs of establishment and population proliferation (albeit at only one site) within the short time frame of this project are encouraging. While factors such as release effort (size and number of releases) are probably important, predation and competition may not play a major role in the establishment of *A. santacruzi*, while climatic conditions may be more important. Consequently, this study should be expanded to include: (i) more releases or redistribution of weevils at more sites in the KZN coastal region to increase the insect's distribution in the province and; (ii) a study of the thermal tolerances of *A. santacruzi*, including climatic matching, to confirm the suspicion that the agent may be constrained by climatic factors.

# CHAPTER 5: GENERAL DISCUSSION AND CONCLUSIONS

## 5.1 Overview

*Solanum mauritianum*, a major environmental weed in South Africa, New Zealand and several other tropical and sub-tropical regions worldwide (Olckers 1999, 2009, 2011, Olckers & Borea 2009), has been targeted for biological control since 1984, with South Africa the first country in the world to import, test and release biological control agents against this weed (Olckers 2011). Following the results of surveys on *S. mauritianum* in its native and introduced habitats (Olckers & Hulley 1989, 1991a, b), it was advocated that the control of *S. mauritianum* would depend on the establishment of biological control agents that were able to limit the weed's excessive levels of fruiting (Olckers 2003, 2009, 2011).

*Anthonomus santacruzi*, a flower-feeding weevil found throughout the native range of *S. mauritianum*, was viewed as the most promising agent capable of limiting the spread of this weed (Olckers 2003, 2009, 2011). This study investigated some aspects that could influence the release and establishment of the weevil namely: (i) an assessment of the host range of the newly imported culture to confirm that it is not different from the originally-tested culture; (ii) an assessment of the suitability of the weevil for release in New Zealand and confirmation of the weevil's safety for South Africa and New Zealand; (iii) an investigation of native arthropods that could interfere with its establishment and proliferation and; (iv) releases and monitoring of the weevil populations at selected release sites in KwaZulu-Natal. This chapter will provide a final discussion of the results of the preceding chapters and draw some conclusions on the potential of *A. santacruzi* for the biological control of *S. mauritianum* in South Africa and New Zealand**.**

### 5.2. Reassessment of the host range of *A. santacruzi*

Even though biological control is a powerful and cost-effective way of managing invasive alien plants (Carson *et al*. 2008), there are major concerns about attacks by introduced biological control agents on non-target plants, particularly those closely related to the target weed (e.g. Simberloff & Stiling 1996; Van Driesche *et al*. 2008). Thorough assessments of the agents' host range prior to their release in the new habitat are a safeguard against such unwanted negative impacts on non-target species (Pearson & Callaway 2003). Following approval for the release of *A. santacruzi* in South Africa in 2007, a primary aim was to reassess the host range of the newly introduced colony in order to confirm that is not different from that of the population originally tested. This study was expanded to include native and cultivated New Zealand *Solanum* species to assess the risks associated with the release of the weevil in New Zealand (Chapter 2).

Several different testing procedures were used in evaluating these non-target species and included: two sets of no-choice tests involving bouquets with floral material (flowers and flower buds) either present or absent; two sets of multi-choice tests in small cages involving bouquets with and without floral material; and two sets of multi-choice tests in large walk-in cages involving potted plants with and without floral material. The results of these trials did not deviate from the results of the original quarantine tests. As was previously experienced, the weevils are capable of surviving and producing progeny on a few non-target species during no-choice tests, but revert to *S. mauritianum* during the choice tests as observed in the small and walk-in cages. Overall, one may conclude that, at worst, two South African native plants may be at some risk, but the risks associated with New Zealand native and cultivated species are very low and *A. santacruzi* can be considered to be safe for release in New Zealand. However, regulators in New Zealand are considerably more risk averse than those in South Africa and may be reluctant to

accept ambiguous host-range data (L. Hayes, Landcare Research, personal communication). Future studies may be required to resolve this issue and could include: (i) open-field trials at field sites in South Africa to determine the insect's realized host range and; (ii) studies on the chemical ecology of *S. mauritianum* and related *Solanum* species to determine the specific phytochemical(s) that attract the weevils. It is expected that these would confirm the contention that none of the South African and New Zealand native *Solanum* species utilized during the tests are at risk.

### 5.3. Interference by native arthropods

Predation is the most common form of biotic interference that limits the impact of arthropod biological control agents (Goeden & Louda 1976, Reimer 1988, Muller & Goeden 1990, Muller *et al.* 1990, Ehler 1998, Snyder & Ives 2001, Pratt *et al.* 2003, Sebolt & Landis 2004). In particular, plants provide suitable habitats for predators as they use them for foraging, mating and shelter (Romero & Vascellos-Neto 2004, 2005a, b, c, Morais-Filho & Romero 2010). Consequently, even though biological control agents are largely freed from specialist predators and parasites, they are often vulnerable to generalist predators that inhabit release sites and disrupt their impact on the target weed (Hunt-Joshi *et al.* 2005).There are several examples where opportunistic predators have limited population increases or prevented the establishment of weed biocontrol agents (Goeden & Louda 1976, Crawley 1986) and determining whether biotic interference is likely to occur with a new agent can increase the predictability of biological control (Chacon *et al*. 2008).

The arthropods associated with *S. mauritianum* were assessed at four field sites that ranged from colder to warmer conditions (Chapter 3) and groups that could impact negatively on *A. santacruzi* were considered. In addition, the potential impact of inflorescence-inhabiting spiders on the persistence and proliferation of *A. santacruzi* populations was investigated in the laboratory. While there were no arthropods that were likely to compete significantly with *A. santacruzi* for resources, several predatory groups could exploit them as prey. These included spiders, predatory bugs (Miridae), ants, thrips and mites, although their incidence and abundance was considered to be too low to have any significant effect on the weevil's establishment. However, it is possible that numbers of predators (e.g. spiders) may increase in response to increases in the weevil's population density. Trials involving inflorescence-inhabiting spiders indicated that, although these may reduce weevil numbers and overall reproductive output, the weevils are capable of surviving and proliferating in their presence. These spiders are therefore unlikely to prevent the establishment of *A. santacruzi.*

### 5.4. Propagation, release and establishment of *A. santacruzi*

Field establishment of natural enemies, which is the ability to produce self-sustaining wild populations (Lodge 1993, Williamson 1996, Williamson & Fitter 1996), is a critical step in classical biological control (Shea & Possingham 2000). Factors that affect the establishment of biological control agents include the number of insects released and the number of releases made (Memmott *et al.* 1998, Grevstad 1999, Kolar & Lodge 2001), predation and interspecific competition, life history of the released agents (Kolar & Lodge 2001) and climate combined with environmental factors (Ulrichs & Hopper 2008). During this study (Chapter 4), 1500 adult weevils were released at four sites ranging from colder to warmer temperatures with

establishment confirmed at only site typified by warm conditions (Umkomaas on the KZN South Coast). Preliminary results suggest that while the size and frequency of releases may be important, competition and predation are not likely to hinder the establishment of *A. santacruzi*. However, climate may play a major role in the establishment of this weevil. Although releases in warmer areas are advocated for the time being, thermal physiology studies could be initiated to confirm the suspicion that *A. santacruzi* is intolerant of colder conditions. Using climate modelling programmes (e.g. Sutherst & Maywald 1985) to determine favourable release sites, future release efforts could include more intensive propagation of the weevils by implementing agencies (e.g. South African Sugarcane Research Institute) and/or redistribution of the weevils from established nursery sites to these sites.

### 5.5. Conclusions and recommendations

The results of this study have provided some insight into the potential of *A. santacruzi* as a biological control agent of *S. mauritianum* but have also raised a number of questions that could be pursued by an extension of this project or by independent studies. These aspects are summarized below.

Although the host-range tests suggested that the new colony of *A. santacruzi* was no different to the originally tested colony and the weevil is also safe for release in New Zealand, additional evidence of the insect's host specificity is required (L. Hayes, Landcare Research, personal communication). Besides open-field trials in South Africa that include the plant species deemed to be most at risk, namely *S.* c.f. *linnaeanum*, *S. aculeastrum*, *S. giganteum*, *S. aviculare* and *S. laciniatum*, a comparison of the chemical ecology of these plants may provide some

useful insight. In particular, identification of the compounds in *S. mauritianum* that attract *A. santacruzi* and an analysis of whether these are present or absent in the above non-target plants may well be able to resolve this uncertainty.

Given the difficulties in propagating *A. santacruzi* in the laboratory, confirmation of the weevil's establishment, albeit at only one site so far, is encouraging. However, releases need to be intensified to increase the number of established populations in the field and it is recommended that these be carried out in warmer areas to maximize the chances of success. Based on these results, the South African Sugarcane Research Institute have very recently intensified release efforts in the KZN coastal region and it is expected that these will be successful. Another recommendation is the demarcation of nursery sites in this region where the weevils can be collected for redistribution to new sites.

Finally, thermal tolerance studies should be initiated to clarify the suspicion that *A. santacruzi* may fail to establish in colder areas. These data can then be used in climate modelling programmes to determine the most suitable release sites, both in South Africa and in New Zealand.

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