DISTRIBUTION AND SEASONAL ABUNDANCE OF THE FLOWERBUD WEEVIL ANTHONOMUS SANTACRUZI HUSTACHE (COLEOPTERA: CURCULIONIDAE) IN KWAZULU-NATAL AND ITS IMPACT ON THE INVASIVE WEED SOLANUM MAURITIANUM SCOPOLI (SOLANACEAE)

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PREFACE

The research described in this dissertation was carried out in the School of Life Sciences (Pietermaritzburg campus) from February 2014 to February 2016 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, this has been duly acknowledged in the text.

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ABSTRACT

*Solanum mauritianum* Scopoli (Solanaceae), native to South America, is an invasive weed of tropical, subtropical and warm temperate regions in many countries including South Africa. The seed-packed fruits are highly palatable to native birds which feed on them throughout the year, vastly aiding in the weed’s dispersal. Research into the biological control of the weed began in the 1980s after chemical and mechanical control efforts proved insufficient and resulted in the release of *Gargaphia decoris* Drake (Hemiptera: Tingidae), a leaf-sucking lace bug, in 1999. *Anthonomus santacruzi* Hustache (Coleoptera: Curculionidae), a flowerbud weevil, was later released in 2008 to reduce the excessive levels of fruiting by *S. mauritianum* populations. Although several thousand weevils have recently been released in KwaZulu-Natal province, where infestations of *S. mauritianum* are particularly severe, to date there has been no post-release evaluation to determine the extent of the weevil’s establishment, seasonal abundance and impact on the weed’s reproductive output.

Twenty four sites with healthy populations of *S. mauritianum* were initially sampled in the KwaZulu-Natal Midlands and coastal regions from February to October 2014 to determine the presence and abundance of *A. santacruzi*. Populations of *A. santacruzi* were recovered at 14 sites, mainly along the coast, with poor establishment recorded in the inland region. A preliminary assessment of the role of climate in the weevil’s establishment suggested that low temperatures may be a constraint. Six sites (three inland and three coastal) with established populations of *A. santacruzi* were subsequently chosen for monitoring across seasons from October 2014 to September 2015. Although seasonally variable, the numbers of flowers and flowerbuds of *S. mauritianum* were high at all sites throughout the monitoring period, indicating no distinct periods of food scarcity. However, the numbers of weevils were relatively low in comparison resulting in low levels of floral damage (up to 26%) and no apparent impact on fruiting. Although higher weevil numbers were recorded at the coastal sites, there was a consistent trend of weevil numbers peaking during the autumn months (April/May), at all six sites. Despite the low population densities of *A. santacruzi*, there were indications of density-dependent relationships between food availability and weevil numbers. At the study sites (i.e. where *A. santacruzi* had established), climatic factors (e.g. monthly temperature) had no significant effect on the abundance of the weevils. Ants were frequently associated with *S. mauritianum* inflorescences at the study sites and displayed a significant positive relationship with the numbers of mature fruits, presumably because of their high sugar content. However, there was no relationship between weevil abundance and the
numbers of ants, suggesting that ants were not interfering with the weevil populations. A preliminary survey for parasitoids failed to provide any evidence that the weevil’s immature stages had recruited native parasitoids.

Only seven years has elapsed since *A. santacruzi* was first released in KwaZulu-Natal. Although the weevil’s establishment and population proliferation has been confirmed at several sites, its impact on *S. mauritianum* populations is currently negligible. Should higher population densities of *A. santacruzi* be realized over the medium to longer term, its impact could become significant. Further monitoring of *A. santacruzi* populations should thus be conducted in KwaZulu-Natal, but also in other provinces, to determine their potential for the biocontrol of *S. mauritianum*.

Keywords: Agent establishment, bugweed, flowerbud-feeding agents, resource availability, seasonal abundance, weed biological control
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1.1 Invasive alien plants in South Africa

Invasive alien plants are exotic plants that have reached levels of uncontrolled spread in a new country or environment. These exotic plants have been introduced purposefully as cultivated or ornamental species, or unintentionally through seeds and other propagative material that were introduced via human travel or imported cargo (Culliney, 2005; Moran et al., 2013). Although there are many quarantine measures at key entry points to prevent new introductions of exotic plants, several invasive species have been present in their new countries for long periods of time (Klein et al., 2011).

Invasive alien plants are a significant and growing threat to many terrestrial, but also aquatic, ecosystems throughout the world and also have negative impacts on a country’s economy (Van Wilgen et al., 2004; Culliney, 2005). Invasive plant species compete with native vegetation, disrupting natural ecosystem processes and impacting negatively on environments (Bright, 1998; McFadyen, 1998; Kaiser, 1999; Van Wilgen et al., 2004, 2011). Few invasive plant species are problematic in their country of origin, as their rapid growth rates and ability to produce high numbers of seeds are often controlled by native natural enemies with which they have co-evolved (Van Wilgen et al., 2004). Due to the absence of natural enemies in their new countries, these invasive alien species exhibit ‘ecological release’ and tend to out-compete native species which are under natural control (Van Wilgen et al., 2004, 2011).

Exotic plant species have been introduced into South Africa from around the 1600s and many of them are classified as invasive weeds today (Moran et al., 2011). There are nine distinct vegetation biomes in South Africa, namely Fynbos, Succulent Karoo, Desert, Nama-Karoo, Grassland, Savanna, Albany Thicket, Indian Ocean Coastal Belt and Forest, each with distinctive climatic and environmental conditions (Rutherford, 1997; Rutherford et al., 2006). This diversity of ecosystems opens up a wider opportunity for alien plant invasion. However, regions with higher rainfall (Schulze, 1997), urban development, and those supporting the cultivation of agricultural and silvicultural crops generally correspond to the areas which have the highest numbers of invasive alien plants, notably the southern, south-western and eastern coastal belts (Henderson, 2007). Areas in South Africa which become invaded by
invasive alien plants largely reflect the climate of the weeds’ country of origin (Henderson, 2006).

There have been many attempts to document all of the invasive alien plant species in South Africa. Originally, Wells et al. (1986) listed approximately 965 weedy plant species (some of which were native), although these were mostly herbaceous species. According to Henderson (2001) invasive weeds in South Africa are largely composed of trees and shrubs (155 species), but also other terrestrial plants that include grasses, reeds, herbs and climbers (73 species), and aquatic species (10 species). However, a total of 601 invasive plant species were later listed in the South African Plant Invaders Atlas (SAPIA) database for South Africa, Lesotho and Swaziland, as a result of surveys carried out during 1979 to 2000 (Henderson, 2007). These species were estimated to be around only half of the weed species present in South Africa. The SAPIA list (Henderson, 2007) included an additional 231 species but also more woody species than reported by Wells et al. (1986). Some 28 species included in the SAPIA list are invasive elsewhere in the world but have remained at low, relatively undamaging levels, but with the potential to become more damaging (Henderson, 2007). The exact number of invasive plant species in South Africa is, therefore, unknown and many additional species have probably remained undetected. Of the species listed by Henderson (2001) some 50% originated in the Americas, with 25% from Europe and Asia and 25% from Australia (Moran et al., 2011).

Of all invasive plant species, some of the most successful are angiosperms which produce fleshy fruit that are eaten by avian frugivores (Cronk and Fuller, 1995; Richardson et al., 2000; Renne et al., 2002; Buckley et al., 2006; Jordaan and Downs, 2012a) allowing their seeds to be dispersed over long distances. Because of their high fruit production relative to native plants, the fruits of alien plants are typically utilized by native bird species as a food source, giving these invaders their competitive edge (Richardson et al., 2000). Several invasive plants set fruit throughout the year, which is seldom the case with native plants; thus making alien plants a more attractive and predictable food source (Gosper, 2004; Gosper and Vivian-Smith, 2010). Several studies have indicated that the fruits of invasive species are small and multi-seeded and offer higher nutritional rewards for frugivores than native plants (Gosper and Vivian-Smith, 2010; Jordaan and Downs, 2012b). The benefits of dispersal by avian frugivores are that seeds can be dispersed over vast distances (Jordano, 1987; Schupp, 1993; Kinnaird, 1998) but also because germination is enhanced by chemical and mechanical
scarification processes in their digestive tracts (Evenari, 1949; McKey, 1975; Agami and Waisel, 1988; Barnea et al., 1990, 1991).

Invasive alien plants are also generally more abundant than their native counterparts as they display higher germination success, growth rates and reproductive output, particularly in disturbed sites (Corlett, 2005; Davis, 2011; Gleditsch and Carlo, 2011). Several theories have been proposed to explain the comparative success of invasive species. One of these is the enemy release hypothesis (ERH) which states that when a plant species is introduced into an exotic region its distribution and abundance increases more rapidly than would be expected in its native range due to the absence of its coevolved natural enemies (Keane and Crawley, 2002). A restoration of natural enemy pressure in the invaded country could thus ensure regulation of, and balance within, an invaded ecosystem and this is the fundamental principle underpinning classical biological control (Keane and Crawley, 2002).

1.2 Biological control of invasive plants in South Africa

There are three main approaches to control the spread and density of invasive plant species, namely chemical, mechanical and biological control (Culliney, 2005). Conventional control methods that involve applications of herbicides or mechanical clearing of alien plant populations, often in combination, are typically expensive and labour intensive as they require follow-up operations (Culliney, 2005; Witkowski and Garner, 2008). Biological control, which involves the release of natural enemies (mostly insects, but also pathogens) from the invasive plant’s native country, is generally considered to be more sustainable over the long term (McFadyen, 1998; Marais et al., 2004; Culliney, 2005). Extensive tests are first carried out to determine the host specificity of the biocontrol agent to ensure that there are no risks of non-target effects on economically important or native plants (McFadyen, 1998; Klein et al., 2011). Once agents have been released, it is imperative to conduct post-release evaluations to assess aspects like their establishment, distribution, seasonal abundance and impact on weed populations (McFadyen, 1998).

Post-release evaluations are important as they determine which biocontrol programmes are successful and which are not and also provide some understanding of the factors that have facilitated or hindered success (Morin et al., 1996; Carson et al., 2008). However, the determination of success or failure does not necessarily guarantee that the factors responsible
for this will be determined (MacFadyen, 1998). Many factors (e.g. climatic incompatibility and predation/parasitism of agents) could render a programme unsuccessful and in some cases the impact of the agent(s) varies between countries (i.e. extensive damage in some but only trivial damage in others) (Winston et al., 2014). Using experimental results, models have been developed to test the theoretical framework of biocontrol programmes in order to allow scientists to predict the impact of releasing new biocontrol agents (Hoffmann, 1990; Lonsdale et al., 1995), although this has proven to be difficult. Biocontrol initially involves deciding on whether the risk of releasing an agent outweighs the risk of leaving an alien invasive plant uncontrolled (Blossey, 1995; McClay, 1996). Using predictive models incorporating agent host-specificity data, scientists can determine whether non-target effects are likely to upset the balance of the indigenous environment (MacFadyen, 1998).

In successful biocontrol programmes, the damage inflicted on the target plant results in a decline in population densities, distribution and rates of spread (Zimmermann et al., 2004). Despite many successes, biocontrol is often a last resort in controlling weeds as chemical and mechanical methods often take precedence, being easier and quicker to implement in the short term (Olckers et al., 1998; Olckers, 2004). High-priority targets for biocontrol are often weed species that have already reached detrimental levels due to the failure of conventional methods to control them, although plants in the early stages of invasion may also be targeted (Olckers, 2004). A major factor that aids in the success of weed biocontrol is initiating the process when the plants are still in the early stages of invasion (Zimmermann and Neser, 1999; Olckers, 2004).

Biological control of invasive alien plants in South Africa dates back to 1913 and, to date, 106 biocontrol agents (invertebrates and pathogens) have been released against 48 invasive plant species making South Africa third in the world in biocontrol activity behind the United States and Australia (McFadyen, 1998; Van Wilgen et al., 2004; Moran et al., 2013). There has been much success in weed biocontrol in South Africa with 75 biocontrol agents becoming established (Moran et al., 2013). Of the 22 invasive alien woody tree species that have been targeted for biocontrol worldwide, 18 are in South Africa with 33 established biocontrol agents (Moran et al., 2004, 2013; Winston et al., 2014). Luckily in South Africa there are organisations that aid in the clearing and control of alien plant species, the most prominent of which is the Working for Water Programme which has also facilitated progress in weed biocontrol by funding several programmes (Olckers, 2004; Moran et al., 2011). The Working for Water Programme was initiated in 1995 as a social development project that
planned to diminish the threat of invasive alien plants to South Africa’s water resources and biodiversity, through increased funding and employment opportunities (Moran et al., 2011). It focuses on reducing the distribution and densities of several prominent alien invasive species through mechanical, chemical and biological control (Moran et al., 2011). Since clearing methods are labour intensive, the programme has created many employment opportunities for unemployed South Africans over the years (Moran et al., 2011).

Despite the many successes reported in South Africa (Moran et al., 2013), not all biocontrol programmes have been successful. There are several reasons for the inability of imported agents to control their target weeds and include: incompatibility with South Africa’s climate, incompatibility with the variety of the weed, disruption by native predators and parasitoids, and lack of synchronization with the weeds’ phenology (McFadyen, 1998; Culliney, 2005). Post-release evaluations have thus become important in quantifying the impact of biocontrol agents and elucidating the factors that are responsible for their success or failure (McFadyen, 1998; Culliney, 2005). Several plants have proved to be difficult targets for biocontrol in South Africa and one of these, *Solanum mauritianum* Scopoli (Solanaceae), a woody tree with a high reproductive output, is the subject of this study.

1.3 *Solanum mauritianum*

*Solanum mauritianum* Scopoli (Solanaceae), is a woody tree, commonly known as bugweed or woolly nightshade, which is indigenous to South America and has become naturalised in Africa as well as Australasia, India and some islands in the Indian, Atlantic and Pacific oceans (Roe, 1972; Moran et al., 2004; Olckers, 2011). Besides being widespread in South Africa (Figure 1.1), the weed has invaded other southern African countries like Mozambique, Swaziland and Zimbabwe, and has also been recorded in western, central and eastern Africa (Olckers, 2003). The spread of *S. mauritianum* outside its native range has been attributed to the Portuguese trade routes in the 16th century and the plant was first noted in South Africa (KwaZulu-Natal) in 1862 and later proclaimed a noxious weed in 1937 (Wright, 1904; Harding, 1938; Roe, 1972; Olckers and Zimmermann, 1991). Bugweed has become a weed of areas within the eastern higher rainfall regions of South Africa including riverine habitats, forestry plantations, conservation areas and agricultural lands (Olckers and Zimmermann, 1991; Olckers, 1998). Within the summer rainfall region of South Africa, *S. mauritianum* is
regarded as one of the top five invasive alien plant species while other African countries also consider it to be one of their worst weeds (Witkowski and Garner, 2008).

Figure 1.1 Distribution of *Solanum mauritianum* across South Africa (from the SAPIA database, 2010; see Olckers, 2011).

The plant is able to grow in numerous habitats, particularly disturbed sites, and can germinate under a wide range of temperatures with seeds persisting in the soil until conditions are favourable (Denslow, 1980; Brokaw, 1985; Swaine and Whitmore, 1988; Barboza et al., 2009). In three of the seven biomes of South Africa, namely Savanna, Forest and Grassland, *S. mauritianum* is considered to be one of the 10 most prominent invasive weed species (Henderson, 2007). Within 2-3 years the plant can reach heights of several metres (Olckers, 2009). The plant has dense foliage with large leaves that are covered in fine, whitish trichomes and inflorescences with lilac-blue flowers which produce green fruit that turn yellow when ripe (Kissmann and Groth, 1997; Henderson, 2001; PIER, 2005; ISSG, 2006; Olckers, 2009). The inflorescences bear flowers and fruit (Figure 1.2) throughout the year.
and plants can produce inflorescences within their first year after germination (Olckers, 2009). Trees often bear inflorescences with flowerbuds, open flowers, immature fruits and mature fruits at the same time (Olckers, 2011; Figure 1.2). The foliage is generally unpalatable to browsing animals and the fruit, especially when immature, are rich in alkaloids and thus toxic to animals and humans (ISSG, 2006; Olckers, 2009).

![Inflorescences of Solanum mauritianum with different floral stages.](image)

Figure 1.2 Inflorescences of *Solanum mauritianum* with different floral stages.

Although bugweed has a generalised pollination system that includes pollination by honeybees, the plant is able to self-pollinate (Rambuda and Johnson, 2004), producing fruits that each contain up to 150 seeds which have a 98% chance of germinating (Campbell and Van Staden, 1983; Olckers, 2011). This ability to self-pollinate has enabled bugweed to colonize new habitats very rapidly as single plants, arising from long-distance seed dispersal, are able to establish populations; this phenomenon (known as “Baker’s Rule”) has been associated with many successful invasive plant species (Baker, 1955, 1967, 1974). Fruits are available year round with individual plants producing 20-80, sometimes more, berries per
inflorescence (Campbell and Van Staden, 1983; Henderson, 2001; Witkowski and Garner, 2008). Plants become reproductive when they reach a height of 1.5 m and trees taller than 3 m are capable of producing between 100 000 to 200 000 seeds per annum (Witkowski and Garner, 2008). However, *S. mauritianum* is also able to reproduce vegetatively through suckering and coppicing (Olckers, 2011). The plant’s very high fruit set in South Africa promotes utilization of the ripe fruit by several frugivorous bird species which disperse the seeds and promote the weed’s spread (Olckers and Hulley, 1989; Olckers, 1996; Henderson, 2007; Olckers, 2009; Jordaan and Downs, 2012a). Surveys on *S. mauritianum* carried out in South America showed high levels of herbivory by foliar and floral herbivores resulting in low levels of fruit set (Olckers, 2003, 2011). In contrast, surveys on bugweed in South Africa showed low levels of herbivory resulting from a poor herbivore community, resulting in high levels of fruiting (Olckers and Hulley, 1989, 1991a, b). These differences in herbivory between the native and introduced range of the plant are consistent with the enemy release hypothesis (Keane and Crawley, 2002).

Several additional factors, besides its environmental impacts, have contributed to the pest status of *S. mauritianum*. During clearing operations, trichomes on the leaves and stems also pose a health risk to labourers who clear the plant, as they become dislodged and cause respiratory difficulties when inhaled (Olckers, 2011). As the weed invades agricultural land (Olckers and Zimmermann, 1991; Olckers, 1998) it exploits nutrients that the crops need which could affect crop yields if the weed populations are high enough. Fruit flies which are agricultural pests of many commercially grown fruit (e.g. plums and peaches) also utilize the fruit of *S. mauritianum*, particularly during months when the former are unavailable, thus facilitating their persistence throughout the year (Olckers and Zimmermann, 1991; Copeland and Wharton, 2006).

The extent of *S. mauritianum* invasions in South Africa is vast and although chemical control is effective, the high costs are aggravated by cleared areas becoming rapidly reinvaded by seedlings arising from bird-dispersed seeds (Olckers, 2011). Since bugweed is able to propagate vegetatively, mechanical clearing is mostly ineffective since plants will coppice from root sections and cut stumps if not treated chemically (Olckers and Zimmermann, 1991; Olckers, 2009). Since 1995, infestations of *S. mauritianum* have routinely been cleared along watercourses by operatives of the Working for Water Programme (Witkowski and Garner, 2008). These operations have involved the cutting down of trees followed by applications of herbicide to the cut stumps (Witkowski and Garner, 2008). However, in some cases these
interventions caused an increase in *S. mauritianum* densities (Van Wilgen et al., 1998) due to coppice growth from stumps that were incorrectly treated and seedling recruitment in the cleared areas (Witkowski and Garner, 2008; Olckers, 2011). Consequently, biological control has been proposed as a more sustainable means of managing *S. mauritianum* infestations over the long term (Olckers, 1998, 2011).

1.4 Biological control of *S. mauritianum*

Although there are several invasive species of *Solanum* worldwide, South Africa was the first country to implement biocontrol on weeds in this genus (Olckers, 1998; Winston et al., 2014). Weeds in the genus *Solanum* are difficult targets for biocontrol because agronomic and native species in the same genus often support non-target feeding during the host-specificity testing of candidate agents, making it hard to justify their release (Cullen, 1990; Olckers, 1998, 2011). In South Africa there are at least 30 native *Solanum* species, three cultivated species that include *S. tuberosum* L. (potato), *S. melongena* L. (eggplant, aubergine) and *S. lycopersicum* L. (tomato), and several other exotic species that are grown as ornamentals (Olckers and Zimmermann, 1991; Olckers, 1996).

South Africa became the first country to introduce natural enemies for the biocontrol of *S. mauritianum* in 1984 (Olckers and Zimmermann, 1991). However, progress was slow with most candidate agents rejected because of attacks on native and cultivated non-target plants during quarantine tests (Olckers, 1999, 2011 and references therein). However, the later release of two agents on *Solanum elaeagnifolium* Cav. (Olckers et al., 1995) and one on *S. sisymbriifolium* Lam. (Hill and Hulley, 1995), despite feeding on non-target *Solanum* species, including native species and cultivated eggplant, in quarantine (Olckers et al., 1995), advanced progress on *S. mauritianum*. These agents were released as the problems caused by the existing infestations of *S. elaeagnifolium* and *S. sisymbriifolium* were deemed to outweigh the minor risks posed to eggplant and other native *Solanum* species. In particular, there were stronger preferences by the agents for the target plants but also other mitigating factors, including no evidence of attacks on non-target species (i.e. crops) by the agents in their countries of origin (Hill and Hulley, 1995; Olckers et al., 1995). Success in resolving such ambiguous host-specificity test results have set a precedent for the interpretation of test results involving *Solanum*-feeding agents in South Africa (Olckers et al., 1995; Olckers, 1998). This sparked a renewed interest in finding agents for *S. mauritianum* and several
potential agents were imported during the 1990s (Olckers, 2011). Leaf-feeding insects with high reproductive rates were initially considered due to their ability to cause extensive defoliation and indirectly decrease plant reproduction (Solbring, 1981; Hartley and Jones, 1997; Barboza et al., 2009). However, several otherwise promising potential agents were rejected due to a lack of host specificity during quarantine testing (e.g. Olckers, 1998, 2000a, 2011).

Despite these setbacks, *Gargaphia decoris* Drake (Hemiptera: Tingidae), a leaf-sucking lace bug that was introduced from Argentina in 1995, was released in South Africa in 1999 as the first biocontrol agent of *S. mauritianum* anywhere in the world (Olckers, 2000b; Olckers et al., 2002; Olckers, 2011). These releases were later augmented in 2002 with new stocks collected from a colder region in south-western Brazil to improve establishment success in areas where colder temperatures may have constrained the original stocks (Olckers and Borea, 2009; Hope and Olckers, 2011). The lace bug has since become widely established in several provinces in South Africa (Olckers, 2011). At high densities *G. decoris* has caused extensive damage to *S. mauritianum* populations, leading to premature senescence, reduced flowering and fruiting and even death of individual plants (Olckers, 2000b; Witt, 2007; Olckers and Borea, 2009). However, outbreaks of *G. decoris* have been sporadic and populations are believed to be constrained by predation of the immature stages (Olckers, 2011; Patrick and Olckers, 2014). The lace bug also appears to persist better in shaded areas but it is unclear as to whether this is due to plant quality, predation, or other factors (Patrick and Olckers, 2014). Based on additional studies in South Africa (Olckers and Borea, 2009) *G. decoris* was released in New Zealand in 2010 and has become established, although its impact is currently not known (Olckers, 2011; Winston et al., 2014).

Given the limited impact of *G. decoris*, attention was focused on agents that could directly reduce the high fruit set of *S. mauritianum* (Olckers, 2003, 2008; Barboza et al., 2009). Flowerbud-feeding weevils in the genus *Anthonomus* (Coleoptera: Curculionidae) were prioritised as it was believed that they were largely responsible for the low fruit set that is typical of *S. mauritianum* populations in their native range (Barboza et al., 2009; Olckers, 2011). One of these, *Anthonomus santacruzi* Hustache (Coleoptera: Curculionidae), was later released in South Africa and is the subject of this study.
1.5 *Anthonomus santacruzi*

The adult weevils are black and about 2 to 3 mm in length (Figure 1.3). They have striated elytra and conspicuous patches of white scales that appear as thin vertical lines on the second to sixth interstrial regions (Clark and Burke, 1996; Olckers, 2003). The eggs are round and clear, are not easily visible to the naked eye and are inserted into the tissues of immature and mature flowerbuds by ovipositing females (Olckers, 2003). The developing larvae, which are white with brown head capsules, consume the floral contents. The closed petals and sepals conceal the feeding larvae which eventually pupate within a frass-lined chamber within the bud. Usually, a single larva is recovered from an infested flowerbud, but two larvae are occasionally found within a single flowerbud. The females display a short pre-oviposition period of approximately four days but will continue to lay eggs for 43 to 114 days (Olckers, 2003). Larval development is relatively quick, with developmental time from oviposition to pupation ranging from 10 to 18 days and the time from pupation to adult emergence ranging from 15 to 25 days (Olckers, 2003). The adults feed on the reproductive tissues of open flowers of *S. mauritianum* but will also feed on young leaves/shoot tips (Figure 1.4) when the availability of floral material is low (Olckers, 2003).

![Figure 1.3 a) Lateral and b) dorsal views of *Anthonomus santacruzi* adults.](image-url)
Although earlier importations of *A. santacruzi* failed to produce healthy laboratory cultures, substantial collections in north-eastern Argentina and south-eastern Paraguay during early 1998 enabled culturing and host-specificity testing to proceed (Olckers et al., 2002). Host-specificity tests were carried out over a period of four years and the agent was proposed for release in 2002 (Olckers, 2003). In the interim, the South African cotton industry had voiced concerns over possible risks posed by *A. santacruzi* to cotton production because of its relatedness to a major pest, the cotton boll weevil *Anthonomus grandis* Boheman (Coleoptera: Curculionidae) (Olckers, 2008). After additional host-specificity tests, that included cultivars of cotton (Olckers, 2008), *A. santacruzi* was deemed safe for release in South Africa. Although the weevil was eventually approved for release in 2007, the original quarantine culture had died out in the interim. Consequently, during 2008-2009, fresh stocks of *A. santacruzi* were imported from the same region in north-eastern Argentina where the original stocks were collected (Olckers, 2011). Additional studies confirmed that the host range of these stocks did not differ from that of the original stocks (Hakizimana and Olckers, 2013a) and the new stocks were considered suitable for release.

Releases of *A. santacruzi* commenced in late 2008 and involved releases of small founder populations at a few sites in KwaZulu-Natal which resulted in establishment (Olckers, 2011). However, since 2011 larger-scale releases have been carried out by the South African Sugarcane Research Institute (SASRI) which, due to its considerable facilities and expertise in the mass-rearing of insects, has been contracted by the Working for Water Programme to
mass-rear and release weed biocontrol agents (Olckers, 2011). Efforts by SASRI have resulted in the release of several thousand weevils at many additional sites in the province, as well as in other provinces. Reports of establishment at several additional sites in KwaZulu-Natal, together with indications that the weevils were spreading (D. Gillespie, pers. comm.) necessitated the initiation of post-release monitoring. Preliminary laboratory and field studies suggested that populations of A. santacruzi were unlikely to be compromised by generalist predators, especially ants and spiders, which are often associated with S. mauritianum inflorescences in the field (Hakizimana and Olckers, 2013b, c). In contrast, climate seemed to play a role as the weevil appeared to be proliferating in the coastal areas of KwaZulu-Natal, but not in the colder inland areas (T. Olckers, pers. comm.).

1.6 Aims of the study

Since the time between the first releases of the weevil and the start of this study was only five years, it is still too early to quantify the impact of A. santacruzi on S. mauritianum populations. However, there were several aspects that needed to be considered as part of a preliminary post-release evaluation and these comprised the aims of the study, as follows:

1. Consolidation of the information on where releases of A. santacruzi have taken place, in relation to where populations have become established, in KwaZulu-Natal. This involved the mapping of release sites and localities where populations have been confirmed. Release site information was based on the records of SASRI and the Working for Water Programme’s weed biocontrol implementation strategy while establishment information was based on the results of field surveys undertaken during this study.

2. Field surveys to select the best sites in the coastal and inland regions of KwaZulu-Natal for seasonal monitoring (see below). These preliminary surveys provided an opportunity to determine whether establishment success was related to climatic variables.

3. Monthly monitoring at selected coastal and inland sites to determine the abundance of the different life stages of A. santacruzi in relation to the phenology of S. mauritianum populations (i.e. floral resource availability).

4. Confirmation of whether the weevils’ abundance was affected by the presence of ants and whether the immature stages of A. santacruzi had acquired any native parasitoids.
CHAPTER 2: Releases and establishment of *Anthonomus santacruzi* in KwaZulu-Natal.

2.1 Introduction

*Solanum mauritianum* Scop. (Solanaceae) commonly known as bugweed, is a woody tree species indigenous to South America that has become invasive in several countries including South Africa (Olckers, 2011). In South Africa it is a major weed of agricultural lands, plantations, conservation areas and the banks of watercourses (Henderson, 2001; ISSG, 2006; Witkowski and Garner, 2008). Biological control research first began in the 1980s as mechanical and chemical control proved ineffective in controlling the spread of the weed over the long term (Olckers and Zimmermann, 1991; Olckers, 2009). Cleared areas also become reinvaded due to seed dispersal by birds and extensive soil seed banks (Olckers, 2011). The seed-packed fruits are highly palatable to native birds and due to their year round production (Campbell and Van Staden, 1983; Henderson, 2001; Witkowski and Garner, 2008) they provide a reliable food source (Gosper, 2004; Gosper and Vivian-Smith, 2010). Avian frugivory thus results in seed dispersal over great distances, thereby facilitating the invasiveness of this weed as it invades all but the drier regions of South Africa (Henderson, 2007; Jordaan and Downs, 2012a).

Many candidate biocontrol agents were introduced into quarantine in South Africa and eventually, after 11 years of host-range testing, the first suitable agent for *S. mauritianum* was found (Olckers, 2000b; 2011). The leaf-sucking lace bug, *Gargaphia decoris* Drake (Hemiptera: Tingidae), which was collected in Argentina in 1995, was first released in South Africa in 1999 (Olckers, 2000b; Olckers et al., 2002; Olckers, 2011). The lace bug has become widely established in several provinces in South Africa (Olckers, 2011), but with limited impact on the weed, and was observed on many occasions during this study. *Anthonomus santacruzi* Hustache (Coleoptera: Curculionidae), a flower-feeding weevil, is the most recent natural enemy to be released against bugweed, following its importation from Argentina in 1998 (Olckers et al., 2002). The low levels of fruit set of bugweed plants which are typical in its native range were attributed to *A. santacruzi*, thus making it a potentially useful agent (Olckers, 2011). At high densities the adult weevils feed on the open flowers including the reproductive material, but also the shoot tips of leaves during times when flowers are scarce (Olckers, 2003). The females oviposit in the flowerbuds and the larvae remain inside the flowerbuds for the duration of their development to adulthood, where they
feed on the reproductive material (Olckers, 2003, 2011). The feeding larvae destroy the floral parts within the flowerbuds, thereby causing their abscission and preventing their development into fruit (Olckers, 2003, 2011). The weevil was approved for release in South Africa in 2007 and the first releases commenced in KwaZulu-Natal in late 2008 (Olckers, 2011). These releases, which involved relatively low numbers of weevils, were carried out by staff of the University of KwaZulu-Natal (UKZN) and continued until 2012. The South African Sugarcane Research Institute (SASRI) has since 2011 released the weevil in much larger numbers at additional sites in KwaZulu-Natal and in other provinces, including the Western Cape, Eastern Cape, Gauteng, Mpumalanga, and Limpopo.

Given that the agent has only recently been released, the establishment success and distribution of *A. santacruzi* in KwaZulu-Natal has not been determined. Therefore, the aims of this study were to consolidate the details of all releases undertaken in the province and gain some insight into where the weevil has established in relation to where it was released. Ultimately, this study was intended as a precursor to the study described in Chapter 3 and was aimed at identifying sites, where the weevil had established, that would be appropriate for longer term monitoring. Quantitative surveys were thus conducted at several sites over several months to acquire a general idea of the weevil’s abundance. Since climate can affect the success of agent establishment and proliferation (e.g. McFadyen, 1998), climatic data for these sites were also acquired to determine whether there were any trends relating to the weevil’s abundance across KwaZulu-Natal. However, since the influence of climate was not tested explicitly, any trends relating to climate should be regarded as tentative.

2.2 Methods

2.2.1 Release records

Data on *A. santacruzi* releases in KwaZulu-Natal that were originally carried out by UKZN staff were updated with additional release data that were obtained from other weed biocontrol implementing agencies, notably SASRI (D. Gillespie, pers. comm.) and the Working for Water Programme (K.J. Hope, pers. comm.).
2.2.2 Study sites

Between February and October 2014, inflorescences of *S. mauritianum* were sampled on a single occasion at 24 sites in KwaZulu-Natal where bugweed populations were present, in order to determine whether *A. santacruzi* was present or not and gain some insight into its abundance (Table 2.1). The limitations of these surveys (single samples at different times of the year) need to be highlighted in the context of the preliminary nature of this study. These surveys included sites where weevils had previously been released or sites that were in relatively close proximity to release sites (see Table 2.2). The surveyed sites supported populations of *S. mauritianum* that comprised at least 10 large trees that contained inflorescences with floral material. Site locations ranged from the warmer coastal region (13 sites) to the cooler, higher altitude midlands region (11 sites). To facilitate a preliminary assessment of the influence of climate, climatic data for each of these sites were provided by the Department of Natural Resources, KZN Department of Agriculture & Rural Development (F. Mitchell, pers. comm.). These data included long-term records (updated every few years) of mean annual temperature, rainfall and humidity that were derived from the Bioresource Groups of KwaZulu-Natal database (Camp, 1997).
Table 2.1 Sites in KwaZulu-Natal where surveys for the presence and abundance of *Anthonomus santacruzi* were carried out.

<table>
<thead>
<tr>
<th>Site</th>
<th>Coordinates</th>
<th>Region</th>
<th>Site code</th>
<th>Date visited</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pietermaritzburg: Chase Valley (CV)²</td>
<td>S29°33'20&quot; E30°21'21&quot;</td>
<td>Inland</td>
<td>Zb2</td>
<td>18 Feb 2014</td>
</tr>
<tr>
<td>Pennington (Pen)</td>
<td>S30°23'59&quot; E30°39'11&quot;</td>
<td>Coastal</td>
<td>Ya12</td>
<td>28 Mar 2014</td>
</tr>
<tr>
<td>Umkomaas: Empisini (Emp)²</td>
<td>S30°12'07&quot; E30°47'06&quot;</td>
<td>Coastal</td>
<td>Ya12</td>
<td>28 Mar 2014</td>
</tr>
<tr>
<td>Park Rynie (PR)</td>
<td>S30°17'50&quot; E30°44'43&quot;</td>
<td>Coastal</td>
<td>Ya12</td>
<td>28 Mar 2014</td>
</tr>
<tr>
<td>Ifafa Beach (Ifa)</td>
<td>S30°27'26&quot; E30°38'07&quot;</td>
<td>Coastal</td>
<td>Ya12</td>
<td>28 Mar 2014</td>
</tr>
<tr>
<td>Hilton (Hilt)²</td>
<td>S29°32'36&quot; E30°17'59&quot;</td>
<td>Inland</td>
<td>Zc7</td>
<td>20 May 2014</td>
</tr>
<tr>
<td>Winterskloof (Win)</td>
<td>S29°34'38&quot; E30°17'15&quot;</td>
<td>Inland</td>
<td>Zc7</td>
<td>20 May 2014</td>
</tr>
<tr>
<td>Pietermaritzburg: Mkondeni (Mko)²</td>
<td>S29°38'30&quot; E30°24'34&quot;</td>
<td>Inland</td>
<td>Tub10</td>
<td>20 May 2014</td>
</tr>
<tr>
<td>Botha’s Hill (BH)</td>
<td>S29°45'55&quot; E30°42'10&quot;</td>
<td>Coastal</td>
<td>Wb16</td>
<td>18 Jun 2014</td>
</tr>
<tr>
<td>Assegay (Ass)</td>
<td>S29°47'30&quot; E30°44'18&quot;</td>
<td>Coastal</td>
<td>Wb16</td>
<td>18 Jun 2014</td>
</tr>
<tr>
<td>Hillcrest (Hil)</td>
<td>S29°47'50&quot; E30°46'31&quot;</td>
<td>Coastal</td>
<td>Yb15</td>
<td>18 Jun 2014</td>
</tr>
<tr>
<td>Ballito (Bal)</td>
<td>S29°31'20&quot; E31°13'19&quot;</td>
<td>Coastal</td>
<td>Ya14</td>
<td>25 Jul 2014</td>
</tr>
<tr>
<td>Verulam (Ver)</td>
<td>S29°40'47&quot; E31°02'07&quot;</td>
<td>Coastal</td>
<td>Ya14</td>
<td>25 Jul 2014</td>
</tr>
<tr>
<td>Tongaat (Ton)</td>
<td>S29°35'17&quot; E31°09'09&quot;</td>
<td>Coastal</td>
<td>Ya14</td>
<td>25 Jul 2014</td>
</tr>
<tr>
<td>Umlaas Road: Tala (Tal)</td>
<td>S29°46'43&quot; E30°30'24&quot;</td>
<td>Inland</td>
<td>Tub11</td>
<td>15 Aug 2014</td>
</tr>
<tr>
<td>Richmond (Ric)</td>
<td>S29°52'45&quot; E30°15'02&quot;</td>
<td>Inland</td>
<td>Yb7</td>
<td>15 Aug 2014</td>
</tr>
<tr>
<td>Hella Hella Drive (HHD)</td>
<td>S29°53'44&quot; E30°09'51&quot;</td>
<td>Inland</td>
<td>Yb14</td>
<td>15 Aug 2014</td>
</tr>
<tr>
<td>Thornville (Tho)</td>
<td>S29°44'09&quot; E30°23'01&quot;</td>
<td>Inland</td>
<td>Vb15</td>
<td>15 Aug 2014</td>
</tr>
<tr>
<td>Stanger (Sta)</td>
<td>S29°20'33&quot; E31°18'18&quot;</td>
<td>Coastal</td>
<td>Ya14</td>
<td>12 Sep 2014</td>
</tr>
<tr>
<td>Mtunzini (Mtu)</td>
<td>S28°56'50&quot; E31°43'58&quot;</td>
<td>Coastal</td>
<td>Za3</td>
<td>12 Sep 2014</td>
</tr>
<tr>
<td>Gingindlovu (Gin)</td>
<td>S29°01'56&quot; E31°35'20&quot;</td>
<td>Coastal</td>
<td>Za3</td>
<td>12 Sep 2014</td>
</tr>
<tr>
<td>New Hanover (NH)</td>
<td>S29°20'34&quot; E30°31'21&quot;</td>
<td>Inland</td>
<td>Wb11</td>
<td>02 Oct 2014</td>
</tr>
<tr>
<td>Harburg (Har)</td>
<td>S29°24'44&quot; E30°44'14&quot;</td>
<td>Inland</td>
<td>Yc21</td>
<td>02 Oct 2014</td>
</tr>
<tr>
<td>Greytown (Gre)</td>
<td>S29°03'36&quot; E30°35'09&quot;</td>
<td>Inland</td>
<td>Wc35</td>
<td>02 Oct 2014</td>
</tr>
</tbody>
</table>

¹ Site codes refer to specific Bioresource Units which are embodied within the different Bioresource Groups (Camp, 1997) and for which long-term climatic data are available.
² Initial *A. santacruzi* release sites.

2.2.3 Sampling procedure

At each site, 10 trees that contained floral material were randomly selected within the population. One inflorescence that typically contained fruit, open flowers and flowerbuds was collected from each tree and placed in a Ziploc™ bag. The samples were frozen to keep the material fresh and prevent the weevils from laying eggs, so as to avoid overestimating the population densities. During the processing of each sample, the number of adult weevils was recorded and the material was separated into the different floral stages, namely mature fruits, immature fruits, flowers, mature flowerbuds, and immature flowerbuds and the numbers of
each stage were recorded. The mature and immature flowerbuds were then dissected under a dissecting microscope to record the numbers of eggs, larvae and pupae of *A. santacruzi*.

### 2.2.4 Statistical analysis

Figures were produced using Microsoft Excel 2010 and statistical analyses were conducted using IBM SPSS Statistics version 23.0. Despite the obvious flaw in the sampling procedure (see 2.2.2), the relationships between the mean numbers of *A. santacruzi* (adults and immature stages combined) per inflorescence at each site and climatic features (namely the mean annual rainfall (mm), mean annual temperature (°C), and mean annual humidity (%) for each site) were determined by correlation analysis, for descriptive purposes. Since the data did not meet the assumptions of normality, the strength of the relationships was tested using Spearman’s rank correlation. A map of KwaZulu-Natal that indicated the presence and absence of *A. santacruzi* in relation to mean annual temperature was created using ArcGIS. This map incorporated a statistical geometric interval that separated the temperature data into ranges that corresponded with their statistical distribution.

### 2.3 Results

#### 2.3.1 Releases of *A. santacruzi*

Between 2008 and 2014, releases of *A. santacruzi* were carried out at 66 sites (Figure 2.1) that fell within 23 quarter degree squares in KwaZulu-Natal (Table 2.2). In total, some 104 releases resulted in the release of more than 28 000 weevils in the coastal and midlands regions of the province. Of these releases, some 59% were undertaken at 29 coastal sites with the remaining 41% at 17 inland sites (Table 2.2). A substantial number of releases were carried out around Durban and along the KwaZulu-Natal South Coast, with fewer releases north of Durban (Figure 2.1).
Table 2.2 *Anthonomus santacruzi* release records per quarter degree square (QDS) in KwaZulu-Natal between 2008 and 2014.

<table>
<thead>
<tr>
<th>Sites1</th>
<th>QDS</th>
<th>Region</th>
<th>Month of release (first – last)</th>
<th>Number of releases</th>
<th>Numbers released</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pietermaritzburg area (Mkondeni/ Chase Valley/ Montrose/ Hilton)2</td>
<td>2930CB</td>
<td>Inland</td>
<td>Dec 2008 – Jun 2013</td>
<td>15</td>
<td>1595</td>
</tr>
<tr>
<td>Umkomaas (Empisini)2</td>
<td>3030BB</td>
<td>Coastal</td>
<td>Jun – Sep 2009</td>
<td>3</td>
<td>302</td>
</tr>
<tr>
<td>Hillcrest2/ Pinetown/ Durban/ Kloof</td>
<td>2930DD</td>
<td>Coastal</td>
<td>Jun 2010 – May 2014</td>
<td>21</td>
<td>4484</td>
</tr>
<tr>
<td>Leisure Bay</td>
<td>3130AA</td>
<td>Coastal</td>
<td>May 2011 – Jun 2012</td>
<td>3</td>
<td>500</td>
</tr>
<tr>
<td>Tongaat/ Mount Moreland/ Phoenix</td>
<td>2931CA</td>
<td>Coastal</td>
<td>Jul 2011 – Jun 2014</td>
<td>9</td>
<td>3220</td>
</tr>
<tr>
<td>Umtentweni</td>
<td>3030CB</td>
<td>Coastal</td>
<td>Aug 2011</td>
<td>3</td>
<td>500</td>
</tr>
<tr>
<td>New Hanover</td>
<td>2930AD</td>
<td>Inland</td>
<td>Aug 2011</td>
<td>1</td>
<td>190</td>
</tr>
<tr>
<td>Southbroom/ Ramsgate/ Margate/ Port Shepstone/ Marina Beach</td>
<td>3030CD</td>
<td>Coastal</td>
<td>Aug 2011 – Nov 2012</td>
<td>13</td>
<td>3000</td>
</tr>
<tr>
<td>Richmond/ Tala Valley</td>
<td>2930CD</td>
<td>Inland</td>
<td>Sep 2011 – Jun 2013</td>
<td>2</td>
<td>1100</td>
</tr>
<tr>
<td>Hibberdene (Glen Echo)/ Sezela</td>
<td>3030AD</td>
<td>Coastal</td>
<td>Sep 2011 – Jun 2013</td>
<td>2</td>
<td>923</td>
</tr>
<tr>
<td>Eston/Shongweni/ Assagay</td>
<td>2930DC</td>
<td>Inland</td>
<td>Oct 2011 – Apr 2014</td>
<td>7</td>
<td>2286</td>
</tr>
<tr>
<td>Harburg</td>
<td>2930BC</td>
<td>Inland</td>
<td>Nov 2011 – May 2012</td>
<td>4</td>
<td>650</td>
</tr>
<tr>
<td>Scottburgh (Mpambanyoni River)</td>
<td>3030BC</td>
<td>Coastal</td>
<td>Jul 2012</td>
<td>1</td>
<td>500</td>
</tr>
<tr>
<td>Umzumbe (Ntshambili River)</td>
<td>3030DA</td>
<td>Coastal</td>
<td>Jul 2012</td>
<td>2</td>
<td>500</td>
</tr>
<tr>
<td>Melmoth (Mtonjaneni area)</td>
<td>2831CB</td>
<td>Inland</td>
<td>Jul – Sep 2012</td>
<td>3</td>
<td>1300</td>
</tr>
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<td>Mtubatuba</td>
<td>2832AC</td>
<td>Coastal</td>
<td>Aug 2012 – Jun 2013</td>
<td>4</td>
<td>1200</td>
</tr>
<tr>
<td>Botha’s Hill</td>
<td>2930DA</td>
<td>Coastal</td>
<td>Sep 2012 – Jun 2013</td>
<td>2</td>
<td>690</td>
</tr>
<tr>
<td>Cramond</td>
<td>2930AB</td>
<td>Inland</td>
<td>Jun 2013</td>
<td>1</td>
<td>2250</td>
</tr>
<tr>
<td>Howick (Nr Midmar Dam)</td>
<td>2930AC</td>
<td>Inland</td>
<td>Jul 2013</td>
<td>1</td>
<td>250</td>
</tr>
<tr>
<td>Greytown</td>
<td>2930BA</td>
<td>Inland</td>
<td>Nov 2013</td>
<td>1</td>
<td>254</td>
</tr>
<tr>
<td>Umhlali</td>
<td>2931AC</td>
<td>Coastal</td>
<td>Jan 2014</td>
<td>1</td>
<td>900</td>
</tr>
<tr>
<td>Newcastle (Normandien)</td>
<td>2729DC</td>
<td>Inland</td>
<td>Mar 2014</td>
<td>1</td>
<td>1100</td>
</tr>
<tr>
<td>Ulundi (Ceza)</td>
<td>2731CD</td>
<td>Inland</td>
<td>May 2014</td>
<td>4</td>
<td>600</td>
</tr>
</tbody>
</table>

1 See Figure 2.1 for more exact locations.
2 Releases carried out by UKZN; the remainder were carried out by SASRI.
Figure 2.1 *Anthonomus santacruzi* release sites in KwaZulu-Natal between 2008 and 2014. See Table 2.2 for site names.
2.3.2 Reproductive phenology of *S. mauritianum*

The mean numbers of the different floral stages of *S. mauritianum* were plotted for each of the 24 survey sites (Figure 2.2) to give an indication of food availability for the weevils. While fruit are not utilized by any of the life stages of *A. santacruzi*, the flowers and flowerbuds provide food for the adults, while only flowerbuds are utilized for oviposition and subsequent larval food. As expected, food availability was variable, with inflorescences at most sites providing sufficient food for the different life stages (Figure 2.2). Mean numbers of flowerbuds per inflorescence ranged from 22.9 ± 5.3 (Stanger) to 131 ± 17.5 (Winterskloof) and flowers ranged from 10.1 ± 1.1 at Verulam to 48.1 ± 5.6 at Winterskloof (Figure 2.2). In addition, the composition of the inflorescences (i.e. proportions of fruit, flowers and flowerbuds) were also plotted for each site (Figure 2.3) to illustrate food availability for the weevils. Inflorescences at 16 of the 24 sites were mostly composed of flowerbuds (more than 50% of inflorescence material), with flowers and fruit in similar proportions (Figure 2.3). These results suggested that in relation to weevil numbers (see below), food and oviposition sites were not a limiting factor for weevil populations at the time that the plants were sampled at the different sites.

![Figure 2.2 Mean numbers of flowerbuds, flowers and fruits per inflorescence of *Solanum mauritianum* at each site. See Table 2.1 for site details.](image-url)
2.3.3 Establishment and population densities of *A. santacruzi*

The mean numbers of weevils per inflorescence, including the immature stages (eggs, larvae and pupae) and the adults, were plotted for each site (Figure 2.4) to determine the best sites for the seasonal study (Chapter 3). The coastal sites displayed the highest numbers of *A. santacruzi* with the weevils present at all but one (Mtunzini) of the 13 coastal sites (Figure 2.4). In contrast, the weevils were present at only two of the 11 inland sites, namely Tala and Thornville, and their numbers were relatively low (Figure 2.4). At sites where the weevil was present, their mean numbers per inflorescence ranged from $0.2 \pm 0.1$ (Umlaas Road: Tala) to a maximum of $7.5 \pm 1.4$ (Ballito) (Figure 2.4). The four sites with the highest weevil abundance were situated along the coast and included Ballito, Tongaat, Umkomaas: Empisini, and Ifafa Beach (Figure 2.4). At the 14 sites where *A. santacruzi* was recorded, immature stages were recovered from the flowerbuds at 10 sites (Figure 2.4), which was presumably influenced by the time of year that the sampling was conducted (Table 2.1).
2.3.4 Climatic influences on *A. santacruzi* abundance

There was no significant relationship (*n* = 24; *r*$_s$ = -0.039; *P* = 0.858) between the mean number of weevils and the mean annual rainfall at the different sites, showing that rainfall did not appear to influence the abundance of *A. santacruzi* (Figure 2.5).

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Figure 2.4 Mean (± SE) numbers of immature stages (eggs and larvae) and adults of *Anthonomus santacruzi* recorded per inflorescence of *Solanum mauritianum* at each site. SE bars are based on the mean numbers of immatures and adults combined. See Table 2.1 for site details.
Figure 2.5 Relationship between the mean numbers of *Anthonomus santacruzi* (all stages) per inflorescence and the mean annual rainfall (mm) at the survey sites.

In contrast, there was a relatively strong, positive, significant relationship ($n = 24$; $r_s = 0.613$; $P = 0.001$) between the mean number of weevils and the mean annual temperature at the different sites, showing that increased temperatures significantly increased the abundance of *A. santacruzi* (Figure 2.6).
This trend was supported when the 24 monitoring sites were mapped according to the presence or absence of *A. santacruzi* in relation to mean annual temperature (Figure 2.7). In this representation, a statistical geometric interval was used to separate the temperature data into ranges corresponding with their statistical distribution. With one exception (Mtunzini), there was a clear temperature divide between the presence and absence sites, indicating that the warmer coastal areas were clearly more suitable for *A. santacruzi* establishment.
Figure 2.7 Presence and absence of *Anthonomus santacruzi* at the 24 survey sites in KwaZulu-Natal in relation to the mean annual temperature (°C) variation in the region.
There was also a relatively strong, positive, significant relationship \((n = 24; r_s = 0.669; P < 0.001)\) between the mean number of weevils and the mean annual humidity at the different sites, showing that the abundance of *A. santacruzi* increased significantly with increased humidity (Figure 2.8). However, when the 24 monitoring sites were mapped according to the presence or absence of *A. santacruzi* in relation to mean relative humidity, the trend was not as apparent and the map was thus not included.

![Figure 2.8 Relationship between the mean numbers of *Anthonomus santacruzi* (all stages) per inflorescence and the mean annual humidity (%) at the survey sites.](image)

2.4 Discussion

From these initial surveys it appears that *A. santacruzi* has established and performed better in the coastal region of KwaZulu-Natal as it was recovered at all but one of the coastal sites. The exception was the site at Mtunzini which was the northernmost site sampled on the KZN North Coast. The absence of the weevil at this site could be an indication that the weevil has not dispersed this far north yet as the general conditions were similar to those at the other coastal sites. In particular, the Mtunzini site was somewhat distant from, and midway
between, the closest release sites at Umhlali (north of Tongaat) and Mtubatuba (the northernmost release site) (see Figures 2.1 and 2.7), suggesting that this is a likely explanation. *Anthonomus santacruzi* appeared to perform poorly in the inland region of KwaZulu-Natal as it was not recovered at most of the inland sites, despite the release of high numbers of the weevil at several inland sites (Olckers, 2011; Table 2.2).

The reason for lower numbers of *A. santacruzi* at the inland sites is presently unconfirmed, but may be related to climatic conditions, notably temperature and possibly also humidity which were positively linked with the weevil’s abundance. Inland areas generally have colder temperatures, a higher incidence of frost and lower humidity than coastal areas, particularly during winter, which may affect the survival and proliferation of the weevil’s populations (Olckers, 2011). However, these results should be interpreted with caution as this study was not designed to test climatic effects. Also, since the study was conducted over several months and across different seasons, it is likely that seasonal effects may have been responsible for low (or no) recoveries at inland sites. Ultimately, a study of the weevil’s thermal tolerances (e.g. upper and lower lethal temperatures) and developmental rates at different temperatures will provide more insight into the role of temperature in the proliferation of *A. santacruzi* populations. Previously it was thought that generalist predators could have a negative impact on the establishment and abundance of *A. santacruzi* but this was not supported by earlier field surveys and laboratory exposure trials (Hakizimana and Olckers, 2013b, c). The possibility of interference by native parasitoids has not been ruled out and will be considered in the next chapter.

These preliminary surveys were carried out to determine whether *A. santacruzi* had become widely established in both the coastal and inland regions of KwaZulu-Natal following the considerable release efforts that were recently undertaken. Therefore, collections at the 24 sites were carried out over a period of eight months and across different seasons. The presence of the weevil during winter, albeit in low numbers, was considered as a positive sign. The different seasons across which the sites were surveyed may well have affected the numbers of flowers and flowerbuds as well as weevils at the sites, thus reducing the accuracy of direct comparisons between the sites. Nevertheless, the numbers of flowers and flowerbuds always greatly exceeded the numbers of *A. santacruzi* (adults and immature stages combined) at all sites. There were always large numbers of fruit on the inflorescences, suggesting that the weevil has so far not been particularly effective in infesting enough flowerbuds. Six of the 24 sites (three coastal and three inland) that were shown to support populations of *A.
*santacruzi* were selected for more intensive seasonal monitoring over a period of one year (see Chapter 3).
CHAPTER 3: Abundance across seasons, response to food availability and possible predation and parasitism of *Anthonomus santacruzi* at sites in KwaZulu-Natal.

3.1 Introduction

It has been 16 years since *Gargaphia decoris* Drake (Hemiptera: Tingidae), a leaf-sucking lace bug, was first released and seven years since *Anthonomus santacruzi* Hustache (Coleoptera: Curculionidae), a flower-feeding weevil, was first released in South Africa as biological control agents of *Solanum mauritianum* Scop. (Solanaceae) (Olckers, 2011). Low intensity releases of *A. santacruzi* began in KwaZulu-Natal in 2008 and continued until 2011 when larger scale releases were undertaken by the South African Sugarcane Research Institute (SASRI). Since 2008 more than 28 000 weevils have been released at various sites in KwaZulu-Natal (see Table 2.2 in Chapter 2), where most releases were carried out. *Anthonomus santacruzi* larvae feed within the flowerbuds of *S. mauritianum* and the adults feed on open flowers but also shoot tips when floral availability is low (Olckers, 2003). Due to the high fruit set of *S. mauritianum* and long-distance seed dispersal by frugivorous birds (Olckers and Hulley, 1989; Olckers, 1996; Henderson, 2007; Olckers, 2009; Jordaan and Downs, 2012a), these flower-feeding weevils were a promising addition to the biocontrol programme as they damage the flowerbuds and flowers and prevent their development into fruit (Barboza et al., 2009; Olckers, 2011).

Although only seven years have elapsed since *A. santacruzi* was first released, there have been several indications of establishment, dispersal and population proliferation (Olckers, 2011; Hakizimana and Olckers, 2013b). However, the weevil’s seasonal abundance and response to flowering by *S. mauritianum* populations has not been determined. A preliminary assessment of the weevil’s establishment and abundance was thus undertaken in KwaZulu-Natal (see Chapter 2) in order to select sites for seasonal monitoring. Six sites (from those surveyed in the preliminary study) were selected for monthly monitoring over one year to compare coastal and inland *A. santacruzi* populations and determine whether climatic conditions and food availability had any effect on their abundance. The results should be beneficial in determining which sites would be most favourable for *A. santacruzi* proliferation.
Other factors which could influence the establishment of *A. santacruzi* include predation and parasitism. Typically, insects introduced into an exotic range for use as biocontrol agents should lack natural enemies and be freed from suppression by specialist predators or parasitoids (Hunt-Joshi et al., 2005). However, this is not always the case as generalist species often exploit newly introduced biocontrol agents as prey or hosts (Hunt-Joshi et al., 2005). These opportunistic predators and parasitoids can thus affect the establishment and proliferation of biocontrol agents (Goeden and Louda, 1976; Crawley, 1986, 1987; Julien and Griffiths, 1998). Julien and Griffiths (1998) examined 488 biocontrol releases of which 105 had failed; of these failures 48% were attributed to predation and parasitism. However, studies carried out by Hakizimana and Olckers (2013b, c) determined that generalist predators, especially ants and spiders, were not particularly abundant on *S. mauritianum* inflorescences and posed no real threat to the weevils and so predation was regarded as a minor risk. However, ants were surveyed during this study to confirm this conclusion. Also, parasitism of the weevil’s immature stages has not been previously studied and a preliminary survey was thus conducted to determine whether any native parasitoids had been acquired.

3.2 Methods

3.2.1 Study sites

Of the 24 preliminary sites evaluated in KwaZulu-Natal (Table 2.1), six were chosen for the current study based on their location and the presence of healthy weevil populations. Three inland sites and three coastal sites were chosen for comparisons of weevil densities under different climatic conditions. The three inland sites were Pietermaritzburg: Mkondeni (S29°38’30” E30°24’34”), Umlaas Road: Tala (S29°46’43” E30°30’24”), and Thornville (S29°44’09” E30°23’01”), and the three coastal sites were Hillcrest (S29°47’50” E30°46’31”), Tongaat (S29°35’17” E31°09’09”), and Umkomaas: Empisini (S30°12’07” E30°47’06”). Samples were taken from each of the six sites on a monthly basis from October 2014 until September 2015. Climatic data for each of these sites were provided by the Department of Natural Resources, KZN Department of Agriculture & Rural Development (F. Mitchell, pers. comm.). These data included long-term mean monthly temperature, rainfall and humidity records that were derived from the Bioresource Groups of KwaZulu-Natal database (Camp, 1997).
3.2.2 Sampling procedure

On each monthly sampling occasion, 10 trees that contained floral material were randomly selected within the *S. mauritianum* population at each site. On each tree, a branch bearing inflorescences was selected and all inflorescences and shoot tips that were present on the first 100 cm of the branch (measured from the tip) were removed and placed in a Ziploc™ bag. The samples were frozen to keep the material fresh and prevent the weevils from laying eggs, so as to avoid overestimating the population densities. During the processing of each sample, the numbers of adult weevils and ants were recorded and the material was separated into the different floral stages, namely mature fruits, immature fruits, flowers, mature flowerbuds, and immature flowerbuds and the numbers of each stage were recorded. The mature and immature flowerbuds were then dissected under a dissecting microscope to record the numbers of eggs, larvae and pupae of *A. santacruzi*. Although sampling was originally planned for eight of the 12 months (i.e. two of the three months per season), this was later amended to monthly sampling. As a result, one month (December 2014) was skipped and sampling was thus carried out over 11 months. Overall, 660 inflorescence-bearing branches were sampled (i.e. 10 branches at 6 sites over 11 months).

3.2.3 Parasitoid survey

In each of the last three months of sampling (July, August and September 2015), one flowerbud-bearing inflorescence from each of five tree samples from each of the six sites was placed within a Petri dish (i.e. 30 dishes per month). A moist sponge was also placed within each Petri dish to prevent desiccation of the floral material and allow any *A. santacruzi* or parasitoid larvae to develop to adulthood. After about seven days the flowerbuds were carefully dissected under a dissecting microscope to recover weevil larvae or pupae. Developing larvae were either left *in situ* if the flowerbuds were healthy or transferred into fresh flowerbuds and left within the Petri dishes for further development. The numbers of emerging adult weevils and any parasitoids were recorded in relation to the numbers of larvae/pupae recovered.
3.2.4 Statistical analysis

Figures were produced using Microsoft Excel 2010 and statistical analyses were conducted using IBM SPSS Statistics version 23.0. The numbers of floral material that were available to the weevils (i.e. flowers and flowerbuds), numbers of weevils (adults and immatures combined) and numbers of ants were compared between months and sites. Since none of these datasets met the assumptions of normality, generalized linear modelling was used to determine the influence of month and site, and their interaction, on these numbers. Since these models analysed count data, they incorporated a Poisson distribution (corrected for over-dispersion) and log link function. Significance (P < 0.05) was assessed using Wald chi-square statistics. The proportions of flowerbuds damaged were also compared between months and sites. Since this model analysed binary data (counts of infested flowerbuds versus available flowerbuds), it incorporated a Binomial distribution and logit link function. Significance (P < 0.05) was also assessed using Wald chi-square statistics. Due to the datasets not meeting the assumptions of normality, Spearman’s rank-order correlations were used to determine the relationships between: the numbers of weevils (all stages) and floral material available for feeding (flowers and flowerbuds) at each site; the mean number of weevils (all stages) and climatic features (namely the mean monthly rainfall (mm), mean monthly temperature (°C), and mean monthly humidity (%)) at inland and coastal sites; the numbers of ants and mature fruits at each site and; the numbers of adult weevils and ants at each site.

3.3 Results

3.3.1 Reproductive phenology of S. mauritianum

The mean numbers of inflorescence material (including flowerbuds, flowers and fruits) per sampled branch of S. mauritianum during each month were plotted for each of the six sites (Figure 3.1). There was considerable variation in mean numbers of the different floral components between the different months but this was largely consistent between the different sites (Figure 3.1). At most of the sites, there was a general trend of reduced floral availability (i.e. flowers and flowerbuds which are utilized by the weevils) between January and February (summer) with peaks in floral availability between March and April (autumn) (Figure 3.1). Overall, there were significant differences in floral availability between months
(χ² = 193.348; df = 10, P < 0.0005) and sites (χ² = 20.454; df = 5, P = 0.001) and the interaction between months and sites (χ² = 83.371; df = 50, P = 0.002) was also significant.

The proportions of inflorescence material per sampled branch of *S. mauritianum* that comprised fruits, flowers and flowerbuds during each month were also plotted for each site (Figure 3.2). During most of the months at each of the sites, more than 50% of the inflorescence material was accessible to the weevils (i.e. comprised flowers and flowerbuds) (Figure 3.2). However, during a few months at the different sites the inflorescence material was largely comprised of fruit (denoted by the black portions of the bars) and was thus less accessible to the weevil populations (Figure 3.2). These results again suggest that, with few exceptions, food and oviposition sites were not a limiting factor for *A. santacruzi* populations at the different sites, since floral material was available throughout the year.
Figure 3.1 Mean numbers of inflorescence material (including flowerbuds, flowers and fruits) per sampled branch of *Solanum mauritianum* at six KwaZulu-Natal sites, namely a) Mkondeni, b) Tala, c) Thornville, d) Hillcrest, e) Tongaat, and f) Empisini, between October 2014 and September 2015.
Figure 3.2 Proportions (%) of inflorescence material (including flowerbuds, flowers and fruits) per sampled branch of *Solanum mauritianum* at six KwaZulu-Natal sites, namely a) Mkondeni, b) Tala, c) Thornville, d) Hillcrest, e) Tongaat, and f) Empisini, between October 2014 and September 2015.
3.3.2 Abundance of *A. santacruzi*

The mean numbers of weevils (all stages) per sampled branch of *S. mauritianum* during each month were plotted for each site (Figure 3.3). There were considerable and highly significant differences in the abundance of *A. santacruzi* between months ($\chi^2 = 279.937$; df = 10, $P < 0.0005$) and sites ($\chi^2 = 30.503$; df = 5, $P < 0.0005$) and the interaction between months and sites ($\chi^2 = 148.993$; df = 50, $P < 0.0005$) was also significant. Weevil abundance consistently peaked around April and May (autumn) at each of the six sites (Figure 3.3), which coincided roughly with peaks in flowerbud availability (Figure 3.1).

The highest weevil numbers (immature and adults) were recorded at Empisini on the KZN South Coast with a maximum mean of $40.4 \pm 10.7$ individuals per sampled branch in May 2015 (Figure 3.3). The lowest weevil numbers were recorded at Hillcrest, situated at a higher altitude on the outskirts of Durban, with its maximum at only $5.7 \pm 1$ individuals per sampled branch in May 2015 (Figure 3.3). Empisini is a typical humid, warm site whereas Hillcrest (although close to the coast) is considerably cooler with morning and evening mist. Maximum population means per sampled branch at the other four sites included $24.3 \pm 8.6$ at Tala in April 2015, $19.9 \pm 3.5$ at Tongaat in April 2015, $14.5 \pm 3.2$ at Thornville in May 2015 and $11.9 \pm 3.4$ at Mkondeni in May 2015 (Figure 3.3). With few exceptions (e.g. during some months at Mkondeni and Hillcrest), *A. santacruzi* was recovered throughout the year (Figure 3.3). Generally, most recoveries of *A. santacruzi* comprised immature stages (larvae and eggs) inside the flowerbuds (denoted by the black portions of the bars) which often exceeded the numbers of adults collected (Figure 3.3). No pupae were recovered inside the buds; presumably because infested buds abscise at the time of larval pupation.
Figure 3.3 Mean (± SE) numbers of adults and immature stages (including larvae and eggs) of *Anthonomus santacruzi* at six KwaZulu-Natal sites, namely a) Mkondeni, b) Tala, c) Thornville, d) Hillcrest, e) Tongaat, and f) Empisini, between October 2014 and September 2015. SE bars are based on the mean numbers of immatures and adults combined.
There were positive, significant relationships between the numbers of *A. santacruzi* and the amounts of floral material available for feeding, at all of the six sites (Figure 3.4). There were relatively weak relationships at two sites, notably Mkondeni (n = 110; \( r_s = 0.273; \) \( P = 0.004 \)) and Empisini (n = 110; \( r_s = 0.290; \) \( P = 0.002 \)). Moderately stronger relationships were found at the remaining sites, notably Tala (n = 110; \( r_s = 0.323; \) \( P = 0.001 \)), Thornville (n = 110; \( r_s = 0.373; \) \( P < 0.001 \)), Hillcrest (n = 110; \( r_s = 0.332; \) \( P < 0.001 \)), and Tongaat (n = 110; \( r_s = 0.324; \) \( P = 0.001 \)).

Despite the year-round availability of flowerbuds for oviposition, the percentage of *S. mauritianum* flowerbuds that were infested by immature weevils at each site was consistently low (Figure 3.5). With few exceptions, monthly infestations amounted to less than 10% (mostly < 5%) of available flowerbuds. With one exception, the highest percentages of flowerbuds infested were recorded in May 2014 and these amounted to 25.4% at Empisini, 17.5% at Tongaat, 6.8% at Mkondeni, 5.2% at Thornville and 3.3% at Hillcrest (Figure 3.5). At Tala, the highest percentage of flowerbuds infested (11.8%) was recorded in January 2015 (Figure 3.5). There were significant differences in the proportions of flowerbuds that were infested by *A. santacruzi* between months (\( \chi^2 = 557.918; \) df = 10, \( P < 0.0005 \)) but not between sites (\( \chi^2 = 0.098; \) df = 5, \( P > 0.05 \)) while the interaction between months and sites (\( \chi^2 = 4106.784; \) df = 49, \( P < 0.0005 \)) was significant.
Figure 3.4 Relationships between the total numbers of *Anthonomus santacruzi* (adults and immatures) and the total numbers of floral material (flowers and flowerbuds) available for utilisation at six sites in KwaZulu-Natal, namely a) Mkondeni, b) Tala, c) Thornville, d) Hillcrest, e) Tongaat, and f) Empisini.
Figure 3.5 Percentages of *Solanum mauritianum* flowerbuds that were infested by immature *Anthonomus santacruzi* at six sites in KwaZulu-Natal, namely a) Mkondeni, b) Tala, c) Thornville, d) Hillcrest, e) Tongaat, f) Empisini, between October 2014 and September 2015.
3.3.3 Climatic influences on A. santacruzi abundance

3.3.3.1 Rainfall

The mean monthly numbers of A. santacruzi were plotted with the mean monthly rainfall to determine any patterns (Figure 3.6). In general, all sites experience increased rainfall during the spring and summer months (September to March) with declines during the autumn and winter months (April to July) (Figure 3.6). At all sites, the numbers of A. santacruzi peaked during periods of lower monthly rainfall (April to May) (Figure 3.6). However, there was no relationship between weevil numbers and rainfall at either the inland sites ($n = 33; r_s = -0.168; P = 0.349$) or the coastal sites ($n = 33; r_s = -0.168; P = 0.349$) (Figure 3.7).
Figure 3.6 Mean monthly numbers of *Anthonomus santacruzi* in relation to mean monthly rainfall (mm) at six KwaZulu-Natal sites, namely a) Mkondeni, b) Tala, c) Thornville, d) Hillcrest, e) Tongaat, and f) Empisini, between October 2014 and September 2015.
3.3.3.2 Temperature

The mean monthly numbers of *A. santacruzi* were similarly plotted with the mean monthly temperatures to determine any patterns (Figure 3.8). In general, all sites experience similar mean monthly temperatures that range from 15°C to 25°C (Figure 3.8). The highest temperatures occur during the late spring and summer months (November to March) with declines during the autumn and winter months (April to July) (Figure 3.8). At all sites, the numbers of *A. santacruzi* peaked during the cooler autumn months (April to May) (Figure 3.8). However, there was no relationship between weevil numbers and temperature at either the inland sites \( (n = 33; r_s = -0.074; P = 0.682) \) or the coastal sites \( (n = 33; r_s = 0.139; P = 0.442) \) (Figure 3.9).
Figure 3.8 Mean monthly numbers of *Anthonomus santacruzi* in relation to mean monthly temperature (°C) at six KwaZulu-Natal sites, namely a) Mkondeni, b) Tala, c) Thornville, d) Hillcrest, e) Tongaat, and f) Empisini, between October 2014 and September 2015.
3.3.3.3 Humidity

The mean monthly numbers of *Anthonomus santacruzi* were finally plotted with the mean monthly humidity to determine any patterns (Figure 3.10). In general, all sites experience similar mean monthly humidity, although the coastal sites typically have 5-10% higher average humidity than inland sites (Figure 3.10). The highest humidity levels occur during the late spring and summer months (November to March) with declines during the autumn and winter months (April to July) (Figure 3.10). At all sites, the numbers of *A. santacruzi* peaked during periods of lower humidity that coincided with the cooler autumn months (April to May) (Figure 3.10). As before, there was no relationship between weevil numbers and humidity at either the inland sites (n = 33; rs = -0.072; P = 0.692) or the coastal sites (n = 33; rs = -0.020; P = 0.910) (Figure 3.11).
Figure 3.10 Mean monthly numbers of *Anthonomus santacruzi* in relation to mean monthly humidity (%) at six KwaZulu-Natal sites, namely a) Mkondeni, b) Tala, c) Thornville, d) Hillcrest, e) Tongaat, and f) Empisini, between October 2014 and September 2015.
3.3.4 Effect of ants on *A. santacruzi*

The mean numbers of ants per sampled branch of *S. mauritianum* during each month were plotted for each site (Figure 3.12). There were considerable and highly significant differences in the abundance of ants between months ($\chi^2 = 55.125; \text{df} = 10, P < 0.001$) and sites ($\chi^2 = 32.706; \text{df} = 5, P < 0.001$) and the interaction between months and sites ($\chi^2 = 94.109; \text{df} = 34, P < 0.001$) was also significant. Ants were present throughout the year at most sites, except at Mkondeni and Tala where they were not recorded during most months of the year (Figure 3.12). Generally, more ants were recorded during the late spring and summer months. The highest numbers of ants were recorded at Hillcrest with a maximum mean of 106.5 ± 47.9 ants per sample in October 2014 (Figure 3.12).

The relationship between the numbers of ants and mature fruits of *S. mauritianum* was positive and significant at all six sites (Figure 3.13). There were weak relationships at Mkondeni ($n = 110; r_s = 0.282; P = 0.003$), Thornville ($n = 110; r_s = 0.272; P = 0.004$), and Hillcrest ($n = 110; r_s = 0.227; P = 0.017$) and moderate relationships at Tala ($n = 110; r_s = 0.373; P < 0.001$), Tongaat ($n = 110; r_s = 0.445; P < 0.001$), and Empisini ($n = 110; r_s =$
0.344; P < 0.001) (Figure 3.13). Generally, as the numbers of mature fruits per sampled branch increased there was a higher number of ants.

There was no significant relationship between the numbers of *A. santacruzi* and ants at five of the six sites (Figure 3.14), including Tala (n = 110; $r_s = -0.178; P = 0.062$), Thornville (n = 110; $r_s = -0.028; P = 0.774$), Hillcrest (n = 110; $r_s = -0.104; P = 0.280$), Tongaat (n = 110; $r_s = 0.006; P = 0.951$), and Empisini (n = 110; $r_s = -0.108; P = 0.262$). There was a weak, negative and significant relationship between *A. santacruzi* numbers and ant numbers at Mkondeni (n = 110; $r_s = -0.201; P = 0.035$) (Figure 3.14). Generally, there was little evidence that the numbers of *A. santacruzi* were affected by the presence of ants on the inflorescences.
Figure 3.12 Mean (± SE) numbers of ants per sampled branch of *Solanum mauritianum* at six KwaZulu-Natal sites, namely a) Mkondeni, b) Tala, c) Thornville, d) Hillcrest, e) Tongaat, and f) Empisini, between October 2014 and September 2015.
Relationships between the total numbers of ants and the total numbers of mature fruits per sampled branch of *Solanum mauritianum* at six KwaZulu-Natal sites, namely a) Mkondeni, b) Tala, c) Thornville, d) Hillcrest, e) Tongaat, and f) Empisini.
Figure 3.14 Relationships between the total numbers of *Anthonomus santacruzi* and the total numbers of ants per sampled branch of *Solanum mauritianum* at six KwaZulu-Natal sites, namely a) Mkondeni, b) Tala, c) Thornville, d) Hillcrest, e) Tongaat, and f) Empisini.
3.3.5 Parasitism

During the three months in which larvae that were collected from the six sites were reared to adulthood, relatively few larvae (only 20 in total) were recovered in the flowerbuds. Larvae were recovered at five sites, but not consistently over the three months (Figure 3.15). Most of the larvae were reared to adulthood and no parasitoids were reared from any of the samples (Figure 3.15). The relatively low larval mortality (Figure 3.15) was presumably caused by injuries sustained during their transfer to fresh flowerbuds.

![Figure 3.15 Total numbers of Anthonomus santacruzi larvae that emerged as adults or died following their collection at six sites in KwaZulu-Natal, during July to September 2015.](image)

3.4 Discussion

Throughout the year, each of the six sites, whether on the coast or inland, supported healthy flowering populations of *S. mauritianum*, ensuring that there was ample food and oviposition sites for *A. santacruzi* to utilise. However, the levels of infested flowerbuds were generally very low with the maximum infestation recorded during any single month reaching only 25.4%. These low levels of flowerbud damage suggest that the impact of *A. santacruzi* on the fruiting of *S. mauritianum* populations is negligible. This is presumably because populations of *A. santacruzi* have not yet reached their full potential, having been established in the field
in KwaZulu-Natal for only a few years. However, there were positive and significant relationships between *A. santacruzi* numbers and resource availability at the different field sites, suggesting a positive response to increased flowering. Such density-dependent relationships could become more meaningful at higher *A. santacruzi* population densities (Stephens and Myers, 2012), suggesting that regulation of *S. mauritianum* populations through reduced fruit set may be possible in the long term. It is likely that the weevil is in the process of expanding its range in South Africa, which may be partially responsible for the low levels of floral infestation currently recorded. Although *A. santacruzi* numbers were generally lower at the inland sites, the strongest and most significant relationship between weevil numbers and resource availability was recorded at Thornville.

The highest weevil densities were recorded at two coastal sites, namely Umkomaas: Empisini on the KZN South Coast and Tongaat on the KZN North Coast. The Umkomaas site was one of the initial three release sites and the first where the establishment of *A. santacruzi* was confirmed (Olckers, 2011) and has thus supported weevil populations for the longest. Although inland sites supported lower numbers of weevils, the lowest *A. santacruzi* densities were recorded at Hillcrest which was included as a coastal site. Despite also being one of the original three release sites (Olckers, 2011), *A. santacruzi* populations did not appear to be thriving at Hillcrest and no individuals were recorded during three months of the year. Since Hillcrest is more inland and at a higher altitude than coastal Durban, it has many similarities with the other inland sites (e.g. lower temperatures due to morning and evening mist). However, the low *A. santacruzi* populations may also have been disrupted by the clearing of some of the trees in the *S. mauritianum* population during the study. The Umkomaas: Empisini and Tongaat sites are typified by less variable climate (e.g. temperature and humidity) during the year which may be more suitable for the weevils.

Although *A. santacruzi* population densities varied between sites and months of the year, population peaks were consistently recorded during the autumn months (April/May), which are typified by lower temperatures, rainfall and humidity. However, within-region assessments (i.e. inland and coastal sites) of the possible effect of monthly rainfall, temperature and humidity on weevil densities did not provide any evidence of the influence of these climatic factors. This does not necessarily imply that climatic factors are not important for *A. santacruzi*, particularly where their failure to establish is concerned (see Chapter 2). Nevertheless, where weevil populations have become established, climate did not seem to be influencing their numbers. Similar population peaks during the autumn months
were previously reported for the leaf-sucking lace bug *G. decoris*, which were followed by sharp declines during winter and slow recoveries during spring and summer (Olckers, 2011). It was proposed that these declines were not caused by climatic effects, but by decreased food quality of the *S. mauritianum* plants during the dry KwaZulu-Natal winters (Olckers, 2011). It is possible that the seasonal fluctuations of *A. santacruzi* may also be caused by fluctuating host plant quality which was observed during sampling, particularly since drier conditions can cause the abscission of flowers and flowerbuds by *S. mauritianum* plants. Depending on their developmental stage, larvae present within the flowerbuds are unlikely to survive in situations of premature abscission. Also, ovipositing females are likely to reject flowerbuds of lower quality (e.g. low moisture content). The winter declines in *A. santacruzi* densities did appear to coincide with reduced numbers of flowers and flowerbuds at the study sites (see Figure 3.1).

Generalist predation, notably by spiders and ants, was previously considered to be a minor risk to the proliferation of *A. santacruzi* populations (Hakizimana and Olckers, 2013b, c). During this study, ants were sometimes collected in large numbers but were mostly found on inflorescences with large numbers of mature fruits; presumably for the high sugar content contained in these (Jordaan and Downs, 2012a). Indeed, there were significant positive relationships between the numbers of ants recorded and numbers of mature fruits at all of the study sites. However, there was no evidence that increased numbers of ants were having a negative effect on *A. santacruzi* numbers, supporting the contention that ants are attracted by the fruit and not the weevils. It thus seems unlikely that ants will pose a threat to *A. santacruzi* populations in the future.

The preliminary parasitoid survey did not provide any evidence that *A. santacruzi* had recruited native larval/pupal parasitoids. Although the survey was carried out during the early months of spring, when parasitoid densities may have been low, their presence should still have been detected. Previously, *A. santacruzi* was deemed unlikely to recruit native parasitoids (Olckers, 2011). This is because surveys of native *Solanum* species (Olckers and Hulley, 1989, 1991a, 1995) did not record any native flower-feeding Curculionidae from which parasitoids could be acquired. However, additional collections of larvae-infested flowerbuds should be undertaken in the field to confirm this contention.

Post-release evaluations on weed biocontrol agents can determine how successful the agent has been at dispersing and proliferating and the extent to which it is able to decrease the
weed’s density or rate of spread (Carson et al., 2008). At this early stage of its deployment, *A. santacruzi* has persisted at many of the original release sites and appears to have dispersed readily from these. This is an optimistic result since any biocontrol agent, given more time, has the potential to have an impact on its target plant (Carson et al., 2008). In its native range, *S. mauritianum* populations have much lower fruit yields which was attributed to *A. santacruzi* (Barboza et al., 2009; Olckers, 2011). However, at this stage, *A. santacruzi* does not appear to be having any impact on the reproductive output of *S. mauritianum* populations in KwaZulu-Natal. Although, weevils (and/or their immature stages) were present throughout the year at the majority of the study sites, their population densities were seasonal and low at the best of times. Populations of *S. mauritianum* produce high numbers of flowers and fruits throughout the year (Campbell and Van Staden, 1983; Henderson, 2001; Witkowski and Garner, 2008) and high levels of flowerbud mortality are consistently required to offset this. However, since this biocontrol programme is still at an early stage *A. santacruzi* populations could increase with time. If higher weevil population densities are realized in the field, their impact could be substantial; especially since weevil densities were positively correlated with flowerbud production by *S. mauritianum* populations (i.e. density dependent). Further monitoring of *A. santacruzi* populations should thus be conducted in KwaZulu-Natal, but also in other provinces, to determine their impact over the longer term.
CHAPTER 4: General discussion and conclusions

4.1 Introduction

Invasive alien plants are changing ecosystems around the world (Bright, 1998; McFadyen, 1998; Kaiser, 1999; Myers and Bazely, 2003; Van Wilgen et al., 2004) and the only economically sustainable method to reduce their considerable impacts is through biological control (McFadyen, 1998; Marais et al., 2004; Culliney, 2005). However, not all weed biocontrol agents are successful and predicting which agents are likely to be effective is very difficult at the pre-release stage (Myers et al., 2009). Although considerable funding is normally invested in pre-release activities (i.e. selecting suitable agents, host-specificity testing and mass-rearing and releasing approved agents), post-release evaluations are typically under-funded (Ding et al., 2006; Myers et al., 2009). Such evaluations are very important as, besides quantifying agent performance and impact, they can highlight which agent taxa and feeding guilds are likely to be the most successful and thereby facilitate future predictions of success (Myers et al., 2009). In this regard, weevils (Curculionidae) have a good track record in weed biocontrol success in several countries (Winston et al., 2014). Better predictions of successful agents would ensure fewer releases of alien organisms into new environments around the world, thereby minimizing the chances of non-target effects (Louda et al., 1997, 2003). In this context, a post-release evaluation of *Anthonomus santacruzi* Hustache (Coleoptera: Curculionidae), a recently established flower-feeding agent of *Solanum mauritianum* Scopoli (Solanaceae) was initiated to provide some insight into its efficacy as a biocontrol agent in South Africa.

*Anthonomus santacruzi* was considered as a biocontrol agent of *S. mauritianum* because plants in their native South American range displayed a much lower fruit set than plants in South Africa that were free of natural enemies (Barboza et al., 2009; Olckers, 2011). Several flower- or flowerbud-feeding agents have been deployed against invasive plants worldwide (Appendix I) and include chewing, boring, galling, sucking and mining arthropod species. In South Africa, flower-feeding biocontrol agents have been used to curb the excessive levels of seed production and dispersal of their target plants but also to preserve the commercially valuable parts of some species (e.g. exotic forestry plants) (Dennill and Donnelly, 1991; Impson et al., 2009, 2013). For example, the flower-galling fly *Dasineura rubiformis* Kolesik (Cecidomyiidae) was released to control the spread of *Acacia mearnsii* De Wild. (Fabaceae)
which has considerable economic value in the South African forestry industry (Impson et al., 2013). This programme has the potential to be very successful as *D. rubiformis* reduces seed set to very low levels without negatively affecting the growth of *A. mearnsii* and hence its commercial value in South Africa (Impson et al., 2013). *Acacia mearnsii* is estimated to be worth US $552 million in the forestry industry (de Wit et al., 2001) based on the production of wood chips, pulp and tanning extracts, and other products (Feely, 2012).

4.2 *Anthonomus* species as weed biocontrol agents

The Curculionidae represent one of the largest insect families and, despite several species being listed as crop pests (Burke, 1976), many species have been successfully deployed as weed biocontrol agents (Winston et al., 2014). The genus *Anthonomus* comprises several species that target the floral tissues, and sometimes the fruits, of their host plants and are generally host specific (Burke, 1976; Chacon-Madrigal et al., 2012). Besides *A. santacruzi*, other species have been considered as potential biocontrol agents of weeds in the family Solanaceae. These include the flowerbud weevils *Anthonomus tenebrosus* Boheman and *A. elutus* Clark for the control of *Solanum viarum* Dunal and *S. tampicense* Dunal, respectively, in the USA (Medal et al., 2002, 2009, 2011). While *A. tenebrosus* was rejected due to perceived threats to eggplant (*Solanum melongena* L.) cultivations (Medal et al., 2011; Diaz et al., 2014), *A. elutus* was considered suitable for release (Medal et al., 2009), but with permission still pending. The flowerbud weevil *A. partiarius* Boheman was considered for the control of *Tibouchina herbacea* Cogniaux (Melastomataceae) in Hawaii (Pedrosa-Macedo et al., 2000; Caxambu, 2003; Pedrosa-Macedo, 2004a, b), while the fruit-feeding weevil, *A. monostigma* Champion was considered for the control of *Miconia calvescens* DC (Melastomataceae) in Costa Rica and the Pacific Islands (Chacon-Madrigal et al., 2012), but neither has yet been released. Since *A. santacruzi* is, to date, the only species in the genus that has been released, there are no precedents to demonstrate the efficacy of *Anthonomus* species as biocontrol agents, anywhere in the world (Winston et al., 2014).

4.3 Flower-feeders as weed biocontrol agents

Some 21 species of flower-feeders have been released as biological control agents worldwide (Appendix I). These comprise species belonging to 12 families of Diptera, Coleoptera, Acari, Lepidoptera and Hemiptera that have targeted weeds in the families Fabaceae, Asteraceae,
Proteaceae, Verbenaceae, Plantaginaceae, Lythraceae, Mimosaceae and Solanaceae (Appendix I). Ten of these species have been released in South Africa with varying degrees of effectiveness, with some species proving to be more effective in other countries (Appendix I). All five orders of flower-feeding agents have at least one species that has caused extensive damage to a weed somewhere in the world (see Appendix I). Two flower-galling flies (Diptera: Cecidomyiidae) have caused extensive damage to two Acacia species; a flower-boring weevil (Coleoptera: Curculionidae) caused extensive damage to two Centaurea species; a flowerbud weevil (Coleoptera: Brentidae) caused extensive damage to Sesbania punicea (Cav.) Benth. (Fabaceae) and; a flower-galling mite (Acari: Eriophyidae), flower-boring moth (Lepidoptera: Pterophoridae), and a leaf and flower-feeding lace bug (Hemiptera: Tingidae) have similarly damaged Lantana camara L. (Verbenaceae) (see references in Appendix I). Of the 10 flower-feeding agent species that have been deployed in South Africa, four have caused extensive damage to four weed species. These include the two flower-galling flies on A. mearnsii and A. cyclops A. Cunn. ex G. Don, a flower-galling mite on L. camara and a flowerbud-feeding weevil on S. punicea (see references in Appendix I). The impacts of the remaining agents have been less impressive, although not all have been fully quantified. Some agents causing trivial damage in South Africa have, however, caused considerable damage to the same weed in other countries (see Appendix I) and this could be due to climatic effects or fewer natural enemies/predators in those countries. Such examples in South Africa include biocontrol agents released against L. camara with the flower-galling moth Crocidosema lantana Busck (Lepidoptera: Torticidae) having caused considerable damage in Guam, the Northern Mariana Islands and Palau (Baars, 2003; Winston et al., 2014) and the flower-mining moth Lantanophaga pusillidactyla Walker (Lepidoptera: Pterophoridae) having caused extensive damage in Guam (Baars, 2003; Zalucki et al., 2007).

The value of flower-feeding agents was illustrated by the programme against the invasive South American tree S. punicea in South Africa (Hoffmann and Moran, 1988, 1991, 1998). The trees, which typically invaded wetlands and riparian zones in the higher rainfall areas of South Africa, grow to about 4 m in height, live for around 15 years and produce high numbers of seeds every summer (Hoffmann and Moran, 1998). The weed was controlled biologically through the combined action of three weevil species that target different parts of the plant; namely the flower-feeding Trichapion lativentre (Bèguin-Billecocq) (Coleoptera: Brentidae), seed-feeding Rhyssomatus marginatus Fahreus (Coleoptera: Curculionidae) and
The biology of *T. lativentre* is similar to that of *A. santacruzi* in that the adults feed on the leaves and flowers and oviposit in the flowerbuds, with the developing larvae destroying the flowerbuds and preventing seed set (Hoffmann and Moran, 1998; Ocklers, 2003). The impact of this agent on its own was considerable as it caused a 98% reduction in seed production and a 40% reduction in the plant’s above-ground biomass (Hoffmann and Moran, 1998). *Trichapion lativentre* populations caused a decrease in the density of immature *S. punicea* populations within 6 years of establishing at the study sites (Hoffmann and Moran, 1998). Despite this, flowers that escaped damage were able to produce sufficient seeds to allow the *S. punicea* populations to replenish and *T. lativentre* was thus unable to bring about complete control in isolation (Hoffmann and Moran, 1998). The addition of *R. marginatus*, whose larvae destroy around 90% of the residual seeds, and *N. quadrivittatus*, whose larvae are able to kill large, mature trees, to the biocontrol programme had a dramatic effect and *S. punicea* is today considered to be of minor importance in South Africa (Hoffmann and Moran, 1998). Nevertheless, *T. lativentre* has remained a very successful biocontrol agent as it vastly diminishes the weed’s annual seed set (Hoffmann and Moran, 1998). Indeed, the selection of *A. santacruzi* as a candidate agent was largely based on the above case history.

In another case history, *Larinus minutus* Gyllenhal (Coleoptera: Curculionidae), a flower-feeding weevil, contributed substantially to the biocontrol of diffuse knapweed, *Centaurea diffusa* Lam. (Asteraceae), an invader of rangelands in Canada (Myers et al., 2009). Twelve agent species have been released against this weed since the 1970s (Bourchier et al., 2002) of which only four have become widely abundant. These include two flower-galling flies *Urophora affinis* Fraenfeld and *U. quadrifasciata* Meigen (Diptera: Tephritidae), a root-boring beetle, *Sphenoptera jugoslavica* Obenberger (Coleoptera: Buprestidae), and the flower-feeding *L. minutus* (Groppe, 1990). It took 30 years to achieve control of *C. diffusa* in Canada and this occurred following the introduction of *L. minutus*, since the other three agents were effective in reducing the weed’s seed set but were unable to kill the plant or decrease its density (Myers et al., 2009). Reductions in seed set typically have limited impact if seedling survival is able to compensate for seed loss (Myers et al., 1990; Powell, 1990; Myers and Risley, 2000; Myers and Bazely, 2003); a factor that needs to be considered in the control of *S. mauritianum*. In the case of *C. diffusa*, the addition of *L. minutus* to the
biocontrol programme caused a significant reduction in weed density due to the combined action of the adults feeding on the leaves of rosettes and seedlings and the parenchyma of bolting stems and the larvae feeding internally on the flowerbuds and preventing seed set (Myers et al., 2009). In this context, the impact of *A. santacruzi* is likely to be less dramatic as adult feeding damage to foliar tissues is negligible and other agents would be required to target other plant parts (Olckers, 2011).

4.4 Use of multiple agents in weed biocontrol

The control of *S. mauritianum* will most likely depend on the establishment of a suite of agents that target a range of plant parts (Olckers, 2011). However, there have been several criticisms of the use of multiple agents with suggestions that the number of biocontrol agents per target weed should be minimized (Denoth et al., 2002). This is because in situations where multiple agents have been deployed against a single target species, usually only one is considered to have contributed to the programme’s success (Myers, 1985; Myers et al., 1989). The release of multiple agents is often referred to as the “lottery approach” because as more agents are introduced there is a greater chance that at least one of them will become successful (Myers, 1985). Also, there have been reports of competition between agents for plant resources, potentially leading to competitive exclusion of agents that would otherwise have been effective (e.g. Ehler and Hall, 1982). In the biocontrol programme against purple loosestrife, *Lythrum salicaria* (L.) (Lythraceae), in the USA, two leaf-feeding beetles *Galerucella calmariensis* (L.) and *G. pusilla* (Duftschmidt) (Coleoptera: Chrysomelidae) typically cause extensive defoliation which results in reduced flowering and thus food shortages for the flower-feeding beetle *Nanophyes marmoratus* Goeze (Coleoptera: Nanophyidae) (Denoth et al., 2002; Winston et al., 2014).

Successful control of *S. mauritianum* will depend on an agent(s) that is capable of reducing the weed’s excessively high fruit set (Olckers and Hulley, 1989; Olckers, 1996; Henderson, 2007; Olckers, 2011; Jordaan and Downs, 2012a). Other agents will need to supplement this damage and it is important that these do not hinder the impact of the fruit-reducing agent(s). Although the leaf-sucking lace bug *Gargaphia decoris* Drake (Hemiptera: Tingidae) is able to reach outbreak levels and cause substantial defoliation that can also reduce flowering and fruit set (and even kill plants), these have proved to be seasonal and sporadic (Olckers, 2009, 2011). It is thus unlikely that *G. decoris* will interfere with populations of *A. santacruzi* and
prevent them from reaching the high densities that are needed to offset seed production. The most successful biocontrol programmes are not those that release the most agent species or necessarily those that locate an agent that single-handedly controls the weed, but rather those that find the correct combination of agents that work together effectively (Hoffmann and Moran, 1998). The release of an agent that is able to reduce a weed’s reproductive output is often regarded as an important first step in achieving biocontrol success (Hoffmann and Moran, 1998) and this is what prompted the introduction of *A. santacruzi* against *S. mauritianum*.

4.5 *Anthonomus santacruzi* as a biological control agent of *S. mauritianum*

The contribution of the flowerbud weevil *T. lativentre* towards the successful control of *S. punicea* in South Africa inspired the release of *A. santacruzi* against *S. mauritianum*. Although both weed species are similarly short-lived, persisting for around 15 years in the field, there are fundamental differences in their phenology which could prevent *A. santacruzi* from achieving the same levels of success. In particular, *S. punicea* populations flower for a limited period in the summer (Hoffmann and Moran, 1998) whereas *S. mauritianum* flowers all year round (Olckers, 2011). While *T. lativentre* populations are well synchronized with *S. punicea* flowering, thereby infesting a high proportion of the floral output, this is currently not the case with *A. santacruzi*. Although *A. santacruzi* populations displayed some response to an increase in floral output by *S. mauritianum*, there was a consistent seasonal effect with population peaks during autumn and decreases during winter. Given the year-round availability of *S. mauritianum* flowers (Campbell and Van Staden, 1983; Henderson, 2001; Witkowski and Garner, 2008), weevil populations need to inflict consistently high levels of floral damage to be effective in reducing fruit set. Since *A. santacruzi* has been established in the field in South Africa for only a few years, the low levels of floral damage currently recorded may be an effect of insufficient time since release.

The positive correlation between weevil numbers and floral availability is, however, a positive sign that suggests that, with more time, population densities could build up to a point where floral damage is more effective. The lack of any evidence of interference by native predators (notably ants associated with *S. mauritianum* inflorescences) or parasitoids may also facilitate weevil population increases. However, there were indications that the weevils are less effective at the cooler, higher altitude inland sites where they appear to be
constrained by cold temperatures. *Anthonomus santacruzi* was originally collected from areas of comparatively lower altitude in Argentina, generally not higher than 200 m (Olckers, 2011), which could explain why it may be less suited to colder areas at higher altitudes. The reasons for the fluctuations in the weevil’s seasonal abundance are also unclear (e.g. host plant quality) and although weevils were recovered throughout the year, it is unclear whether (and where) the adults overwinter. Although, *G. decoris*, which has been established in the field for some 15 years (Olckers, 2011), was encountered at many of the *A. santacruzi* monitoring sites, their densities were too low to have any negative effect on the weevil or augment its damage.

The Working for Water Programme has been mechanically clearing *S. mauritianum* from watercourses in South Africa for more than 20 years (Witkowski and Garner, 2008). The weed is also routinely cleared by other agencies and landowners, since bugweed is one of the more notorious weed species in South Africa. Normally these activities are disruptive to biocontrol agents, as has been observed with *G. decoris* at several sites (Olckers, 2011). Since *A. santacruzi* has better dispersal abilities and a shorter life cycle than *G. decoris* (Olckers, 2003, 2011), the weevils may be less affected by clearing operations. Indeed, during this study, plants at the Mkondeni, Umlaas Road: Tala and Hillcrest sites were cleared without any major impact on *A. santacruzi* populations. Nevertheless, clearing operations need to be properly integrated with biocontrol to minimize disruption of agent populations.

4.6 Conclusion and recommendations

Flower-feeding agents can be an important component of biocontrol programmes targeting weeds with high reproductive outputs, as evidenced by the programme against *S. punicea* in South Africa. However, these need to inflict very high levels of floral damage (Hoffmann and Moran, 1998) in order to have a substantial impact. The numbers of *A. santacruzi* and levels of infested flowerbuds were low at all sites in KwaZulu-Natal that were surveyed and, compared to the floral output of *S. mauritianum*, the impact of the weevil is currently negligible. However, *A. santacruzi* was released only seven years ago (Olckers, 2011) and has been established in the field in KwaZulu-Natal for even less time. In contrast, *S. mauritianum* has been regarded as an invasive weed in many parts of South Africa for more than 100 years (Wright, 1904; Harding, 1938; Roe, 1972; Olckers and Zimmermann, 1991). It is thus likely that *A. santacruzi* populations need more time to build up and spread
throughout the range of *S. mauritianum* in KwaZulu-Natal where releases were first carried out.

Based on the results of this study, the following recommendations are made:

(i) Additional studies are required to investigate some ecological aspects of *A. santacruzi*, notably the factors that are responsible for the patterns of seasonal abundance that were observed.

(ii) The influence of climatic factors (notably temperature) on the establishment success of *A. santacruzi* should be confirmed with field surveys that are conducted at inland and coastal sites, during periods when weevil population densities are at their highest (i.e. April/May). Temperature-tolerance trials should also be conducted to determine the weevil’s thermal limits.

(iii) In the interim, further releases of *A. santacruzi* should best be confined to coastal or low altitude regions in order to maximize establishment success and population proliferation.

(iv) The patterns described in this thesis (e.g. low weevil population densities and levels of floral damage) should be confirmed by future field studies, once populations of *A. santacruzi* have been established over a longer time frame.
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Appendix I: List of flower-feeding arthropods released as biological control agents of invasive alien plants worldwide.

<table>
<thead>
<tr>
<th>Plant species Origin</th>
<th>Agent species</th>
<th>Feeding guild</th>
<th>Countries where released</th>
<th>Status</th>
<th>Damage inflicted</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acacia cyclops A. Cunn. ex G. Don (Fabaceae) Australia Substantial</td>
<td>Dasineura dielsi Rübsaamen (Diptera: Cecidomyiidae)</td>
<td>Flower galler</td>
<td>SA</td>
<td>Established</td>
<td>Extensive</td>
<td>Adair, 2005; Impson et al., 2009, 2011</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>NZ</td>
<td>Established</td>
<td>Unknown</td>
<td>Winston et al., 2014</td>
</tr>
<tr>
<td>Centaurea diffusa Lam. (Asteraceae) Eurasia Substantial</td>
<td>Larinus minutus Gyllenhal (Coleoptera: Curculionidae)</td>
<td>Flower feeder</td>
<td>USA, Can</td>
<td>Established</td>
<td>Extensive</td>
<td>Story et al., 2006; Winston et al., 2014</td>
</tr>
<tr>
<td></td>
<td>Larinus obtusus Gyllenhal (Coleoptera: Curculionidae)</td>
<td>Flower feeder</td>
<td>USA</td>
<td>Established</td>
<td>Unknown</td>
<td>Winston et al., 2014</td>
</tr>
<tr>
<td>Centaurea jacea L. ssp. pratensis W.D.J. Koch Celak (Asteraceae) Europe Unknown</td>
<td>Larinus minutus Gyllenhal (Coleoptera: Curculionidae)</td>
<td>Flower feeder</td>
<td>USA</td>
<td>Established</td>
<td>Trivial</td>
<td>Winston et al., 2014</td>
</tr>
<tr>
<td></td>
<td>Larinus obtusus Gyllenhal (Coleoptera: Curculionidae)</td>
<td>Flower feeder</td>
<td>USA</td>
<td>Established</td>
<td>Considerable</td>
<td>Winston et al., 2014</td>
</tr>
<tr>
<td>Centaurea jacea L. ssp. jacea (Asteraceae) Europe Unknown</td>
<td>Larinus obtusus Gyllenhal (Coleoptera: Curculionidae)</td>
<td>Flower feeder</td>
<td>USA</td>
<td>Established</td>
<td>Considerable</td>
<td>Winston et al., 2014</td>
</tr>
<tr>
<td><strong>Centaurea jacea</strong> L. ssp. nigra L. Bonnier and Layens (Asteraceae) Europe Unknown</td>
<td><strong>Larinus obtusus</strong> Gyllenhal (Coleoptera: Curculionidae)</td>
<td>Flower feeder USA</td>
<td>Established Considerable</td>
<td>Winston et al., 2014</td>
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<td><strong>Centaurea solstitialis</strong> L. (Asteraceae) Southern Europe (Mediterranean) Negligible</td>
<td><strong>Bangasternus orientalis</strong> Capiomont (Coleoptera: Curculionidae)</td>
<td>Flower feeder USA</td>
<td>Established Trivial</td>
<td>Gutierrez et al., 2005; Winston et al., 2014</td>
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<td><strong>Centaurea stoebe</strong> L. sl. (Asteraceae) Eurasia Unknown</td>
<td><strong>Larinus curtus</strong> Hochhut (Coleoptera: Curculionidae)</td>
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<td>Established Moderate</td>
<td>Sobbian and Fornasari, 1994, Wilson et al., 2003</td>
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<td><strong>Centaurea virgate</strong> Lam. ssp. squarrosa Boiss. Gugler (Asteraceae) Eurasia Unknown</td>
<td><strong>Larinus minutus</strong> Gyllenhal (Coleoptera: Curculionidae)</td>
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<td>Winston et al., 2014</td>
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<td><strong>Cirsium arvense</strong> L. Scop. (Asteraceae) Eurasia Substantial</td>
<td><strong>Larinus planus</strong> Fabricius (Coleoptera: Curculionidae)</td>
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<td>Louda and O’Brien, 2001; Winston et al., 2014</td>
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<td>Insect</td>
<td>Pest Type</td>
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<td>Gordon, 1999; Gordon and Fourie, 2011</td>
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<td><em>Schrad. and J.C. Wendl.</em></td>
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<td><em>Lantana camara</em> L.</td>
<td><em>Aceria lantanae</em> Cook</td>
<td>Flower gallers</td>
<td>Aus, SA, USA</td>
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<td>Extensive (some varieties)</td>
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<td>Established</td>
<td>Unknown</td>
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<td>Flower gallers</td>
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<td>MI</td>
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<td>Moderate</td>
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<td><em>Lantanophaga pusillidactyla</em> Walker (Lepidoptera: Pterophoridae)</td>
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<td>HK, Mya, NZ</td>
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<td>Unknown</td>
<td>Zalucki et al., 2007</td>
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<td>Flower feeder</td>
<td>Aus, Mic, Fij, SA</td>
<td>Established</td>
<td>Trivial</td>
<td>Winston et al., 2014</td>
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<td>Winston et al., 2014</td>
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<td>Aus, Mic, Fij, SA</td>
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<td>Fij, Haw</td>
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<th>Flower feeder</th>
<th>USA, Can</th>
<th>Established</th>
<th>Trivial</th>
<th>Winston et al., 2014</th>
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<td>Eurasia</td>
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<td><strong>Lythrum salicaria L.</strong></td>
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<td>**Mimosa pigra L. (Mimosaceae)</td>
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<td>South America</td>
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<td><strong>Senecio jacobaea L.</strong></td>
<td><em>Tyria jacobaeae</em> L. (Lepidoptera: Erebidae)</td>
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<td><strong>Sesbania punicea</strong></td>
<td><em>Trichapion lativentre</em> Béguin- Billecocq</td>
<td>Flowerbud and leaf feeder</td>
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1Refer to definition of terms (degree of control)
2Refer to country abbreviations
3Refer to definition of terms (damage inflicted)
**Definition of terms** (after Klein, 2011)

Degree of control – The effectiveness of the agent in reducing the numbers or spread of the target plant

- Complete: Agent has completely controlled the plant; no other control methods necessary
- Substantial: Most control accomplished by the agent; other control methods still required
- Negligible: Agent not shown to be effective in controlling the target plant; still able to spread or no reduction in numbers
- Unknown: No information given on the effectiveness of the agent or the agent is still under investigation

Damage inflicted – Determined by the percentage of flowers damaged by the agents upon emergence

- Extensive: High levels of damage; few plants survive
- Considerable: More than 50% of seed production reduced
- Moderate: Less than 50% of seed production reduced
- Trivial: Few flowers are damaged
- Unknown: Agent has not been evaluated yet

**Country abbreviations:**

| AI | Ascension Islands | Mau | Mauritius | SH | St. Helena |
| Aus | Australia | MI | Marshall Islands | SI | Solomon Islands |
| Can | Canada | Mic | Micronesia | Swa | Swaziland |
| Fij | Fiji | Mya | Myanmar | Tan | Tanzania |
| FP | French Polynesia | Nam | Namibia | Tha | Thailand |
| Gha | Ghana | NC | New Caledonia | TL | Timor Lester |
| Gua | Guam | Niu | Niue | Ton | Tonga |
| Haw | Hawaii | NMI | Northern Mariana Islands | Uga | Uganda |
| HK | Hong Kong | NZ | New Zealand | USA | United States of America |
| Indi | India | Pal | Palau | Van | Vanuatu |
| Indo | Indonesia | Phi | Philippines | Zam | Zambia |
| Ken | Kenya | PNG | Papua New Guinea | Zim | Zimbabwe |

| Mad | Madagascar | SA | South Africa | Sam | Samoa |